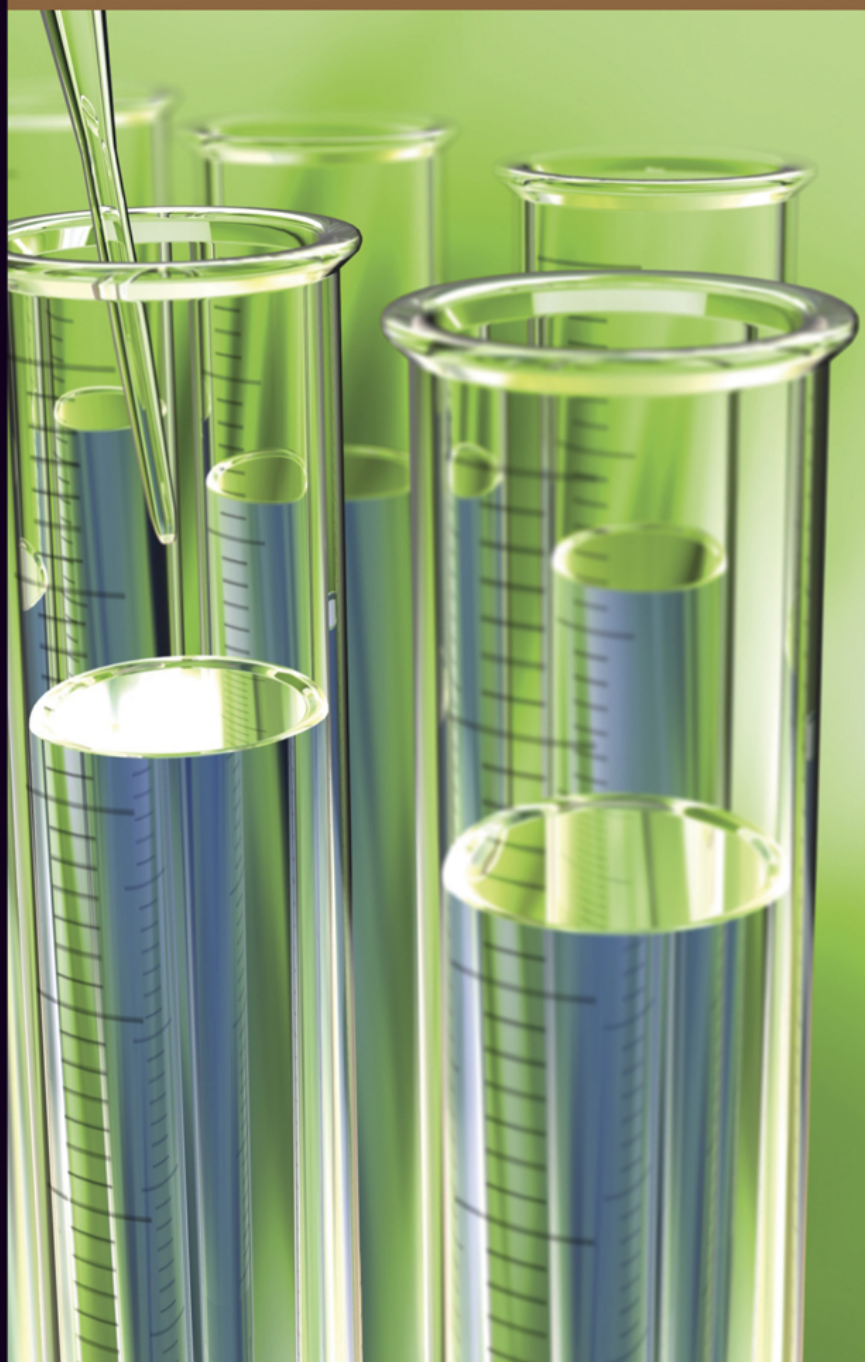
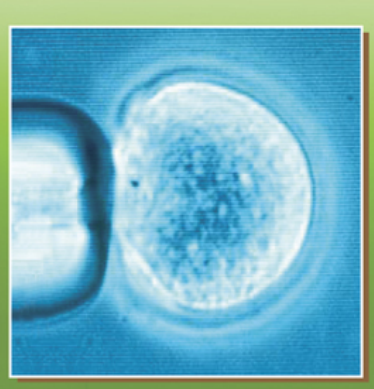
FACTS ON FILE SCIENCE LIBRARY

ENCYCLOPEDIA OF LIFE SCIENCE

KATHERINE CULLEN, PH.D.





ENCYCLOPEDIA OF LIFE SCIENCE

VOLUME I

ENCYCLOPEDIA OF
LIFE SCIENCE

VOLUME I

KATHERINE CULLEN, Ph.D.

Facts On File
An imprint of Infobase Publishing

ENCYCLOPEDIA OF LIFE SCIENCE

Facts On File, Inc.
An imprint of Infobase Publishing
132 West 31st Street
New York NY 10001

Library of Congress Cataloging-in-Publication Data

Cullen, Katherine E.
Encyclopedia of life science / Katherine Cullen.
p. cm.
Includes bibliographical references and index.
ISBN-13: 978-0-8160-7008-4
ISBN-10: 0-8160-7008-3
1. Life sciences—Encyclopedias, Juvenile. I. Title.
QH309.2.C85 2009
570.3—dc22 2008016224

Facts On File books are available at special discounts when purchased in bulk quantities for businesses, associations, institutions, or sales promotions. Please call our Special Sales Department in New York at (212) 967-8800 or (800) 322-8755.

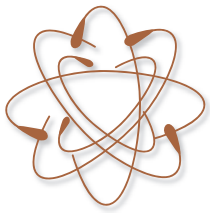
You can find Facts On File on the World Wide Web at http://www.factsonfile.com

Text design by Annie O'Donnell
Illustrations by Richard Garratt, Chris and Elisa Scherer, Melissa Ericksen, and Sholto Ainslie
Photo research by Suzanne M. Tibor

Printed in China

CP Hermitage 10 9 8 7 6 5 4 3 2 1

This book is printed on acid-free paper.



Contents

Acknowledgments vii

Introduction viii

Entries Categorized by National Science Education Standards for Content (Grades 9–12) x

Entries A–Z 1

Feature Essays:

"The Colony Collapse Disorder Crisis for Honeybees" 15
by Zachary Huang, Ph.D.

"Bioinformatics: Sequence Alignment Algorithms" 84
by Jason S. Rawlings, Ph.D.

"Structure-Function Relationships in Life Science" 100
by Lisa M. Dorn

"Partners in Protection: Cooperative Approaches to Protecting Wide-Ranging Species" 222
by Rachel Mazur, Ph.D.

"The Effects of Global Change on Tropical Forests" 296
by Kenneth J. Feeley, Ph.D.

"Adaptation or Not: How to Tell the Difference" 334
by James Wagner, Ph.D.

"Gene Therapy and Cystic Fibrosis" 372
by Caleb Hodson, Ph.D.

"Epigenetics: Regulation above the Genome" 473
by Alexandra Silveira, Ph.D.

"The Biology of ADHD" 576
by Peggy Shadduck Palombi, Ph.D.

"The Childhood Obesity Epidemic" 608
by Sharon Brown, Ph.D.

"Model Organisms for Life Science Research" 674
by Michael L. Goodson, Ph.D.

"Stem Cells: The Next Major Medical Breakthrough?" 750
by Jacob P. Harney, Ph.D.

Appendixes:

I. Chronology 763

II. Glossary 769

III. Further Resources 797

IV. Periodic Table of the Elements 802

V. Common Conversions from U.S. Customary to Metric Unit Values 804

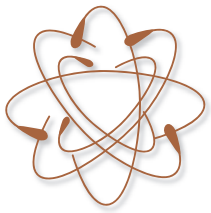
VI. Conversions within the Metric System 805

VII. Temperature Conversions 806

Index I-1

Acknowledgments

I would like to express appreciation to Frank K. Darmstadt, executive editor, for his critical review of this manuscript, wise advice, patience, and professionalism. Thank you to Richard Garratt, Melissa Ericksen, Chris Scherer, and Elisa Scherer who created the illustrations that accompany the entries in this work, and to Suzie Tibor, who performed the photo research. I would like to express my sincere gratitude to Ann E. Hicks for her constructive comments and suggestions regarding the entries throughout this text. The guest essayists deserve recognition for generously donating time to share their expert knowledge in the numerous highlighted areas of interest. The staff of the main branch of the Medina County District Library was extremely helpful in obtaining hundreds of documents and print resources to aid me in my research, as were Alison Ricker and Jennifer Schreiner in showing me around the impressive science library at Oberlin College and introducing me to the available electronic resources. Appreciation is also extended to the production and copyediting departments and the many others who helped in the production of this project. Thank you all.



Introduction

Encyclopedia of Life Science is a two-volume reference intended to complement the material typically taught in high school biology and in introductory college biology courses. The substance reflects the fundamental concepts and principles that underlie the content standards for life science identified by the National Committee on Science Education Standards and Assessment of the National Research Council for grades 9–12. Within the category of life science, these include the cell; the molecular basis of heredity; biological evolution; interdependence of organisms; matter, energy, and organization in living systems; and the behavior of organisms. The National Science Education Standards (NSES) also place importance on student awareness of the nature of science and the process by which modern scientists gather information. To assist educators in achieving this goal, other subject matter discusses concepts that unify the life sciences with physical science and Earth and space science: science as inquiry, technology and other applications of scientific advances, science in personal and social perspectives including topics such as natural hazards and global challenges, and the history and nature of science. A listing of entry topics organized by the relevant NSES content standards and an extensive index will assist educators, students, and other readers in locating information or examples of topics that fulfill a particular aspect of their curriculum.

Encyclopedia of Life Science provides historical perspectives, portrays science as a human endeavor, and gives insight into the process of scientific inquiry by incorporating biographical profiles of people who have contributed significantly to the development of the sciences. Processes that shape the natural world and life within it are also discussed. Instruments and methodology-related entries focus on the tools and procedures used by scientists to gather information, conduct experiments, and perform analyses. Other entries summarize the major branches and subdisciplines of life science or describe selected applications of the information and technology gleaned from life science research. Pertinent topics in all categories collectively convey the relationship between science and individuals and science and society.

The majority of this encyclopedia comprises more than 200 entries covering NSES concepts and topics, theories, subdisciplines, biographies of people who have made significant contributions to the life sciences, common methods, and techniques relevant to modern science. Entries average more than 2,000 words each (some are shorter, some longer), and most include a cross-listing of related entries and a selection of recommended further readings. In addition, one dozen guest essayists contributed essays covering a variety of subjects—contemporary topics of particular interest and specific themes common to the life sciences. Approximately 150 photographs and 150 line art illustrations accompany the text, depicting difficult concepts, clarifying complex processes, and summarizing information for the reader. A chronology outlines important events in the history of the field, and a glossary defines relevant scientific terminology. The back matter of *Encyclopedia of Life Science* contains a list of additional print and Web resources for readers who would like to explore the discipline further. Readers can find a periodic table of the elements and common metric and temperature conversions in the appendixes.

I have been involved in research and teaching life sciences for almost two decades. After obtaining my doctorate in molecular biology from Vanderbilt University, I became a postdoctoral fellow in the department of biochemistry at Chandler Medical Center of the University of Kentucky. After spending several years on the biology faculty at Transylvania University, I moved to northern Ohio, where I currently teach as a visiting faculty member at Oberlin College. Throughout the years I have taught numerous life science subjects, including general biology, microbiology, genetics, cell and molecular biology,

immunology, and human reproductive biology. My career has allowed me to continue to explore the exciting and constantly evolving life sciences. I hope that this encyclopedia serves you as a valuable reference, and that you will learn as much from referring to it as I have from writing it.

Entries Categorized by National Science Education Standards for Content (Grades 9–12)

When relevant, an entry may be listed under more than one category. For example, Sidney Altman, who studies RNA, is listed under both Life Science Content Standard C: The Molecular Basis of Heredity and Content Standard G: The History and Nature of Science. Biographical entries, topical entries, and entries that summarize a subdiscipline may all appear under Content Standard G: The History and Nature of Science when a significant portion of the entry describes a historical perspective of the subject. Subdisciplines are listed separately under the category Subdisciplines, which is not a NSES category, but are also listed under the related content standard category.

SCIENCE AS INQUIRY (CONTENT STANDARD A)

bioinformatics
cell culture
centrifugation
chromatography
cloning of DNA
data presentation and analysis
dissection
DNA sequencing
electrophoresis
metric system
microscopy
polymerase chain reaction
radioactivity
recombinant DNA technology
RNA interference
scientific investigation
scientific theory
spectrophotometry
X-ray crystallography

LIFE SCIENCE (CONTENT STANDARD C): THE CELL

aging
Altman, Sidney
biochemical reactions
biochemistry
biological membranes
biomolecules
Boveri, Theodor
Brock, Thomas
Calvin, Melvin
cancer, the biology of
cell biology
cell communication
cellular metabolism
cellular reproduction
chromosomes
Duve, Christian de
embryology and early animal development
enzymes
eukaryotic cells
gene expression
genetics
Golgi, Camillo
Ingenhousz, Jan
Just, Ernest
Leeuwenhoek, Antoni van
McClintock, Barbara
Mendel, Gregor
molecular biology
Morgan, Thomas Hunt
nutrition
organic chemistry, its relevance to life science
origin of life
Pasteur, Louis
Pauling, Linus
photosynthesis
plant form and function
Priestley, Joseph
prokaryotic cells
RNA interference
Schleiden, Matthias
Schwann, Theodor
Virchow, Rudolf
water, its biological importance
Wilmut, Sir Ian

LIFE SCIENCE (CONTENT STANDARD C): THE MOLECULAR BASIS OF HEREDITY

Altman, Sidney
Avery, Oswald
Boveri, Theodor
Cech, Thomas
Chargaff, Erwin
Chase, Martha
chromosomes
Crick, Francis
deoxyribonucleic acid (DNA)

Franklin, Rosalind
gene expression
genetics
genomes
Griffith, Frederick
Hershey, Alfred
inheritance
MacLeod, Colin Munro
McCarty, Maclyn
McClintock, Barbara
Mendel, Gregor
molecular biology
Morgan, Thomas Hunt
point mutations
RNA interference
sex determination
Stevens, Nettie
variation, genetic variation
Watson, James D.
Wilkins, Maurice H. F.

LIFE SCIENCE (CONTENT STANDARD C): BIOLOGICAL EVOLUTION

algae
Archaea
Aristotle
Bacteria (Eubacteria)
biodiversity
biological classification
botany
Brock, Thomas
Buffon, Georges-Louis Leclerc, comte de
Cuvier, Georges, Baron
Darwin, Charles
Dobzhansky, Theodosius
Eukarya
eukaryotic cells
evolutionary biology
evolution, theory of
fungi
Geoffroy Saint-Hilaire, Étienne
Gould, Stephen Jay
Gray, Asa
Haeckel, Ernst
history of life
Hooker, Sir Joseph Dalton
human evolution
Humboldt, Alexander von
Hyman, Libbie Henrietta
invertebrates
Ivanovsky, Dmitri
Lamarck, Jean-Baptiste
Linnaeus, Carl
Margulis, Lynn
microbiology
Miller, Stanley
origin of life
plant diversity
prokaryotic cells
protozoa
Prusiner, Stanley
slime molds
Thomson, Sir C. Wyville
variation, genetic variation
vertebrates
viruses and other infectious particles
Wallace, Alfred Russel
Woese, Carl
zoology

LIFE SCIENCE (CONTENT STANDARD C): THE INTERDEPENDENCE OF ORGANISMS

Bigelow, Henry
biodiversity
biogeochemical cycles
biogeography
biomes, aquatic
biomes, terrestrial
biosphere
Carson, Rachel
community ecology
conservation biology
ecology
ecosystems
endangered species
environmental concerns, human-induced
environmental science
Humboldt, Alexander von
hydrothermal vents
Margulis, Lynn
population ecology
Whittaker, Robert

LIFE SCIENCE (CONTENT STANDARD C): MATTER, ENERGY, AND ORGANIZATION IN LIVING SYSTEMS

anatomy
animal form
biochemical reactions
biochemistry
bioenergetics
biomolecules
cellular metabolism
chemical basis of life
circulatory system
digestive system
ecosystems
endocrine system
enzymes
eukaryotic cells
excretory system
Golgi, Camillo
Harvey, William
homeostasis
host defenses
human reproduction
immune system disorders
integumentary system
Malpighi, Marcello
musculoskeletal system
nervous system
nutrition
organic chemistry, its relevance to life science
photosynthesis
physiology
plant form and function
population ecology
prokaryotic cells
reproduction
respiration and gas exchange
sensation

LIFE SCIENCE (CONTENT STANDARD C): THE BEHAVIOR OF ORGANISMS

animal behavior
animal cognition and learning
Banting, Sir Frederick G.
cell communication
diabetes
endocrine system
ethology
Frisch, Karl von
homeostasis
human reproduction
Levi-Montalcini, Rita
Lorenz, Konrad
nervous system
nutrition
sensation
social behavior of animals
sociobiology
Tinbergen, Nikolaas
Turner, Charles Henry

SCIENCE AND TECHNOLOGY (CONTENT STANDARD E)

agriculture
antimicrobial drugs
assisted reproductive technology
bioinformatics

biological weapons
bioremediation
biotechnology
cloning of DNA
cloning of organisms
DNA fingerprinting
DNA sequencing
Fleming, Sir Alexander
forensic biology
gene therapy
genetic engineering
Human Genome Project
Mullis, Kary
polymerase chain reaction
radioactivity
recombinant DNA technology
RNA interference
vaccines
water and sewage treatment
Wilmut, Sir Ian

SCIENCE IN PERSONAL AND SOCIAL PERSPECTIVES (CONTENT STANDARD F)

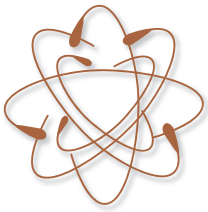
acquired immunodeficiency syndrome (AIDS)
addiction, the biology of
aging
agriculture
antimicrobial drugs
assisted reproductive technology
biodiversity
biogeochemical cycles
bioremediation
cancer, the biology of
cloning of organisms
conservation biology
diabetes
DNA fingerprinting
eating disorders
endangered species
environmental concerns, human-induced
Environmental Protection Agency
environmental science
epilepsy
gene therapy
genetic disorders
Human Genome Project
human reproduction
immune system disorders
infectious diseases
population ecology
sexual and reproductive health
vaccines
water and sewage treatment

HISTORY AND NATURE OF SCIENCE (CONTENT STANDARD G)

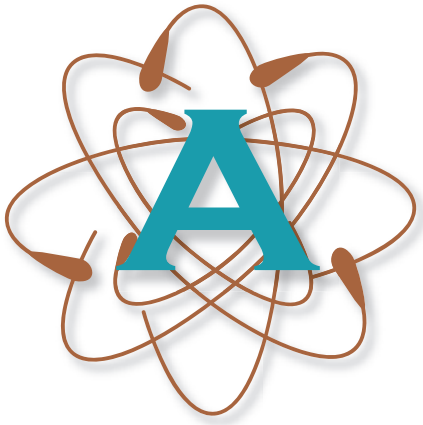
Altman, Sidney
anatomy
Aristotle
Avery, Oswald
Banting, Sir Frederick G.
Beebe, William
Bigelow, Henry
biochemistry
biogeography
botany
Boveri, Theodor
Brock, Thomas
Buffon, Georges-Louis Leclerc, comte de
Calvin, Melvin
Carson, Rachel
Cech, Thomas
cell biology
Chargaff, Erwin
Chase, Martha
Clark, Eugenie
cloning of organisms
Crick, Francis
Cuvier, Georges, Baron
Darwin, Charles
Dobzhansky, Theodosius
Duve, Christian de
Earle, Sylvia
ecology
evolution, theory of
Fleming, Sir Alexander
Franklin, Rosalind
Frisch, Karl von
genetics
Geoffroy Saint-Hilaire, Étienne
germ theory of disease
Golgi, Camillo
Gould, Stephen Jay
Gray, Asa
Griffith, Frederick
Haeckel, Ernst
Harvey, William
Hershey, Alfred
Hodgkin, Dorothy
Hooke, Robert
Hooker, Sir Joseph Dalton
Humboldt, Alexander von
hydrothermal vents
Hyman Libbie Henrietta
Ingenhousz, Jan
Ivanovsky, Dmitri
Just, Ernest
Koch, Robert
Lamarck, Jean-Baptiste
Leeuwenhoek, Antoni van
Levi-Montacini, Rita
Linnaeus, Carl
Lorenz, Konrad
MacLeod, Colin Munro
Malpighi, Marcello
Margulis, Lynn
marine biology
McCarty, Maclyn
McClintock, Barbara
Mendel, Gregor
Miller, Stanley
molecular biology
Morgan, Thomas Hunt
Mullis, Kary
Pasteur, Louis
Pauling, Linus
Priestley, Joseph
Prusiner, Stanley
RNA interference
Schleiden, Matthias
Schwann, Theodor
scientific investigation
spontaneous generation
Stevens, Nettie
Thomson, Sir C. Wyville
Tinbergen, Nikolaas
Turner, Charles Henry
Virchow, Rudolf
Wallace, Alfred Russel
Watson, James D.
Whittaker, Robert
Wilkins, Maurice H. F.
Wilmut, Sir Ian
Woese, Carl

SUBDISCIPLINES

anatomy
biochemistry
biogeography
bioinformatics
biology
botany
cell biology
conservation biology
ecology
environmental science
ethology
evolutionary biology
forensic biology
genetics
marine biology
microbiology
molecular biology
organic chemistry, its relevance to life science
physiology
sociobiology
zoology



Entries A–Z



acquired immunodeficiency syndrome (AIDS) Since it was first reported in 1981, acquired immunodeficiency syndrome (AIDS) has become a worldwide epidemic. Caused by the human immunodeficiency virus (HIV), the life-threatening disease affects the specific immune system, destroying the host's ability to fight infections and developing cancers. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) 2007 AIDS epidemic update, in 2007 an estimated 33.2 million people were living with HIV, 2.5 million people became newly infected, and 2.1 million people died of AIDS. Though a person can live unaffected by HIV for many years, most do eventually develop AIDS. No cure for AIDS currently exists, though drugs that help fight HIV infection and its associated diseases are available. Because the viral particles are present in the semen, vaginal secretions, and blood of infected persons, HIV is transmitted through intimate sexual contact, contact with tainted blood, or sharing contaminated needles or syringes during intravenous drug use. The virus can also cross the placenta during pregnancy and infect an unborn fetus and is secreted in breast milk. Avoiding all contact with contaminated bodily fluids is the only sure way to prevent infection.

Stark discrepancies exist in global trends for the HIV/AIDS pandemic. The UNAIDS estimates that 22.5 million people infected with HIV (68 percent of the global total), including 90 percent of the children infected with HIV, live in sub-Saharan Africa. In comparison, 1.3 million (4 percent of the global total) live in North America. In sub-Saharan Africa, 61 percent of affected adults are women compared with 50 percent globally, and in numerous countries, more than 20 percent of pregnant women visiting prenatal clinics are infected. Without treatment, 35 percent of the children born to those women will become infected. Antiviral therapy effectively reduces mother-to-child transmission, but only 5 percent of women receive this therapy. Across Asia and Eastern Europe, infections due to injected drug use and commercial prostitution are increasing. Socioeconomic factors such as women's being forced to have sex or the refusal of men to wear a condom during sex, particularly among married women whose husbands have extramarital sex, also contribute.

MOLECULAR BIOLOGY OF HIV

Two HIV variants exist, HIV-1 and HIV-2: the first is prominent in the United States and Europe whereas the second occurs mostly in West Africa. Both are enveloped ribonucleic acid (RNA) viruses that encode a total of nine genes. The envelope contains surface glycoproteins (gp) called spikes. One that is 160 kilodaltons (gp 160) can be broken down into two smaller fragments, one that is 120 kilodaltons (gp 120) and another that is 41 kilodaltons (gp 41). The gp 120 spike is easily shed by the virus and interacts with the CD4 receptor on the T lymphocytes to gain entry. The smaller glycoprotein, gp 41, is embedded in the envelope. HIV is a retrovirus, one of a family of viruses that reproduce by synthesizing deoxyribonucleic acid (DNA) from the RNA genome, then inserting that DNA into a host chromosome. In addition to the RNA genome, the viral particles contain specific enzymes inside their capsids, including reverse transcriptase, which can read an RNA template and synthesize DNA from it. As soon as the virus penetrates the host cell, the genome is converted to DNA that is subsequently inserted into the

host genome, where it can remain in the latent state for years. Stimulated by an unknown cause, the cell becomes activated and starts replicating the virus. Newly assembled virions are released and seek other cells to infect. This process destroys the T cells. At first, the body compensates by stepping up its synthesis of new T cells, but the virus can replicate faster than the body can replenish its T cells. The virus can also infect monocytes, macrophages, and B cells but does not kill these cells, so they serve as an additional and continual source of new virions.

SYMPTOMS

Within a few weeks of becoming infected, a person might develop flulike symptoms including a fever, headache, sore throat, and swollen lymph nodes. Since these symptoms are associated with numerous infections, the person might not be aware he or she is infected with HIV. Once inside the body, HIV attacks white blood cells, specifically T helper cells that have CD4 receptors. B and T lymphocytes mount a strong immune response and practically clear the virus from circulation, but the virus persists in the lymph nodes. During a period called clinical latency, years can pass without the infected person's experiencing any additional symptoms, but the number of healthy T lymphocytes decreases, until symptoms such as swollen lymph nodes, fever, diarrhea, weight loss, and fatigue develop and persist. HIV destroys the very cells whose job it is to fight the virus. The person loses the ability to fight infections and is said to have full-blown AIDS when he or she develops an opportunistic infection and has a CD4 T cell count of less than 200 per cubic millimeter of blood (normal range is between 500 and 1,800). An opportunistic infection is one that is caused by a microorganism that is pervasive in the environment and to which people are generally resistant, but that affects someone with an impaired immune system. Common opportunistic infections found in AIDS patients include toxoplasmosis, histoplasmosis, *Pneumocystis* pneumonia, herpes, hepatitis C, candidiasis, bacterial diarrheas, and tuberculosis. Because T lymphocytes are involved in fighting cancers, certain types of cancers including Kaposi's sarcoma, cervical cancer, and lymphoma are also frequently found in AIDS patients.

DIAGNOSIS AND TREATMENT

Infection with HIV is diagnosed by screening a sample of blood for the presence of antibodies against the virus. The enzyme-linked immunosorbent assay (ELISA) is one method that detects antibodies specific for viral proteins, but it takes a few weeks to get results. The production of a detectable level of antibodies by the specific immune system can take up to eight weeks after the initial exposure to the virus, so this test is not very informative if performed immediately after infection. Another procedure called a western blot analysis can detect the presence of HIV proteins in the blood and is used to confirm a positive antibody screen result. Two newer tests that can be performed in the doctor's office require a blood sample obtained from a finger prick or a swab sample of the fluids around the gum tissue. Results from these tests can be obtained in only 20 minutes.

Once infection is diagnosed, the physician will perform additional tests to assess the progression of the disease. One such test measures the viral load, the amount of viral particles present in the blood. A lower viral load correlates with a better prognosis for the patient.

When AIDS first appeared in the early 1980s, there were not any drugs to treat it and very few drugs to treat the numerous associated opportunistic infections. There is still no cure for AIDS and the drugs that are now available cause serious side effects and are enormously expensive, but the available treatments have increased the quality of life and the life expectancy or those infected with HIV. One category of drugs that targets HIV is the group that inhibits transcription of the viral genome. Reverse transcriptase inhibitor drugs work by inhibiting the enzyme and halting the life cycle of the virus. The nucleoside reverse transcriptase inhibitors are recognized by the enzyme, bind the active site, and are incorporated into DNA, but once incorporated, they do not allow additional nucleotides to become bonded to it. Azidothymidine (AZT), an example of a drug that blocks reverse transcriptase, was the first drug approved for treating HIV infection. Unfortunately, many strains of HIV have developed resistance to the drug. Nonnucleoside inhibitors bind the enzyme at a site other than the active site and inactivate it. Another group of drugs, the protease inhibitors, work by interfering with a viral protein called HIV protease. Without this protein the viral particles are not assembled properly and are noninfectious. One new type of drug are the fusion inhibitors. These work by inhibiting the viruses from fusing with the host cell membranes, stopping viral replication. In August 2007 the U.S. Food and Drug Administration approved a new drug called Selzentry that works by blocking a receptor, CCR_5, that the virus often uses to gain entry into the host's white blood cells. The long-term effects of this drug made by Pfizer are unknown. The recommended treatment is a cocktail, a combination of three or more drugs with different mechanisms of action. Called highly active antiretroviral therapy (HAART), this strict regimen is followed to overcome potential resistance. HIV mutates very rapidly, so it can quickly become resistant to a once-effective drug. The virus can only bear so many mutations at once

without losing its infectiveness. Another difficulty in developing an effective chemotherapeutic approach is the fact that HIV persists in resting memory T cells. Since the current drugs all slow or stop viral replication rather than inactivate all viral particles, complete eradication is not possible.

To date, no vaccine to prevent AIDS has been approved. Besides the problem of the rapid mutation rate for the virus, antibodies do not seem effective against HIV. Consider that diagnosis of infection is based on the presence of antibodies against HIV proteins. Diagnosed individuals produce antibodies, but the presence of antibodies does not prevent AIDS. Many scientists around the world are involved in the development of a preventative vaccine and a therapeutic vaccine to boost the immune system of HIV-positive individuals. Clinical trials are under way for both, but success does not seem very near.

Most recently, in January 2008, researchers from Harvard Medical School published results from a study led by the geneticist Stephen J. Elledge that led to the identification of 273 proteins that HIV requires for survival and replication in human cells. Previous studies had only identified 36 human proteins necessary for the virus to enter host cells and replicate; thus these new findings open the door for potential new drug targets.

See also HOST DEFENSES; IMMUNE SYSTEM DISORDERS; INFECTIOUS DISEASES; VIRUSES AND OTHER INFECTIOUS PARTICLES.

FURTHER READING

Bardham-Quallen, Sudipta. *AIDS*. Farmington Hills, Mich.: Thomson Gale, 2005.

Centers for Disease Control and Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Division of HIV/AIDS Prevention. "Living with HIV/AIDS." Available online. URL: http://www.cdc.gov/hiv/resources/brochures/livingwithhiv.htm. Updated June 21, 2007.

Libman, Howard, and Harvey J. Makadon, eds. *HIV*. 2nd ed. Philadelphia: American College of Physicians, 2003.

Marlink, Richard G., and Alison G. Kotin. *Global AIDS Crisis: A Reference Handbook*. Santa Barbara, Calif.: ABC-CLIO, 2004.

Matthews, Dawn D. *AIDS Sourcebook*. 3rd ed. Detroit: Omnigraphics, 2003.

MayoClinic.com. "HIV/AIDS." Available online. URL: http://www.mayoclinic.com/health/hiv-aids/DS00005. Updated June 20, 2008.

U.S. Department of Health and Human Services. "AIDS *info*." Available online. URL: http://aidsinfo.nih.gov/. Accessed January 14, 2008.

U.S. National Library of Medicine, National Institutes of Health. "HIV/AIDS Information." Available online. URL: http://sis.nlm.nih.gov/hiv.html. Updated July 16, 2008.

Watstein, Sarah Barbara, and Stephen E. Stratton. *The Encyclopedia of HIV and AIDS*. 2nd ed. New York: Facts On File, 2003.

addiction, the biology of

Although the definition of *addiction* is a compulsion to engage in any sort of behavior despite detrimental consequences (e.g., gambling, shopping, instant messaging), the term most commonly refers to physical dependence on a habit-forming chemical substance such as alcohol, heroin, or nicotine. Whereas an addict initially makes a choice whether or not to drink alcohol, use a drug, or smoke a cigarette, the phenomenon of addiction is a biological problem. Considered a disease, addiction stems from the attempt to avoid or overcome a negative consequence from not using the chemical substance. For example, smoking crack cocaine produces a pleasurable feeling called a high. After the drug wears off, the person experiences a low that another dose of the drug will prevent. Substance addiction not only changes a person's behavior, but also alters the cellular physiological characteristics of the brain in a manner that increases the body's tolerance to the substance and fosters continued use of it.

According to the National Institute on Drug Abuse (NIDA), the abuse of illegal drugs and alcohol and tobacco contribute to the deaths of more than 540,000 people in the United States every year. The psychiatric disorder of addiction impacts the lives of many more people, as children, spouses, siblings, friends, and parents of addicts suffer as well. Substance abuse negatively affects a person's health, but it can also damage a person's social life, family life, economic situation, and legal record. So why do people use potentially habit-forming substances? Reasons vary depending on the person and the situation, but common causes include desire to feel good, peer pressure, relief of anxiety or stress, for energy, for relaxation, out of curiosity, for pain relief, emotional problems, or mental illness. In the beginning users often feel they are in control and can stop using at any time, but the physiological changes that make quitting difficult can occur quickly.

Though no race, gender, ethnicity, socioeconomic status, religious affiliation, or other similar factor is impervious to addiction, chemical substances do not affect all individuals equally. Children do not inherit alcoholism or an addiction to pain pills, but evidence suggests that the tendency to become dependent on chemical substances runs in families. Certain genes have been found to play a role in addiction. According to the Genetic Science Learning Center at the

University of Utah, the following several studies have shown that certain genes play a role in addiction:

- A certain allele of the dopamine receptor is more common in people addicted to alcohol or cocaine.
- Expression of higher levels of the *Mpdz* gene in mice reduces withdrawal symptons from barbiturates.
- Absence of the serotonin receptor gene *Htr1b* is associated with increased attraction to cocaine and alcohol.
- Mice with low levels of neuropeptide Y or with a defective *Per2* gene drink more alcohol.
- Mice lacking the *Creb* gene develop morphine dependence less often.
- The reward response to morphine or cocaine is reduced in mice lacking the cannaboid receptor gene *Cnr1* or a gene encoding part of the nicotinic cholinergic receptors.

NIDA reports that between 40 and 60 percent of an individual's vulnerability to developing an addiction may be genetic. Thus two people who have taken the same drugs in the same doses the same number of times may not be equally affected. One may become addicted and the other not. Age does seem to play a role—people who start using a chemical substance as a child or young adult are more likely to become addicted to it. Scientists believe this partly relates to the fact that the brain continues to develop through adolescence into early adulthood. Besides biological risk factors, someone's upbringing, home situation, social factors, and other events that are going on in a person's life can also affect one's ability to control substance use.

DRUG TYPES

A drug is a chemical that affects the body's structure or function, and only some drugs are addictive. Addictive drugs fall into the following seven major classes:.

- Nicotine is a stimulant present in tobacco leaf and is found in cigarettes and chewing tobacco.
- Alcohol, barbiturates, benzodiazepines, and volatile substances used in sniffing can cause direct damage to the brain. Inhalants are particularly toxic and can damage the heart, kidneys, and lungs in addition to the brain. They are more harmful than addictive and can cause death within minutes.
- Opiates include opium, morphine, heroin (made by chemically treating morphine), codeine, and synthetic opiates used as painkillers (such as oxycodone and Demerol). These drugs cause a feeling of pleasure followed by a sense of well-being or calmness and are highly addictive.
- Cocaine and amphetamines are stimulants. The effects of cocaine are short-lasting; thus abusers often binge, or take several doses within a single session. Amphetamines cause feelings of euphoria and alertness. The effects last longer.
- Cannabis, including any of the preparations made from the herb hemp, such as marijuana or hashish, is the most commonly abused illicit substance. The effects are impaired memory and learning, inability to focus, and lack of coordination.
- Caffeine, most commonly found in coffee, tea, and soft drinks, is the only addictive substance whose use is not prohibited in children.
- Hallucinogens include many naturally occurring products (such as psilocybin in magic mushrooms and mescaline in cactus) and synthetic compounds (such as lysergic acid diethylamide [LSD], MDMA or ecstasy, and phencyclidine [PCP] or angel dust). These drugs cause mind-altering effects, and the effects are unpredictable. In the short term, blood pressure, body temperature, and heart rate all increase, and sweating, appetite loss, dry mouth, and tremors can also result.

Because nicotine, alcohol, and caffeine are legal and therefore more socially acceptable, the term *addict* is rarely used to refer to a person who habitually uses these substances. Society may refer to such a person as a heavy smoker, an alcoholic, or a heavy coffee drinker rather than an addict, but the biological causes and effects of the addiction to these substances and to prescribed medications are the same as for illegal substances.

Different drugs have different routes for entering the body: injection directly into a vein, a muscle, or underneath the skin; ingestion though the mouth; inhalation into the lungs; or intranasal, in which the person snorts the substance, and it enters the bloodstream through the nasal mucosa. Drugs that are inhaled, injected, or snorted generally produce an effect more rapidly than ingested substances, and their effects wear off more quickly. The difference in how one feels before and after using the substance is very noticeable. Because of this, inhaled or injected drugs are typically more addictive than ingested substances.

EFFECTS ON THE BRAIN

The brain is the least understood organ in the human body. Made up of neurons and supporting cells, the brain serves as the control center of the nervous system. The brain stem, located at the base of the back of the head, regulates many involuntary physiological activities such as the heartbeat and respiration. The cerebral cortex is the convoluted surface layer of the cerebrum, which is the largest portion of the brain. Different regions of the cerebral cortex are responsible for specific functions, such as sensory processing, thinking, making decisions, and problem solving. The limbic system is a group of structures including the hippocampus, the hypothalamus, and the amygdala that are located underneath the cortex and control emotions and feelings of pleasure and motivation. Drugs that alter mood target this area of the brain.

The neurons that carry sensory input to the brain, within the brain, and from the brain to different parts of the body communicate with one another through chemical signals. Individual neurons connect to other neurons to form neural pathways and networks. The space between the end of one neuron and the start of another is called the synapse. When stimulated to do so, a neuron will release chemicals called neurotransmitters into the synapse. By diffusion, the neurotransmitters will reach the next neuron in the pathway and bind to specific receptors on its cell membrane. The binding triggers a chain of events in the postsynaptic neuron that will cause a particular response depending on the type of neurotransmitter. The action of the neurotransmitter may be stimulatory or inhibitory and might trigger muscular contraction or the release of certain hormones, additional neurotransmitters, or other chemical substances that have a specific physiological effect—for example, an increase in heart rate or blood sugar levels or slowed reflexes. Shortly after the release of the neurotransmitter, transporters bind and carry the neurotransmitter back into the neurons that released it, or, in some cases, enzymes degrade the neurotransmitters. Either way, the signal is terminated.

One neurotransmitter that plays a key role in addiction is dopamine, which belongs to the family of three neurotransmitters called catecholamines because they consist of a six-carbon catechol ring and an amino group. The other two catecholamines are epinephrine and norepinephrine, and all are synthesized from the amino acid tyrosine. Dopamine plays a role in motor coordination, but evidence suggests that it also functions in motivation, reward, and behavior reinforcement. The latter function relates to the importance of dopamine in substance addiction.

Prescribed and illicit drugs both interfere with the natural brain chemistry described. A physician may prescribe a drug for medical reasons. For example, an inadequate response by the brain to stimulation by the neurotransmitter serotonin may result in a psychiatric disorder such as anxiety or depression. Treatment may involve medications (called SSRIs, for selective serotonin reuptake inhibitors) that inhibit the reuptake of serotonin by binding to the transporters that carry serotonin back into the presynaptic cell, giving the serotonin a longer period to stimulate the receptors on the postsynaptic neurons. Another example of a medical use for drugs may be to prevent the perception of pain by the nervous system during surgery.

Addictive drugs work by either mimicking or blocking the effect of a natural neurotransmitter or by increasing the levels of neurotransmitter in a neural synapse. Drugs that mimic natural neurotransmitters resemble the neurotransmitter structurally such that the specific target receptors bind the drug and activate the postsynaptic neuron. By this mechanism, a drug can induce a response in the absence of the normal physiological stimulus. The effect of the drug, however, can differ from that of the natural neurotransmitter; for example, the duration may be longer or other neural pathways may be activated simultaneously, leading to an altered combined effect. Opiates are an example of a type of addictive drug that works by mimicking the structure of a natural neurotransmitter. Endogenous opioids (i.e., endorphins, enkephalins, dynorphins) are natural neural polypeptides that bind to pain receptors located along sensory pathways. At the point where the neural pathway reaches the spinal cord of the central nervous system, neurons release a peptide neurotransmitter called neurokinin that stimulates the pain pathway leading to the brain, where the stimulus is perceived as pain. The receptors that normally bind the endogenous opioids also recognize and bind morphine and other opiates. When these drugs bind, they block the pain pathway at the spinal cord. These drugs also work in the brain by causing an indifference to pain, so the patient may be aware of pain but not care about it. Another mechanism by which drugs act is by stimulating the inappropriate release of higher than normal levels of an endogenous neurotransmitter or preventing its reuptake. Either of these mechanisms results in an amplified signal to the postsynaptic neuron. Amphetamines and cocaine work in this manner.

In order for a person to seek and administer a drug without a medical reason, the drug must generate an enjoyable effect: in other words, the result must reinforce the habit of use. Neurobiologists have

identified a specific area deep within the middle of the brain that produces a pleasurable and satisfying feeling when electrically stimulated. When electrodes were placed in different locations within rat brains, and the rats had access to a lever that stimulated the electrodes when pressed, the rats repeatedly pressed the lever when the electrodes penetrated this so-called reward pathway. This experimental method is called intracranial self-stimulation (ICSS), and similar experiments have been performed on humans during research on epilepsy. The tract of nerves believed to be stimulated is called the mesolimbic dopaminergic pathway. The neurons originate in an area called the ventral tegmental area (VTA) and extend into the nucleus accumbens (NAc) of the frontal cortex. When stimulated, the participating neurons release dopamine in the frontal cortex, where dopamine receptors are located. All addictive drugs, whether acting directly or indirectly, have been shown to increase synaptic dopamine levels in the NAc region of the brain; thus this phenomenon may explain the powerful reinforcement that leads to addiction, which can be viewed as self-initiated stimulation of this dopaminergic pathway. Scientists have also found evidence that blocking the dopamine receptors in the NAc prevents the reinforcement effect. This pathway normally functions in reinforcing behaviors that have positive survival and reproductive value, such as eating palatable food and engaging in behaviors that promote successful mating. Conditions such as hunger and sexual arousal increase the motivation and the likelihood of carrying out behaviors that will result in satiating this pathway. Addictive drugs are believed to act in the same way—when taken, they stimulate this reward pathway, and the early symptoms of drug withdrawal cue the behaviors that will result in the consequence of seeking and obtaining the drug.

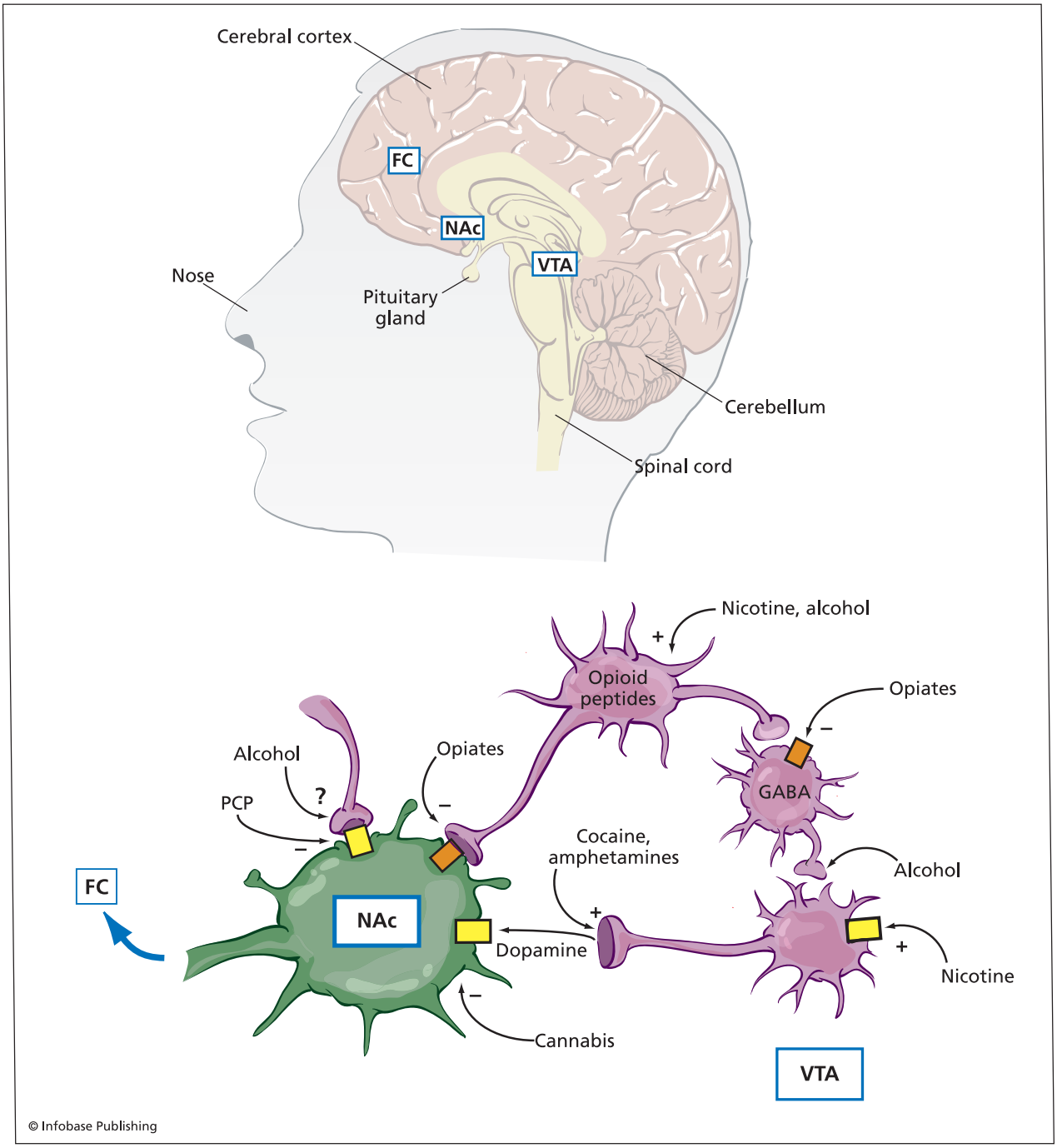
The location of action of many addictive drugs has been mapped. Cocaine acts at the end of the neurons that release dopamine by blocking the transporters that normally return the neurotransmitter to the interior of the cell. Some evidence suggests that cocaine may also act elsewhere, but how and where are not known. Amphetamines enter dopamine neurons through the reuptake transporters and cause dopamine to be released into the synapse. One long-term effect of overstimulation by dopamine from prolonged drug use is a decreased production of dopamine. Opiates act earlier in the pathway on inhibitory receptors called mu opioid receptors, and they prevent the release of other neurotransmitters. The specific effect depends on the particular neurotransmitter. For example, this may result in the inhibition of the release of a neurotransmitter that controls or limits dopaminergic neuron stimulation. Nicotine and alcohol may activate the reward pathway at the VTA by either stimulating the release of natural opioid peptides or directly stimulating dopaminergic neurons. Cannabis can act in the VTA or directly on the NAc. Hallucinogens, such as PCP, can also act directly on the NAc.

After repeated administration, an individual may develop homeostatic adaptations such as tolerance, or reduction in the response to the drug. The brain adjusts to the continued exposure of a drug to the point where the stimulus eventually is unnoticed, just as a person who buys a house near a railroad track adjusts to the whistle of oncoming trains. As one's tolerance for a drug increases, the individual must use higher dosages to achieve the same desired effect. This is due to physiological and biochemical changes that result from the flooding of dopamine in certain areas of the brain. These changes can affect areas that control judgment, decision making, learning and memory, and behavior control.

Neuroscientists have characterized the long-term physiological changes that chronic use of some drugs causes. For example, chronic morphine use increases the levels of certain cellular enzymes (adenylyl cyclases) that play a role in cellular signaling. These changes have a significant effect on cellular physiology and increase the intrinsic excitability of some neurons.

ENDING THE ADDICTION

In addition to struggling with the compulsion to continue using an addictive substance, an individual trying to stop taking a drug often suffers a range of negative physiological and emotional symptoms. In some cases, such as with opiates, the nerve cells in the brain become so used to the presence of the drug that they cannot function normally in its absence—the cells become overactive in an attempt to compensate. When the individual has developed a physical dependence, stopping the drug abruptly can cause withdrawal sickness. The urge to take another dose becomes very intense as the levels of the addictive chemical in the blood gradually decrease during the withdrawal period. The nature and intensity of the withdrawal symptoms vary depending on the specific drug and the synapses and neural circuits affected, but the range includes increased heart rate, increased blood pressure, sweating, tremors, depression, insomnia, fatigue, irritability, nausea, restlessness, general malaise, and muscle pain. In the case of alcohol, barbiturates, and benzodiazepines, abrupt withdrawal can be fatal. In most cases, though the withdrawal period may cause extreme discomfort, pain, or misery, the body does eventually recover. Images of brains of methamphetamine addicts show that



Many addictive drugs are known to act on the mesolimbic dopaminergic pathway, also called the reward pathway, that naturally plays a role in reinforcing certain behaviors that aid in survival.

although the amount of dopamine transporter was significantly lower than in a normal, healthy brain, the levels appeared near normal after 14 months of abstinence. Even after detoxification is complete—meaning all traces of the drug and its metabolites are absent from the body—a recovering addict must also deal with or change behavioral patterns and environmental conditions that contributed to the development of the addiction. This process may take months, years, or a lifetime.

Addiction is a treatable but chronic disease—relapses occur among 40 to 60 percent of drug-addicted patients. The most effective treatment includes a combination of medication (when available) and behavioral therapy. The goal of some medications is to reduce the withdrawal symptoms so

the individual is more likely to continue abstaining from use. Other medications help reduce the cravings for the drug while the brain gradually adjusts to its absence.

Recent research that has demonstrated a physiological basis for substance addiction has changed the way society views addicts and health care professionals approach treating addiction. Though much progress has been made in understanding the chemical and neural effects of addictive chemicals, the molecular, cellular, systems, and behavioral levels are still unknown. Medical researchers are also actively investigating novel pharmacological agents that have the potential to help addicts gain control of their compulsions, reduce the effects of withdrawals, and change the brain chemical processes to alter the effect of the substance to make it undesirable. Because prevention is the best strategy for dealing with addiction, increased educational efforts about the harmful nature of drugs and dangers of addiction must accompany the improved understanding of the biology of addiction if the number of lives that are affected by addiction is to be decreased.

See also CELL COMMUNICATION; NERVOUS SYSTEM.

FURTHER READING

Goldstein, Avram. *Addiction: From Biology to Drug Policy*. 2nd ed. New York: Oxford University Press, 2001.

Hyman, Steven E., Robert C. Malenka, and Eric J. Nestler. "Addiction: The Role of Reward-Related Learning and Memory." *Annual Review of Neuroscience* 29 (2006): 565–598.

National Institute on Drug Abuse home page. Available online. URL: http://www.nida.nih.gov/. Accessed January 22, 2008.

U.S. Department of Human Health and Human Services. Substance Abuse and Mental Health Services Administration home page. Available online. URL: http://www.samhsa.gov. Updated January 22, 2008.

aging Aging is the normal process of growing older. Numerous adverse effects often accompany the aging process. Some signs of aging, such as wrinkles, hair loss, or graying hair, may not be desirable, but they do not have any deleterious effects on someone's health. On the other hand, harmful conditions and diseases, such as arthritis, Alzheimer's disease, cataracts, cancer, bone fractures, hypothermia, forgetfulness, cardiovascular disease, high blood pressure, strokes, slowed reflexes, decreased strength, vision and hearing loss, decreased immune system function, and osteoporosis, occur more frequently as individuals age. Some of these develop as a consequence of the accumulation of years of damage from poor health habits including improper diet, drug or alcohol misuse, or lack of exercise, but many healthy people who have lived exemplary lifestyles and followed preventative health care recommendations still experience problems commonly associated with aging. Because deterioration in cell number and function manifests itself in symptoms experienced at the organism level, gerontology, the study of aging and age-related problems, involves research on molecules, cells, tissues, and organ systems. Research on the complex biological nature of aging and age-related diseases is active. By studying aging at several levels, gerontologists hope to achieve a better understanding of the causes of aging and perhaps learn how to delay or prevent the common adverse effects.

Senescence, the progressive deterioration caused by biochemical and physical changes associated with aging, increases the risk of mortality as one gets older. Effects of senescence accumulate, affecting body functions, and eventually result in death. According to death registration data collected by the Centers for Disease Control and Prevention and the National Center for Health Statistics, a person born in the United States in the year 1900 had a life expectancy of 47.3 years. That number rose to 77.8 years for people born in the year 2004. While expecting such a large jump over the course of the 21st century seems unrealistic, life expectancies continue to rise slowly as medical researchers learn more about the chronic diseases that ultimately lead to death.

PROGRAMMED THEORIES OF AGING

Gerontologists believe that, to some degree, biochemical, genetic, and physiological characteristics all contribute to the aging process. Many intrinsic factors seem to play a role in determining life span. Just as hormonal and other chemical signals control and coordinate other life cycle stages including embryogenesis, development, and puberty, they may also bring about senescence. Genes also clearly play a role, as different animal species have different average life spans. The gastrotrich, a type of aquatic animal, lives only three days, shrews live slightly more than one year, while giant tortoises live an average of 177 years. In addition, evidence suggests that longevity runs in families. Individuals with parents and grandparents who lived long lives have a greater probability of living long lives themselves. Environmental and ecological factors also contribute to aging and affect life span; extrinsic factors such as climate, food availability, and the niche an organism fills in an ecosystem affect an animal's survival. Gerontologists study all the factors that affect one's life span, but only recently have they begun to make significant advances toward understanding the intrinsic

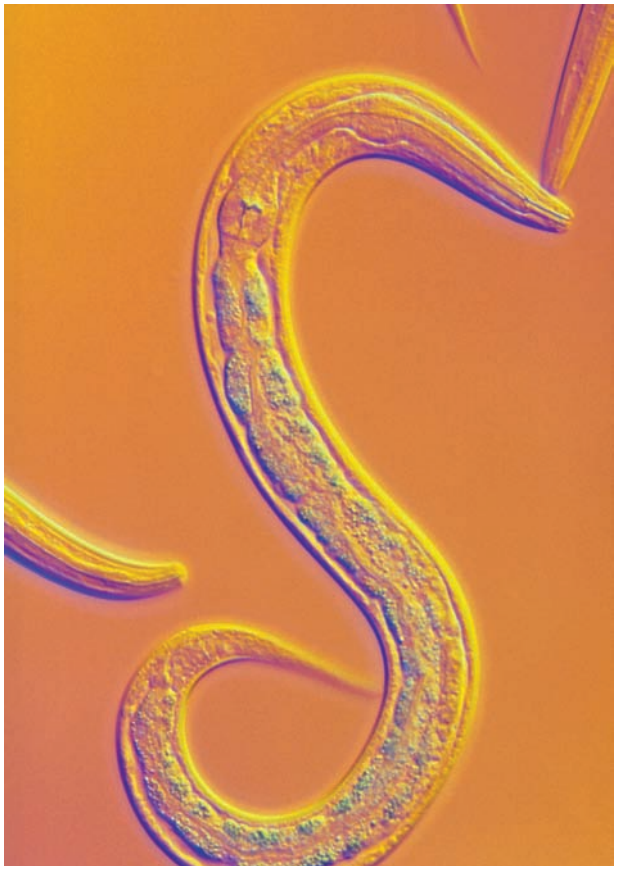
NORMAL EFFECTS OF AGING

What Is Affected	General Change
arteries	lose elasticity, require more force to move blood through circulation
bladder	capacity declines
body fat	gradually increases until middle age, stabilizes until late in life, then decreases
bones	bone mineral is lost; bones weaken
brain	loss of some axons, diminished function
hearing	decline in hearing
heart	muscle thickening, diminished function
kidneys	less efficient at filtering wastes from blood
lungs	vital capacity decreases
muscles	muscle mass declines in absence of regular exercise
sight	difficulty focusing on near or fine objects, increased susceptibility to glare
skin	becomes thinner and loses elasticity

mechanisms of the physical decline associated with aging—in other words, the cellular events that lead to the symptoms characteristic of aging. Different theories proposed to explain aging generally fall into one of two categories: programmed theories or error theories. The programmed theories have in common the basic notion that biology controls the life span of a cell.

As a starting point, researchers have identified several genes that appear to affect longevity. One way to approach this avenue of investigation is to look for genes that are expressed either more or less as an organism grows older. One gene, *lag-1,* characterized in yeast, affects the number of cell divisions yeast can undergo before dying off. The average number of generations is about 21, but yeast that expressed higher than normal quantities of this gene averaged 28 generations. Current research aims to clone the related gene in humans and to study the function of the gene product. Overexpression of the *sir2* gene, which functions to stabilize DNA in yeast, increases the life span of fruit flies. Extrinsic factors purported to increase life span, such as caloric restriction and exposure to the antioxidant resveratrol, act by increasing levels of the Sir2 protein. The homologue for this gene in humans, *sirt1,* has been found to function similarly, though through more complex mechanisms. The protein helps cells withstand periods of stress that would normally lead to apoptosis, or programmed cell death. Another gene called *INDY* (for I'm Not Dead Yet), also studied in fruit flies, nearly doubles their normal life span from 37 to 70 days or longer when mutated. The mechanism by which the mutant gene extends life seems to be by decreasing the normal activity of the fly by restricting the calories absorbed by the cells. A gene from the roundworm *Caenorhabditis elegans, daf-2,* encodes a protein similar to the human insulin receptor. The hormone insulin plays a role in glucose utilization in metabolism, which is another important avenue of research for understanding senescence. Another longevity gene appears to regulate gene expression in *C. elegans*; the gene product is an enzyme that changes the structure of DNA and, as a result, turns off the synthesis of proteins encoded by other genes. Researchers have identified many other longevity genes in model organisms, and current research goals include finding more of those genes, characterizing the protein products of the genes, investigating the regulation of these genes, and examining the function of the proteins.

According to the programmed longevity theory of aging, cells have predetermined life spans. Cells do have finite life spans when grown in vitro; after a limited number of cell divisions, replication stops. The cells continue to be active metabolically, but they no longer proliferate. They change their gene expression patterns, and in some cases their presence becomes harmful. The cellular mechanism that limits the number of cell divisions before senescence sets in is currently unknown, but continued research on longevity genes may shed light on this. One explanation involves telomeres, looped structures located at the ends of linear chromosomes that contain numerous repeated copies of a sequence of DNA. In humans and other vertebrates this sequence is TTAGGG. Its



Researchers use animals such as the roundworm *Caenorhabditis elegans,* shown here, to study the aging process. *(Sinclair Stammers/Photo Researchers, Inc.)*

purpose is to permit replication of the DNA to proceed all the way to the end. DNA is a double-stranded molecule, resembling a ladder with the rails on the sides representing each strand. Unlike ladder rails, however, the two strands of DNA are antiparallel, meaning the two strands run in opposite directions, as if one rail on the ladder has a supportive base at the bottom and a cushioned tip at the top, but the other rail has the reverse—the supportive base at the top and a cushioned tip on the bottom. This is significant because the enzyme that replicates DNA only works in one direction and the replication system's mechanism that compensates for this requires extra length at one end of the double-stranded DNA molecule. Without telomeres, one of the strands would lose a bit of its sequence at the end during each round of replication. The telomeric repeats do not encode any protein; they just ensure that genes located at the ends of chromosomes do get replicated. With each round of DNA replication that precedes cell division, the telomeres shrink, eventually becoming too small to perform their job. At this point, the cell is said to have reached replicative senescence and will no longer divide. While these findings initially generated much excitement among gerontologists, no correlation between cellular senescence from decreasing telomere length and longevity of a whole organism has been established. In fact, some animals with short telomeres have longer life spans than other animals with longer telomeres. Many cell biologists continue to pursue research in this area, however, because of its initial promise and the as-of-yet unexplained connection of cellular senescence and aging.

The endocrine system may be an important regulator for mechanisms involved in programmed aging. Specialized glands and tissues produce and secrete chemicals called hormones that travel throughout the circulatory system and act on other target tissues or cells. For example, the pituitary gland located at the base of the brain secretes hormones that act on the ovaries and uterus in females to coordinate the complicated events of the menstrual cycle. Hormones regulate nearly all aspects of growth, metabolism, development, reproduction, and homeostasis, so it is logical that they would regulate aging as well. Some events associated with aging are definitely under hormonal control. For example, as a human female ages, she loses her ability to reproduce. This process is called menopause and results when the hypothalamus ceases to release the necessary stimulatory hormones. The concentration of many hormones decreases with age, but replacement therapy does not stop aging. Hormonal imbalance seems to explain many phenomena associated with aging, but medical researchers have yet to turn this information into practices that halt the aging process.

The immunological theory of aging states that self-destruction is programmed into the immune system. In addition to preventing and fighting infections, finding and removing foreign substances from the body, and repairing damaged tissues, the immune system functions to survey the body for cells that are abnormal, such as potential cancerous cells or cells that have been damaged by viruses, and then destroys them. In order to perform this task, the immune system must be able to differentiate between the body's normal cells and abnormal or foreign cells (such as pathogenic microorganisms). According to the immunological theory of aging, the immune system loses this ability over time and begins to attack its own healthy cells and tissues. As the immune system function declines, the body also becomes more vulnerable to infections and cancer.

ERROR THEORIES OF AGING

The error theories of aging purport that senescence results from damage to cells or loss of function over time due to continuous exposure to environmen-

tal assaults such as radiation, toxic chemicals, and free radicals. The chromosomes located inside the nucleus of the cells that make up a living organism contain the organism's genetic information. Genes composed of deoxyribonucleic acid (DNA) encode all the information necessary for the cells to make proteins and other biomolecules that compose cells and tissues and that perform all the functions necessary for life, such as growth and metabolism. DNA is copied every time the cell divides to create new cells as a normal part of growth, repair, and tissue maintenance. Regions that encode genes along a molecule of DNA contain specific sequences of four alternating nucleotide bases (abbreviated *A, C, G,* and *T*) that encode for specific sequences of amino acids, the subunits from which proteins are synthesized. Alterations in the DNA sequence called mutations can lead to changes in the amino acid sequence of the proteins. Cells have mechanisms for repairing the majority of mutations, but given the sheer enormity of the task of replicating the DNA during cellular reproduction—the sequences within the nucleus of each cell in humans are 3 billion nucleotides long—inevitably, a number of the mutations become permanent. Some mutations are minor and have no noticeable effect, but others cause significant problems, even resulting in cell death. Error theory states that over time, the number of mutations accumulates to dangerous levels, killing cells and, eventually, the entire organism.

Atoms are most stable when all the electrons are paired. Free radicals, also called reactive oxygen species, are atoms or molecules that have an unpaired electron, making them unstable. Though some cells use free radicals in a controlled manner to perform useful functions such as destroying pathogens, free radicals are often dangerous to living cells because they readily combine with other molecules or strip off electrons from other molecules including DNA or proteins in order to pair their lone electron. If a free radical steals an electron from one molecule, that molecule then may try to replace it and may take the electron off another molecule, initiating a chain reaction inside the cell. Damage from free radicals causes cells and eventually tissues and organs to lose their ability to function. Free radicals are created during the production of adenosine triphosphate (ATP), the form of energy used by cells, from oxygen and organic molecules during aerobic respiration. Sunlight, tobacco smoke, and other substances ingested with food or inhaled with air also cause the formation of free radicals. Mitochondria, the cellular organelles that carry out cellular respiration and ATP synthesis, are particularly vulnerable to damage, and damaged mitochondria are less efficient. These organelles contain their own DNA, which encodes for proteins that function in cellular respiration. The constant exposure of the mitochondrial DNA to free radicals causes mutations in the mitochondrial genes, leading to an overall decrease in efficiency of ATP production over the years. Because cellular respiration is a necessary metabolic process, free radicals are unavoidable, but cells have some means for dealing with them. In particular, one enzyme called superoxide dismutase catalyzes the neutralization of oxygen free radicals produced during aerobic respiration. Fruit flies that have been genetically engineered to express higher than normal quantities of this enzyme live longer than normal fruit flies, suggesting there might be a link between free radicals and senescence. Unfortunately, the cellular defenses are not foolproof, and some free radicals escape them and cause damage. Evidence correlating the action of free radicals with senescence includes the association of genetic defects that accelerate the accumulation of free radicals with premature aging. *C. elegans* that had this genetic defect exhibited normal life spans when exposed to a synthetic antioxidant drug. Antioxidants are substances that can inhibit oxidation reactions (reactions involving the loss of electrons) by safely donating their own electrons to the radicals without incurring damage in the process. This makes the radical less reactive. Free radicals may also contribute to the development of cancer, neurodegenerative diseases, cataracts, and atherosclerosis. Many antiaging remedies include dietary supplements or lotions that contain antioxidants such as the vitamins C and E.

The rate of living theory closely relates to the free radical theory of aging. Caloric restriction, the only demonstrated means of extending life in mammals, prolongs life spans by slowing an individual's metabolic rate. The goal is to reduce total calories but not to let the body become dangerously emaciated, and maintaining a balanced diet is still important to prevent malnutrition. Basically, severely reducing the amount of calories an individual takes in decreases the activity level, and the individual lives longer. One idea behind this is that reducing the amount of food reduces the levels of exposure to toxic metabolic by-products, such as oxygen free radicals. Mice that consume a healthy diet containing 30 percent fewer calories than other mice live 40 percent longer. Researchers have found that all of the body's physiological systems exhibit delayed age-related degeneration as a result of caloric restriction. Studies on the effects of caloric restriction on aging in primates are currently in progress. Even if caloric restriction is proved to delay aging and improve health in humans, it is not a realistic antiaging therapy, as people are very resistant to drastic changes in their eating habits, as shown by the inability of many to maintain long-term restricted diets in order

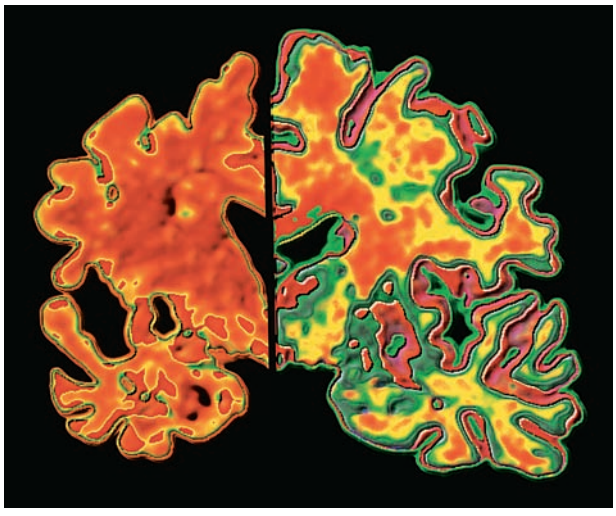
to improve cardiovascular health or to lose weight. However, understanding the mechanism by which caloric restriction works could lead to other therapies or treatments of age-related deterioration.

Cross-linking of proteins occurs when glucose molecules attach themselves to proteins, causing them to bind together. The accumulation of cross-linked proteins can stiffen tissues, causing them to lose elasticity and flexibility. Tissues that have lots of collagen, a common component of connective tissue, are particularly affected. Vision becomes blurred as collagen in the eye lens becomes more rigid. Arteries lose elasticity, leading to an increase in blood pressure. Lungs cannot expand as far, reducing their maximal capacity. Tendons lose their flexibility, limiting range of motion, stiffening joints, and increasing risk of injury. Thus cross-linking leads to the deterioration of several body functions that are associated with aging.

AGE-RELATED DISEASES

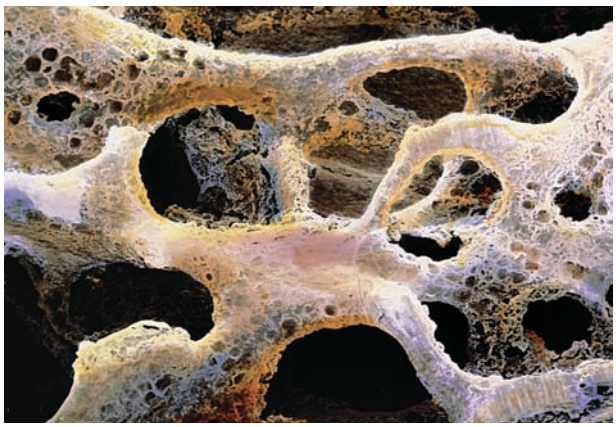
Some illnesses or diseases become more common as people age. The causes of some are known and others are unknown. Alzheimer's disease, cancer, arthritis, osteoporosis, diabetes, and cardiovascular disease are examples of conditions that result from impaired function due to deterioration of the body's tissues with age.

According to the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics (NCHS), Alzheimer's disease rose to the fifth leading cause of death among people aged 65 and older in 2003. Alzheimer's is a degenerative brain disease of unknown origin that typically appears in people over the age of 60, and the risk increases with increasing age. The symptoms typically begin as mild then progressively worsen and include memory loss, impaired thinking, disorientation, mood disturbances, and personality changes. Abnormal clumps called amyloid plaques and tangled webs of nerve fibers in the cerebral cortex are characteristic signs of Alzheimer's. Several genetic mutations have been associated with the development of Alzheimer's. One factor appears to be a protein that carries cholesterol in blood circulation and is encoded by the apolipoprotein E (ApoE) gene. A team of researchers led by Huntington Potter from the University of South Florida found that ApoE speeds up the formation of amyloid plaques in the brain by converting harmless amyloid-beta protein into the toxic fibrous deposits and that this conversion is associated with the cognitive decline. No cure for Alzheimer's exists, but medications are available that slow the progression in some cases.



Alzheimer's disease causes degeneration of nerve tissue and nerve cell death, leading to shrinkage, as shown here on the left, compared to a normal brain on the right *(A. Pakieka/Photo Researchers, Inc.)*

Cancer, when cells grow out of control to form tumors or neoplasms, can strike a person of any age, but as one grows older, its risk increases. According to the NCHS, malignant neoplasms were the second leading cause of death in 2003 for persons 65 years of age or older. The goal of many antiaging therapies is to increase the life spans of cells, but the body has evolved natural mechanisms for limiting cellular life span for a good reason. The greater number of times a cell line undergoes DNA replication and division, the greater the number of accumulated genetic mutations. Cancer results from the accumulation of mutations in genes that control the cell cycle. Proteins that stimulate cell division and those that inhibit it normally work together to ensure that cells replicate in a highly regulated and appropriate manner. Biochemists have identified and characterized numerous genes that play a role in cell cycle control. Tumor suppressor genes encode proteins that limit or inhibit cell proliferation under appropriate circumstances. For example, a cell should not reproduce when its DNA contains a lot of unrepaired mutations because cancer can result from the accumulation of numerous genetic mutations. Thus the normal function of tumor suppressor genes is to prevent the reproduction of cells that have a high potential for developing into tumors. The retinoblastoma gene is one tumor suppressor gene that acts by preventing mitosis; when mutated, the protein product of this gene (often abbreviated RB) prevents cellular senescence and leads to tumor formation. The protein p53, also encoded by a tumor suppressor gene, acts by binding to DNA and altering the expression patterns of numerous other proteins. More than half of all cancers are associated with a mutation in the gene encoding the p53 protein that leads to the protein's inactivation. Studying antiproliferative genes will help cell biologists to understand not only



Scanning electron microscopy of bones affected by osteoporosis reveals numerous round dark areas void of bone tissue. *(Professor Pietro M. Motta/Photo Researchers, Inc.)*

cancer, but the process of cellular senescence. A connection also exists between cancer and telomeres. In cancerous cells, the telomeres behave abnormally and do not shrink with each round of replication. An enzyme called telomerase keeps replacing the telomeric sequences, preventing this mechanism from causing cellular senescence, allowing the cancer cells to divide uncontrollably.

Arthritis includes a number of diseases that affect the joints and are the leading cause of disability in elderly people. The most common type in older people is osteoarthritis, which results when the cartilage that cushions the bones at joints becomes worn as a result of extensive use and gravity. This degenerative disease causes joint pain, inflammation, and stiffness. Other common forms of arthritis include rheumatoid arthritis, which results from the immune system's attacking the lining of the joints, and gout, which results from the accumulation of uric acid crystals in the joints and connective tissue. Treatments for arthritis depend on the particular type of arthritis and include exercises and medications to reduce pain and swelling.

Osteoporosis is a disease that causes the bones to weaken and easily break. Throughout one's life, bone tissue breaks down and is replaced with new bone tissue, but as one grows older, more breaks down than is replaced, and strength decreases. Taking vitamin D and calcium supplements, getting plenty of exercise, and not smoking help prevent the breakdown of bone tissue and assist in building new bone tissue.

Diabetes results when the level of glucose (a simple sugar) in the blood is too high. The pancreas produce insulin, a hormone that helps cells to absorb glucose from the bloodstream. Type I diabetes is typically seen in children, young adults, or people below the age of 30, when their body stops producing insulin. In Type II diabetes, which is most common in people over the age of 40, the individual produces insulin, but the body stops responding to it properly. High blood glucose levels can lead to serious medical problems, such as heart disease and stroke. Diabetes is the seventh leading cause of death in people over the age of 65 according to the NCHS. Fat buildup, a normal effect of aging, also increases the levels of glucose in the blood; thus weight loss is one means to control Type II diabetes. Monitoring one's diet and increasing physical activity are also ways to manage this disease, and medications also help many people control their diabetes.

Cardiovascular disease includes numerous different conditions affecting the heart and blood vessels and can strike anyone, but the risk increases with age. Heart disease consistently ranks as the leading cause of death in persons of all ages, and stroke ranks third. The heart muscle weakens with age, and the blood vessels lose their elasticity, making it harder to propel the blood throughout the body. Atherosclerosis is a disease in which plaque deposits of dead cells and cholesterol build up in the arteries, narrowing the diameter of the vessel. Proper diet and exercise can prevent or reduce the risk of cardiovascular disease, which is a common, but not an inevitable consequence of aging. Many effective medications are also available.

See also CANCER, THE BIOLOGY OF; CHROMOSOMES; POINT MUTATIONS.

FURTHER READING

Clark, W. R. *A Means to an End: The Biological Basis of Aging and Death*. New York: Oxford University Press, 2002.

Dollemore, Doug. *Aging under the Microscope: A Biological Quest*. Bethesda, Md.: National Institute on Aging, National Institues of Health, June 2006.

Panno, Joseph. *Aging: Theories and Potential Therapies*. New York: Facts On File, 2005.

Scientific American Special Edition: The Science of Staying Young. Special edition. 14 (3) (June 2004).

Sinclair, David A., and Lenny Guarente. "Unlocking the Secrets of Longevity Genes." *Scientific American* 294 (March 2006): 48–57.

U.S. National Institutes of Health, National Institute on Aging home page. Available online. URL: http://www.nia.nih.gov/. Accessed January 17, 2008.

agriculture Agriculture is the science or process of producing food, feed, and fiber (cotton, wool, flax, and hemp) through the systematic cultivation of plants and raising of domesticated animals. In addition to food products for humans and animals, agriculture includes cut flowers, ornamentals, timber,

fertilizers, leather, chemicals, fuels, and drugs such as tobacco, opium, and biopharmaceuticals. More than 40 percent of the world's population works in the field of agriculture.

Agriculture is an application of life science as it depends on and utilizes many principles from botany, zoology, genetics, microbiology, molecular biology, and more. Other sciences, such as chemistry and engineering, also contribute to agriculture. While the term *agriculture* refers to the activities performed to transform the environment for raising animals or growing crops for human use, agricultural science is a multidisciplinary field that encompasses the study of numerous subjects that are related to agriculture. Agronomy is the research and development related to studying and improving field-crop production and soil management. An agricultural scientist might research methods for improving crop yields, determine the best way to package dairy products, design and develop new pesticides, study waste management, or predict nitrogen needs of a field based on the basis of ecological models. Related disciplines include fields as varied as agricultural engineering, aquaculture, food science, environmental science, soil science, agrophysics, horticulture, agricultural economics, animal science, and irrigation management.

Archaeologists and anthropologists have traced the origins of agricultural practices to approximately 9500 B.C.E. in the area of the Middle East including Egypt, Mesopotamia, and the Levant. The first domesticated crops were cereal grains. Root crops and legumes were first cultivated 8,000 to 9,000 years ago, followed by vegetables, and oil, fiber, and fruit crops. Plants used for decoration and drugs were not domesticated until about 2,000 years ago. The gradual development of agriculture was a crucial step in the development of civilizations. Without having to wander in search of animals and plants for food, people could settle in a single location, an action that led to the formation of towns or cities. By 5000 B.C.E. the Sumerians, a civilization in the southern part of Mesopotamia, were cultivating land on a large scale, monocropping, and using labor and irrigation. The invention of the plow around 4000 B.C.E. was another major technological advance that led to crop planting in rows and increased efficiency, allowing populations to become denser. The earliest simple plows were simply forked sticks and timbers that later developed into heavy plows that had a coutler for cutting a thin strip in the earth, a share to slice the soil, and a mouldboard that turned the soil. Later wheels and seats were added. In the Middle Ages, the Persian Muslims made several advances that led to the methods used in more modern farming. These revolutionary techniques included the development of sophisticated irrigation, or watering systems; the use of the scientific method to improve farming techniques; giving incentives to and rewarding productive laborers with land and shares in the harvest; and the introduction of new crops, such as sugar cane, citrus fruits, and cotton, that were attractive as commercial products to people in other geographical locations. The later development of more specialized plows and the concept of crop rotation continued to improve farming efficiency. During the late 15th and 16th centuries, crops spread by exchange between the Old and New Worlds. Tomatoes, squash, maize, peanuts, potatoes, and tobacco were introduced to Europe. The Americas began to depend on wheat, rice, coffee, cattle, horses, and sheep. Agriculture played a significant role in the Industrial Revolution of the 18th and 19th centuries. New means of transportation facilitated the shipping of farm products and reduced the incidence of starvation. In turn, the increasing populations helped to meet the demand for new laborers in all the growing industries, including agriculture. Inventions such as the cotton gin, the mechanical reaper, threshing machines, mowing machines, and better plows further advanced farming. In the 20th century, tractors and trucks, refrigeration, the development of hybrid crops, and genetic engineering to improve varieties have all benefited agricultural industries.

Today crop improvement remains a major goal of agricultural science. Continued advancements in traditional farming methods include technology that has led to newer equipment. Better machines have improved yields while requiring less effort. Agronomists continue to search for new ways to control pests and weeds. Understanding the relationships among living organisms has led to natural mechanisms of pest control. The concept behind natural pest control is to identify the natural enemy of the crop-destroying pest. For example, ladybugs consume aphids, whiteflies, mealybugs, scales, mites, and many other soft-bodied insects in addition to bollworms, broccoli worms, cabbage moths, and tomato hornworms. The bacteria *Bacillus thuringiensis* produces a paralytic toxin (Bt toxin) that, when ingested by insects, paralyzes their digestive tract and eventually kills the pests. Though the term *natural* implies these methods cannot be harmful, ecologists must carefully consider the effect that introducing new species or altering the numbers of present species will have on the ecological community before implementing biological means of pest control. Animal husbandry, the breeding and raising of animals for their meat or products such as eggs or wool, uses selective breeding programs to create organisms that yield either larger quantities of products or more desirable products. Plant breeding programs accelerate the natural process of evolution

(continues on page 19)

THE COLONY COLLAPSE DISORDER CRISIS FOR HONEYBEES

by Zachary Huang, Ph.D.
Michigan State University

Honeybees (*Apis mellifera*) not only produce honey, the natural sweetener that is considered wholesome and healthy, but also beeswax, pollen, venom, royal jelly, and propolis. Beeswax is used for candle making, batik, and sculpturing; pollen, royal jelly, and propolis are used as nutraceuticals or health foods. Venom can be used for pharmacological studies or for treating autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. While these products from honeybees are impressive, the most significant contribution of honeybees to human society is actually the pollination services they provide for agriculture. Approximately 130 crops grown in the United States depend on honeybees for pollination. Most fruits and vegetables need honeybees either to set fruit or to produce better fruit (more symmetric fruits, sweeter, or with a higher oil content) or to allow seed production (most vegetables). Examples of bee-pollinated fruits include almonds, apples, cherries, blueberries, pears, peaches, strawberries, raspberries, and the melons (squashes, cucumbers, pumpkins, and watermelons). Vegetables that require bees for seed productions include broccoli, onions, cabbages, and radishes. A Cornell study from 2001 estimated the total value of pollination to these crops in the United States to be about $15 billion per year. The total value of honeybee products (honey, pollen, beeswax, etc.) ranges from about 1/50 to 1/80 of the value of pollination. The almond crop alone in California requires about 1.2 million colonies each year, about 50 percent of the total number of colonies in the country. The almond growers paid about $150–$200 per colony in the year of 2007 for pollination, usually at two to three colonies per acre. Typical prices for pollinating apple and cherry orchards are about $50–$70 per colony.

THREATS TO HONEYBEES

Honeybees, as do most other organisms, face the threat of diseases and pests attacking them. The main threats to honeybees are pests, diseases, and pesticides, but other threats also exist. Global trade has complicated beekeeping by introducing new pests and diseases that have required new pesticides or antibiotics for control. The stagnant honey prices and increased cost for maintaining bees have caused the downward trend of colony numbers in the United States.

Pests

By far the worst threat to honeybee health, in the United States and worldwide, is the ectoparasite varroa mite (*Varroa destructor*). This mite, formerly referred to as *Varroa jacobsoni* in the literature, was introduced into the United States around 1987. Apiculturists initially thought the parasite was *V. jacobsoni* but later found that all mites in North America differed genetically from the real *V. jacobsoni* by about 8 percent. Denis Anderson named the newly identified North American species *V. destructor.* Within a few years almost all feral honeybees (i.e., unmanaged bees that nest inside tree holes and in the walls of people's houses) died off because of the mites. Managed honeybees rely upon pesticide use to reduce the mite population, or they too will die after one to two years. The mites suck blood from adult and immature bees but only reproduce on bee pupae, which are "capped" by a layer of wax by worker bees. Varroa mites have been known to transmit many types of bee viruses (e.g., deformed winged virus, cloudy wing virus, Kashmir virus, slow paralysis virus, and acute bee paralysis virus). One theory is that mites do not kill bees: the viruses they harbor and transmit do the killing.

Another mite pest is the tracheal mite (*Acarapis woodi*), an internal parasite that lives and reproduces inside the breathing tubes (tracheas) of the adult bees. First introduced to the United States around 1983, the tracheal mite also wiped out about 50 percent of managed bees during the first few years. The mites weaken the bees so that they live shorter lives and have trouble returning from their cleaning flights in early spring. Bees normally do not defecate inside hives; they wait for a 55°F (12.8°C) day in February or March to fly out and defecate. Typical symptoms of this mite are small clusters or no bees at all in the early spring when most or all bees die while away from the colony (flying out and not returning).

Diseases

Honeybees also suffer diseases that are caused by viruses, bacteria, parasites, or fungi. American foulbrood is a larval disease caused by *Paenibacillus larvae,* the endospores of which can survive decades on bee combs. Infected larvae die at late larvae stage, just before they pupate, or during the pupation process (under the sealed cells). The antibiotic tetracycline (marketed as Terramycin®) used to treat the infection effectively, but the bacterium is now mostly resistant to it. A new chemical, tylosin (marketed as Tylan®), was registered in 2006 for the control of *Paenibacillus. Nosema apis,* a protozoan parasite that infects the midgut cells of bees, causes nosema disease. This parasite forms spores that can survive many years inside honey and wax and only germinates inside the midgut of bees. Infection causes death of epithelial cells of the midgut, basically rendering bees unable to digest pollen to absorb proteins. Colonies often die during winter because sick bees defecate inside the hive and spread the disease. In the summer, infected workers forage earlier and live shorter lives, causing reduced honey yield. In 1996 a new species of *Nosema* was discovered in Asia and named *Nosema ceranae* because entomologists thought at the time that it only infected

(continues)

(continued)

the Asian hive bees, *Apis cerana.* In 2005 scientists in Taiwan found that it can also infect *Apis mellifera.* Soon European scientists found the same species (*Nosema ceranae*) infecting their bees and thought the new disease was the main culprit causing massive bees die-offs, especially in Spain.

Pesticides

There are two routes for pesticides to get into honeybees. One route is pesticides applied internally to the colonies by beekeepers to kill pests and disease organisms. Many types of pesticides are used against the varroa mites, partly because of resistance developed by the parasite. Apistan™, a pyrethroid (a type of insecticide), has been used since the late 1980s and currently is largely ineffective against the mites. Checkmite +, an organophosphate (which is more toxic to humans than pyrethroid), was introduced about 10 years ago, and mites are also becoming resistant to this. Other treatments include organic acids (acetic acid, formic acid, and oxalic acid) and essential oils (thymol in both ApiGard and Apilife Var). Other pesticides that are applied externally to the hive, those used on crops and on ground covers or water in an attempt to control other insects, also affect honeybees. Pesticides sprayed onto flowers directly can be picked up by bees on flowers; systemic insecticides are absorbed by plants and then transported to nectar and pollen and collected by bees. French beekeepers observed the mysterious "mad bee disease" around 1999, when foragers became disoriented and simply could not find their home. Beekeepers suspected a pesticide, Gaucho (imidacloprid), that was used for seed treatment for crops such as sunflowers. At high concentrations, the chemical can disrupt learning and memory of bees, but numerous studies between 2000 and 2003 concluded that there is not enough imidacloprid in the nectar and pollen from the seed-treated plants to have caused behavioral changes or toxic effects in bees.

Other Threats

In addition to pests, diseases, and insecticides, other stresses abound for the honeybees. There are concerns of decreased genetic variation among *A. mellifera* simply because of the well-developed queen rearing system in the United States. Queens are mass-produced from a few "queen-producers" (all closely related to one another), sometimes tens of thousands per year, and distributed around the country. As a result of this practice, if a particular line of bees is vulnerable to certain diseases or pests, they can represent a sizable proportion of the total bees in the country and be wiped out. Beekeepers also transport bees long distances for pollination purposes. For example, bees from Michigan or New York travel by truck all the way to California for almond pollination. Bee colonies are stacked together (as many as 400 of them) onto the same truck, covered with nets, and transported across four time zones. Moving the bees in this manner stresses them. Bees do not even have good tempers when moved two miles away—they dislike vibrations from cars or trucks and being confined. (Imagine being stuck in a black box elbow to elbow with 40,000 siblings!) Other unanswered questions include whether bees get jetlag (yes, foragers do sleep at night) and whether moving them makes them more prone to damage from diseases and pests.

THE CCD CRISIS

As if not enough assaults are directed at honeybees, the single most important pollinator nationally and globally, they are currently suffering from yet another problem. Starting around October 2006, some beekeepers observed the rapid loss of bees in many colonies. One beekeeper, for example, lost 90 percent of his 9,000 colonies. The symptoms were very different from those caused by known pests, diseases, or pesticide kills. Instead of a gradual weakening, in which case the colonies would have very few brood and adult bees left, the new ailment seems to deplete bees very quickly. A colony full of 40,000 workers on one day after four to eight days may have only one queen and a few hundred young bees left, with five to six frames of sealed brood (immature stages of bees, including eggs, larvae, and pupae). No dead adult bees are found inside the hive or in close proximity. Furthermore, opportunistic pests, such as wax moths or small hive beetles, which attack weak colonies or empty hives, do not invade these colonies. Nor are the very weakened colonies "robbed" by nearby honeybees. Robbing is a behavior by honeybees to remove honey from nearby, usually weaker, colonies when resources are scarce. Honeybee scientists termed this ailment CCD, shortened from *colony collapse disorder,* because it was not clear what caused these symptoms and whether it was a disease or simply a symptom of stresses or pesticide poisoning.

While to most people these symptoms are totally new, digging back into old papers one finds a report published in 1987 describing "disappearing disease," and yet another paper describes colonies with only brood left, but it was published in 1897! If what is happening today is the same thing as in 1987 and 1897, then it suggests the causes are not genetically modified organisms (GMOs), or pesticides, or varroa. Unfortunately, no samples were saved during the 1987 episode so researchers might never know whether the "disappearing disease" is the same as CCD.

NONCAUSES

In order to solve the problem of disappearing honeybees, scientists have eliminated several potential causes.

- *Cell phones.* Cell phones can be safely excluded from the list of potential causes of CCD. One German study placed a base set of a wireless phone under a honeybee colony and noticed some behavioral changes. This observation was blown up, leading people to claim that cell phones must cause disorientation in honeybees, preventing them from being able to return to their hives, resulting in CCD. The CCD working group did not find any association between cell phone towers

(which have much stronger signals than cell phones) and the cases of CCD.

- *Tracheal mites.* When tracheal mite infestation is serious, colonies can dwindle in the fall, and sometimes no bees are left in the colony come springtime. This seems similar to CCD, but in tracheal mite–infested colonies, there is no brood and sometimes there is a small cluster of dead bees left in the colony (occasionally there are no bees). The disappearance of bees due to tracheal mites happens only in early spring, when mite-infested bees fly out and die in the field.
- *Nosema disease. Nosema*-infected colonies also show much higher winter mortality rates, but usually there is a small cluster of bees inside the hive and the symptoms do not occur in the fall. After learning that a new species of *Nosema* found in Europe and Taiwan was attacking the Western honeybees, scientists thought that *Nosema ceranae* might be the culprit. Usually when a new disease or parasite strikes a new host, it causes massive die-offs (as the varroa and tracheal mite did) because the host has not evolved to have adaptations for resisting the new disease or parasite and therefore the virulence is much higher. However, by using a highly specific method (polymerase chain reaction, PCR), most *Nosema* samples in the United States have been identified as *Nosema ceranae.* Not only that, but examination of *Nosema* samples collected seven or eight years ago revealed that they also were *Nosema ceranae.* Scientists do not know when *N. ceranae* replaced *N. apis* in the United States, but because *N. ceranae* has been here longer than five years, it is unlikely that it suddenly is causing new symptoms like those of CCD.
- *Varroa mites.* Colonies severely infested with varroa mites usually develop parasitic mite syndrome (PMS), characterized by spotty brood and gradual weakening over a longer period, not within four to six days, as CCD does. However, it still is possible a new type of virus is transmitted by varroa mites and is causing CCD.
- *Genetically modified organisms (GMOs).* Scientists found no obvious association between CCD occurrences and regions where a certain type of genetically modified plants are grown. Plants containing genes responsible for the production of the insecticidal protein known as Bt (named for *Bacillus thuringiensis*) are safe for honeybees, mainly because the Bt endotoxins are highly specific against certain groups of insects (e.g., moths and beetles), and no known Bt acts against hymenopterans (ants, bees, and wasps).
- *Food supplements.* Beekeepers feed honeybees honey substitutes such as cane or beet sugar, high-fructose corn syrup (either spring or fall), and pollen substitutes in the early spring. The CCD working group did not find any association of this practice with CCD. However, high-fructose corn syrup can shorten the life of caged bees (Zachary Huang, unpublished results) and may not be the optimal diet for honeybees.

WHAT IS LEFT?

After eliminating all of the possibilities discussed, a limited number of possibilities remain. Currently scientists who study CCD list four possible causes.

- *New or reemerging pathogens.* A paper published in the highly regarded journal *Science* in September 2007 (Cox-Foster et al.) showed that a new virus, IAPV (Israeli acute paralysis virus), was a "marker" for the occurrence of CCD. IAPV was first described in Israel in 2004 and was said to cause bees to have shivering wings, paralysis, and then death outside the hive. The Cox-Foster paper did not conclude that the virus was causing CCD because no healthy colonies were inoculated to show that the virus causes the exact symptoms. This step is needed to identify any cause of disease and is part of the so-called Koch's postulates. The *Science* paper caused a controversy because it implicated the new virus and because the route of the virus was suspected to be the importation of Australian package bees. A senator called for an embargo of Australian bees, and Australian beekeepers were not happy they were blamed. A more recent paper published in the December 2007 issue of *American Bee Journal* provided evidence that the Australian bees were not to blame, and importation was allowed for spring 2008. This paper found that IAPV was in the United States prior to the Australian bee importation. Scientists continue to debate the importance of this virus because the authors of the *Science* paper found that the IAPV that was present in United States was slightly different from those found in Australian bees.
- *New bee pests or parasites. Nosema ceranae* does not seem to be the cause since it has been in the country for at least eight to nine years. Again if the cause of CCD is a new insect pest, which would be relatively large, it should have been observed and identified by now.
- *Environmental and/or nutritional stresses.* Bees are trucked long distances across many time zones, climate is changing through global warming, bees are pushed very hard to pollinate multiple crops, and some of the crops perhaps do not provide adequate nutrition (especially protein) to bees. Beekeepers also feed bees high-fructose corn syrup, which in some instances has high amounts of hydroxymethylfurfuraldehyde (HMF), a chemical that is toxic to bees. The list goes on and on. Could these stresses cause bees simply not to return to their colonies? This is plausible but unlikely.
- *Pesticides.* Beekeepers have been putting all kinds of pesticides, including pyrethroids, organophosphates, organic acids, essential oils, and

(continues)

(continued)

who-knows-what else, into bee colonies. Some of these chemicals will remain stable inside beeswax for a long time and be slowly released into larval food, affecting bees. Studies have shown that insecticides inside beeswax can cause reduction in both the survival rate and the body weight of newly emerged queens and reduced sperm numbers in drones. It is not clear how these chemicals affect worker behavior, especially their learning and memory.

It is most likely that a combination of different factors causes the CCD problem. For example, bees may be stressed from an inadequate pollen supply, fed high-fructose corn syrup, be exposed to many kinds of pesticides reaching a high enough level inside the colonies, and face existing pathogens or parasites (such as *Nosema* or viruses transmitted by the varroa mite), and suddenly workers are affected such that they fly out of the hive and die en masse out in the field.

LESSON LEARNED?

As important as the honeybees are for agriculture, the United States has not really invested much on them. The U.S. Department of Agriculture has four honeybee laboratories (stationed at Beltsville, Maryland; Weslaco, Texas; Baton Rouge, Louisiana; and Tucson, Arizona), with a total funding of about $10 million per year. This is a small number compared to investment in other animals (such as poultry and cattle) and considering that bees are valued at about $15 billion per year. The CCD crisis is a wakeup call for the scientists and the general public that they cannot take the honeybees for granted. CCD generated many reports in the news media, and the general public now has a much better understanding of the importance of honeybees to our food supply. As a result of the CCD crisis, Congress is considering several bills to help fund research on honeybees and other pollinators.

Because the honeybee is an introduced species to North America, it is unlikely that any native plant species depends totally on honeybees for pollination. In other words, honeybees are not technically needed for the "natural" ecosystem in North America. However, people do need honeybees for agricultural purposes, precisely because agriculture is no longer "natural." This author has seen hundreds of other bees (mostly andrenids, some halictids, and megachilids), if not thousands of them, hovering above a single plum tree at the Michigan State University campus. The same thing was seen on flowering cherry trees in front of a botanic garden in South Carolina. Enough diversity and abundance of those solitary bees exist for a few trees in a backyard orchard. The problem is that with hundreds of acres of almonds, apples, cherries, and blueberries, with very few other flowers around (a result of herbicides, tilling, and mowing), there will never be enough of these alternative pollinators. The problem lies in the way of modern agriculture—monoculture on a grand scale. Increasing the diversity of pollinators for crops would be helpful, so that farmers do not rely so heavily on one species of insect that is not even native here. But the truth is, as long as this manner of large-scale monoculturing is maintained, fruit and vegetable growers will continue to rely heavily on honeybees for pollination. The leafcutter bees (*Megachile rodundata*) can do a great job of pollination, but they are specialized for seed production in alfalfa. Farmers have begun to use the blue orchard bees (*Osmia lignaria*) or horn-faced bees (*Osmia cornifrons*) for cherry and apple pollination in some states; however, it will take many years, if it ever happens, to culture them so as to reach the numbers needed for the 600,000 acres of almonds in California alone (valued about $2 billion in 2005). Honeybees are ideal for the modern way of agriculture: large in numbers (30,000 per hive), easily movable (one can transport 400 hives per truck), efficiently managed because much of their biology is known, and relatively easy population to rebuild, even if 50 percent of colonies are lost.

That is, if only scientists can find out what causes CCD and prevent future bee losses.

FURTHER READING

Almond Board of California home page. Available online. URL: http://www.almondboard.com. Accessed February 13, 2008.

Chen, Yanping, and Jay D. Evans. "Historical Presence of Israeli Acute Paralysis Virus in the United States." *American Bee Journal* (December 2007). Available online. URL: http://www.dadant.com/documents/ChenandEvansarticlefromDec07ABJ.pdf. Accessed February 14, 2008.

Cox-Foster, D. L. et al. "A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder." *Science* 318, no. 5848 (2007): 283–287.

Huang, Zachary. Cyberbee.net home page. Available online. URL: http://www.cyberbee.net. Accessed February 13, 2008.

———. "Zach's Bee Photos." Available online. URL: http://photo.bees.net/gallery/Disease-and-Pests. Accessed February 13, 2008.

Maori, E., E. Tanne, and I. Sela. "Reciprocal Sequence Exchange between Non-Retro Viruses and Hosts Leading to the Appearance of New Host Phenotypes." *Virology* 362, no. 2 (June 5, 2007): 342–349.

Mid-Atlantic Apiculture, Research and Extension Consortium. "Colony Collapse Disorder." Available online. URL: http://www.ento.psu.edu/MAAREC/ColonyCollapseDisorder.html Updated November 20, 2007.

Official Web site of Barbara Boxer, United States Senator from California. "Senators Introduce Legislation to Maintain and Protect American's Bees and Native Pollinators." Press release dated June 26, 2007. Available online. URL: http://boxer.senate.gov/news/releases/record.cfm?id=277777.

Wikipedia. "Nosema ceranae." Available online. URL: http://en.wikipedia.org/wiki/Nosema_ceranae. Accessed February 13, 2008.

(continued from page 14)
by using artificial selection to create new plant varieties that are more resistant to certain pests and diseases, have improved yields, and have higher tolerances to environmental stresses.

Newer methods including biotechnology and genetic engineering have transformed agriculture. Laboratory researchers can genetically modify plants to suit their purposes, a much faster process than artificial selection. Transgenic plants are created by introducing one or more new genes into an organism, a process called transformation. In the past this was accomplished by hybridization, cross-pollinating different plant types. Recombinant deoxyribonucleic acid (DNA) technology now accomplishes the same task and even allows for the introduction to plants of genes from organisms belonging to completely different kingdoms. The new gene can be inserted either by gold particle bombardment or by insertion of the new gene into a plasmid vector and infecting the plant with an engineered *Agrobacterium tumefaciens* strain that carries the vector. The use of recombinant DNA technology has led to varieties with longer shelf lives, disease resistance, herbicide resistance, pest resistance, drought resistance, resistance to nitrogen starvation, and nutritional improvement.

With all the benefits agricultural advancements have given to society, agricultural technology has also caused some problems. Widespread use of chemicals as fertilizers, pesticides, and herbicides has raised concern for the health of the environment and its natural resources. While hunger still threatens certain populations around the globe, other populations have overgrown, forcing the cultivation of lands that once supported lively diverse biological communities and leading to the deterioration of many ecosystems. Another concern voiced by society is the escape of transgenes into the environment. For example, could herbicide-resistant genes move into the natural weed populations, eliminating their effectiveness for treating crops? The gene that encodes Bt toxin can be inserted into plant

Agricultural researchers perform controlled experiments in laboratory settings, in greenhouses, and in the field. These investigators are examining the effects of herbicides and fertilizers on crop size. *(Maximilian Stock Ltd./ Photo Researchers, Inc.)*

genomes so farmers would not have to spray them, but would the increased exposure lead to the selection for insect populations that are resistant to it?

Governments have developed policies regarding the goals and methods used in agriculture in order to correct some problems and prevent other potential future problems. Many of the policies are concerned with food safety, the production of sufficient quantities for a given population, food quality, conservation, environmental impact of farming practices, and economic stability. President Abraham Lincoln founded the United States Department of Agriculture (USDA) in 1862 to assist America's farmers and ranchers. Today the role of the USDA has expanded to include many other responsibilities such as developing anti-hunger programs, ensuring the safety of the food supply and of drinking water, and maintaining the health of the land through sustainable management. The concept of sustainable agriculture has been accepted and is being integrated into traditional agricultural practices. Sustainable agriculture considers the health of the environment, economic profitability, and economic equity while balancing the current needs with the needs of the future generations.

See also BIOTECHNOLOGY; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED; ENVIRONMENTAL SCIENCE; GENETIC ENGINEERING; PLANT DIVERSITY; PLANT FORM AND FUNCTION; POPULATION ECOLOGY.

algae Algae are a diverse group of aquatic photosynthetic eukaryotic organisms that make up one of two subkingdoms in the traditional kingdom Protista. (The other subkingdom is Protozoa.) Newer classification schemes place some algae in their own kingdoms and others in the plant kingdom. Algae are characteristically phototrophic, meaning they obtain their energy from sunlight, but vary widely in terms of cell structure and organization, size, and habitats. Algal cells contain the structures and organelles typical of eukaryotic cells, including a nucleus, mitochondria, an endomembrane system, and plastids (organelles that carry out photosynthesis). Some types of algae are unicellular, while others are filamentous or live in colonial arrangements. Algae are a main component of plankton, the mass of mostly microscopic organisms that float freely in aquatic environments, but they also live in soil, on rocks, or even in symbiotic relationships with other organisms.

Algae possess pigments that capture light of specific wavelengths, which the cells convert into chemical energy in the form of organic compounds. All algae contain chlorophyll a, the same pigment found in plants, but different types of algae contain different amounts of accessory pigments that absorb light of different wavelengths.

Algae are like photosynthetic plants but contain no true leaves, stems, roots, flowers, or veins and require moist or aquatic habitats. *(Simon Fraser/ Photo Researchers, Inc.)*

ALGAL CLASSIFICATION

The familiar term *algae* refers to all photosynthetic organisms that are not plants or bacteria. Unlike bacteria, algae are eukaryotic, and unlike plants, algae lack roots, leaves, and flowers. The more than 22,000 distantly related algal species have traditionally been categorized into seven main divisions as a matter of convenience rather than biological or evolutionary significance: Chrysophyta, Pyrrophyta, Euglenophyta, Chlorophyta, Rhodophyta, Phaeophyta, and Xanthophyta. Considerations for categorizing the algae include the combination of photosynthetic pigments contained in the plastids, the cellular structure and organization, composition of the cell wall, the presence or absence of flagella, reproductive means, and motility. The field of modern systematics depends on evolutionary relationships to classify organisms, but comparison of the deoxyribonucleic acid (DNA) sequences of the many diverse groups of photosynthetic protists shows that they have deeply divergent ancestries.

More modern classification schemes reflect the belief that eukaryotic algae developed by three different endosymbioses. The first evolutionary line includes the algae with chloroplasts that possess two membranes, Glaucophyta, Rhodophyta, and Chlorophyta. The second line includes algae with chloroplasts that are surrounded by an additional membrane of chloroplast endoplasmic reticulum, Euglenophyta and Dinophyta. The third line includes algae that possess two membranes of chloroplast endoplasmic reticulum, continuous with the outer membrane of the nuclear envelope: Cryptophyta, Bacillariophyta, Chrysophyta, Prymnesiophyta, Xanthophyta, Eustig-

matophyta, Rhaphidophyta, and Phaeophyta. A brief overview of some of the most common algal divisions follows.

Members of the division Chrysophyta, commonly known as the golden algae, often contain the photosynthetic pigments chlorophyll c and carotenoids such as fucoxanthin that give them a yellowish brown color in addition to the chlorophyll a that is common to all algae. Most golden algae are unicellular and free-swimming, but colonial and filamentous forms also exist. The cell walls of these mostly marine organisms contain silica compounds and pectin in addition to cellulose. In the absence of light or in the abundance of food, chrysophytes can become facultatively heterotrophic and feed on bacteria and diatoms. Closely related to Chrysophyta are the diatoms, organisms with two protective overlapping shell halves, like a box with a lid, that form, what is called diatomaceous earth over millions of years. The glassy shells contain pores that allow material to flow between the internal and external environments of the cells and give the organisms intricate decorative patterns. Chrysophytes store their food reserves as oil droplets, giving buoyancy to the structures positioning them close to the surface of a body of water so they can capture sunlight to fulfill their energy needs. With sufficient energy and nutrients, diatoms can reproduce asexually daily; however, this leads to progressively smaller progeny. Occasional sexual reproduction allows a full-sized organism to develop.

The division Pyrrophyta (fire algae) consists of the dinoflagellates, unicellular algae that also possess chlorophyll c but that more closely resemble ciliated protists than other algae. As their name suggests, the dinoflagellates have a set of perpendicularly arranged flagella, one at the posterior end and another located within a groove and that spins as the cell swims. As diatoms do, these organisms produce oil droplets, but also store energy as starch. Some dinoflagellates are free-swimming, but some live in symbiotic relationships. For example, many corals have dinoflagellates living inside their tissues. The dinoflagellates undergo photosynthesis, releasing the organic products and oxygen to the corals, which, in turn, excrete waste products from which the dinoflagellates extract nutrients such as phosphates and nitrates in addition to carbon dioxide for their own metabolism. Dinoflagellates are known for their tendency to form algal blooms, a phenomenon that results from nutrient and light conditions that support overgrowth of the organisms. Dinoflagellates live in both marine water and freshwater and mostly reproduce by asexual reproduction.

Members of Euglenophyta, the third strictly unicellular division of algae, are mostly freshwater and contain chlorophylls a and b and carotenoids. This branch of life surprised early biologists, who believed that all life-forms belonged in one of two categories, plants or animals. These organisms have a flagella, a light-sensitive eyespot that helps the organism seek an environment conducive to photosynthesis, and numerous chloroplasts. They store their food as starch. Individual Euglenid cells have been known to survive after losing their chloroplasts. They absorb nutrients from their environments and produce nonphotosynthetic, colorless progeny.

The division Chlorophyta contains the most diverse members and is perhaps the most widely known of the algae. As do plants, these green algae have both chlorophylls a and b and β-carotene as their photosynthetic pigments. Most of the green algae are unicellular, freshwater organisms, but some are multicellular or marine, and others live in moist terrestrial environments. This form of algae is the type that coats the surfaces of ponds. Filamentous green algae form mats that float on top of water. One such organism, *Spirogyra,* undergoes sexual reproduction by conjugation in order to prevent death from dehydration or freezing temperatures. Strands of filaments line alongside one another, form connections, and produce zygotes that can withstand the unfavorable conditions until the next season.

Red algae, belonging to the phylum Rhodophyta, contain phycobilins, photosynthetic pigments that capture green and blue light that penetrates deep into the water, allowing the red algae to survive at greater depths than other algae. Some red algae have cell walls made of calcium carbonate and play a role in building coral reefs. Their life cycles are complex; mature sporophytes undergo meiosis to produce haploid spores that grow into haploid gametophytes, which produce either eggs or sperm. Fertilization results in a diploid sporophyte body, completing the cycle.

Phaeophyta, the brown algae, contain chlorophylls a and c and fucoxanthin and have structures similar to those of plants. Holdfasts anchor the brown algae to seabeds or rocks, stemlike stipes bend with the waves, and blades, also called fronds, resemble leaves and contain the photosynthetic organelles. Gas-filled floats provide buoyancy to keep the blades nearer to the sunlight. Brown and red algae together constitute seaweed. Some brown algae can reach up to 100 feet (30 m) in length.

The Xanthophyta, or yellow-green algae, primarily inhabit freshwater. They lack chlorophyll b and the brown pigment fucoxanthin but do have chlorophyll c. The composition of the cell wall in Xanthophytes is unclear, but the shape often consists of two overlapping cylindrical halves. Many yellow-green algae are unicellular and possess flagella, but colonial and filamentous forms also exist.

ALGAL REPRODUCTION

Algae exhibit a variety of reproductive strategies. Asexually, some employ mitosis, while others produce new organisms by fragmentation of cells from colonies or from multicellular aggregates or by producing spores that develop into mature organisms. Flagellated motile spores are common to algae that live in aquatic environments, and nonmotile spores are characteristic of terrestrial algae. Sexual reproduction involves meiosis, the production of haploid gametes that combine to form diploid zygotes, a process that results in greater genetic variation. Isogamous unions occur when identical gametes combine during fertilization. During heterogamous fertilization, distinct male and female gamete types combine. Some algae undergo a process called alternation of generations, during which haploid generations, gametophytes, alternate with diploid generations, sporophytes.

ECOLOGICAL SIGNIFICANCE

As primary producers, algae fill a vital niche in many ecosystems. Not only do they play an important role in aquatic food webs and serve to produce most of the planet's oxygen, they also provide a significant source of iodine and protein for many human societies. Given certain conditions, the beneficial role played by algae can turn threatening when a sudden increase in the availability of nutrients supports their overgrowth and upsets the delicate ecological balance of marine organisms in the community by disturbing the food webs. As the algae die, other aquatic microorganisms decompose the organic matter, absorbing much of the available oxygen in the process. The increased oxygen demand by the microbial decomposers decreases the availability of oxygen for the other inhabitants, resulting in the death of other community members.

The organism *Gymnodinium breve,* a dinoflagellate, causes outbreaks of a phenomenon known as red tide. During the spring and fall, the waters of the shores of the Pacific, Gulf Coast, and New England states churn, carrying an abundance of nutrients to the surface. These conditions allow *Gymnodinium breve* to thrive, giving the water a reddish appearance. The algae secrete products that are toxic to fish and other marine organisms and that concentrate in shellfish. Though the toxin is harmless to shellfish, it can be harmful to humans who ingest it, causing neuromuscular problems such as numbness.

ECONOMIC IMPORTANCE

Algae are economically important in a number of ways. Many Eastern cultures use red and brown algae as a food source. In Asia, nori, a red alga, is used in sushi and also as a component of soups. Some algae are eaten directly as a vegetable or added to sweetened jellies. The brown alga commonly known as kelp is a good source of iodine, and other seaweeds provide necessary minerals and vitamins. Rhodophyta such as *Gelidium* and *Gracilaria* are an important source of agar, a substance that microbiologists use to thicken culture media and also to produce the gelatin capsules that contain drugs or vitamins. Extracts from a purple seaweed, *Carrageen,* are used in the food production, cosmetics, and pharmaceutical industries. Kelps also provide algin, a gelling agent used in many foods such as ice cream and in substances such as toothpaste.

See also BIOLOGICAL CLASSIFICATION; EUKARYA; EUKARYOTIC CELLS; PHOTOSYNTHESIS; PLANT DIVERSITY; PLANT FORM AND FUNCTION; PROTOZOA; REPRODUCTION.

FURTHER READING

Jackson, John, ed. *Encyclopedia of the Aquatic World.* Vol. 1. New York: Marshall Cavendish, 2004.

Phycological Society of America home page. Available online. URL: http://www.psaalgae.org/. Accessed January 13, 2008.

Sigee, David C. *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment.* Hoboken, N.J.: John Wiley & Sons, 2005.

Altman, Sidney (1939–) Canadian and American *Molecular Biologist* Sidney Altman received the 1989 Nobel Prize in chemistry, shared with the American molecular biophysicist Thomas Cech, for discovering that ribonucleic acid (RNA) had catalytic abilities. Biologists previously thought the role of RNA was simply to convey the information embedded in deoxyribonucleic acid (DNA) to the protein synthesis machinery. The revolutionary finding that RNA actively performs biochemical catalysis not only forced life scientists to redefine enzymes, but also led to a greater understanding of the evolution of the current roles of DNA, RNA, and proteins in living cells.

EDUCATION

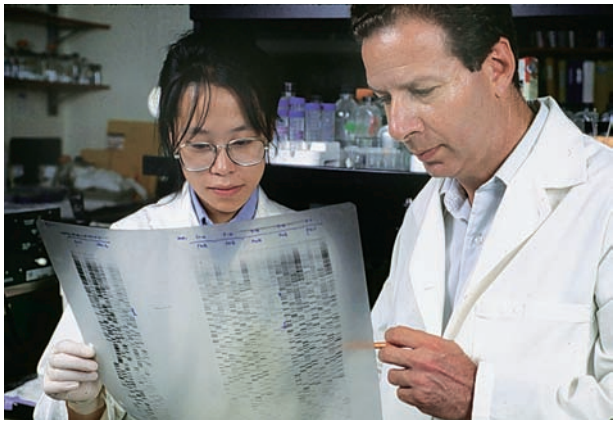
Sidney Altman was born on May 7, 1939, in Montreal, Quebec, Canada, to parents who encouraged reading and supported education as a means of creating opportunity. The atomic bomb sparked an interest in science in six-year-old Sidney, and as a teenager he became enthralled by the predictive power of science after reading *Explaining the Atom* by Selig Hecht. At the age of 17, he moved to Cambridge to study science at the Massachusetts Institute of Technology. He completed his bachelor of science degree

in physics in 1960, having written his senior thesis on nuclear physics. During his last semester he enrolled in an introductory course in molecular biology, not knowing that his own future contributions would help to shape this emerging field.

After graduation Altman began a graduate program in physics at Columbia University, but he left after one and one-half years and later ended up in a biophysics program at the University of Colorado Medical Center in Denver. He worked in the laboratory of Leonard Lerman, where he studied the effects of derivatives of the chemical mutagen acridine on DNA replication of bacteriophage T4. After completing his doctoral degree in 1967, Altman performed postdoctoral research in the laboratory of Matthew Meselson at Harvard University, studying the role of an endonuclease (an enzyme that cleaves within a segment of nucleic acid) in the replication and recombination of T4 DNA. He moved to Cambridge, England, in October 1969 to join the team of Sydney Brenner and Francis Crick at the Medical Research Council (MRC), a venture he later described as "scientific heaven." Altman thought his research project at MRC would involve the structure determination of transfer RNA (tRNA) using biophysical methods. During protein synthesis, tRNA molecules carry amino acids to the ribosome, where they become incorporated in the nascent polypeptide. Each tRNA has an anticodon, a sequence of three nucleotides that recognizes and specifically binds to a triplet codon on the messenger RNA (mRNA), ensuring that the correct amino acid is added to the growing chain.

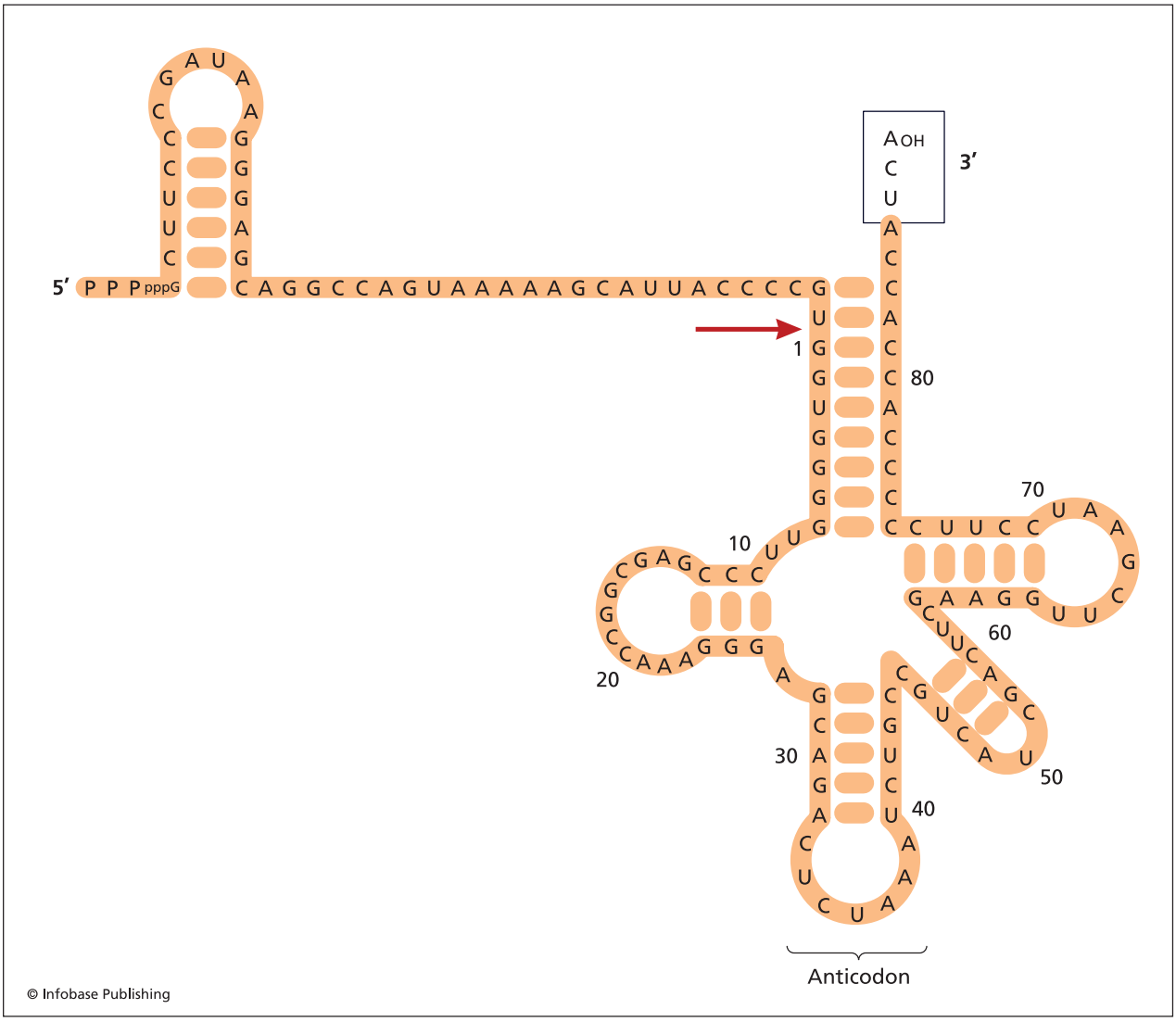
RESEARCH LEADING TO NOBEL PRIZE

By the time Altman arrived at Cambridge, another group had solved the basic structure of a yeast transfer RNA (tRNA), one that was available in large enough quantities for crystallographic studies, and Altman had to pick another research topic. In order to gain supporting evidence for the proposed tRNA structure, Altman decided to take a genetic approach to study the structure and function of bacterial $tRNA^{Tyr}$, the tRNA that carries the amino acid tyrosine. By examining acridine-induced tRNA mutants and suppressors of those mutants, he started working down a pathway that would eventually lead to his receiving the Nobel Prize. Mutation studies provide useful information for structure-function studies. When a mutation causes the loss of a particular function, studies of the associated suppressor mutations provide clues to the mechanism by which the gene product functions. The tRNA molecules in the original mutants did not function normally during translation. Suppressor mutations are mutations that fully or partially restore the function lost as a result of the first mutation. The information gained can support or guide complementary structural studies. Altman hoped to learn whether or not altering the spatial relationships in the tRNA by adding or deleting nucleotides (a change that acridine induces) would alter the function of the molecule.



Sidney Altman shared the 1989 Nobel Prize in chemistry for his work on catalytic RNA. *(Michael Marsland/Yale University)*

Molecular biologists knew that the synthesis of tRNA involved a processing step during which part of the 5' and 3' ends were removed, resulting in the release of a shorter mature tRNA molecule. One group of suppressor mutants that Altman created by treatment with the mutagenic chemical acridine never generated mature $tRNA^{Tyr}$; only the long precursor strands were present. Properties of the mutants suggested to Altman that the tRNA gene might be duplicated, since the mutants he used did not contain the usual suppressor tRNAs. He also thought that the bacteria transcribed the mutant gene and that isolating the mutant transcript would help elucidate the pathway for tRNA biosynthesis. In 1970 Altman successfully isolated and purified a homogeneous preparation of the transcript for the precursor $tRNA^{Tyr}$ from mutant cells that had been grown in the presence of radioactive phosphate. Subsequent treatment of the precursor tRNA molecules with bacterial cell extracts supplied by coworker Hugh Robertson resulted in shorter radiolabeled tRNA molecules, suggesting an enzymatic activity that was present in the extracts removed a portion of the precursor molecule. They named the activity that removed a portion of the 5' end of the precursor tRNA ribonuclease P (RNase P). Ribonucleases are enzymes that cleave RNA, and the *P* stood for "precursor." This accomplishment helped Altman obtain a faculty position the following year at Yale University in New Haven, Connecticut, in the Department of Molecular, Cellular, and Developmental Biology, where he continued his research.



During tRNA biosynthesis, the enzyme ribonuclease P removes a portion of the 5' end by cleaving at the position indicated by the red arrow.

The RNase P isolated by Altman and Robertson would eventually lead Altman into his Nobel Prize–winning research. Characterization revealed that the enzymatic activity was specific, in contrast to other known ribonuclease activities that nonspecifically cleaved nucleotides one at a time from the end of an RNA molecule. Robertson, Altman, and another colleague from MRC named John Smith published their initial characterization of RNase P in 1972, having noted that even their purest fractions contained nucleic acid. Because all known enzymes were proteins, the purification procedure had been optimized for isolating a protein. When RNA appeared in the purified enzyme preparations, everyone assumed the RNA was a contaminant. A few years later, a graduate student in Altman's lab, Benjamin Stark, showed that a high-molecular-weight RNA molecule copurified with the enzyme, and that this RNA was essential for the enzymatic activity. When nucleases digested all the RNA, the enzyme no longer functioned, suggesting that RNA played a critical role in the catalysis. This RNA was named M1 RNA. Others showed that RNase P also participated in the metabolic pathways for the synthesis of other tRNA molecules in *Escherichia coli,* and that RNase P activity was present in other organisms including humans. In 1983 Altman's laboratory published research demonstrating that purified bacterial M1 RNA had catalytic activity under certain buffer conditions and that it exhibited other characteristics of typical enzymes, such as being unchanged during a reaction, and that it had a rate dependent on substrate concentration, was only necessary in small concentrations, and was stable. This was the first demonstration of biological

catalysis performed by RNA. Not all enzymologists accepted this finding at first. In eukaryotic cells the RNA component of RNase P is not sufficient for catalysis; protein components are necessary for the enzymatic activity.

At about the same time, Thomas Cech, a molecular biophysicist at the University of Colorado in Boulder, discovered that a specific RNA molecule isolated from *Tetrahymena,* a ciliated protozoan, catalyzed its own cleavage in vitro in the absence of proteins. The RNA removed a segment of itself, then rejoined the remaining pieces. In 1989, the Karolinska Institute in Stockholm, Sweden, awarded Altman and Cech the Nobel Prize in chemistry for their work revealing the catalytic properties of RNA.

EXTERNAL GUIDE SEQUENCES

After performing extensive analysis of the mechanism by which M1 RNA catalyzed cleavage of the 5' end of precursor tRNA transcripts, Altman turned his expertise on RNA function beyond its serving as a passive messenger of genetic information to the problem of bacterial resistance to antibiotics. Physicians are having a difficult time treating many infections because the bacteria responsible for them have developed a resistance to antibiotics that once successfully destroyed the pathogenic bacteria. In some cases, the cause of the bacterial resistance to the antibiotics is the expression of a specific protein that has the ability to inactivate the antibiotic. In order to synthesize that protein, the bacteria must first transcribe the gene from the DNA to produce an mRNA transcript, which then is translated into a polypeptide. Altman and his colleagues made plasmids containing synthetic genes that encoded for small pieces of RNA that they called external guide sequences (EGSs) that were specifically designed to recognize the 5' untranslated region of the mRNAs from the antibiotic resistance genes. They inserted these plasmids into *E. coli* strains that exhibited resistance to antibiotics. These EGSs bound the target mRNA, forming a structure that the bacterial RNase P then hydrolyzed; thus the protein was not synthesized. Though the bacteria were originally resistant to antibiotic treatment, after transformation with the plasmids containing the EGS, they exhibited antibiotic sensitivity. This research was published in the prestigious journal *Proceedings of the National Academy of Sciences* in 1997.

The following year Altman's lab published similar results describing the use of EGSs directed against viral RNA. Viral infections result when a virus enters a host cell and directs that cell to synthesize viral proteins in order to replicate itself. In order to synthesize the viral proteins, the cell must first transcribe the viral genes to produce mRNA transcripts, which are then translated into polypeptides. The insertion into cultured mouse cells of synthetic genes for EGSs specific for the polymerase and nucleocapsid genes of influenza virus led to cleavage of the mRNAs for those genes by endogenous RNase P and efficiently inhibited viral replication. The goal of this research, which is also being carried out in cultured human cells, is to apply this strategy to treat viral illnesses, such as influenza, the common cold, or other diseases caused by a genetic mutation or the inappropriate expression of a protein. Interest in RNase P has also risen because of the presence of antibodies against two of its subunits in humans with autoimmune diseases, in which the immune system begins to attack the patient's own tissues.

ALTMAN'S INFLUENCE

Altman's pioneering work on the unique properties of RNA molecules opened the door for others to make discoveries just as amazing as RNA catalysis. Molecular biologists are learning that posttranscriptional processing is an important means of regulating the expression of many genes. The American molecular biologists Andrew Fire and Craig Mello received the 2006 Nobel Prize in physiology, or medicine, for their discovery of RNA interference, a means by which double-stranded RNA silences genes in nematodes, plants, and animals. RNA elements, called riboswitches, in the 5' untranslated region of mRNA fold into structures that interact with metabolites to interfere with the completion of transcription or with translation. One such riboswitch mechanism involves self-cleavage of the mRNA in response to the metabolite binding.

Since joining the faculty at Yale in 1971, Altman served as chairperson of the Department of Molecular, Cellular, and Developmental Biology from 1983 to 1985, and then as dean of Yale College from 1985 to 1989. Today Altman continues his research at Yale, where he holds the chair of Sterling Professor of Biology, as well as being a professor of chemistry. The broad goal of research in his laboratory is gene regulation by posttranscriptional RNA processing. In addition to the Nobel Prize, Altman received the Rosenstiel Award for Basic Biomedical Research and the National Institutes of Health Merit Award in 1989 and the Yale Science and Engineering Association Award in 1990.

Altman married Ann Köner in 1972, and they have two grown children, a son named Daniel and a daughter named Leah.

See also BIOMOLECULES; CECH, THOMAS; GENE EXPRESSION; RNA INTERFERENCE.

FURTHER READING

Altman, Sidney. "The Road to RNase P." *Nature Structural Biology* 7, no. 10 (October 2000): 827–828.

Altman, Sidney, and Leif Kirsebom. "Ribonuclase P." In *The RNA World*. 2nd ed., edited by Raymond F. Gesteland, Thomas R. Cech, and John F. Atkins, 351–380. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1999.

Guerrier-Takada, Cecilia, Kathleen Gardiner, Terry Marsh, Norman Pace, and Sidney Altman. "The RNA Moiety of Ribonuclease P is the Catalytic Subunit of the Enzyme." *Cell* 35, no. 3 (December 1983): 849–857.

Guerrier-Takada, Cecilia, Reza Salavati, and Sidney Altman. "Phenotypic Conversion of Drug-Resistant Bacteria to Drug Sensitivity." *Proceedings of the National Academy of Sciences USA* 94 (August 1997): 8,468–8,472.

Jarrous, Nayef, Paul S. Eder, Cecilia Guerrier-Takada, Christer Hoog, and Sidney Altman. "Autoantigenic Properties of Some Protein Subunits of Catalytically Active Complexes of Human Ribonuclease P." *RNA* 4, no. 4 (1998): 407–417.

Kresge, Nicole, Robert D. Simoni, and Robert L. Hill. "Ribonuclease P and the Discovery of Catalytic RNA: The Work of Sidney Altman." *Journal of Biological Chemistry* 282, no. 7 (February 16, 2007): e5–e7.

The Nobel Foundation. "The Nobel Prize in Chemistry 1989." Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1989/index.html. Accessed January 22, 2008.

Plehn-Dujowich, Debora, and Sidney Altman. "Effective Inhibition of Influenza Virus Production in Cultured Cells by External Guide Sequences and RNase P." *Proceedings of the National Academy of Sciences USA* 95 (June 1998): 7,327–7,332.

Robertson, Hugh D., Sidney Altman, and John D. Smith. "Purification and Properties of a Specific *Escherichia coli* Ribonuclease which Cleaves a Tyrosine Transfer Ribonucleic Acid Precursor." *Journal of Biological Chemistry* 247, no. 16 (1972): 5,243–5,251.

Stark, Benjamin C., Ryszard Kole, Emma J. Bowman, and Sidney Altman. "Ribonuclease P: An Enzyme with an Essential RNA Component." *Proceedings of the National Academy of Sciences USA* 75 (August 1, 1978): 3,717–3,721.

anatomy Anatomy is the branch of life science concerned with the structure of organisms. Because structure is closely associated with function at all levels of hierarchy in the life sciences, knowledge of anatomy allows one to understand better how organisms carry out life's necessary functions such as respiration, metabolism, and reproduction. Physiology, the branch of life science that deals with the functions of organisms or life processes, complements anatomy, and the two fields are often taught in concert.

Anatomy can focus on the morphology of different types of organisms, such as plant anatomy, vertebrate anatomy, or more specifically human anatomy. Health care professionals in fields such as medicine, dentistry, and physical and occupational therapy, and those who work in veterinary medicine, must study human or animal anatomy in order to understand disease and to help their patients remain healthy. Anatomic anomalies are deviations from normal patterns for a species. For example, while the normal pattern for a human includes 10 fingers and 10 toes, approximately 0.2 percent of children are born with more, a condition called polydactyly. Individuals with an extra digit may experience different degrees of affected mobility, but the condition is not dangerous. Other anatomic anomalies can prevent normal function and cause harm to a patient or may indicate a serious medical condition. For example, Marfan syndrome results from a mutant gene for the protein fibrillin, a component necessary for the formation of elastic fibers in connective tissue. One dangerous complication caused by the improperly formed connective tissue is a leaky mitral or aortic valve in the heart, which can lead to an irregular pulse, shortness of breath, fatigue, and a potentially fatal aortic aneurism. Individuals with Marfan syndrome have atypically long, slender limbs and fingers, a symptom that in itself is not harmful in any way. But recognition of this characteristic anatomical feature could aid a physician in making a timely diagnosis of this disorder.

Even when studies are limited to a particular organism, such as humans, the field of anatomy can be divided into specialties focusing on a specific region or system or on different levels of organization. Gross anatomy is the study of anatomy on the macroscopic level, the organs and aspects of the tissues that can be examined without the aid of a microscope. Conversely, cytology, the study of cellular architecture, and histology, the study of tissues, depend heavily on microscopy. A developmental anatomist studies the successive changes in body structure as an organism progresses from a fertilized egg to an embryo (a subspecialty called embryology) and then into a fetus and even into adulthood. In regional anatomy, one focuses on a limited region such as the head or the abdomen. Systemic anatomy includes study of physiological systems such as the integumentary, skeletal, muscular, nervous, endocrine, cardiovascular, lymphatic, respiratory, digestive, excretory, and reproductive systems.

Comparative anatomy involves the study and comparison of the structures of animal species and often examines the relationships among different anatomical strategies animals employ for carrying out life processes and the difference in their environments in which they live or the niches they fill. For example, consider that both aquatic and terrestrial

animals require oxygen to undergo cellular respiration, but the means by which they obtain oxygen gas from their external environment and transport it to their body tissues differ greatly. Different morphologies have evolved, depending on the needs of that particular species. The structure of a cell, tissue, organ, or organ system determines the function that structure can accomplish, but over long periods, different environments select for slightly modified structures that are better suited for those conditions. Thus structure determines the immediate potential function, but in the long term, the suitability of different functional adaptations will determine which structures prevail.

One means to study an organism's anatomy is by dissection, the systematic process of separating, taking apart, or exposing the parts for scientific examination. Teaching laboratories in high schools, universities, and medical colleges rely on dissection as a means to teach anatomy. Though preserved specimens are still a far cry from living organisms, dissections provide more realistic anatomical information than learning from observing two-dimensional sketches in a textbook or even three-dimensional images on a computer.

Anatomic imaging allows physicians to examine internal structures without surgery. X-rays are a form of electromagnetic radiation with short wavelengths. When an X-ray source is aimed at a region of the body, bones and other dense material in the path absorb some of the radiation, but it passes through softer tissues and reaches a piece of X-ray film positioned behind the body part of interest. The X-rays expose the film, and after developing the film, bones will appear white, and the exposed areas of the film will appear darker, creating a two-dimensional image for a radiologist to examine. Ultrasound imaging produces sonograms using high-frequency radio waves emitted from a device held next to the skin. The handheld device both transmits and receives the sound waves, which bounce off internal body structures and are analyzed by a computer. Ultrasound is frequently used to examine muscles, tendons, and other internal structures as well as to visualize a fetus during pregnancy. Computed tomography (CT), formerly known as computed axial tomography (CAT), generates a three-dimensional image by processing a series of subsequent or stacked two-dimensional X-ray images taken at repeated interval angles and reconstructing a digital image of the internal structures. Another method for obtaining internal structural information, magnetic resonance imaging (MRI), is better for examining softer tissues than CT scans, as it uses radio frequencies rather than ionizing radiation to collect data. The patient is placed in a large magnetic field and subject to radio waves, causing the orientation of the numerous hydrogen protons (mostly from water molecules) to align, a process that occurs at different rates for different types of tissues. A computer analyzes this information to generate images of cross sections of the body. All of these technologies allow medical personnel to examine the inside of a patient's body to look for structural abnormalities or even tumors that may be associated with particular conditions or diagnoses.

See also ANIMAL FORM; DISSECTION; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; PHYSIOLOGY; PLANT FORM AND FUNCTION.

FURTHER READING

Bailey, Jill. *Animal Life: Form and Function in the Animal Kingdom*. New York: Oxford University Press, 1994.

Melloni, June L. *Melloni's Illustrated Review of Human Anatomy*. 3rd ed. Cambridge: Cambridge University Press, 2006.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. New York: McGraw Hill, 2006.

animal behavior

Animal behaviors are the actions performed by an individual or species in response to its environment, including almost everything an animal does. Many behaviors involve a muscular activity, such as running away from a predator or building a nest. Other actions that are not as easily observed, such as the secretion of hormones to attract a mate or associating a certain color with danger, are also considered behaviors. Some behaviors are innate, or genetically determined, while others are learned. Ethology, the study of animal behavior, aims to understand the evolution, development, and control of the many behaviors exhibited by animals.

Understanding the mechanism by which an animal responds to a stimulus, in addition to the reason for it, helps form a more complete picture of the behavior. Ultimate questions address why an animal behaves in a specific way and examine the relationship of the behavior to fitness, or the reproductive success of an organism. Proximate questions address the physical adaptation that allows for the response, such as the responsible genes, biochemical pathways, anatomical structures, or physiological mechanisms, and the influence of development and maturation of the animal on the behavior. The answers to both ultimate and proximate questions make sense in light of the evolutionary process of natural selection. To summarize, animals exhibit different characteristics, including behaviors, determined by their genetic makeup. Individuals pass on some of their characteristics to their offspring via genes. Characteristics that improve one's success at transmitting his or her genes

to the next generation relative to other members of the species will become more common in the population over time. Natural selection favors behaviors that increase an organism's chance of surviving to adulthood, finding and selecting a fit mate, producing offspring, and raising those offspring to adulthood.

The environment, both the physical environment and other members of the ecological community, profoundly influences the traits that persist in a population. Resources, such as nutrients, or hormones affect the development of a behavioral trait, often by altering the expression of certain genes at key times during development. For example, the level of the hormone testosterone present during embryogenesis of male mice has been shown to affect aggression levels exhibited later in life. Sensory experiences, such as olfaction, can also affect the development of a behavioral trait. Exposure to a certain nest smell soon after the emergence of a paper wasp from a brood cell allows one to recognize individuals that shared that nest. Later in life, the wasp treats differently individuals that do and do not exhibit that specific scent, acting more aggressively toward individuals from a different nest.

The execution of any behavior involves sophisticated neural and hormonal controls. The animal must initially obtain information from the environment, such as hearing a song or seeing a color, a task often involving sensory reception. After the brain processes the input, the animal then responds, perhaps by pursuing a mate or fleeing a possible predator. The control of such behavioral responses often involves the expression of specific gene products during a learning experience or the activity of certain neural pathways that generate a motor response.

FIXED ACTION PATTERNS AND IMPRINTING

Fixed action patterns (FAPs) are innate, stereotypical responses triggered by a well-defined simple stimulus, called a sign stimulus or a releaser. Once initiated, the pattern continues until reaching completion. Web building in spiders and egg retrieval by geese are examples of fixed action patterns. An example of a FAP in humans is yawning; seeing another person yawning triggers yawning in the observer. As instincts, these behavior patterns require no previous experience with the stimulus cues and are functional from the first time they are performed.

Imprinting, on the other hand, involves learning, usually early in life during a sensitive period. Exposure to a certain stimulus influences the behavioral development of an individual. While the tendency to respond is innate, the stimulus is environmental. In a classical example, baby geese formed an association with the pioneering ethologist Konrad Lorenz, whom they observed moving away and calling for them soon after hatching. This experience somehow affects the development of the geese's nervous system. Later in life, the male geese developed a preference for humans, like Lorenz, as sexual mates. One can surmise the ultimate cause to be that young who imprint on and follow their mother will receive parental care and learn species-specific survival behaviors and skills. Thus imprinting leads to a greater chance of survival and reproductive fitness.

MOVEMENTS

Directed movements include genetically controlled simple movements that cover very short distances, perhaps a fraction of an inch, to those covering thousands of miles. Kinesis is a simple movement that lacks direction. An organism that typically moves in a random direction, such as by turning, will continue turning until it reaches favorable conditions. Adverse conditions can increase the movement, which increases the chance of finding a more favorable environment. Kinesis differs from taxis such as chemotaxis (movement toward a chemical or nutrient) or phototaxis (movement directed toward or away from light), because in kinesis, the sensors do not distinguish the direction from which the stimulus originates. Sow bugs, a type of terrestrial crustacean, demonstrate kinesis by responding to dry conditions with increased random movement. When the sow bugs encounter moist conditions, their activity decreases, increasing their chance of staying in the moist environment, which their bodies prefer. Taxis, on the other hand, is directed movement. For example, maggots have light-sensitive eyespots on both sides of their head. When light intensity is greater on one side than the other, the maggot moves away from the source of illumination until both sides are equally stimulated.

Taxis and kinesis are behaviors exhibited by animals over a short distance. Migration, while still genetically determined, is more complex and involves the seasonal movement from one biome to another, covering distances of several hundred to thousands of miles. Researchers have shown that genetic differences help explain the migratory behavior in birds. The behavior improves the animal's reproductive success, since the migratory patterns are often associated with breeding and nesting in addition to finding food and provide optimal environmental conditions for the different stages of the animal's life cycles. Examples of migratory animals include birds, termites, fish, crabs, wildebeest, turtles, monarch butterflies, and dolphins.

COMMUNICATION

The development and exhibition of many animal behaviors involve communication, the transmission

Millions of western sandpipers stop over at Grays Harbor, Washington, during their spring migration from the lagoons and coastal estuaries from California to Peru to the tundra of western Alaska for nesting. *(F. Stuart Westmorland/Photo Researchers, Inc.)*

of information. Animals usually accomplish this via visual, auditory, chemical, electrical, or tactile signals. Successful communication requires sending, receiving, interpreting, and responding to a signal. Honeybees put on an elaborate visual display to communicate information to nest mates about the direction, distance, and odor of newly discovered floral patches. The bees that witness the dance receive the visual sensory input and translate it to locate the food source. Mating songs produced by male fruit flies vibrating their wings are an example of an auditory signal. The female fruit flies recognize the structure of the songs from members of their own species by distinguishing such details as the length of time between the pulses of the wing vibrations. Chemical signals often include pheromones, odor-emitting stimuli. Domestic cats use pheromones contained in their urine to mark their territory, sometimes causing a problem for the pet owners, but animals in the wild often leave behind pheromones to mark the boundaries of their territories as well. When other animals detect the smell, they know the territory is already claimed. Structures called ampullae of Lorenzini found on marine sharks and rays aid in navigation and can detect weak electrical fields generated by other creatures moving nearby. Tactile communication includes behaviors that involve touching, such as grooming in primates, a behavior that promotes bonding.

FEEDING/FORAGING BEHAVIOR

One would expect natural selection to favor successful foraging behaviors, those that aid in finding and obtaining or capturing food. Much of an animal's anatomy and physiology depends on how that species procures its nutrition. Any movement performed by the animal to obtain the food is considered feeding behavior. A variety of mechanisms for drawing in small particles for food include using cilia to sweep food particles toward the body opening, as in sponges; secreting mucus to trap small particles, as in some snails; and using tentacles to paralyze and pull in prey, as in jellyfish. Animals can obtain larger particles by a variety of methods. For example, earthworms burrow, snakes catch and swallow small organisms whole, mammals and crustaceans catch and chew, spiders and sea starts catch and begin digestion externally and then ingest much smaller particles, and snails use their radula to scrape algae off rocks and pull them into their mouth. Fluid

Primates, such as these bonobos, groom each other as a form of bonding. *(Connie Bransilver/Photo Researchers, Inc.)*

feeders exhibit unique behaviors, such as mosquitoes piercing and sucking or just sucking as butterflies do. Feeding behaviors can also be classified on the basis of the degree of discrimination of food sources. Filter feeders take in everything of the appropriate size, whereas selective feeders use sensory input to make food choices based on available selections.

No matter what mechanisms an animal employs to obtain food, it costs energy to seek and obtain nutrition. Moving greater distances to find prey or to capture prey, having to work harder to break open a shell, or breaking down a larger mass of food to extract the energy from it all will influence what food sources an animal will pursue. The animal has no choice whether or not to expend energy but can make decisions regarding the amount of energy spent to obtain certain types of food. Optimal foraging theory purports that organisms will consume the most energy while expending the least amount of energy, predicting that evolution will select for foraging behaviors that optimize this ratio. One factor that affects foraging behavior is the need to minimize the risk of predation. This risk may affect behavioral decisions such as whether an animal forages during the day or night, under shelter or out in the open; how far from safety an animal will venture; how often an animal eats; and whether foraging is performed individually or in a group. Other behaviors related to feeding behaviors include social behaviors such as feeding as part of a courtship ritual, communication about food sources, cooperating to cultivate or obtain a food source, or feeding as part of parental caring for young.

COURTSHIP AND MATING

Many behaviors are involved in attracting potential mates and successfully mating with them. Songs, complicated dances, postures, athletic displays, and other similar rituals are examples of courtship behaviors intended to convince a potential partner of the individual's worthiness for mating. Male bowerbirds build elaborate structures called bowers by clearing an area of ground; padding it with moss, leaves, and the like; or building a teepeelike bower from twigs and sticks. The bird collects colorful trinkets such as flowers, shells, and berries to adorn the bower and then waits for a female to approach. When she does, he dances in front of his bower and shows off his decorations. If she is impressed, they will mate inside the bower. Afterward, he cleans up by returning all his objects to their designated places and waits for another female to approach, mating with as many as he can attract. Feeding behaviors are often incorporated into a courtship ritual; for example, male European nursery spiders capture prey, wrap it in silk, hold it in their teeth for presentation to a female. If the food gift is acceptable, she will bite into it, and while she eats it, the male will mate with her. Domestic cocks call and peck at the ground, even when food is not present, in order to attract a female. Aggressive (or agonistic) displays warn others off, such as when a strange male approaches the territory of another. Just as animals often fight to gain access to a particular territory or food resource, males also often fight to compete for access to a female partner.

Different species employ different mating strategies. Whether a species is mostly monogamous, meaning an individual only mates with one other individual, or polygamous, meaning an individual mates with many partners, influences the types of behaviors that attract mates. Whether or not a male is sure that his sperm fertilized the female's eggs, something that is more difficult to know in the case of internal fertilization than external fertilization, also influences mating behavior. To ensure his own genes are passed on to the offspring, a male might guard or protect his female mating partner from other males or even physically remove preexisting sperm from a female's reproductive tract before mating. This also affects the degree to which a male participates in the parental care of offspring. Males will participate in parental care more often when they are certain the offspring are their own.

Sexual selection is a special type of natural selection that works on characteristics that improve an organism's ability to compete for mates against other members of the same species. Sexual selection results in dimorphic species, in which the males and females differ in their appearance beyond the necessary differences in their primary sexual reproductive organs. For example, males birds are often more colorful than females of the same species. Understanding why natural selection would favor a characteristic that

would make an organism more visible to predators is difficult. Why would a female prefer such a mate? Some ethologists have suggested that colorful plumage might be an indicator of good health, something a female would desire in the father of her offspring in order for her offspring also to be healthy and to continue passing on her genes. By selecting such a mate, whatever the reason, the genes that influenced the female's decision to mate with males displaying that characteristic, as well as the genes for that characteristic itself, are passed on to the next generation.

PARENTAL CARE

Selection favors adaptations that improve the transmission of an individual's genes to future generations. An individual not only needs to reproduce, but needs the offspring to survive and also reproduce. In some species, both parents participate in caring for the offspring; in others only the mother or father does. The benefit to an individual in ensuring the survival of offspring is increased fitness, as the offspring pass on their genes to the grandchildren, and on to future generations, but this care has a great cost in terms of time and energy. Evolution has created a variety of different strategies for parental care. Most involve the mother more than the father. One reason for this is that fathers do not always know for sure that the offspring resulted from eggs fertilized by their sperm or sperm from other males. Also, males generally increase their fitness by reproducing with several mates, and the time spent caring for offspring is time they cannot spend seeking and obtaining additional mates.

Exceptions do exist, however, in which males of a species invest more in parental care than the females. Male bluegills build nests to attract females, then after mating, they stay there for a week to protect the fertilized eggs, occasionally fanning them to ensure they receive enough oxygen. While female emperor penguins go in search of food, the fathers guard their eggs between their feet under a fold of loose skin that blankets the egg from the Antarctic cold. The males all huddle together to help stay warm and forgo feeding for two months, guarding their eggs until their mates return.

Other examples of parental care include mother scorpions, who, after giving birth to their live young, carry their offspring around on their backs until they molt for the first time, up to two weeks after birth. Many frogs carry their broods in pouches on their backs or on the sides of their bodies. Male Darwin's frogs stay near the eggs they fertilize for three weeks. When the embryos begin to rotate in the translucent eggs, the male encloses them in his mouth, and they slide down into his vocal sac. The eggs soon hatch while inside the vocal sac, and the tadpoles continue to develop while the father provides nourishment and a safe, comfortable environment. After the young metamorphosize into frogs, they crawl into the father's mouth, causing him to gag, and the young frogs hop out. Australian social spider mothers perform the ultimate sacrifice for their young. After their offspring hatch, they give them food in the form of large insects, often weighing up to 10 times the mother's weight. When winter arrives and food becomes scarce, the mother allows her spiderlings to feed on her nutrient-rich blood, and then they inject her with venom and cannibalize her. While some examples are more extreme than others, parental care involves considerable investment, but one that yields a worthwhile evolutionary return, increased fitness.

See also ANIMAL COGNITION AND LEARNING; ECOLOGY; ETHOLOGY; EVOLUTION, THEORY OF; SOCIAL BEHAVIOR OF ANIMALS.

FURTHER READING

Alcock, John. *Animal Behavior: An Evolutionary Approach*. 8th ed. Sunderland, Mass.: Sinauer, 2005.

Crump, Marty. *Headless Males Make Great Lovers*. Chicago: University of Chicago Press, 2005.

Dugatkin, Lee Alan. *Principles of Animal Behavior*. New York: W. W. Norton, 2003.

Forsyth, Adrian. *A Natural History of Sex: The Ecology and Evolution of Mating Behavior*. Ontario, Canada: Firefly Books, 2001.

McFarland, David. *Animal Behavior: Psychobiology, Ethology, and Evolution*. 3rd ed. Harlow, England: Pearson, 1999.

animal cognition and learning Broadly defined, cognition is the ability of an animal's nervous system to perceive, store, process, and use information gathered through the senses. An animal's behavior depends on its cognitive experiences. Animal learning is the acquisition of a behavioral tendency based on specific experiences. When discussed in the context of human behavior, cognition often refers to a mental or intellectual awareness, and learning implies the gain of knowledge or a skill, but as a subfield of zoology, animal cognition and learning focus on actions and behaviors that can be experimentally examined and explained in terms of adaptive value within the context of an evolutionary framework. Most learned behaviors in animals are adaptations that help the animal find food, escape predators, or reproduce.

Habituation is a simple type of learning in which animals become accustomed to repeated exposure to a stimulus that does not provide any useful information. They become used to the stimulus and stop

responding to it. This can occur at different levels of nervous system function. The sensory organs may stop sending stimulatory signals to the brain, or the animal may continue to perceive the stimuli, but the brain stops initiating a physiological response. Ethologists, scientists who study animal behavior, depend on habituation in order to observe animals in their natural environments. At first the animals may act cautious or afraid of the researcher, but after continued exposure, the animals begin to ignore the presence of the investigator. The ultimate causation for this behavior, or the evolutionary explanation habituation exists, may be to preserve the animal's energy for real dangers.

Spatial learning refers an animal's ability to recognize and distinguish differences in the spatial structure or details of arrangement within a particular environment. Nikolaas Tinbergen, one of the founders of the science of animal behavior, performed a well-known experiment examining the ability of a digger wasp to recognize her nest. To test his hypothesis that the female wasp used visual landmarks to locate her underground nests, he placed a ring of pinecones around a hole in the ground that served as an entrance to a nest. After the mother flew away, he moved the pinecones and set them up in the same ringed orientation but to the side of the nest. When the female wasp returned, she flew to the center of the ring of shifted pinecones, demonstrating that she used visual cues to remember the location of her nests. In this illustration, the adaptive value of spatial learning is to increase reproductive success. Cognitive mapping is another strategy animals use to navigate their environments. More complicated than spatial learning, cognitive mapping involves the creation of an internal representation, such as a mental image, of spatial relationships among objects in an environment. For example, some birds collect and store food in hundreds of different hiding places. Rather than remember visual cues about every single cache, the bird may follow a general rule, such as that the food is always stored at the base of a tree or under a rock. Though spatial learning and cognitive mapping are difficult to distinguish experimentally, the advantage of having the ability to form a cognitive map would be reducing the amount of detail to memorize while still being able to locate food stores more efficiently than by having to search for them randomly. This would be similar to recognizing a pattern to an arithmetic sequence and applying a formula to determine a number at a given position in the series rather than memorizing the entire sequence of numbers.

Associative learning occurs when an animal begins to recognize a connection between two events, such as a behavior and a reward. Classical conditioning is a type of associative learning in which an animal develops a response to a previously neutral condition through repeated combined presentation. Unconditioned or primary stimuli are stimuli such as food or pain to which an animal reacts without training. The response is completely inherent and physiological. Conditioned or secondary stimuli are usually arbitrary stimuli that an experimenter or animal trainer has associated with an unconditioned stimulus. If the unconditioned and conditioned stimuli are presented together repeatedly, then the animal will become conditioned to associate the two stimuli and over time will respond to the unconditioned stimulus when presented with the conditioned stimulus. For example, a cat owner may want to train the cat to stay off the kitchen counters when hearing fingers snapping. When the owner sees the cat on the counter, he squirts water at it and snaps his fingers at the same time. Being squirted with water naturally startles a cat (no training necessary for this response), so the water is an unconditioned stimulus. The sound of snapping fingers means nothing to the cat initially, but over time the cat becomes conditioned to associate the snapping with an oncoming squirt of water. After repeated simultaneous exposure to both the unconditioned and the conditioned stimulus, the sound of snapping fingers eventually produces the startled response even in the absence of water.

Operant conditioning is similar to classical conditioning in that both are associative learning processes, but whereas classical conditioning results in the association of two stimuli, operant conditioning results in an animal's associating a behavior with a consequence, either a reward or a punishment. In classical conditioning, the animal responds to a stimulus, but in operant conditioning the animal learns that a consequence follows a voluntary response and thus affects whether or not the behavior will happen again. Reinforcements are actions that increase a behavior by either presenting a reward or removing an unpleasant stimulus. Punishments decrease the frequency of a behavior by either presenting an unpleasant stimulus or taking away a favorable stimulus. If a bear turns over a rock and finds numerous ants to eat, the bear will learn that the behavior of looking under rocks will bring about the food reward of ants and will turn over rocks more frequently. On the other hand, if an animal eats a plant that tastes bitter or makes it sick, the animal will learn to recognize and avoid eating that plant in the future.

Research has shown that some animals have advanced cognitive abilities that allow them to perform tasks such as categorizing objects and solving problems. For example, after training baboons to match alphanumeric characters displayed in different fonts or typefaces, the animals could correctly recognize and place the same letter or number into the

appropriate category when presented in a new font. Animal brains have adapted to deal with specific problems that their ancestors encountered; cognitive adaptations would have increased their ability to find food or survive challenges in order to reproduce successfully. Such abilities are demonstrated in pigeons, squirrel monkeys, baboons, gorillas, and chimpanzees, which have been shown to learn how to categorize through training as in the preceding example, but some studies show that other animals have this innate ability, as do humans. Researchers have observed young chimpanzees and infant macaques spontaneously sorting objects, such as models of animals and furniture or vehicles, according to perceptual properties. One can imagine that this adaptation would allow an animal to categorize an animal never seen before into a "dangerous" category and allow it to respond appropriately at the first encounter, or to recognize a potential new food source by its similarities to other familiar food sources, thus giving it an advantage if the familiar food source becomes scarce. The nervous systems of many animals allow them to solve complex problems, something that requires integrating prior experiences, observation, and insight. Different animals are capable of solving problems that require different degrees of problem solving skills. For example, if food is placed behind a barrier, can the animal figure out how to go around the barrier? Can an animal figure out how to get at food hanging from a string? The use of tools also demonstrates advanced cognitive abilities—creating a mental representation of an object to plan how to use it to achieve a goal or solve a problem. For example, a chimpanzee may stack boxes to climb and reach food placed high out of reach or a woodpecker finch may use a cactus spine to pry underneath the bark of a tree to access grubs hiding underneath.

Animals learn some of these complex behaviors by observing other animals performing similar tasks. Inherited or innate abilities and environmental influences both affect the ability of an animal to learn a complex behavior. The degree to which genetics or environment plays a role varies from behavior to behavior and from species to species. Genetics may exert control by setting a sensitive period during which an animal must learn a certain behavior, such as a species-specific song of a bird. For example, without being exposed to either real or recorded sparrow songs during the first 50 days of life, a white-crowned sparrow will not learn the adult song of its species. Other birds, however, such as New World flycatchers, develop the species-specific song even if raised in isolation; the behavior is completely innate.

Whether animals are capable of thinking is difficult to determine experimentally. Because they cannot communicate with humans through language, scientists have limited means to explore this aspect of animal behavior. Some people have taught gorillas and chimpanzees to use sign language to communicate, and the animals can convey basic needs and wants using signs. Some claim that the animals have expressed hopes and desires as well. Other investigators think that chimpanzees demonstrate self-recognition and awareness when they look in mirrors and perform actions on themselves, such as picking something out of their hair while looking in the mirror. Studies exploring concepts such as thought, self-awareness, and consciousness extend beyond connections between an animal's nervous system and its behavior and therefore beyond the realm of life science research and into the social science field of psychology. Though understanding human behavioral stimuli and responses can be useful in generating hypotheses concerning animal behaviors, life science researchers must be careful not to anthropomorphize, or attribute human behaviors, motivations, and feelings to animals.

See also ANIMAL BEHAVIOR; ETHOLOGY.

FURTHER READING

Dugatkin, Lee Alan. *Principles of Animal Behavior.* New York: W. W. Norton, 2003.

McFarland, David. *Animal Behavior: Psychobiology, Ethology, and Evolution.* 3rd ed. Harlow, England: Pearson, 1999.

Shettleworth, Sara J. *Cognition, Evolution, and Behavior.* New York: Oxford University Press, 1998.

Vauclair, Jacques. "Categorization and Conceptual Behavior in Nonhuman Primates." In *The Cognitive Animal: Empirical and Theoretical Perspectives on Animal Cognition,* edited by Marc Bekoff, Colin Allen, and Gordon M. Burghardt. Cambridge, Mass.: MIT Press, 2002.

animal form The kingdom Animalia includes more than 35 recognized phyla of multicellular organisms as diverse as jellyfish, dragonflies, and elephants. Despite apparent differences in size and shape, animals share many characteristics, as they are derived from a common evolutionary ancestor. Most animals are diploid, meaning they contain two copies of each chromosome. They reproduce by sexual reproduction, and during embryogenesis they form distinct layers of cells that develop into specialized tissues. As heterotrophs, animals fulfill their nutritional requirements by eating other organisms or by ingesting organic molecules made by other organisms. They have no cell walls and are capable of movement.

Though animals from various phyla or classes may appear very different, the most striking differences

are in size and shape. Animals exhibit a limited number of basic body plans, or programmed patterns of development, with slight variations among lineages. The environment and natural physical laws constrain or place limits on the ranges of overall body structures. For example, active living cells must exist in an aqueous environment, or at least be bathed in such a solution to allow the efficient exchange of nutrients, gases, and other substances. This necessity demands that larger and more complex animals must have extensive invaginations or branches within their bodies to allow for the exchange of nutrients and gases in all tissues. Furthermore, the volume that a given surface area across which exchange occurs can support limits the size of individual cells and ultimately the entire organism. Environmental constraints or the conditions of a particular habitat also influence the degree and types of variations an animal's body plan can tolerate.

HIERARCHICAL LEVELS OF ORGANIZATION

One difference between animal groups is the level of organizational complexity. The earliest life-forms were unicellular organisms, which exhibit the simplest level of organization. All of the functions necessary for life, such as obtaining of nutrition, metabolic processes, elimination of wastes, and reproduction, were carried out by individual cells, as they are today by prokaryotic organisms and unicellular eukaryotic organisms. The next level of organization builds upon the simplest level and is characterized by groups or aggregations of cells that share labor for a collective good. For example, *Volvox* is a green alga that lives in colonies shaped like hollow spheres consisting of hundreds or thousands of individual cells. The individuals must cooperate to reproduce. In asexual colonies, only cells in one region of the colony can divide to form new colonies. For sexual reproduction, male colonies release sperm, and cells within female colonies develop into eggs. The third level of organizational hierarchy is characterized by the presence of tissues, organized aggregates of cells that have similar structures and that perform similar functions. Some biologists consider sponges to have this level of organization, while others believe that sponges do not have any true tissues. Cnidarians like jellyfish do have a nerve net, a specialized tissue, but many of their cells are not organized into tissues. Organs are assemblies of several types of tissues that work together to perform a specific function; for example, the heart is an organ that pumps blood in many animals, such as earthworms, octopuses, and insects. At the highest level of structural organization within an organism, the organ-system level, several organs work together to accomplish a physiological function. For example, the stomach and the intestines are organs that function in conjunction with other organs and tissues as part of the digestive system, a physiological system that carries out digestion and absorption of nutrients.

TISSUE TYPES

After fertilization, the zygote undergoes cleavage, repeated mitotic divisions that result in a cluster of cells called a morula that eventually takes the shape of a hollow ball of cells called a blastula. The enclosed, fluid-filled cavity of a blastula is called the blastocoel. In a process called gastrulation, the cells of the blastula rearrange themselves, usually by first folding inward and then expanding out. Gastrulation results in a structure called a gastrula that comprises an outer layer of cells called the ectoderm and an inner layer called an endoderm. Diploblastic animals such as cnidarians (including hydra, jellyfish, sea anemones, and corals) and ctenophorans (comb jellies) only have these two germ layers. In triploblastic animals, a third layer, mesoderm, forms between the ectoderm and the endoderm. The blind pouch formed during gastrulation and lined with endoderm makes up the archenteron, and the opening where the initial infolding occurred is the blastopore. The archenteron and the blastopore will develop into the animal's digestive tract.

The ectoderm gives rise to the outer layer of skin, the nervous system, and the sensory organs. The endoderm forms the lining of the digestive tract, the respiratory system, the urinary bladder, the digestive organs, the liver, and several glands. The mesoderm develops into most of the skeleton, muscles, circulatory system, reproductive organs, and excretory organs. All of the differentiated tissues that make up the organs and structures of the organ systems are composed of four main tissue types that develop from the primary germ layers: epithelial, connective, muscular, and nervous tissues.

Epithelial tissues are sheets of cells that cover external body parts or line body cavities and ducts. Tight packing of the cells protects the underlying tissues and prevents the loss of fluids and the entry of potentially pathogenic microorganisms. Many cells on the surface of epithelial tissues produce and secrete substances such as digestive enzymes, sweat, or lubricating substances. The number of layers and the shape of the cells distinguish types of epithelial tissue. Simple epithelia have a single layer of cells, and stratified epithelia are multilayered. The shapes of the epithelial cells may be squamous (flattened to permit rapid diffusion), cuboidal (boxlike and often involved in secretion or absorption), or columnar (tall, rectangular prisms that often have microvilli to increase their absorptive surface area). In stratified squamous epithelium, the basal layer

continually undergoes mitotic divisions to replace cells from above that are damaged or that have died and sloughed off. Transitional stratified epithelium is adapted to stretching. A supportive basement membrane underlies all epithelial tissues and consists of substances secreted by both the overlaying epithelial tissue and neighboring connective tissue.

Connective tissue, derived from mesoderm, serves a supportive role and consists of a few cells and protein fibers embedded in a matrix, or ground substance. The fibers may be either loosely or densely packed. Loose connective tissue is found throughout the animal body and holds organs together and in place. Fibroblast cells within loose connective tissue form three types of fibers, collagenous, elastic, or reticular fibers. Macrophages, cells of the immune system that phagocytose old cells and foreign materials, wander through the ground substance. Dense connective tissue contains closely packed fibers, making tissues like tendons (that attach bones to muscles) and ligaments (structures that attach bones to other bones) very strong. Blood, lymph, fluids within the body tissues, cartilage, bone, and adipose tissue (which stores fat) are also considered connective tissues.

Muscular tissue, also derived from mesoderm, consists of elongated cells called muscle fibers that contain myofibrils, the units capable of contraction. Vertebrate animals contain three types of this abundant tissue: skeletal, cardiac, and smooth. Both skeletal and cardiac muscle tissues have striations, alternating thick or thin bands that run perpendicular to their length. The distance between these bands decreases during contraction. An animal can voluntarily contract skeletal muscles, resulting in movement, but cardiac muscle, found only in the heart, is under involuntary control. Contraction of smooth muscles surrounding the stomach helps churn the food contents. Smooth muscle also encircles the intestines, esophagus, blood vessels, bronchi, and other passageways where constriction controls the diameter, which in turn affects the rate by which substances move.

Nervous tissue is specialized to transmit, conduct, and receive nervous impulses, electrical signals that travel throughout the body via nerves. In animals that exhibit cephalization, most nerves lead either to or from the brain, which processes the information transmitted by nervous tissue. Nervous tissue consists of nerve cells called neurons and neuroglia, cells that insulate and support nerve cells.

Epithelial, connective, muscular, and nervous tissue make up the organs of all animal bodies, except for sponges, which only exhibit organization up to the tissue level. Different tissues and organs work together to carry out different physiological functions and are dependent on one another as components of the same organism.

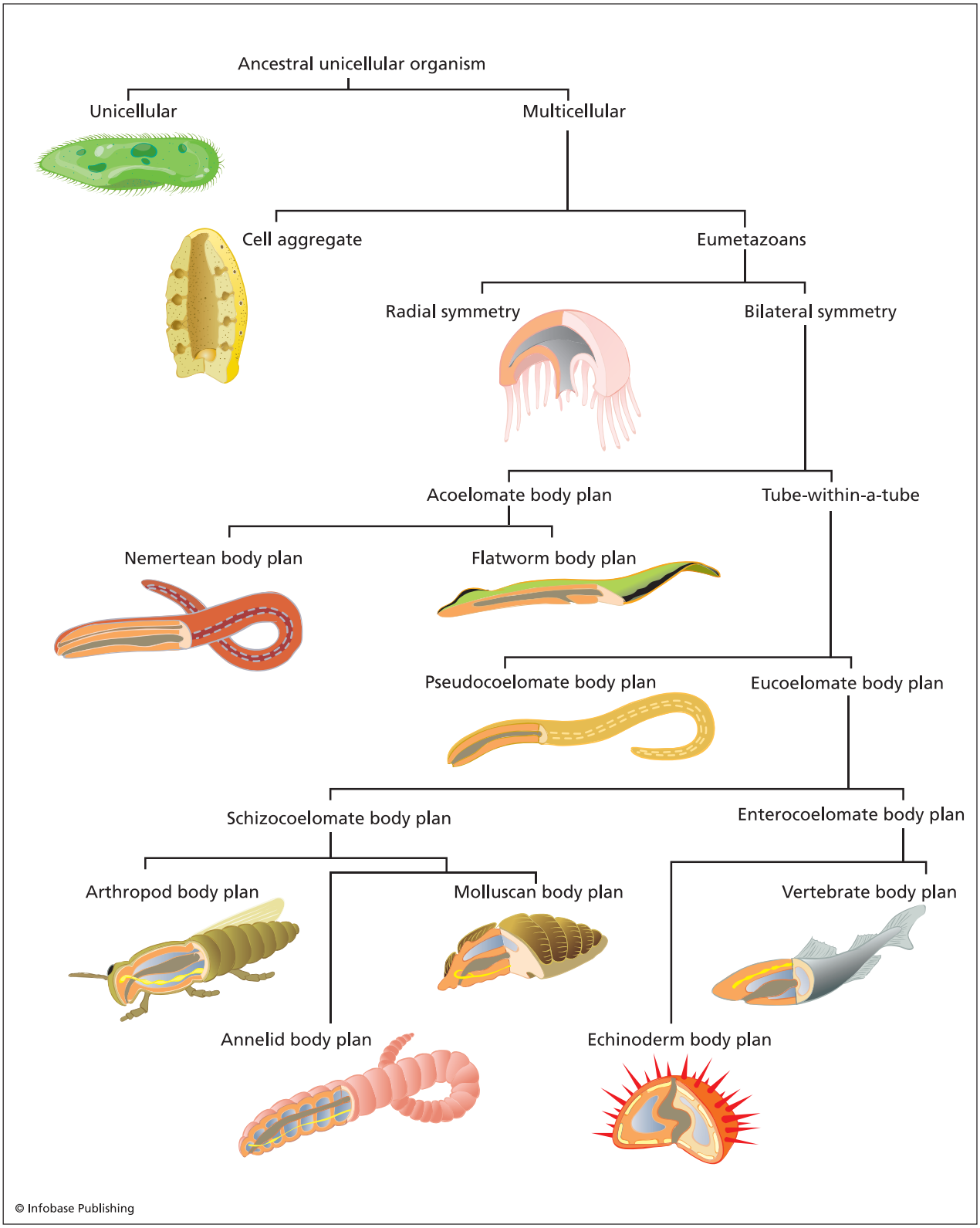
BODY PLANS

An animal's body plan refers to its developmentally programmed general shape, type of symmetry, and internal organization. Biological symmetry refers to a balance in the size, shape, and relative proportions of body parts on opposite sides of a median plane or center. With the exception of sponges, which are asymmetrical, animals exhibit either radial or bilateral symmetry. In radial symmetry, more than one plane passing through a central axis divides the animal into symmetric halves. Cnidarians and echinoderms (such as starfish and sea urchins) are radially symmetric. Animals with bilateral symmetry have one plane of symmetry that divides the body into distinct right and left halves. Bilaterally symmetric animals have nonsymmetrical dorsal (top) and ventral (bottom) halves occurring at the ends of the axis, and anterior (front) and posterior (back) sides in addition to right and left sides. The development of bilateral symmetry led to cephalization, the concentration of neural and sensory organs at the anterior portion, the head, of the body. Having eyes, ears, smell, or other sensory organs on the head provides an advantage for organisms as they travel forward: they can be aware of the environment they are entering and move toward food sources or away from danger.

Another evolutionary adaptation found in some bilaterally symmetric animals is a fluid-filled space. In coelomate animals, mesoderm completely lines this body cavity and forms a peritoneum, a sheet of connective tissue that wraps around and suspends the internal organs. This "tube-within-a-tube" arrangement provides extra space for body organs, allows for more body tissues to exchange nutrients and wastes, and allows animals to grow larger. Two different methods give rise to a coelom. In schizocoely, the mesoderm forms from cells near the blastopore, then splits to form the coelom. In enterocoely, the mesoderm forms from pouches of the archenteron. Both methods lead to the formation of a complete coelom with a peritoneum. Acoelomates, such as flatworms, have no body cavity; all of their body cells are in contact with other cells. Pseudocoelomates, such as roundworms, also have the tube-within-a-tube arrangement but do not have a peritoneum. In other words, their body cavity is merely a blastocoel left over from embryogenesis.

Bilateral animals can also be divided into groups based on the fate of the blastopore, a characteristic related to the method by which the coelom forms. After the formation of the archenteron during gastrulation, another opening forms at the end of the

embryo opposite the blastopore, where the infolding began. One of the holes becomes the mouth and the other becomes the anus in a one-way digestive tract. In coelomate protostomes, the coelom forms by schizocoely, and the mouth develops from or near the blastopore. In deuterostomes, which are all



Though animals have a variety of shapes and sizes, all are variations of a few basic body plans.

coelomates, the coelom forms by enterocoely, and the anus forms from or near the blastopore. The division of bilateral animals into protostomes and deuterostomes is somewhat controversial because some phyla exhibit qualities of both groups.

Metamerism, or segmentation, is the condition of having the body divided into a linear series of metameres, or repeated segments. Annelids (including earthworms), arthropods (including insects), and chordates (including vertebrate animals) all possess segmented bodies, though in some animals the segmentation is not as apparent as in others. For example, evidence of segmentation exists in vertebrate embryos and in insect larvae, but segments are not as obvious in the adult forms. In animals such as earthworms, many of the internal organs within each segment also repeat.

See also ANATOMY; ANIMAL FORM; CIRCULATORY SYSTEM; DIGESTIVE SYSTEM; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; ENDOCRINE SYSTEM; EXCRETORY SYSTEM; HOMEOSTASIS; HOST DEFENSES; HUMAN REPRODUCTION; INTEGUMENTARY SYSTEM; INVERTEBRATES; MUSCULOSKELETAL SYSTEM; NERVOUS SYSTEM; REPRODUCTION; RESPIRATION AND GAS EXCHANGE; SENSATION; VERTEBRATES.

FURTHER READING

Hickman, Cleveland P., Jr., Larry S. Roberts, Susan L. Keen, Allan Larson, Helen I'Anson, and David Eisenhour. *Integrated Principles of Zoology*. 14th ed. New York: McGraw-Hill, 2008.

antimicrobial drugs

Antibiotics are substances that are made by one microorganism that inhibit the growth of or kill another microorganism. Semisynthetic antibiotics are antibiotics derived from a substance produced by a microorganism. Many diseases result from infection by a pathogenic microorganism. The use of chemicals in the treatment of disease is called chemotherapy, the goal of which is to destroy the responsible microorganism while causing minimal harm to the host. In the early 1900s, the German physician Paul Ehrlich conceived of a "magic bullet," an agent that would selectively target and kill a disease-causing organism. Ehrlich's research led to his discovery of salvarsan, the first chemotherapeutic agent, used to treat syphilis. Though salvarsan did serve as an effective chemotherapeutic agent for syphilis, it is not considered an antibiotic since a microorganism does not produce it. In 1928 Sir Alexander Fleming, a British bacteriologist, serendipitously discovered the first antibiotic, penicillin, when he observed that a substance produced by the mold *Penicillium notatum* inhibited bacterial growth in culture. Sir Howard Florey and Ernst Chain from Oxford University performed the first successful clinical trials of penicillin to treat an infection in 1940. These events led to the age of antibiotics, during which microbiologists have discovered thousands of antibiotics. Many potentially fatal infectious diseases became easily treatable, leading to a significant decline in the mortality rate from infectious diseases. Antibiotics are not without problems, however; many induce negative side effects and can be toxic, and the growing resistance of many microorganisms to different antibiotics is cause for alarm.

MECHANISMS OF ACTION OF ANTIBACTERIAL ANTIBIOTICS

Most antibiotics are only effective against bacteria. Infections by fungi, protozoa, helminthes, or viruses necessitate different types of chemotherapeutic agents. Antibiotics work by either inhibiting the growth of a microorganism or killing the microorganism. If an antibiotic prevents further growth of a pathogen, the body's immune system can often eliminate any microorganisms that are already present. The major mechanisms of action for antibiotics include inhibition or destruction of the cell wall, inhibition of protein synthesis, destruction of the cell membrane, inhibition of nucleic acid synthesis, and inhibition of synthesis of other essential metabolites.

The purpose of the bacterial cell wall is to give shape to the cell and protect it against adverse changes in the external environment, such as a decrease in solute concentration, which could result in cell lysis from a buildup of water pressure inside the cell. The main component of most bacterial cell walls is peptidoglycan, composed of repeating disaccharide subunits made of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic (NAM) acid. Polymers of these repeats are linked by cross-bridges consisting of tetrapeptides, chains of four amino acids, forming a sheetlike layer. Prokaryotic cells are grouped into two main categories, gram-negative or gram-positive, based on differences in how they respond to a particular staining method called the Gram stain. Gram-negative cells have as little as a single layer of peptidoglycan, but the cell wall of gram-positive cells can contain as many as 40 stacked layers. Some antibiotics, such as penicillin and its derivatives, prevent the synthesis of peptidoglycan, and therefore the construction of cell walls, in actively growing cells. These antibiotics will not, however, kill any cells that already have completely formed cell walls and that are not growing. Because gram-positive cells contain much more peptidoglycan, these antibiotics are most effective against gram-positive bacteria. Antibiotics that only work on limited types of bacteria, such as penicillin, are said to have a narrow spectrum of activity. Other antibiotics that work by preventing cell wall synthesis are the cephalosporins, bacitracin,

and vancomycin. Because human cells do not have cell walls, this class of antibiotic does not harm host cells.

Many antibiotics destroy bacteria by interfering with the process of protein synthesis. Cells that are actively growing must constantly synthesize new proteins. Ribosomes perform the function of reading a messenger ribonucleic acid (mRNA) transcript and linking the correct sequence of amino acids to construct a new polypeptide chain that may or may not combine with other polypeptides to form a complete protein. Ribosomes consist of two subunits, one large and one small. In prokaryotic cells, the subunits are 50S and 30S, which join to form a 70S ribosome; in eukaryotic cells, the subunits are 60S and 40S, which together make an 80S ribosome. (The *S* refers to the way the subunits sediment when centrifuged, a characteristic dependent on their size and overall shape.) Though prokaryotic cells and eukaryotic cells synthesize proteins by a very similar process, the differences in the structure of the ribosomes are significant enough for antibiotics to target prokaryotic ribosomes without affecting eukaryotic protein synthesis. By inhibiting the synthesis of new proteins, the antibiotics prevent cells from growing and dividing and from maintaining sufficient levels of so-called housekeeping proteins. Some antibiotics inhibit ribosome function by binding to the 50S portion of the ribosome—for example, chloramphenicol and erythromycin. Others, such as tetracyclines, streptomycin, and gentamicin, interact with the 30S subunit. Most antibiotics that interfere with ribosome function affect a wide range of bacterial species; in other words, they have a broad spectrum of activity.

Because the construction of gram-negative cell envelopes differs from construction of gram-positive cell envelopes, even though some antibiotics can bind to the ribosomes of all prokaryotes, they cannot penetrate gram-negative cell walls and thus are ineffective for treating infections caused by gram-positive bacteria. Although 80S ribosomes carry out most of the protein synthesis in eukaryotic cells, the mitochondria contain 70S ribosomes that synthesize proteins from mitochondria genes. Antibiotics that interact with prokaryotic ribosomes also interact with mitochondrial ribosomes and therefore can cause negative side effects such as fatigue.

Another group of antibiotics works by damaging the cell membrane. Disruption of the integrity of the cell membrane results in the loss of control over what enters and leaves the cell. If the injury is very large, the cell lyses and releases all its contents, causing immediate cell death. Antibiotics such as polymyxin B attach to the phospholipids that compose the cell membrane, making it more permeable and allowing important nutrients to escape.

Antibiotics that inhibit nucleic acid synthesis either interfere with deoxyribonucleic acid (DNA) replication or transcription, the process of making an mRNA transcript from a gene on the DNA. Transcription must occur prior to translation, the synthesis of polypeptide chains by ribosomes, as a crucial step in new protein synthesis. The processes of DNA replication and transcription are similar in prokaryotic and eukaryotic cells; thus antibiotics intended to harm the pathogenic bacteria may also harm the host. Rifampin, quinolones, nalidixic acid, nofloxacin, and ciprofloxacin are examples of DNA replication inhibitors. Rifamycins inhibit transcription.

The last group of antibiotics includes those that are competitive inhibitors for enzymes that catalyze steps of important metabolic pathways. A competitive inhibitor is a chemical that binds to an enzyme and thus prevents the natural substrate from binding. If the substrate cannot bind, then the enzyme cannot catalyze the reaction at that step in the metabolic pathway. For example, para-aminobenzoic acid (PABA) is a metabolite that bacteria chemically convert into the vitamin folic acid. Bacteria need this vitamin in order to make components of nucleotides, the building blocks of the nucleic acids DNA and RNA. If a cell cannot synthesize these components, transcription and DNA replication will cease. Sulfanilimide, a sulfa drug, mimics the structure of PABA and competes for binding to the enzyme that normally binds PABA. When this happens, the cell cannot make folic acid. Even though humans also need folic acid to remain healthy, they obtain it through diet rather than synthesize it, so the host is not affected. Sulfones and trimethoprim also work by preventing the synthesis of important metabolites.

ANTIFUNGAL, ANTIPROTOZOAN, AND ANTIHELMINTH DRUGS

Most antibacterial antibiotics are useful because they selectively target characteristics of prokaryotic cells such as peptidoglycan or 70S ribosomes; thus they only harm the bacteria in or on the host's body—in other words, they are selectively toxic. Finding chemotherapeutic agents that are selectively toxic for pathogenic fungi, protozoa, or helminths (parasitic worms) is more difficult since they are eukaryotic. Chemicals that adversely affect parasitic eukaryotes often also harm the host cells. Antifungal drugs such as polyenes and azoles often work by targeting sterols, a type of lipid present in fungal cell membranes. Their therapeutic value is limited, however, because human cells also have sterols in their cell membranes. Polyenes, such as amphotericin B, bind to sterols in the membrane and make it more permeable. Azoles (for example, imidazole, triazole, clotrimazole, miconazole, and fluconazole) inhibit the synthesis of sterols.

Quinine, a chemical originally isolated from the Peruvian cinchona tree, and its synthetic derivatives are used to treat the malaria caused by the protozoan *Plasmodium*. Some drugs used to treat tapeworm infestation act by inhibiting adenosine triphosphate (ATP) synthesis or changing the membrane permeability. Other helminth infections are treated by chemicals that cause the worms to have muscular spasms, disrupting microtubule action and interfering with mobility, or by paralyzing the worms.

ANTIVIRAL DRUGS

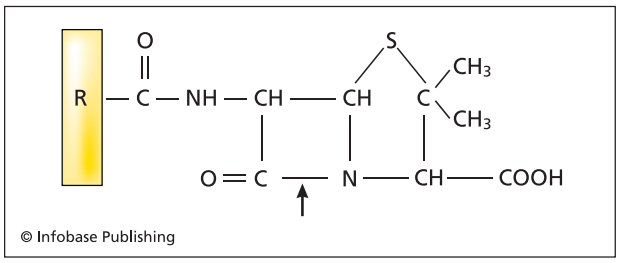
Viruses are not living entities; thus no drug can kill a virus. Any drugs used to treat viral infections must work by preventing further viral replication. Because viruses depend on host machinery to replicate, finding or designing drugs that inhibit viral DNA replication or transcription without also affecting the host cell activities is sometimes challenging. Antiviral drugs can target replication of the viral nucleic acid or the transcription of its genes when the virus carries specific genes required for these processes. Antiviral drugs often work by interfering with attachment of the viral particle to the host cell, preventing penetration, inhibiting uncoating of the viral nucleic acid once it enters the cell, or preventing the assembly of new viral particles. Some antiviral medications, including acyclovir, ribavirin, and zidovudine (AZT), mimic the structure of nucleotide bases. The DNA polymerase incorporates these base analogs into the viral nucleic acid, then cannot proceed with synthesis because the analog has no attachment site for the next nucleotide. Ideally, the virus encodes its own DNA polymerase, which is selectively inhibited by the base analogs. Retroviruses, such as human immunodeficiency virus, are viruses that have an RNA genome and depend on an enzyme called reverse transcriptase to synthesize DNA from RNA. Some drugs interfere with this enzyme's activity. Drugs called protease inhibitors inhibit enzymes necessary for the assembly of new viral particles and are also used to treat human immunodeficiency virus (HIV) infections. Enzyme inhibitors are used to treat influenza also. Another antiviral chemical is interferon, a natural substance produced by cells in response to infection by a virus that inhibits the virus from infecting neighboring cells. Physicians often prescribe interferon for viral hepatitis.

RESISTANCE

When bacteria do not respond to treatment with a particular antibiotic, the bacteria are said to be resistant, or insensitive, to that antibiotic. In some cases, the mechanism of action is simply not effective for a particular type of bacteria, but in other cases, bacteria that once were sensitive have developed a resistance to the antibiotic. The global increase in antibiotic resistance is a major current public health concern. As resistant populations replace the normal sensitive populations, infections grow increasingly difficult to treat.

Resistance results from the phenotypic expression of a gene, so the development of resistance in bacteria that previously were sensitive results from a change in genotype, the genetic makeup of an organism. Alterations in genotype occur by spontaneous mutations or by acquiring of a new gene through the processes of transformation, conjugation, or transduction. The gene product can lead to antibiotic resistance by one of several different mechanisms. Point mutations usually affect the cellular target of the antibiotic, and acquired genes usually encode enzymes that either inactivate the antibiotic or affect its ability to enter and stay in the cell. One mechanism of resistance works by destroying the antibiotic. Penicillin and its derivatives all contain a unique structure called a β-lactam ring. The enzyme β-lactamase cleaves the β-lactam ring, inactivating it. Expression of these enzymes, generally referred to as penicillinases, is the most common mechanism of resistance to this class of antibiotics. Another common means of resistance involves a mutated membrane transport protein that prevents the antibiotics from gaining entry into the cell. A slight change in the structure of the target of an antibiotic can prevent the drug from recognizing and binding it. For example, a substitution of an amino acid in one of the ribosomal polypeptides can prevent an antibiotic from binding to that ribosomal subunit and inhibit protein synthesis. Alternatively, a cell could up-regulate the expression of the target molecules, so that the concentration of the target increased, bound up all of the available antibiotic, and still had enough left over to carry out the normal function. Depending on the mechanism of action of an antibiotic, down-regulation of a bacterial protein can also lead to resistance. For example, if an antibiotic makes use of a bacterial protein to gain entry into the cell, a decrease in the amount of that protein can decrease the antibiotic's effectiveness. A bacterial cell can become resistant by developing a means for ejecting the antibiotic from the cell before it can interfere with the bacterial cell processes. Enzymes called translocases pump the drugs out of the cell and are relatively nonspecific; thus they cause bacteria to be resistant to several drugs.

Numerous factors have contributed to the alarming increase in resistance to antimicrobials, the most significant being indiscriminate use. Simply put, the presence of antibiotics selects for bacterial strains that are resistant, so frequent use increases the numbers of resistant bacteria. Viruses are the most common cause of colds and sore throats, but many patients



Penicillin and its derivatives share the black-and-white portion of this structure and have variable R groups. The enzyme β-lactamase cleaves the β-lactam ring structure at the covalent linkage indicated by the arrow. Bacteria that synthesize the enzyme β-lactamase destroy penicillin and its derivatives, making them resistant to that class of antibiotics.

demand and many physicians prescribe antibiotics to treat such maladies, though antibiotics do not affect viruses in any way. Viruses do not have cell walls, 70S ribosomes, or prokaryotic molecular machinery, common targets of antibiotics. Hospitals harbor many drug-resistant bacterial strains, an unfortunate fact since many hospitalized patients are already immunocompromised or have wounds that facilitate the entry of pathogens into the host's body. Since the 1950s, strains of *Staphylococcus aureus* found in hospitals went from almost all being sensitive to almost 100 percent being resistant. Methicillin-resistant *S. aureus* (MRSA), a strain that is resistant to most antibiotics, is prevalent in hospitals and other health care facilities and a frequent cause of potentially fatal staph infections. The powerful antibiotic vancomycin was the last resort drug to treat such infections until recently, when vancomycin-resistant strains started appearing. Vancomycin-resistant *Enterococcus* (VRE) is another danger in health care facilities. Though VRE exists as part of the normal human flora, in immunocompromised individuals (infants, the elderly, and very ill people), VRE can overgrow, resulting in diarrhea.

To limit the continued spread of resistant strains, physicians should only prescribe antibiotics when appropriate, and patients should follow instructions regarding dosage and duration of treatment. The simultaneous treatment with more than one antibiotic decreases the chance that a resistant strain will survive. Researchers should continue to search for new antimicrobials with structures that do not lend themselves to easy destruction or avoidance. Measures to decrease the use of antimicrobials in animal feed worldwide will also help to curb the spread of resistance.

See also BACTERIA (EUBACTERIA); ENZYMES; FLEMING, SIR ALEXANDER; FUNGI; GENE EXPRESSION; INFECTIOUS DISEASES; MICROBIOLOGY; PROKARYOTIC CELLS; PROTOZOA; VIRUSES AND OTHER INFECTIOUS PARTICLES.

FURTHER READING

Centers for Disease Control and Prevention, Department of Health and Human Services. "General Information about Antimicrobial Resistance." Available online. URL: http://www.cdc.gov/drugresistance/general/index.htm. Accessed January 13, 2008.

Tortora, Gerald J., Berdell R. Funke, and Christine L. Case. *Microbiology: An Introduction*. 9th ed. San Francisco: Benjamin Cummings, 2007.

U.S. National Library of Medicine, National Institutes of Health, Department of Health and Human Services. "Medline Plus: Drugs, Supplements, and Herbal Information." Available online. URL: http://www.nlm.nih.gov/medlineplus/druginformation.html. Updated August 28, 2008.

Archaea According to the five-kingdom classification scheme proposed by Robert Whittaker, the kingdom Monera comprised all prokaryotic organisms. In 1977 Carl Woese from the University of Illinois at Urbana-Champaign performed an extensive molecular analysis of several types of prokaryotic organisms and obtained surprising results. Comparison of the sequences for the 16S ribosomal ribonucleic acid (RNA) genes showed that some of the prokaryotes differed tremendously from others, enough so that they warranted their own superkingdom on the tree of life. He proposed the formation of three domains of living organisms: Eukaryota, which contains the protists, fungi, plants, and animals; Bacteria, or Eubacteria, which includes the more familiar prokaryotic organisms; and Archaea, which includes the most recently recognized forms of prokaryotic life.

The domain Archaea is named for the Greek word meaning "ancient," because archaea live in conditions similar to those on Earth 3.5 billion years ago. Most of the planet was covered in water that contained harsh chemicals and often reached boiling temperatures. Microbiologists do not yet know nearly as much about archaea, which have only been recognized for a few decades, as they do about bacteria. Archaea often live in extreme environmental conditions; such organisms are called extremophiles, and their unusual growth requirements make it more difficult to study them in the laboratory. Not all archaea are extremophiles, and not all extremophiles are archaea. Thermophiles are extremophiles that live at very high temperatures, and psychrophiles live in extreme cold. Acidophiles and alkalinophiles live

in acidic or basic environments. The halophiles ("salt lovers") live in very salty environments. Another major group of archaea are the methanogens, which produce methane gas (CH_4) as a by-product of their metabolism.

ARCHAEAN CHARACTERISTICS

Archaea are unicellular, prokaryotic organisms that have a variety of shapes, such as rods, cocci, and other unusual forms including triangles. Members of this domain share similarities with both bacteria and eukaryotic organisms. As prokaryotes, archaea lack internal compartmentalization. The single chromosome that makes up their genome is not bound by a nuclear envelope. Like bacteria, archaea can be gram-negative or gram-positive, but the composition of their cell walls differs. Bacterial cell walls consist of peptidoglycan, made of chains of alternating subunits of *N*-acetylglucosamine and *N*-acetylmuramic acid and linked together by short peptide bridges. Gram-negative archaea have a proteinaceous layer associated with their cell membranes. In some gram-positive archaea, the thick cell wall consists of pseudomurein, which contains *N*-acetylalosaminuronic acid in place of *N*-acetylmuramic acid in addition to different types of amino acids. Other archaea contain different complex polysaccharides. Because of this, antibiotics that are harmful to bacteria, such as penicillin, and chemicals, such as lysozyme, that target peptidoglycan are ineffective against archaea.

The membrane lipids of archaea are also distinctive. The lipids contain branched isoprenes rather than fatty acids, and the lipids are linked to glycerol through ether bonds rather than ester bonds as in bacteria and eukaryotic organisms. Sometimes the

COMPARISON OF ARCHAEA, BACTERIA, AND EUKARYA

	Archaea	Bacteria	Eukarya
nuclear envelope	absent	absent	present
membrane-bound organelles	absent	absent	present
cell wall	composed of proteinaceous subunits (in most), in some made of pseudomurein	made of peptidoglycan	if present, composed of a variety of substances such as cellulose or chitin
lipids in the cell membrane	branched carbon chains made of isoprene derivatives, ether-linked	straight chain fatty acids, ester-linked	straight chain fatty acids, ester-linked, contain sterols
initiator tRNA	methionine	N-formyl-methionine	methionine
introns	absent	present in some genes	present
sensitive to the antibiotics streptomycin, kanamycin, and chloramphenicol	no	yes (for most)	no
DNA bound by histones	yes	no	yes
chromosome	single, circular	single, circular	linear, number varies
microtubule cytoskeleton	no	no	yes
ribosomes	70S	70S	80S
plasmid DNA	present	present	rare
ribosomes sensitive to diphtheria toxin	yes	no	yes
polycistronic mRNA	present	present	absent

branched portions cyclize, providing more rigidity to the membrane.

Archaea contain a single, circular chromosome, but the sequence of the archaean genome is genotypically distinct from that of bacteria and eukaryotes. Histonelike proteins bind the chromosomes of archaea in a manner similar to the way they bind to eukaryotic chromosomes. As bacteria do, archaea have polycistronic genes that contain no introns, or intervening sequences between coding regions of messenger RNA (mRNA). The initiator transfer RNA (tRNA) is methionine, as in eukaryotic organisms. Their ribosomes are 70S, as in bacteria, but the ribosomes' shape differs and they are insensitive to antibiotics that inhibit bacterial ribosomes. Also, the RNA polymerases are more similar to eukaryotic RNA polymerases than to bacterial RNA polymerases.

Archaea exhibit a variety of means for obtaining their energy and nutrients. Scientists have identified both aerobic and anaerobic species, and autotrophs and heterotrophs. Hydrogen gas (H_2), carbon dioxide (CO_2), or sulfur provides energy for some archaea, and some are photosynthetic. Their metabolisms vary greatly among the different groups of archaea.

ARCHAEAN CLASSIFICATION

Sequence similarities of 16S rRNA genes group the archaeans into four main clades: Euryarchaeotes, Crenarchaeotes, Korarchaeotes, and Nanoarchaeotes. The Euryarchaeota include the methane producers and the halophiles (salt lovers). Some extreme thermophiles are also in Euryarchaeota, but most thermophiles belong to Crenarchaeota. Methanogens, one type of Euryarchaeota, oxidize hydrogen gas (H_2) for energy and are the most common of the Archaea. Molecular oxygen (O_2) is toxic to methanogens; instead they use an inorganic substance such as CO_2 as the oxidizing agent, in the process reducing it to methane gas (CH_4), which they release into the environment. In addition to CO_2, methanogenic species convert other substances, such as formate, methanol, acetate, carbon monoxide, and methylamine, into methane. Methanogens inhabit marshes, producing swamp gas; live as endosymbionts of cattle and termites; or reside in the human gut. Sewage treatment facilities utilize methanogens to help decompose organic matter in wastewater, and some industrial plants harvest the gas given off to utilize as a source of energy. At least 17 methanogenic archaean genera and 93 species have been identified.

The extreme halophiles, which grow in very salty conditions, also belong to Euryarchaeota. Organisms that live in environments that have a higher concentration of solutes than inside the cell must have special adaptations that prevent the cell from dehydrating. Natural tendencies cause the water to diffuse from inside the cell to the outside, but the loss of too much water causes cell death. Some halophiles overcome this obstacle by transporting other solutes, such as potassium, into the cell to balance the osmolarity with the external environment and prevent excessive water loss. Extreme halophiles require a high percentage of salt, a minimum of 9 percent but on average 12–23 percent (for comparison, normal seawater contains 0.9 percent salt). One type of halophile is photosynthetic. The purple pigment bacteriorhodopsin, found in its cell membranes, harvests light energy from the Sun and uses it to generate adenosine triphosphate (ATP). Scientists have identified 20 species of extreme halophiles so far.

The Crenarchaeota includes most of the organisms that live in extreme temperatures, both hot and cold, and the organisms that can tolerate extreme acidity. Most bacteria and eukaryotic organisms are mesophiles, meaning their optimal growth temperature ranges from 68°F to 113°F (20°C–45°C). Bacteria that live in symbiotic relationships with humans or that are pathogenic to humans must be mesophiles, since normal body temperature, 98.6°F (37°C) falls within this range. Thermophiles thrive at a range of 104°F–176°F (40°C–80°C). Extreme thermophiles (hyperthermophiles) prefer temperatures even higher, with an optimum around 212°F (100°C), the temperature at which water boils at standard pressure. Thermophiles and hyperthermophiles have special enzymes and molecules that prevent denaturation at such high temperatures. Thomas D. Brock at the University of Wisconsin at Madison first discovered thermophiles in 1966 in hot springs at Yellowstone National Park. Since then, scientists have discovered microbes that can withstand surprising conditions. In 2003 Derek Lovley and Kazem Kashefi of the University of Massachusetts at Amherst identified a spherical, flagellated archaean species that can thrive at 250°F (121°C), the current record held by a living organism, and, interestingly, the temperature at which autoclave ovens sterilize the equipment and media used in most microbiological research. They discovered these archaea, named Strain 121, near some black smokers 200 miles from Puget Sound and one and one-half miles deep in the Pacific Ocean. Strain 121 respires using iron as the final electron acceptor, forming magnetite in the process. This microbe "breathes" iron, in comparison to aerobic organisms, which "breathe" oxygen. At the other end of the spectrum, psychrophiles can grow at temperatures as low as 32°F (0°C), but they grow best between 68°F and 86°F (20°C–30°C). These organisms can spoil food quickly, even when it is kept in a refrigerator. Some have been found living in an

Antarctic lake of ice. Not very much is known about the psychrophiles.

Most organisms grow best at a neutral pH, around 7.0. Acidophiles, also in Crenarchaeota, have the rare quality of growing best in acidic conditions, and alkalinophiles grow best in basic conditions. Molecules inside the cell, such as the DNA, cannot withstand a pH of this level; thus the cells have mechanisms for maintaining a neutral internal pH despite the harsh external conditions that the cell surface molecules must tolerate.

Korarchaeota is a relatively new clade, first recognized in 1996 when an organism living in a hot spring in Yellowstone National Park was found to be very different from the Euryarchaeota or the Crenarchaeota. Korarchaeota includes members that are more distantly related to the other archaea. Scientists believe that the korarchaeote species more closely resemble the ancient ancestral life-form common to prokaryotes and eukaryotes than the other archaea. The newest clade, Nanoarchaeota, discovered in 2002, contains organisms that are tiny, even by prokaryotic standards. Approximately 1.57×10^{-5} inch (0.4 μm) in diameter, nanoarchaeote genomes only contain 500,000 base pairs, smaller than any other known organism.

See also Bacteria (Eubacteria); biogeochemical cycles; Brock, Thomas; microbiology; prokaryotic cells; Woese, Carl.

FURTHER READING

American Society for Microbiology. "Microbe World." Available online. URL: http://www.microbeworld.org. Accessed January 13, 2008.

Dyer, Betsey Dexter. *A Field Guide to Bacteria*. Ithaca, N.Y.: Cornell University Press, 2003.

Madigan, Michael T., and John M. Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle, N.J.: Prentice Hall, 2006.

Margulis, Lynn, and Karlene V. Schwartz. *Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth*. 3rd ed. New York: Henry Holt, 2002.

Aristotle (384–322 b.c.e.) Greek *Philosopher*

Aristotle was an ancient Greek philosopher who contributed to numerous broad fields, ranging from politics and cosmology to physics and life science. He has been called the first true scientist and his school the first research institution with the first academic library. Though many of his ideas have been disregarded, his work formed the basis of many scientific fields, including the life sciences.

Aristotle was born in 384 b.c.e. in Stagira, in northern Greece. His father, Nichomachus, was a physician for the royal family in Macedonia. Both of his parents died when he was a boy, and at age 17, he went to Athens to study philosophy at Plato's Academy. Plato was a student of Socrates, and both men were brilliant Greek philosophers; thus Aristotle's educational pedigree was of the highest quality. During the 20 years he spent in Athens, he became known as Plato's brightest pupil. Whereas Plato was a renowned philosophical thinker, Aristotle was more of a natural philosopher, who saw reality in objects formed of matter.

After Plato died in 347 b.c.e., Aristotle left the academy, possibly because he was disappointed that he was not named its head, a position given to Plato's nephew. He started his own school in Assos, in northern Asia Minor (now Turkey). While there, Aristotle married Pythias, the niece of the city's ruler, Hermias, who was also his school's benefactor. They had one daughter, whom they named Pythias, after her mother. In 344 b.c.e. Hermias was overthrown, and Aristotle moved to the island of Lesbos. Two years later he returned to Macedonia, where he tutored the future Alexander the Great, son of Phillip II, the king of Macedonia. In 339 b.c.e. Aristotle moved home to Stagira, accompanied by a group of devoted students. Some historians believe Aristotle met and married Herpyllis during this time. Whether they married or not, together they had a son, named Nichomachus, after Aristotle's father. In 335 b.c.e. he moved back to Athens and started his own school, the Lyceum, also known as the Peripatetic School because he often lectured while walking along the covered walkways (called peripatus) on the grounds.

In Assos, Aristotle began to work on *Historia animalium (The History of Animals),* a venture that served as a reference for his other works in zoological science. At the Lyceum, Aristotle continued work on this treatise but also branched out into other sciences, the humanities, and history. His student followers wrote essays on these subjects, and over the years newer students updated them. These documents became a valuable source of historical information, and Aristotle's systematic collection of observations and information became the basis of natural philosophy, which evolved into modern science, as well as mathematics, astronomy, and medicine. Most of the manuscripts that have survived are not Aristotle's own writings, but interpretations or revisions of his notes or lectures that others have arranged and edited. This has led to some inconsistencies in his works and makes it difficult to follow the evolution of his thoughts and ideas. Because he did collect knowledge in the form of written books and maps, the Lyceum was the first school to resemble a modern academic institution.

Aristotle's greatest contribution to science may have been his attempt to classify and organize into

hierarchies all the organisms known at the time. He started by dividing life-forms into plants and animals, and then further categorized them on the basis of shared physical characteristics, such as whether or not they had blood. (He mistakenly thought insects did not have blood, so his categories resembled the modern groups of invertebrates and vertebrates.) Other features that defined Aristotle's classification scheme included the distinction between aquatic and terrestrial habitats, ecological roles such as predator and prey, mode of reproduction, number of legs, and other shared anatomical characteristics.

Believing observation was key to understanding the natural world, he dissected animals to look at their insides, something most philosophers at the time saw as dirty work that was beneath the role of an intellectual. Aristotle systematically examined the development of chick embryos by incubating several eggs of the same age and dissecting one each day to see what new features had formed. By dissecting cattle, he found that their digestive tracts contained more than one stomach chamber. He noted observations such as that no single-hoofed animal had horns, and no horned animal also had tusks. He discovered that beehives contained a single queen, whom all the other bees in the hive assisted. His observations demonstrated that hyenas existed as separate sexes—male and female—in contrast to the belief at the time that they were hermaphrodites. Sea life particularly fascinated Aristotle, who was the first to recognize that dolphins nurtured their developing young through a structure called a placenta, something no fish did. Because of this unique feature, Aristotle sagely grouped dolphins with terrestrial animals that had the same structure (now called placental mammals). His books included the description of many marine invertebrates, such as octopuses, cuttlefish, and crabs. His comparison of sea urchin mouths to a five-sided lantern called a horn lantern led to the term *Aristotle's lantern* to portray the arrangement of five teeth around the circular mouth. For almost 2,000 years, natural philosophers relied upon and added to the system Aristotle developed for classifying living organisms. The system worked until the 1700s, when the Swedish naturalist Carl Linnaeus proposed and implemented a new scheme to accommodate the large increase in the number of species discovered as exploration to other lands became more common.

Historia animalium includes observations and descriptions of about 500 different species, including their anatomy, habitats, and behaviors. Collecting the data for this intended reference spanned several years, and it probably was not written in complete form until after many of his other zoological treatises, whose content was based on the observations recorded in *Historia animalium*. When Alexander ruled as king of Macedonia, he sent men far away to obtain all sorts of animals (and plants and rocks), which he gave to Aristotle, whose scientific collections may have formed the first zoo and museum of natural history. The main goals of zoology at the time were to identify and classify animals and to define their purpose or function in nature. Aristotle's early zoological treatises examined the design of tissues and anatomical structures and discussed physiological processes such as movement, respiration, aging, and death. He also explored inheritance, inessential characteristics such as color, and the relationship between composition or structure and reproduction, realizing that living organisms were more than a collection of parts. He observed that offspring often resembled distant relatives more than they resembled their parents and that parents who had suffered injuries or lost limbs did not pass on these malformations to their children. From this he surmised that parents passed on the potential to exhibit certain characteristics rather than the characteristics themselves. He also mistakenly believed that inherited characteristics were passed on through the blood.

In *Historia animalium,* Aristotle described the heart and blood vessels in some detail and even divided animals into two general classes, based on whether they had blood or not. This led to categories similar to those with a backbone and those without. He was fascinated by the beating heart of a chick embryo and observed that all the blood vessels originated at the heart; he did not differentiate between arteries and veins. He studied the vessels by starving the animals so they would become so thin that he could visualize the vessels of the living organism, then sacrificed them by strangulation so the blood would remain inside the vessels for more accurate observations. This method led to some artifactual structural abnormalities, however, such as a swollen right side of the heart, with the atrium and ventricle appearing as one continuous chamber, into which the apparently widened vena cava delivered blood. Though he was a keen observer, he did foster several erroneous beliefs about animal physiology. Aristotle thought that the heart was the center of life and that it warmed the blood, whereas the brain served as a cooling organ for the blood. He also believed that heat generated from the heart caused the lungs to expand, drawing in air, which then cooled the lungs before exhalation. Aristotle was a proponent of preformation, the idea that reproduction occurred when a male deposited a complete, "preformed" miniature individual into a female, in whose body it grew until birth. He thought some animals spontaneously formed from mixtures of mud and water. Physicians and physiologists propagated many of Aristotle's incorrect ideas for hundreds or thousands of years

before being challenged. Some of his other zoological works include *De generatione animalium (On the Generation of Animals), De motu animalium (On the Motion of animals), De incessu animalium (On the Progression of Animals),* and *De partibus animalium (On the Parts of Animals).*

Having spent so much time comparing animals and observing slight structural differences, he envisioned a progression in their form, a precursor notion to evolution. He placed different types of animals on an imaginary ladder, with higher rungs representing increasing ranks of perfection. The ladder consisted of plantlike animals (corals and anemones) and mollusks at the bottom, followed by worms, egg-laying animals, and eventually mammals, with humans at the top. Aristotle believed in teleology—the philosophy that life-forms have end goals, and their forms and body processes are means of achieving that purpose. Today scientists know evolution is a nondirected process; thus Aristotle's belief in teleology was contrary to modern evolutionary theory, but his system of classification based on natural characteristics and his collection and organization of his numerous observations did, provide a framework upon which future biologists could structure their thinking. Charles Darwin, the British naturalist who proposed the theory of evolution by means of natural selection in the mid-1800s, praised Aristotle's intellect and foresight.

Though Aristotle's inclusion of humans with other animals was revolutionary, he did believe humans were special. In accordance with teleological principles, he thought all living things had a purpose, and therefore the potential to achieve that goal. For example, a seed's purpose was to develop into a complete new plant, but this was the maximum of its potential, as plants had the most primitive of "souls." Animals, on the other hand, had souls with greater potential, as they could move and use their senses to perceive events in the surrounding world. Humans had the ability to think and reason, and Aristotle believed this gave them the capacity to become divine. Through seeking and gaining knowledge and using logic to understand that knowledge, which he believed was the aim of science, one's soul could become eternal.

Another contribution Aristotle made to the development of modern science was his overall approach toward studying natural philosophy, which led to the development of science as a discipline distinct from logic or politics. His reliance on universal scientific principles and formulation of hypotheses based on observable data and facts helped establish scientific knowledge as true or real knowledge. This empirical means of achieving scientific knowledge in a particular field led to an explosion of knowledge in subjects such as botany, medicine, and astronomy. The artificial manipulation of variables during scientific experiments occurred later.

In addition to biology, Aristotle significantly influenced the field of cosmology by attempting to explain how the stars and planets moved. He proposed that a series of concentric spheres surrounding the Earth carried the celestial bodies. The spheres were composed of a transparent substance called ether, and the vapor form of this substance filled empty space. He called the outermost sphere the prime mover. To explain the pathways of the planets, he had to add more spheres to his system, which became more and more complex. In contrast, modern science seeks the simplest possible explanations for natural phenomena.

Aristotle thought that everything in the Earth consisted of different amounts of four basic substances: earth, fire, water, and air. Elements had a tendency to return to their natural state; for example, solid objects fell to the Earth, and bubbles floated to the surface of a body of water, where it contacts the atmosphere. Aristotle also delved into the topic of motion. Just as his ideas about planetary motion had to wait until the 17th-century German astronomist Johannes Kepler provided a better explanation, Aristotle's ideas about projectile motion had to wait for the Italian scientist Galileo Galilei.

After the death of his former pupil, Alexander the Great, anti-Macedonian sentiments forced Aristotle to retire to his mother's former estate in Chalcis. He died the following year, in 322 B.C.E., at the age of 62. After his death, his lecture notes and writings were collected, assembled, and edited in multiple volumes.

FURTHER READING

Adler, Robert E. *Science Firsts: From the Creation of Science to the Science of Creation.* Hoboken, N.J.: John Wiley & Sons, 2002.

Cooper, Sharon Katz. *Aristotle: Philosopher, Teacher, and Scientist.* Minneapolis: Compass Point Books, 2007.

Shields, Christopher. *Aristotle.* New York: Routledge, 2007.

assisted reproductive technology The 2005 Assisted Reproductive Technology (ART) Report, published by the Centers for Disease Control and Prevention (CDC) of the U.S. Department of Health and Human Services, states that in 2005 more than 52,000 children were born as a result of successful ART treatments. The term *ART* refers to any procedure during which both eggs and sperm are handled to help a couple conceive, such as in vitro fertilization (IVF) or IVF-based procedures.

INFERTILITY

According to the 2002 National Survey of Family Growth (the most current data available at the time of this writing), published by the National Center for Health Statistics (of the CDC), an estimated 12 percent of women of reproductive age had difficulty either in becoming pregnant or maintaining a pregnancy in 2002. The number of couples seeking treatment for infertility, medically defined as the inability to conceive after one year of unprotected intercourse or the inability to carry a pregnancy to term, has steadily increased since ART was introduced in the United States in 1981. In order to achieve a successful pregnancy without medical intervention, at least one egg must fully mature and escape from a woman's ovaries, the egg must travel unimpeded down the oviduct (also called the fallopian tube), a mature sperm must fertilize the egg, the developing embryo must implant into the lining of the uterus, and the uterus must maintain the pregnancy for nine months while the embryo develops into a fetus and then a child capable of sustaining life outside the mother. This complex series of events offers much opportunity for conditions to go wrong.

Infertility in males can result from a low sperm count, a condition in which the number of sperm is lower than the number typically required to conceive. In other cases the sperm do not develop or mature properly, resulting in deformed or immotile sperm that are incapable of traveling up the woman's reproductive tract and fertilizing an egg. Having low sperm numbers or poor-quality sperm can result from structural abnormalities of the male reproductive organs, general poor health, a specific disease, or exposure to certain environmental factors such as cigarette smoke, alcohol, toxins, or radiation. In females, poor general health, physical problems with the uterus or oviducts, or a history of sexually transmitted diseases can lower fertility, but the most common cause is ovulation problems. As a woman ages, she ovulates less frequently and eventually stops ovulating altogether. Because more women are waiting until later in life to have children, age is a common factor contributing to infertility.

An evaluation performed by a physician can help determine the cause of infertility. Semen analysis will reveal whether the sperm have any structural abnormalities, whether they do not move properly, or whether the numbers are simply too low. In a woman, hormone levels in the blood in combination with an ultrasound examination of the ovaries might indicate problems with ovulation. Injection of a special dye through the vagina allows a physician to examine the uterus and the oviducts for abnormalities or blockages that could prevent the sperm and egg from finding each other or prevent the embryo from implanting in the uterus. Laparoscopic examination of the pelvic cavity can help diagnose diseases such as endometriosis that interfere with reproduction. Finding a likely cause for infertility helps the physician and the couple to determine the most appropriate and potentially effective course of treatment.

TYPES OF ART

Though 85–90 percent of infertile couples may be treated successfully by the administration of drugs or by surgical repair of reproductive organs, others require more complicated treatment to conceive and deliver a live baby. Assisted reproductive technology (ART) generally refers to procedures that involve the handling of both eggs and sperm, so procedures such as the administration of medicine by itself or simple artificial insemination, in which sperm are injected into a woman's cervix, are not included. ART typically involves removing the eggs from the woman's body, combining the egg or eggs with sperm, and placing the eggs and sperm or the fertilized egg or eggs back into the woman's body. Four common ART procedures are IVF, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and intracytoplasmic sperm injection (ICSI).

IVF, a procedure pioneered by Dr. Patrick Steptoe and Robert G. Edwards in the 1970s, forms the basis of all four techniques and accounts for 98 percent of ART procedures. Since 1978, when the first child conceived by IVF was born, the procedure has benefited thousands of women who have blocked or absent oviducts and men who have low sperm counts. In a typical IVF procedure, the woman is given ovulation-inducing drugs to stimulate the ripening or maturation of multiple eggs within her ovaries. After two intense weeks of carefully orchestrated treatments with fertility drugs, the physician retrieves the eggs by aspiration through a needle guided by ultrasound. The father provides a fresh sperm specimen that is washed and added to a dish containing the harvested eggs in a specially designed culture medium, and the gametes are incubated together for 14 to 18 hours. By this time, the sperm have penetrated the eggs, and the zygotes are placed in a new culture medium optimized for cell division. After one and one-half to five days, depending on the clinic, the physician uses a microscope to select the healthiest embryos and places them inside the uterus using a catheter inserted through the vagina and cervix. Administration of the steroid hormone progesterone helps prepare the uterine lining for implantation.

GIFT begins the same way as IVF; the woman is given drugs to induce ovulation, and the eggs are retrieved and combined with a prepared sperm sample. The difference is that in GIFT, immediately

after egg retrieval, the eggs and sperm are combined and placed together in the oviduct, where fertilization naturally occurs, using a laparoscope. Of course, the woman must have at least one open oviduct for this to work. Using GIFT, a physician cannot know whether fertilization has occurred unless the woman becomes pregnant.

In ZIFT, also known as tubal embryo transfer, fertilization occurs in the laboratory, and then the egg is returned to the oviduct before undergoing cell division. ZIFT differs from IVF in that the zygote is returned to the oviduct rather than the uterus, usually earlier than with IVF. If the procedure does not result in a pregnancy, the physician cannot know whether the zygote developed into a normal blastocyst.

When lack of a sufficient number of mature sperm hinders conception, a physician might recommend ICSI. The sperm are examined closely under a microscope, and one that looks normal and healthy is gently drawn into a pipette and then injected into the cytoplasm of a mature egg. After cell division begins, the embryo can be returned to either the oviduct or the uterus.

When the woman's ovaries do not ovulate, donor eggs can be retrieved from another woman. Donor sperm can also be used. Gamete donation is sometimes used when the man or woman carries a gene that causes a disease.

Miscarriages occur in more than 50 percent of pregnancies in women over the age of 42, most resulting from chromosomal abnormalities. After age 42, the likelihood that a woman will have a child born with a chromosomal abnormality reaches one in 39. Preimplantation diagnosis (PGD) reduces this risk. In PGD, a single cell is removed from a three-day-old embryo that has between eight and 10 cells total. The chromosomal makeup of the removed cell is examined. Normally, a human cell contains 46 total chromosomes. Chromosomal analysis will reveal whether too many or too few chromosomes are present or apparent structural abnormalities affect any of the chromosomes. After PGD, only embryos that appear to have a normal set of chromosomes will be implanted. This procedure reduces but does not eliminate the risk of genetic birth defects.

Since the first successful IVF procedure, an estimated 1.2 million children have been born worldwide as a result of ART. The success rate of ART depends on the age and general health of the parents, the condition of the embryos used, the ART procedure, and the particular clinic. Clinics must report their success rates to the CDC. For 2005, the average percentages of ART cycles that resulted in the birth of a healthy baby were 37.3 percent for women under the age of 35, 29.5 percent for women between the ages of 35 and 37, 19.7 percent for women between the ages of 37 and 40, and 10.6 percent for women between the ages of 41 and 42. Because ovulation-inducing drugs stimulate the maturation of multiple eggs, and because physicians often implant several embryos into a woman undergoing an ART procedure in order to increase the chance for success, multiples such as twins, triplets, or higher numbers occur more frequently than in pregnancies that occur without any medical intervention.

See also HUMAN REPRODUCTION; SEXUAL AND REPRODUCTIVE HEALTH.

FURTHER READING

Edwards, R. G., and Patrick Steptoe. *A Matter of Life: The Story of a Medical Breakthrough*. New York: William Morrow, 1980.

Falcone, Tommaso, with Davis Young. *Overcoming Infertility: A Cleveland Clinic Guide*. Cleveland, Ohio: Cleveland Clinic Press, 2006.

Sher, Geoffrey, Virginia Marriage Davis, and Jean Stoess. *In Vitro Fertilization: The A.R.T. of Making Babies, Assisted Reproductive Technology*. 3rd ed. New York: Facts On File, 2005.

U.S. National Library of Medicine and the National Institutes of Health. Infertility. "MedlinePlus." Available online. URL: http://www.nlm.nih.gov/medlineplus/infertility.html. Updated November 18, 2008.

Vliet, Elizabeth Lee. *It's My Ovaries, Stupid!* New York: Scribner, 2003.

Avery, Oswald (1877–1955) Canadian-American *Bacteriologist* Oswald Avery helped pave the way for the molecular biological revolution when, with Colin MacLeod and Maclyn McCarty, he demonstrated that deoxyribonucleic acid is the molecular basis for the transmission of genetic information. This scientific development was one of the most important of the 20th century.

Oswald Theodore Avery was born on October 21, 1877, in Halifax, Nova Scotia. His family moved to New York City in 1887 when his father accepted an invitation to serve as pastor of a church in the Lower East Side. Avery attended Colgate University, where he made good grades and won several oratorical competitions. After earning a bachelor of arts degree in the humanities in 1900, he proceeded to Columbia University College of Physicians and Surgeons and received his medical degree in 1904. After practicing as a general physician for three years, Avery switched his career focus to bacteriological and immunological research. He took one part-time job with the Board of Health and another performing bacterial counts on milk samples. In 1907 he joined the Hoagland Laboratory, a private bacteriological research institute in Brooklyn, where he became

associate director of the division of bacteriology. At Hoagland, Avery researched many topics, including the bacteriology of yogurt and fermented dairy products, immunological proteins, vaccines, and secondary infections in pulmonary tuberculosis. His research on bacterial strains that caused pneumonia, a disease in which the lungs become inflamed and fluid-filled, leading to coughing and difficulty breathing, impressed Rufus Cole, the director of the Hospital of the Rockefeller Institute for Medical Research, who hired Avery in 1913.

At the Rockefeller Institute, Avery concentrated on identifying and characterizing bacterial strains that caused pneumonia. A landmark study performed in 1917 by Avery and his coworker Alphonse Dochez described the presence of an immunologically specific soluble substance in the culture medium of pneumococcus. They subsequently identified this substance as a polysaccharide, a surprising result because chemists generally believed the antigenic agent had to be a protein on the surface of the bacterial cells. Though some scientists suspected Avery's findings resulted from protein contamination, Avery proved otherwise and went further to show that the form of this antigenic polysaccharide varied among pneumococcal types, results that led to the development of immunochemistry as a new field of research. This research led to Avery's nomination for a Nobel Prize in physiology or medicine almost annually from the 1930s until his death in 1955.

For nearly three decades, Avery contributed significantly to the fields of bacteriology and immunology, collaborating with many reputable scientists. His research led to an understanding of how specifically to treat pneumonia cases on the basis of the specific causative bacterial strain. He became a member of the Rockefeller Institute for Medical Research in 1923. The National Academy of Sciences elected Avery to membership in 1933, but the extraordinary findings he published in 1944 earned him a most distinguished place in the history of genetics.

Colin MacLeod, a physician who joined Avery's lab in 1934, and Maclyn McCarty, who joined the lab in 1941, collaborated with Avery. They were interested in pneumococcal transformation, a phenomenon discovered by the British researcher Frederick Griffith in the 1920s. Accumulated evidence showed a correlation between the presence of a smooth capsule surrounding one strain of bacteria and its virulence, or its ability to invade and multiply within host tissues. The capsule prevented host white blood cells called phagocytes from ingesting and destroying the invasive bacteria. Griffith was studying similar phenomena. He had successfully converted a nonvirulent, nonencapsulated strain (designated R) of *Streptococcus pneumoniae* into a virulent encapsulated strain (designated S), by adding heat-killed virulent bacteria to a culture of the nonvirulent strain and injecting mice with the mixture. Though the heat-killed bacteria alone did not make the mice ill, when they were injected simultaneously with the live, harmless, nonencapsulated strain, the mice became sick and died. Avery and his team duplicated Griffith's experiment, showing that a chemical substance from the dead S strain of bacteria was able to confer the ability to produce a smooth coating to a living R strain of bacteria. They prepared extracts from the bacterial cells by repeatedly freezing and thawing them and achieved similar results in vitro.

For 15 years, Avery systematically tried to identify the chemical substance from the extracts responsible for inherited variation in bacteria cells, or, more broadly, to identify the hereditary material of life. Assuming it would be a protein, they obtained many negative results through the years. Surprisingly, a nonprotein substance purified from the crude extracts did show promise in its ability to transform R into S strain bacteria. Avery, MacLeod, and McCarty found the ratio of nitrogen to phosphorus in the substance similar to that of deoxyribonucleic acid (DNA). They wondered whether DNA from the virulent S bacteria could be the transforming material. When they treated the extract with enzymes that destroyed proteins, polysaccharides, or ribonucleic acid, the ability of the material to transform R into S types was not hindered. However, treatment with enzymes that specifically attacked DNA decreased the transformation efficiency. From these results, the trio concluded that the active transforming factor responsible for the formation of polysaccharide capsules in the previously rough strain of bacteria was DNA. Being not only cautious, but modest, Avery waited until he had convincing evidence before publishing the results. Even then he downplayed the enormity of their discovery in the conclusions of their paper, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III" published in the *Journal of Experimental Medicine* in 1944. He preferred simply to report his findings objectively and to let others draw their own conclusions.

The report provoked much opposition from scientists who believed that DNA, generally thought to be a simple tetranucleotide, was too basic a molecule to have such profound influence. Once again, other scientists including colleagues at his own institution believed that Avery's extracts were contaminated with protein and that those proteins were the true transforming factor. Some scientists made it their goal to prove Avery wrong, attacking his methods

for purifying the transformation material and his chemical analyses, but DNA-dependent transformations performed by others eventually confirmed their findings. The revolutionary conclusion that DNA was the hereditary material was later supported by experiments performed by Alfred Hershey and Martha Chase using bacteriophage (a virus that infects bacteria) in 1952.

Avery became a naturalized citizen of the United States in 1918. He had enlisted as a private in the U.S. Army the year before and was promoted to captain soon after becoming naturalized. Though Avery became a professor emeritus of the Rockefeller Institute in 1943 at age 65, he continued his research there until 1948, when he retired to Nashville, Tennessee, to be closer to his family. The Royal Society of London awarded Avery their prestigious Copley Medal in 1945, and the Lasker Foundation gave him the Albert Lasker Award for Basic Medical Research in 1946. He died on February 20, 1955, of liver cancer.

See also BIOMOLECULES; DEOXYRIBONUCLEIC ACID (DNA); GRIFFITH, FREDERICK; HERSHEY, ALFRED; MACLEOD, COLIN MUNRO; MCCARTY, MACLYN.

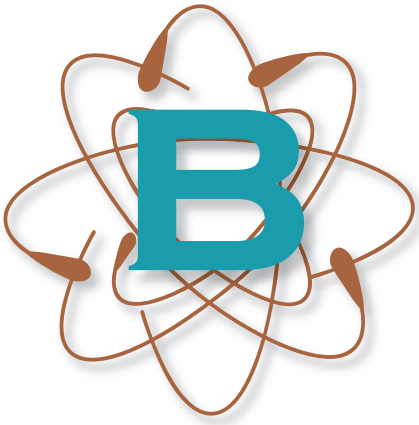
FURTHER READING

Avery, Oswald T., Colin M. MacLeod, and Maclyn McCarty. "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III." *Journal of Experimental Medicine* 79 (1944): 137–158.

Dochez, A. R. "Oswald Theodore Avery (October 21, 1877–February 20, 1955)." In *Biographical Memoirs. National Academy of Sciences*. Vol. 32. Washington, D.C.: National Academy of Sciences, 1958.

Dubos, René. *The Professor, the Institute, and DNA*. New York: Rockefeller University Press, 1956.

U.S. National Library of Medicine. *Profiles in Science*. Available online. URL: http://www.profiles.nlm.nih.gov/CC/. Accessed January 13, 2008.



Bacteria (Eubacteria) The group of organisms referred to as bacteria once encompassed all the prokaryotic organisms, until phylogenetic analysis demonstrated that prokaryotes consisted of two distinct groups, the Bacteria, also called the Eubacteria or true bacteria, and the Archaea. These groups are now recognized as domains or superkingdoms, a classification level higher than kingdom. Bacteria includes the microorganisms with which most people are familiar—the types that scientists use for research, the strains that inhabit the human intestinal tract, the ones used in food production, pathogenic bacteria, photosynthetic bacteria, and the rest of the Bacteria that inhabit moderate environments. Archaea are prokaryotes that often live in extreme environments such as those characterized by very high or very low temperatures, extreme acidity, or high solute concentrations and methane-producing strains. The names of the domains *Bacteria* and *Archaea* are capitalized, whereas the terms *bacteria* and *archaean* are not capitalized when referring to microorganisms within the domain. Bacteria differ structurally, biochemically, and physiologically from archaeans.

GROWTH AND ADAPTATION

As prokaryotic cells, bacterial cells lack compartmentalization. They reproduce by binary fission, in which one cell divides into two, each containing a copy of the parental chromosome. During optimal growth conditions, replication of this chromosome often limits the generation time, the period required for a population of cells to double. Under optimal conditions, generation times vary between species from 20 minutes to a few hours. In a natural setting, conditions never remain optimal as nutrients become depleted and metabolic waste accumulates, poisoning the microbes. Some bacteria can form endospores, tough dormant structures that can withstand harsh environmental conditions and then germinate when the conditions improve.

Bacterial growth is measured by the number of cells or the size of the population rather than looking at an individual organism. Bacteria growing in a liquid broth culture or in an aquatic environment cloud the liquid as the numbers increase. On a solid growth medium, a single bacterial cell grows and divides repeatedly, piling up to form a raised, circular collection of cells called a colony. All of the cells in a colony contain copies of the same chromosome.

Though clonal populations of bacteria all receive identical copies of the chromosome, bacteria do have mechanisms for creating genetic variation that allows evolutionary adaptation to occur. Growth conditions such as temperature, nutrient availability, pH, oxygen concentration, and the presence of certain chemicals can select for spontaneous or induced mutations, causing them to become fixed. Prokaryotes can also obtain new genes by conjugation, a process in which a hollow tubular structure called a pilus forms a bridge between two cells, allowing for the transfer of deoxyribonucleic acid (DNA). In transformation, bacterial cells uptake pieces of DNA from the environment through their cell membranes. Viruses that infect bacteria, called bacteriophages, can also carry genes from one bacterial cell to another, a process called transduction.

DIVERSITY

The domain Bacteria includes tremendously diverse organisms, making them a very successful group. One area of variation is their nutritional mode—

how they obtain their energy and what they use as a carbon source. Some organisms are phototrophs, meaning they obtain their energy from sunlight, and others are chemotrophs, meaning they obtain their energy from chemicals. Organisms that use carbon dioxide (CO_2) as their sole carbon source are called autotrophs, and organisms that utilize organic molecules as their main carbon source are called heterotrophs. These terms can be combined to give more detailed information about an organism's nutritional requirements. For example, a photoautotroph uses light as its energy source and CO_2 as its carbon source. Bacteria exist that represent all the major nutritional modes: photoautotrophs, chemoautotrophs, photoheterotrophs, and chemoheterotrophs. Bacterial requirements for oxygen (O_2) also vary: obligate aerobes require oxygen to survive; facultative aerobes use oxygen if it is present but can ferment in its absence; and oxygen is toxic to obligate anaerobes. Nitrogen is a necessary nutrient for synthesizing amino acids of proteins and nitrogenous bases in nucleic acids, but bacteria have different capabilities with respect to nitrogen metabolism. Some can fix nitrogen, meaning they can convert nitrogen gas (N_2) to ammonia (NH_3), a form that can be readily incorporated into organic molecules. Bacteria capable of nitrogen fixation play an important role in the cycling of this nutrient in ecosystems. Bacteria with unique metabolic requirements often live in close association with organisms that have complementary metabolisms.

BACTERIAL CLASSIFICATION

One means for categorizing bacteria is by evolutionary relationships. Because organisms that are closely related have fewer mutations between them compared with organisms that diverged a long time ago, the molecular differences provide insight into the evolutionary distance between types of organisms. Analysis of the nucleotide sequence of certain ribosomal ribonucleic acid (rRNA) genes divides Bacteria into five major groups (and many more phyla) that share a common evolutionary ancestor: the proteobacteria, chlamydias, spirochetes, cyanobacteria, and gram-positive bacteria. Morphologies or physiological characteristics within groups can be extremely variable since the groups are defined by molecular relatedness.

The phylum Proteobacteria, the largest of the Bacterial clades, includes more than 1,600 identified species of gram-negative bacteria of all nutritional modes and oxygen requirements. Bacteria are termed gram-negative if they do not retain the crystal violet stain during the Gram staining procedure, and gram-positive if they do retain it. The differential reaction depends on the structure of the cell envelope. Some proteobacteria are pathogenic, some are free-living, and others live in symbiotic relationships. Proteobacteria are divided into five subgroups, named for the first five letters of the Greek alphabet. The class Alphaproteobacteria includes *Rhizobium* and *Agrobacterium* species. *Rhizobium* lives in a symbiotic association with legumes; it resides in nodules of the roots and fixes nitrogen. The plants, belonging to the pea and bean family, benefit from the supply of usable nitrogen and the bacteria benefit from the water and nutrients taken in through the plant's root system. *Agrobacterium* causes tumor formation in its hosts. Because of its known ability to transfer DNA between itself and the plant, plant geneticists use it to improve crops. Many *Agrobacterium* species have recently been reclassified as *Rhizobium* species. The rickettsias, including the human pathogen *Rickettsia,* are alphaproteobacteria that can only survive as endosymbionts of other cells. Scientists believe the organisms that evolved into eukaryotic mitochondria by endosymbiosis originated from alphaproteobacteria.

The class Betaproteobacteria includes many soil and wastewater species, but also some human pathogens such as *Neisseria,* which causes gonorrhea and a form of meningoencephalitis. Many betaproteobacteria are facultative aerobes, a few are phototrophs, and some have unique metabolisms. The soil microbe *Nitrosomonas* plays an important role in the cycling of nitrogen by oxidizing ammonium (NH_4^+) for energy and producing nitrite (NO_2^-) as a waste product.

Gammaproteobacteria includes many medically significant species as well as the sulfur bacteria. Many familiar species belong to this group. *Escherichia coli,* one well-known species of gammaproteobacteria, is part of the normal human flora and is used extensively in research. The causative organisms for cholera (*Vibrio cholerae*), the foodborne illness salmonellosis (*Salmonella enteriditis*), typhoid fever (*Salmonella typhus*), the plague (*Yersinia pestis*), and an opportunistic human pathogen (*Pseudomonas aeruginosa*) that often infects the pulmonary tract, the urinary tract, or burns are all members of the class Gammaproteobacteria. The purple sulfur bacteria are capable of photosynthesis and live in hot sulfur springs or stagnant water. They oxidize hydrogen sulfide (H_2S) rather than water as plants and algae do and produce granules of elemental sulfur as a product.

Members of Deltaproteobacteria include the unusual myxobacteria, sulfate- and sulfur-reducing bacteria, and other anaerobic bacteria. Myxobacteria live in the soil, move by gliding in swarms, and produce fruiting bodies when growth conditions are unfavorable. The fruiting bodies release resistant spores

that germinate when conditions improve. Sulfate-reducing bacteria use sulfate (and sometimes other oxidized sulfur compounds) as an oxidizing agent, reducing it to sulfide, but do not incorporate it in organic compounds. This anaerobic process is called dissimilatory sulfur reduction. Sulfur-reducing bacteria obtain energy by reducing elemental sulfur to H_2S with hydrogen or organic compounds. Some deltaproteobacteria reduce other oxidized inorganic compounds, such as ferric iron.

Members of the class Epsilon Proteobacteria live in animal digestive tracts and sometimes are pathogenic. Examples include *Campylobacter,* which can cause gastroenteritis in humans, and *Helicobacter pylori,* which causes stomach ulcers.

Bacteria that belong to the phylum Chlamydiae are nonmotile obligate parasites, meaning they cannot complete their life cycle without an animal or protozoan host. Their metabolic capabilities are extremely limited, and their cell walls lack peptidoglycan. One example is *Chlamydia trachomatis,* a bacterial species that causes the common sexually transmitted disease nongonococcal urethritis as well as trachoma, a leading cause of blindness in humans.

The phylum Spirochaetes includes bacteria that are characteristically long and helical and possess axial filaments. These flagellalike filaments run the length of the cell between the wall and the cell membrane and twist, causing a bacterium to move by rotating like a corkscrew. Most spirochetes are anaerobic and free-living, but a few are parasitic. *Treponema pallidum* causes the sexually transmitted disease syphilis, *Borrelia burgdorferi* causes Lyme disease, and *Leptospira* causes leptospirosis.

The cyanobacteria are unique in that they are the only prokaryotic organisms that undergo oxygenic photosynthesis, meaning that water serves as the electron donor and oxygen is produced as a by-product. Evidence strongly suggests that chloroplasts in plants and algae evolved from endosymbiotic cyanobacteria. Also called blue-green algae because they were once thought to be algae, these aquatic prokaryotes can be unicellular, filamentous, or colonial. Some cyanobacteria can also fix nitrogen, reducing it to NH_4^+, which can be incorporated in cellular metabolism.

The fifth group of Bacteria, the gram-positive bacteria, includes all the bacteria that retain crystal violet when stained by the Gram procedure. The cell walls contain as many as 40 layers of the carbohydrate-protein complex peptidoglycan, and teichoic acids are present in the cell membrane. Gram-positive bacteria include free-living and parasitic forms and consist of two major phyla: the Firmicutes and the Actinobacteria.

The largest phylum of gram-positive genera is the Firmicutes, whose members have a low percentage composition of the nucleotides guanine (G) and cytosine (C) in their nucleic acid. Firmicutes include two spore-forming genera—*Clostridium,* which includes the species that cause gas gangrene and botulism, and *Bacillus,* which includes the species that causes anthrax—in addition to beneficial species that serve as a source of antibiotics and that are used as natural pesticides. Firmicutes also comprise many non-spore-forming genera: *Staphylococcus,* a normal inhabitant of human skin that can also be pathogenic; *Streptococcus,* including *Streptococcus pyogenes,* which causes strep throat and rheumatic fever; *Lactococcus,* which produces lactic acid as an end product of fermentation; and *Enterococcus,* which can cause urinary tract infections, bacterial endocarditis, diverticulitis, and meningitis. The last group of Firmicutes is the mycoplasmas, tiny cell wall–less bacteria that evolved from gram-positive bacteria. *Mycoplasma pneumoniae* causes pneumonia.

The other major phylum of gram-positive bacteria is Actinobacteria, whose members have a high G-C content. Soil-dwelling actinomycetes play an ecologically important role in the decomposition of organic matter such as cellulose and chitin and are also the source of numerous antibiotics. Some actinobacteria are filamentous and resemble mold. Another type of actinobacteria, the coryneform bacteria, exhibit unique shapes, sometimes resembling the letter Y or V. *Corynebacterium diphtheriae* can cause the disease diphtheria. Members of the genera *Mycobacterium,* characterized by the presence of mycolic acids in the cell membrane, cause tuberculosis and leprosy. *Propionibacterium* species are used in the production of swiss cheese, and some cause acne.

See also Archaea; biogeochemical cycles; infectious diseases; Leeuwenhoek, Antoni van; Margulis, Lynn; microbiology; Pasteur, Louis; photosynthesis; prokaryotic cells; spontaneous generation.

FURTHER READING

American Society for Microbiology home page. Available online. URL: http://www.asm.org. Accessed January 13, 2008.

Dyer, Betsey Dexter. *A Field Guide to Bacteria.* Ithaca, N.Y.: Cornell University Press, 2003.

Madigan, Michael T., and John M. Martinko. *Brock Biology of Microorganisms.* 11th ed. Upper Saddle, N.J.: Prentice Hall, 2006.

Margulis, Lynn, and Karlene V. Schwartz. *Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth.* 3rd ed. New York: Henry Holt, 2002.

Singleton, Paul. *Bacteria in Biology, Biotechnology, and Medicine.* 6th ed. New York: John Wiley & Sons, 2004.

Banting, Sir Frederick G. (1891–1941) Canadian *Physician* Diabetes is a disease characterized by the body's inability to produce or utilize insulin. Without insulin, an excess of glucose collects in the bloodstream and is excreted in the urine, and the body does not obtain the energy it needs, leading to a slow death by starvation. For the victims of diabetes whose bodies cannot make insulin, injections of the hormone help regulate the levels of sugar in the blood. The Canadian physician Sir Frederick Banting is venerated for his discovery of insulin and its utility as a treatment for diabetes.

CHILDHOOD, EDUCATION, AND EARLY CAREER

Frederick G. Banting was born on November 14, 1891, in Alliston, Ontario. He was the youngest of five children and grew up in a deeply religious household on a farm. An average student and a decent athlete, he spent his childhood exploring around the farm and its riverbank. When he was a teen, one of his childhood friends became gravely ill, became very thin, lost all of her energy, and died of a then-mysterious illness called diabetes at the age of fourteen. This memorable event tremendously impacted the course Fred would pursue in his future.

After graduating from the local public school in 1910, Banting enrolled at Victoria College, a liberal arts institution in Toronto. To please his parents, Banting planned on majoring in theology and becoming a minister, but he was fascinated with medicine. In the autumn of 1912, he registered as a medical student at the University of Toronto. While a student, Banting saved to purchase a microscope for $57.50, a considerable sum in those days. In his free time he studied his own blood under the microscope, perfected his tissue preparation skills, and conducted experiments in the laboratory. At the nearby Hospital for Sick Children, Banting specialized in orthopedic surgery, the surgical correction of skeletal deformities. After World War I broke out in 1914, licensed doctors were scarce in the city. The school accelerated the medical students' courses, and they graduated six months early.

In December 1916, Banting entered the Canadian Army Medical Corps as a lieutenant. Banting first went to England, and then France, where he witnessed the suffering of many wounded soldiers and gained extensive surgical experience. During action in 1918, a piece of shrapnel seriously injured Banting's forearm and severed an artery, but he continued providing medical assistance to other soldiers for 17 hours. Later, when the doctors wanted to amputate, he refused to let them and determinedly strove to rehabilitate his arm. He received the Military Cross for his brave conduct.

In 1919 Banting returned to Toronto, where he accepted an orthopedic surgery residency at the Hospital for Sick Children. He specialized in the mechanical correction of childhood deformities such as clubfeet and twisted limbs. After one year, he attempted to start his own surgical practice in London, Ontario, but was unable to attract enough patients, so he accepted a part-time instructorship in anatomy, physiology, and clinical surgery for the medical school at Western University (now the University of Western Ontario). Banting was popular with students and delivered meticulously prepared lectures, but he missed performing medical research. He often joined the chief of physiology, Dr. Frederick R. Miller, in his neurophysiologic investigations. Together the physicians showed that the cortex of the brain was sensitive to outside stimulation.

THE PANCREAS AND HORMONE "X"

Banting spent a lot of time reading medical journals in order to include the latest reports and discoveries in his lectures. In autumn 1920, he began preparing for an upcoming lecture on the pancreas. The pancreas is a large abdominal gland that produces digestive enzymes that travel through a duct to the small intestine, where they chemically break down proteins, lipids, and carbohydrates into simpler molecules that the body can readily absorb. Removal of the pancreas leads to increased levels of sugar in the blood and urine, and death results. While searching the medical literature to learn more about this gland, Banting found descriptions of diabetes symptoms dating back 4,000 years. Victims suffer unquenchable thirst and hunger, high sugar levels in the blood and urine, an acetone-like odor on the breath, tiredness and depression, extreme weight loss, and eventually a coma leading to death. Though the disease had been recognized since ancient times, no treatment or cure had been discovered. Banting wondered why so little was known about treating diabetes.

In 1869 a medical student named Paul Langerhans identified groups of cells in pancreatic tissue, later named islets of Langerhans, that looked different from the regular pancreatic cells that secreted digestive enzymes and did not lead to the small intestine via a duct. In 1889 German researchers, Josef Von Mehring and Oskar Minkowksi, removed the pancreas of a dog, which developed acute diabetes mellitus and died within two weeks. The islets of Langerhans in the pancreatic tissue from deceased diabetics appeared atrophied. Some scientists thought these cells produced an undiscovered hormone that helped the body burn sugar for energy. Several physicians, including Dr. John James Richard Macleod, the head of physiology at the University of Toronto,

claimed that there was no proof for the existence of this unknown "hormone X."

On the evening of October 30, 1920, the day before Banting's lecture on the pancreas, he visited the library to search for additional material to include in his lecture. That morning, a new issue of *Surgery, Gynecology, and Obstetrics* had arrived. The journal contained a 12-page article by Dr. Moses Barron titled "The Relation of the Islets of Langerhans to Diabetes, with Special Reference to Cases of Pancreatic Lithiasis." The article said that sometimes an autopsy revealed gallstones blocking the pancreatic duct. In these cases, the pancreatic cells that produce digestive juices had disintegrated, but the Langerhans cells all looked normal and healthy, and the patients showed no symptoms of diabetes. Barron also reported that this effect could be recreated in dogs by surgically tying off the pancreatic duct. After several weeks, the entire pancreas shriveled up except the Langerhans cells.

The information that the Langerhans cells were somehow associated with diabetes interested Banting, who believed that the cells made an unknown hormone X that helps the body burn sugar. Past attempts using pancreatic extracts to relieve diabetic symptoms had been unsuccessful, however. Banting thought digestive enzymes made by the pancreas destroyed the unknown hormone during extraction and thought that tying off the pancreatic duct to destroy the enzyme-making cells before extract preparation would preserve the hormone's activity during extraction.

When Banting shared his idea for a method of obtaining active hormone X with Miller, Miller suggested that Banting speak with the well-known endocrinologist Macleod at the University of Toronto. Several other physicians also deferred to Macleod as the leading expert on blood sugar chemical processes. Banting knew that Macleod did not believe in the existence of hormone X but scheduled an appointment and drove to Toronto anyhow.

Macleod politely listened to Banting but was unimpressed with Banting's lack of research experience on blood chemistry and turned away the discouraged young doctor. Worried that his nervousness interfered with his ability to present a strong case for proceeding with the anticipated research clearly, Banting spent that night typing up a written proposal. The next morning he returned, and Macleod agreed to provide Banting with 10 dogs, an assistant proficient in biochemistry, and laboratory space for eight weeks.

STUDIES DIABETES IN DOGS

A few months later, Banting moved back to Toronto and was joined by an assistant, Charles H. Best, a recent physiology and biochemistry graduate who had previous research experience using chemical procedures, to measure sugar levels in blood and urine. On May 16, 1921, Banting began surgery on the dogs, tying off the pancreatic ducts in hopes of destroying all the pancreatic tissue except the islets of Langerhans. The following week, he attempted to remove the pancreas of one dog using a two-step procedure, but the dog died of shock and infection. Using his surgical experience, he refined a technique to remove the pancreas completely in one operation. As expected, the dog developed diabetes. During the six- to eight-week waiting period for the pancreas of the duct-tied dogs to atrophy, Banting named the unknown hormone that they hoped to find "isletin."

On July 6, 1921, the men opened up two dogs whose pancreatic ducts had been tied and were dismayed to find healthy pancreas glands inside. Examination revealed that Banting had tied the ducts too tightly, and new pathways had formed around the ligature. To correct for this, Banting retied the ducts more loosely than before, but in three different places to ensure digestive juices could not flow through them. In a few dogs, degeneration was occurring, but the men decided to let it progress for two more weeks. Banting was worried that they soon would hear from Macleod, who was vacationing in Scotland for the summer. He had originally promised them eight weeks in his lab, and their time was up. They were also broke, so Banting sold his car to buy more dogs.

They removed a pancreas from another dog, which promptly became diabetic. As it approached the coma stage, they cut open a duct-tied dog and removed its now degenerated pancreas. The islets of Langerhans still appeared healthy, so they crushed the gland in a chilled, buffered saline solution. After filtration, the extract was injected into the neck vein of the dying dog. In an hour, the dog began to lift its head. Within a few hours it was sitting up, wagging its tail, and its blood sugar level had dropped to almost normal. Within five hours, the urine was completely void of sugar. These were exactly the results Banting and Best had anticipated. Isletin had been used successfully to treat diabetes.

Unfortunately, the next morning the dog was dead. Isletin was a treatment, but it was not a cure. They removed the pancreas from a second dog and waited for it to become ill. Then they made more pancreatic extract from another duct-tied dog, but this time they also made extracts from the liver and spleen to demonstrate the previous success was due to a substance specifically from the pancreas. When they injected the liver and spleen extracts into the sick dog, nothing happened, but when they injected the pancreatic extract, again the dog perked up, and

within hours the urine contained no sugar. They managed to keep this dog alive for three days.

Though these results pleased Banting, he was disturbed by having to kill healthy animals to obtain extracts that only treated diabetic animals for a short time. He wondered how he could maximize the amount of extract produced while minimizing the number of animals that had to be sacrificed. One technique they tried was exhausting the pancreas by overstimulation with another hormone, but this still yielded limited amounts.

Macleod returned from vacation, and while he was not overly impressed with their progress, he allowed Banting and Best to continue using his laboratory facilities. To ease financial burdens, Banting assumed a position as a demonstrator in the pharmacology department. His responsibilities were minimal, and the small salary was just enough to allow him to continue his studies.

DISCOVERS INSULIN

One day Best came across a paper that said that the pancreas of newborns was richer in Langerhans cells than that of adults. Since fetuses do not digest their own food in utero, they also would not be producing digestive juices. Banting thought slaughterhouses ought to have a sufficient supply of calf embryos from which they could isolate the pancreas. By noon the following day they had obtained nine embryonic calves, from which they extracted isletin. The isletin from calf embryos also reduced the blood sugar levels to normal when given to diabetic dogs. While this method provided more extract than using duct-tied dogs and did not require the sacrifice of otherwise healthy animals, the supply was still limited.

Banting and Best devised a chemical extraction method from adult cattle pancreas involving a combination of acid and alcohol. To ensure it would not cause any undesirable side effects in sick patients, they injected the extract into each other and observed no harmful effects, but of course, neither of them was diabetic. They had a potent extract and were ready for a real human trial.

The opportunity for a human trial presented itself on January 11, 1922, when a 14-year-old boy was admitted to Toronto General Hospital with a severe case of diabetes. His body had wasted away to a mere 65 pounds and death was imminent. A dose of isletin reduced the boy's blood sugar. They worked to purify the extract further and optimize the dosage, miraculously restoring his health. This surprising success attracted the attention of Macleod, who promptly stopped work on his own research and dedicated his entire staff to assisting in the isletin research. He suggested a name change to *insulin,* since it was easier to pronounce. A biochemist by the name of Dr. J. B. Collip and a recent graduate named E. C. Noble joined Best in the perfection of a technique called fractional alcoholic precipitation to purify the insulin from pancreatic extracts.

In February 1923, a former classmate of Banting's from medical school, Joe Gilchrist, visited Banting. He had developed diabetes during the war and volunteered to act as a human guinea pig for their new extract preparations. Respiration tests showed Gilchrist's body was not burning any sugar. They injected him with insulin, and a few hours later he was producing sugar-free urine. Once he accidentally overdosed with insulin but recovered after drinking a nearby beaker of glucose solution. A few months later, Banting obtained permission from the Canadian government to use Toronto's Christie Street Hospital for Returned Soldiers to begin clinical trials. Later they expanded their testing to Toronto General Hospital.

All the clinical tests taught them that although insulin worked wonders, diet was still an important factor in treatment. They determined proper doses of insulin and observed that injections worked best if administered 20 to 30 minutes before a meal. They learned to recognize the signs of insulin overdose and discovered that administering glucose could prevent insulin shock. Before these tests, six of every 10 diabetics died of coma, and every child was doomed. With the availability of insulin, the death rate dropped considerably.

Banting and Best published their findings, "The Internal Secretion of the Pancreas," in the November 1921 issue of the *Journal of Laboratory and Clinical Medicine.* The results were announced publicly at a medical meeting in New Haven, Connecticut, in late 1922. Banting was not an experienced or polished speaker, but Macleod, who was chairing the meeting, spoke next. He did a much better job telling the story but failed to emphasize who actually performed the work; as a result, many believed that Macleod headed the research. The American Association of Physicians in Chicago asked Macleod to speak. Again, he failed to clarify who led the research that led to the discovery of insulin.

Continued clinical trials gave excellent results, and soon commercial drug companies were given the instructions on how to prepare insulin extracts from bovine pancreas, but people flocked to Toronto to see the "miracle physician." Banting temporarily opened an office to treat diabetic patients, only charging minimal fees. With Best and Collip, Banting patented the process for insulin production and used all the profits to fund continued diabetes research. He received numerous medals, awards, and honorary degrees from several nations. In 1923 Banting was appointed the first full professor of medical research

in the history of the University of Toronto. The chair was named the Banting and Best Chair of medical research. The greatest honor bestowed on Banting was the 1923 Nobel Prize in medicine or physiology. The Nobel committee named Macleod and Banting corecipients but did not include Best. This unjust omission angered Banting so much that he initially planned to refuse the award. Banting did accept the award to honor his nation of Canada, and he publicly acknowledged Best's contribution by pledging half of his monetary award to his colleague. This act motivated Macleod to share his award with Collip. Banting also insisted the order of names be changed to Banting and Macleod.

On June 4, 1925, Banting married Marion Robertson, a radiology technician whom he had met briefly before being discharged from the army. They had one son, William Robertson Banting, in 1929, but his marriage ended in divorce in 1932. Banting received custody of Bill.

RESEARCH ON SILICOSIS

After spending so much time touring, lecturing, and treating diabetic patients, Banting was eager to return to laboratory research. He now had a secretary, a laboratory technician, two graduate students, and his own cramped laboratory. Banting was a good teacher in the lab. He forced his students to think for themselves and taught them to speak simply and to get to the heart of the matter. His research interests included cancer, chemical treatments of mental disorders, and royal jelly (the food of the queen honeybee), but he made the most progress examining silicosis.

Silicosis is a lung disease that affected mostly miners. Symptoms included shortness of breath and a persistent cough, and the disease often resulted in total disability. Banting and his workers identified the cause as inhalation of silicon dioxide, which dissolved in the lungs to form silicic acid. The acid irritated the lining of the lungs and caused hardening, or fibrosis, to occur. Filtering the dust from the air was too cost prohibitive, so the Banting team explored other methods to prevent silicosis. They found that dispersal of a fine dust of aluminum powder into the air successfully prevented the formation of silicic acid in the miners' lungs.

HONORS AND LATER CAREER

The world continued to bestow new honors and responsibilities on Banting. In 1930 the University of Toronto opened a new research center, the Banting Research Institute. In 1934 he was created a Knight Commander of the Civil Division of the Order of the British Empire. As chairman of the Medical Research Committee of the National Research Council of Canada, he surveyed the national medical facilities and recommended the formation of a committee of Aviation Medical Research, which he chaired.

In 1937 he married Henrietta Ball, who worked at the Banting Research Institute on chemotherapy and tuberculosis research. In 1939 right before Canada declared war, Banting rejoined the army as a major. The government gave him the task of organizing and administering a major research program that included studies on decompression, the development of an antidote for mustard gas, and the invention of a protective flight suit for airmen. In February 1941, Banting was flying to England to present findings on the newly developed flight suits. The plane's engine failed during flight, and Banting died after a crash landing over Newfoundland. Thousands visited as Banting lay in state at the University of Toronto's Convocation Hall. Sir Frederick Banting was given full military honors at his funeral.

In recognition of Banting's major contributions to developing treatments for diabetes, the International Diabetes Federation established the Banting and Best Memorial Lectureship, and the American Diabetes Association established the Banting Medal and Memorial Lectureship. The Banting Research Foundation continues to commemorate Banting's discovery of insulin by supporting medical research for young Canadian scientists. The Banting and Best Diabetes Centre at the University of Toronto supports and advances diabetes research, education, and patient care.

As a result of Banting's efforts, millions of people afflicted with diabetes are living healthy, enjoyable lives. Though so-called experts on sugar metabolism believed the disease was hopeless, the dedicated, unpaid medical researcher toiled away in a hot, cramped, borrowed attic lab. Because Banting maintained hope, the miracle hormone insulin was discovered.

See also BIOTECHNOLOGY; DIABETES; DIGESTIVE SYSTEM; HOMEOSTASIS; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

Bliss, Michael. *The Discovery of Insulin.* Toronto: University of Toronto Press, 2000.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1923." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1923/index.html. Accessed January 13, 2008.

Beebe, William (1877–1962) American *Marine Biologist* William Beebe was one of the first men to venture into the ocean depths and record the remarkably diverse array of marine organisms. He pioneered

the use of the diving helmet and the bathysphere for biological research, paving the way for the exploration of oceanic life.

BECOMES ZOO CURATOR

Charles William Beebe was born on July 29, 1877, in Brooklyn, New York, and moved to East Orange, New Jersey, during his early childhood. Before entering East Orange High School, he dropped the *Charles* from his name. He completed four years of Latin as well as two years of German, languages that would help him later in his career. In addition, he took several courses in the natural sciences. He was a strong student and very physically active. In his spare time he watched and listened to birds, memorized the local wildflowers, collected butterflies, and built up a bird skin collection. Before he even graduated, he had his first scientific publication, a letter to the editor of *Harper's Young People* about a bird, the brown creeper.

After high school Will matriculated as a special student at the University of Columbia, where he took many classes and attended several lecture series. Though he never received his degree, he did make the acquaintance of people who helped advance his career. One noteworthy man was the paleontologist Henry Fairfield Osborn, who was a professor at Columbia and was the curator of the American Museum of Natural History. Osborn was a founder of the New York Zoological Society (today called the Wildlife Conservation Society) and in 1895 became its first president. The society opened a Zoological Park (the present-day Bronx Zoo) in 1899.

In October 1899, the zoo hired Beebe as the first assistant curator for birds. Though he lacked formal training, popular magazines had already published several of his articles, and scientific publications such as *Science, The Auk,* and later *Zoologica* were beginning to do the same. In 1902 the zoo promoted Beebe to curator, and he piloted campaigns to build a spacious bird house and an enormous flying cage.

TRAVELS AND WRITES

Mary Blair Rice became Beebe's wife in 1902. She traveled with Beebe and collaborated on many of his writing projects. Together they traveled to Mexico during the winter of 1903 and 1904 with the goal of identifying and collecting Mexican bird specimens, especially those not indigenous to the southern United States. Mary was well educated and a talented writer in her own right. She assisted in writing Will's first published work, *Two Bird Lovers in Mexico* (1905).

Field research dominated Beebe's interests, and writing occupied his time. In 1906 Beebe published two books: *The Bird, Its Form and Function,* an introduction to ornithology, and another popular book intended to inspire amateur naturalists, *The Log of the Sun.* The poetic manuscript included essays on topics ranging from the life sciences to meteorology and was composed of 52 chapters, one for each week of the year. In 1910 Will and Mary wrote *Our Search for a Wilderness,* describing two expeditions they took together, one to northeastern Venezuela in 1908 and another to British Guiana (present-day Guiana) in 1909. Beebe observed the exotic wildlife and took home some birds for the Zoological Park. He captured 40 birds of 14 different species in Venezuela and 280 birds of 51 species in British Guiana. One interesting encounter was with the hoatzin, a strange bird whose young have claws on the back of their wings for climbing trees.

Beebe traveled with his wife to eastern Asia from 1909 to 1911 to study over 20 different pheasant genera. They visited 20 countries in over 17 months. They divorced shortly after returning, and Beebe took a privately funded five-year leave to pursue museum research and to complete the major scientific publication of his career, *A Monograph of the Pheasants.* Only 600 sets of this very expensive book were printed. The series was as popular among painters for the beautiful photographs and sketches of pheasants as it was among naturalists for the extensive knowledge it contained concerning pheasants. General information about each species, its distribution, description, and life history, was presented. Unusually for scientific writing, Beebe used the first-person singular and very colorful prose. He vividly shared his own personal adventures in searching for the birds.

The first volume of this project was published in 1918, but World War I delayed publication of the remaining three volumes. Almost 40 years old, Beebe volunteered for service during the war, though the nature of his service is somewhat unclear. He served through the French Aviation Service rather than the United States. While enlisted, he learned to fly and instructed other volunteers. After one year, he returned to the United States, but tastes of his wartime experiences peppered his future writings.

In 1926 and 1927, less scientific abridged versions of the four-volume *Monograph* were published. These editions, titled *Pheasant Jungles* and *Pheasants, Their Lives and Homes,* were aimed at a more general audience, and the latter contained fictionalized accounts of his actual experiences. Some scientists scoffed at Beebe's popular writings, claiming they detracted from his reputation as a respectable scientist, and they accused him of exaggerating many of the adventurous claims he recorded in his popular texts. Beebe continued to publish objective scientific accounts of his field research as well as write successful creative books for the general population.

After returning from his five-year pheasant sabbatical in 1915, Beebe traveled to Brazil to collect bird specimens for the thriving Zoological Park. While there, he was amazed at the number and variety of organisms located within a small region underneath one huge cinnamon tree. He pioneered the method of studying one small designated location for an extended period. Significantly, he discovered 76 different types of birds and over 500 total organisms within a few square feet. Beebe's interests switched from birds to tropical research.

In 1916 Beebe established the New York Zoological Society's first tropical research station at Kalacoon, near Georgetown, British Guiana, in the northeastern region of South America. The staff shared their quarters with scorpions, tarantulas, and vampire bats. Beebe found and studied 281 bird species while at Kalacoon. The Zoological Society published many of his findings and those of two other scientists from this research station in *Tropical Wildlife in British Guiana* in 1917. This book included observations on the bright-billed toucans, the reptile-like hoatzins, and the ground-dwelling tinamous. Beebe added to this account in *Jungle Peace* (1918), for which the former president, Theodore Roosevelt, wrote the introduction.

After returning from his war service, Beebe was given the title of honorary curator of the department of birds. In addition, in 1918 the society created a department of tropical research, which Beebe directed until he retired. When his staff returned to Kalacoon, the ecology of the region had changed as a result of the number of rubber trees that had been cut down for war supplies. They moved the research station to nearby Kartabo, at the junction of the Cuyuni and Mazaruni Rivers. Beebe wrote several scientific papers describing the flora and fauna of the jungle there. *Edge of the Jungle* (1921) and *Jungle Days* (1925) were both inspired by Kartabo. They focused on the ecology of jungle life. Beebe's professional interests were expanding once again. A 1926 issue of *Zoologica* included a paper he wrote on the three-toed sloth.

THE GALÁPAGOS ISLANDS

In spring 1923, Beebe journeyed to the Galápagos Islands. He spent two and one-half months at sea on the *Noma* and 100 hours on the islands themselves. The tameness of all the wildlife there, including the mockingbirds that ran up to welcome him rather than flying away, enamored the naturalist. Immersed in birds, sea lions, and iguanas, he pondered the irregular variations among the island species. The expedition spent some time anchored in Darwin Bay, which they named, where they were surprised to find bits of coral on the beach. Beebe was harmlessly attacked there by a two-foot (0.6-m) moray eel, and they took pleasure in watching birds fight over prime nest-building sticks.

Soon after returning to New York with plant and animal species gathered for the Zoological Park and the American Museum of Natural History, Beebe ventured out again, this time on a steam yacht called *Arcturus*. His main focus now was oceanography, in particular creatures of the sea. He also planned to study the Sargasso Sea south and east of Bermuda and the Humboldt Current, which moves up the Pacific coast of South America toward the Galápagos. The *Arcturus* departed Brooklyn in February 1925 for a six-month trip. Though storms stirred up the Sargasso Sea too much for study to be useful and the Humboldt Current was unexpectedly absent, they collected much valuable information. After five weeks out, the ship needed some repairs, so they rested at Fort Sherman, Panama, for a while. Beebe took pleasure in examining the wildlife there.

Next they anchored at their previous lodging, Darwin Bay, and continued exploring and collecting. Beebe began to use a copper diving helmet as an integral part of his field research. The helmet allowed him to remain submerged for long periods. A leather tube ran from the helmet to a vessel above the surface, and a person hand-pumped fresh air down the tube to the diver. Using a helmet, he was able to collect specimens that had never before been identified and to view marine life in the natural environment. Animals were taken above the surface for further live study in his aquariums or by dissection.

While they were stationed at Darwin Bay, the crew happened to observe volcanic fires from Albemarle, the largest island of the Galápagos archipelago. They set out in that direction. When Beebe crazily decided to explore up close with his foremost assistant, John Tee-Van, the gas and smoke made them nauseous, and Beebe temporarily lost some sight and speech. After stumbling back to the ship, he was severely dehydrated and exhausted, but he recovered. When passing by again nine weeks later, they were amazed to witness the red hot lava flowing into the ocean waters. The hot waters killed many fish that swam too close. Animal scavengers became ill from unknowingly approaching the gaseous exhalations and died as well. The crew watched one sea lion tragically jump high out of the hot water right into the lava.

When Beebe left the Galápagos in June 1925, he had plenty of research material. As usual, he shared highlights of his experiences as well as scientific information through his writing. *Galápagos: World's End* and *The Arcturus Adventure* were published in 1924 and 1926, respectively. At the time, there was still much debate over the origin of the islands. Some

believed, as scientists do today, that the archipelago was formed from a volcanic hotspot that spewed out hot magma that piled up over time to form the individual islands. In *Galápagos: World's End,* Beebe stated his belief that the islands were originally one continuous landmass that sank, leaving portions above the sea surface, thus creating the archipelago. This would explain the similarities between species on the islands, yet allow for evolution of unique characteristics by adaptation over time. Beebe also believed that a land bridge formerly existed connecting the Galápagos Islands to the Cocos Ridge. This would explain how terrestrial animals and insects and spiders originally arrived on the islands. Others who believed in the idea of a former land bridge thought it probably connected with Ecuador, which would have been closer. However, Beebe always remained open-minded if proof otherwise was presented. Two years later, on the *Arcturus* expedition, Beebe himself made observations that led him to believe that terrestrial creatures could have entered and inhabited the islands in the absence of a land bridge.

During his *Arcturus* expedition, Beebe set up a sea station halfway between the Galápagos Islands and Central America, 60 miles (97 km) south of Cocos Island. A sea station was a temporary designated area where the boat remained stationary or slowly circled in order to gather data. While in that one spot, the staff hauled up nets, dredged, took bottom samples, recorded temperatures, and made other observations. At this one station, named number 74, they captured a remarkable 136 fish species and more than 50 crustacean species in a 10-day period. In this location, Beebe took surface hauls every 30 minutes for an entire day. This allowed him to observe that some fish only surfaced during the daytime, while others surfaced only at night. This was useful information for marine biologists so that if they wanted to study a particular type of fish, they knew when they were most likely to find it. He also figured out that data on luminescent fish were best gathered at nighttime, and he was able to make rare observations of living luminescent fish.

HAITI AND BERMUDA

By July the expedition had returned to New York, leaving Beebe to sort through his data and numerous specimens. Hooked on marine biology, the following year he set out for Haiti on a schooner chartered by the New York Zoological Society. A biological station was set up on board the *Lieutenant* in the Bay of Port-au-Prince, where they stayed for four months. The goal was to identify fish in Haitian waters and explore the coral reefs. From this expedition he wrote *Beneath Tropic Seas* (1928). A list of 270 species was published in *Zoologica* in 1928 and increased to 324 species when the list was supplemented in 1934. To study coral reef life Beebe depended on his diving helmet. In more than 300 dives Beebe observed diverse life-forms of the coral reef, then classified them by their ecological niche.

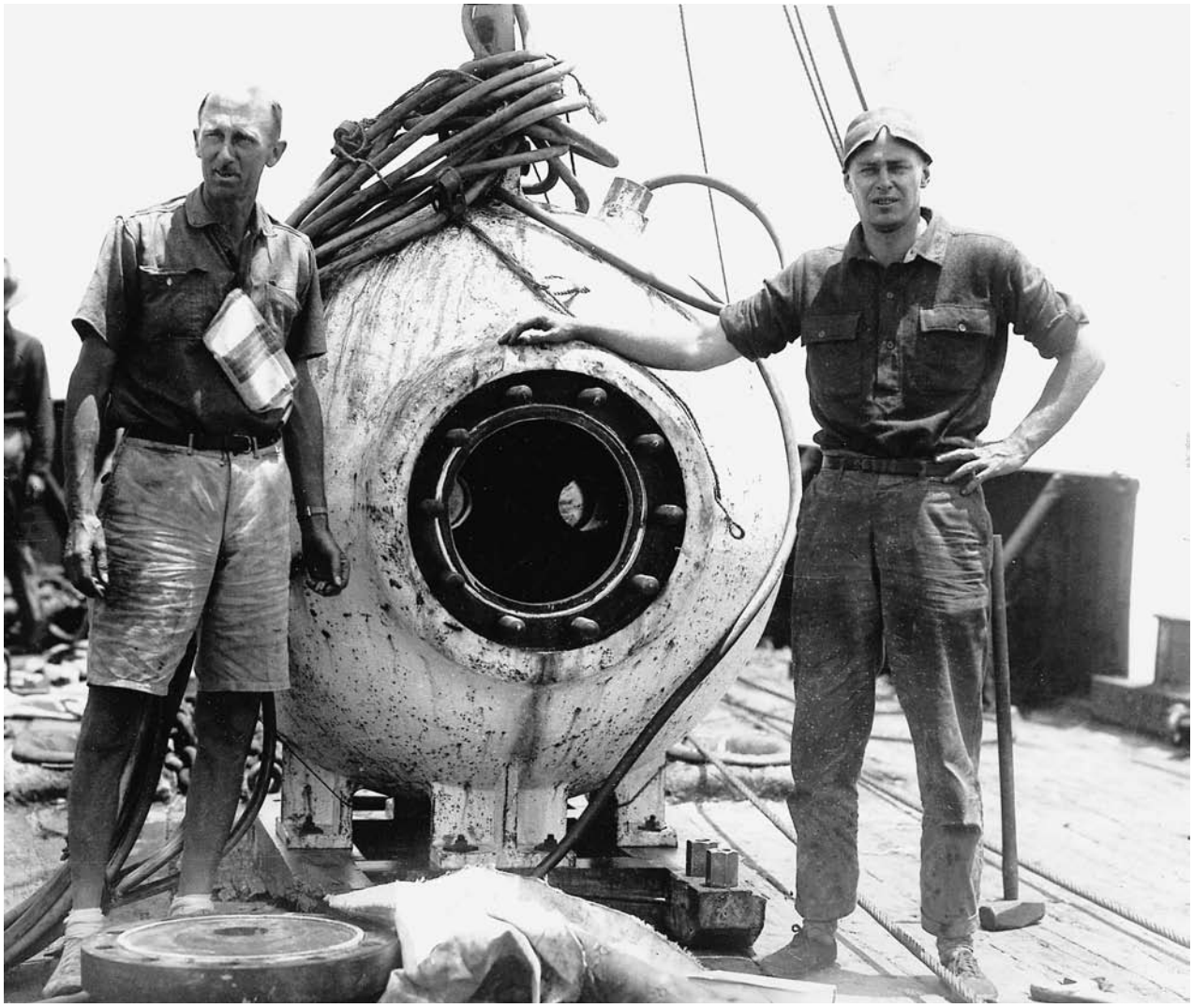
While in Haiti, Beebe met Elswyth Thane, a writer for newspapers and motion picture studios. They married in 1927. The two traveled together and separately, both of them involved in their own research.

In 1928 Beebe obtained permission from the British government to carry out studies on the island of Nonsuch in Bermuda. The region of focus was eight miles (12.9 km) in diameter and ranged from 6,000 to 8,000 feet (1,829 to 2,438 m) deep. Most of Beebe's research over the next 11 years was carried out here, including his most famous deep sea dives. Of course, the goal was to study fish from the deep sea as well as from the shores. Beebe and Tee-Van published *Field Book of the Shore Fishes of Bermuda* (1933). They used many of the common research methods that had been practiced for decades, including trawling, dredging, and hauling silk nets, but Beebe found these means limiting.

SETS RECORDS IN BATHYSPHERE

Beebe had been the first to use a diving helmet as an integral part of his field research rather than just for pleasure, but its utility was limited. Though the helmet was fine for dives between 15 and 50 feet (4.6 to 15.2 m) deep, by 100 feet (30.5 m) it became unsafe. Beebe contemplated alternative means of underwater exploration. In 1928 he teamed up with a young man named Otis Barton, a trained engineer, who drew up blueprints for a sphere-shaped vessel and funded its construction. One major feat of the vessel design was to withstand the enormous pressure from the ocean depths. The proposed round bathysphere was well suited to distribute the extreme external pressure evenly.

The bathysphere weighed 5,000 pounds, the outer diameter of the newly named "bathysphere" was four feet nine inches (145 cm), and the walls were one and one-half inches (3.8 cm) thick. The circular door was a mere 14 inches (35.6 cm) in diameter, barely enough for a grown man to wedge himself through. The door was fastened by 10 bolts. There were three window ports, two of which were filled with eight-inch (20.3-cm) fused quartz disks, three inches (7.6 cm) thick. The third was plugged. There were four legs on the bottom to which wooden skids were attached. Inside were pans of calcium chloride to absorb moisture and soda lime for absorbing excess carbon dioxide. Of course, oxygen tanks supplied air for breathing. To circulate air inside the chamber, the two men carried palm-leaf fans.



The bathysphere was specially outfitted for deep underwater exploration of marine life. *(© Wildlife Conservation Society)*

An electrical line and a communications line were wrapped together in a cable one and one-half inches thick and fed into the top of the sphere. The 3,500 feet (1,067 m) of steel cable necessary to lower the bathysphere into the water weighed 4,000 pounds. Two steam winches on deck moved the enormous hollow ball.

Unmanned test descents commenced in early June 1930. The first test resulted in a tangled mess of communication lines. After adjustments and another unmanned descent, the first manned descent occurred on June 6. Despite a minor leak and a pop from an electric switch at around 300 feet (91.4 m), Beebe and Barton achieved a depth of 800 feet (244 m). Beebe's most amazing observation concerned the colors viewed below the surface. Whereas the water began a light greenish color, as they descended it turned bluish green, then a pale blue, then a blackish blue. On June 10, Beebe and Barton made another attempt, but at 250 feet (76.2 m) the communications line went out. Without being able to hear the human voice from the surface through Beebe's headphones, the men felt very isolated. The crew pulled them up immediately.

After cutting off 300 feet (91.4 m) of cable, they went down again the following day. They descended slowly, making verbal observations every foot of the way. Beebe's experience in ichthyologic identification qualified him for this task. He was thrilled to observe many specimens that had previously only been seen dead in net hauls. However, many were brand new, and Beebe and Barton relished the opportunity to observe them swimming in their natural environment. At 1,426 feet (435 m), they paused and returned to the surface. Barton donated the bathysphere to the New York Zoological Society that fall.

Two years later history was made again, but this time the world was invited along. The National Broadcasting Company arranged a live radio broadcast of a dive one Sunday afternoon in September 1932. The weather had caused delays, and the sea was still rougher than would normally be acceptable, but the world was waiting. Beebe and Barton descended on their 20th deep dive into the sea, and their eager reports to Gloria Hollister aboard the barge were relayed to America. The British Broadcasting Corporation was also connected by short-wave radio, increasing the listening audience.

In the bathysphere all light had disappeared by 1,700 feet (518 m), but as they continued descending, the numbers of luminescent fish increased. They turned around after dangling momentarily at 2,200 feet (671 m), and on the way back up Beebe spotted two six-foot- (1.8-m-) long fish that he named *Bathysphaera intacta* (untouchable bathysphere fish). He claimed their teeth were luminous, and a linear formation of lights ran along their sides. Later, others doubted Beebe really saw these, believing perhaps they were a few fish swimming end to end. On this trip, a spiny lobster had been tied to the outside of the bathysphere. They expected it to be crushed and act as bait to attract fish to the submerged bathysphere for observation. Astonishingly, the lobster survived the thousands of tons of pressure and went on to live in Beebe's aquarium.

The following year, the bathysphere was displayed at the Century of Progress Exposition in Chicago. The president of the National Geographic Society asked Beebe to consider one more bathysphere expedition. The National Geographic Society would sponsor it, and they did not stipulate an attempt at a new depth record. This sounded attractive to Beebe, who later stated that it was the lack of a request for a new record that made him determined to set one.

The new record dive occurred on August 15, 1934. They reached 3,028 feet (923 m), over one-half mile deep. This record was unbeaten for 15 years. Actually, Beebe and Barton had descended to 2,510 feet (765 m) a few days prior, but Beebe felt that the second dive was totally different despite the exact same location. Several new species were named. Again, Beebe noted the increased number of luminescent fish in deeper regions, as well as that larger creatures were more prevalent.

The bathysphere experiences were invaluable not only because they set records and revealed undiscovered species, but because they challenged oceanographers to develop better methods for undersea studies. Beebe emphasized that what was viewed directly was so much different from what was inferred from deep trawling or dredging or net hauls. Observing marine life in the natural environment was much more informative. Many brand new species were identified and others seen live for the first time. Organisms such as siphonophores (including the Portuguese man-o-war) could be viewed in their true form, rather than as a tangled-up mess of debris pulled to the surface. Information on vertical distribution and relative abundance of different species of fish could also be obtained.

After this season, the bathysphere was retired. Just as a diving helmet did, the sphere had to remain tethered to a surface vessel. Though it had descended over 3,000 feet (914 m), its depth were still limited, as was its lateral mobility. Beebe continued studying oceanography on a yacht named *Zaca*. He continued to use his diving helmet and was as zestful and enthusiastic at the age of 60 as he was at 25 years old. His last sea voyage departed in April 1938.

RETIREMENT

During World War II, Beebe was unable to continue his research off Bermuda, so he returned to jungle research at Caripito, Venezuela. Beebe's last sea book, *Book of Bays,* was published in 1942. In it he expressed concern over man's threat to the world's ecosystems. In 1945, he established another research station, at Rancho Grande in Venezuela, then yet another in 1950, at Simla, in the Arima Valley of Trinidad. He personally purchased this land and lived there during the wintertime. He retired in 1952 and eventually sold this land and its 200 associated acres to the New York Zoological Society for one dollar. In 1955 Beebe made one last trip to Asia to check on the pheasant populations he had studied 45 years before.

Though his spirit remained robust, his health failed during the last three years of his life. He was no longer able to ride his bike around the Zoological Park commanding visitors to go check out the new bird exhibit as he had in his younger days. Unable to tolerate the cold of New York, he spent the months of October through May at Simla. He grew physically weaker and his speech sporadically was slurred. Though he expressed hopes of dying of heart failure from viewing an unexpected amazing natural phenomenon, Will Beebe died of pneumonia on June 4, 1962. He was buried in Trinidad.

Simla was renamed the William Beebe Tropical Research Station, but it was functional for only a few more years. The football field–sized bird house at the Zoological Park was replaced by a newer complex in 1972. The bathysphere is on display at the New York Aquarium, which is managed by the Wildlife Conservation Society. During his lifetime, Beebe was criticized for making up such fantastical, outlandish tales of extravagant undersea creatures. Not until years

later did advancements in underwater photography enable others to verify his claims. Vindicated, he was ultimately recognized for discovering hundreds of new species.

Beebe's scientific articles and notes are recorded in scores of *Zoological Society's Bulletin* issues and other scientific journals. He conveyed his passion for deep sea life in his two dozen popular books of naturalist adventures, inspiring a new wave of scientists and naturalists including Sylvia Earle and Rachel Carson.

See also CARSON, RACHEL; EARLE, SYLVIA; MARINE BIOLOGY.

FURTHER READING

Barton, Otis. *The World beneath the Sea*. New York: Crowell, 1953.

Beebe, William. *Adventuring with Beebe: Selections from the Writings of William Beebe*. New York: Duell, Sloan and Pearce, 1955.

———. *Half Mile Down*. New York: Harcourt, Brace, 1934.

Welker, Robert Henry. *Natural Man: The Life of William Beebe*. Bloomington: Indiana University Press, 1975.

Yount, Lisa. *Modern Marine Science: Exploring the Deep*. New York: Chelsea House, 2006.

Bigelow, Henry (1879–1967) American *Zoologist*

Henry Bigelow was a pioneering ocean researcher of the 20th century. As the first person to perform a comprehensive study of the Gulf of Maine, not only did he collect vast amounts of data during his research, but as a result, he recognized and emphasized the importance of physical, chemical, and biological unity in studying the sea. His recognition of the need to study the complex interdisciplinary nature of the sea led to the establishment of the esteemed Woods Hole Oceanographic Institution, which has supported leading oceanographic research for 75 years.

EDUCATION AND TRAINING

Henry Bryant Bigelow was born to a banker, Joseph Smith Bigelow, and Mary Cleveland Bryant Bigelow on October 3, 1879, in Boston, Massachusetts. As a youth, he often traveled to Europe with his family and developed a love for the outdoors and for sports. The family spent summers at the quaint harbor town of Cohasset, Massachusetts. Henry graduated from the Milton Academy in 1896 and then took courses at the Massachusetts Institute of Technology while working at the Boston Museum of Natural History. He enrolled at Harvard University in 1897 and graduated cum laude four years later. Though in his memoirs he reported that he did not have much of a social life during college, he made contacts with influential individuals that shaped his career.

Bigelow's early scientific interest was birds. He went on a trip to the Canadian province of Newfoundland and Labrador in 1900. His first scientific publication on the American eider (a northern sea duck with soft down), "A Virginia Record for the American Eider," was published in 1901 in the respected ornithology journal *Auk* while he was still an undergraduate. The following year he published a more substantial article, "Birds of the Northeastern Coast of Labrador," also in *Auk*.

In 1901 he was invited to accompany a Harvard professor who was also the director of Harvard's Museum of Comparative Zoology (MCZ), Alexander Agassiz, on an expedition to the oceanic island group the Maldives, located in the Indian Ocean, southwest of Sri Lanka. Bigelow's responsibility was caring for the jellyfish and the siphonophores they collected. He enjoyed the fieldwork during this experience, which sparked an interest in marine invertebrates and taught him the basics of taxonomy, the classification of species. He published papers on the

Henry Bigelow, shown here on the deck of the USS *Grampus* while exploring the Gulf of Maine, was a systematic zoologist who merged the physical and life sciences in his pioneering studies of the ocean. *(National Oceanic & Atmospheric Administration/Department of Commerce)*

medusas of the Maldive Islands in 1904 and 1909, establishing himself as a knowledgeable marine biologist. During the period 1904–05, Bigelow traveled to the eastern tropical Pacific with Agassiz, and in 1907, to the West Indies.

In 1904 Bigelow earned his master's degree from Harvard and in 1906, his doctorate. The topic of Bigelow's doctoral dissertation was the nuclear cycle of *Gonionemus vertens,* a hydrozoan that attaches to eelgrass or sea lettuce using adhesive disks on its tentacles. This experience studying cellular structure and function impressed upon Bigelow the necessary discipline for laboratory research.

Bigelow married Elizabeth Perkins Shattuck in 1906, and they eventually had four children together. His wife often accompanied him on his research travels.

GULF OF MAINE STUDIES

After obtaining his doctorate, Bigelow was given a position as an assistant at Harvard's MCZ, where he catalogued specimens. A visit by John Murray, a Scottish oceanographer who specialized in studying the ocean bottom, prompted Bigelow to investigate the virtually unknown Gulf of Maine. In 1912 he borrowed the schooner *Grampus* from the U.S. Bureau of Fisheries and began an extensive study of the Gulf of Maine that lasted for 12 years and was supported jointly by the MCZ and U.S. Bureau of Fisheries. This work was revolutionary because it was so comprehensive—Bigelow studied everything related to the sea in the area. He collected over 10,000 net hauls of marine organisms, sent out more than 1,000 drift bottles to study the water's currents and flow, and set up hundreds of stations to measure water temperature and salinity levels. He became an expert on fishes and coelenterates, which are aquatic invertebrates with a radially symmetric saclike body and a single internal cavity. Examples include jellyfish and hydras.

The extensive data and results from these studies were published in 1924 in three monumental monographs: *Fishes of the Gulf of Maine, Physical Oceanography of the Gulf of Maine,* and *Plankton of the Offshore Waters of the Gulf of Maine*. In the latter, Bigelow described his fortune in having a "veritable *mare incognitum* lay before us" as they set out on the first oceanographic cruise in the gulf to examine not only the pelagic fauna (the animals living in the open sea), but their ecological role, geographical variations, seasonal successions and migrations, and temperature preferences. According to Alfred C. Redfield, the author of Bigelow's memoir for the National Academy of Sciences (NAS), Bigelow's research on the Gulf of Maine made it the most thoroughly studied body of water of comparable size in the world. During these years, Bigelow switched his research focus from cytology and zoology to oceanography and developed an appreciation for the need to understand all of the natural sciences in order to comprehend the complex cycle of the sea. Throughout his career, he would impress this conviction onto his colleagues and students, paving the way for modern oceanography.

HYDROGRAPHY AND OCEANOGRAPHY WORK

In 1919 Bigelow took a temporary break from his Gulf of Maine studies to teach navigational skills and to serve as a navigation officer aboard the U.S. Army Transport *Amphion*. He also assisted the U.S. Shipping Board and was a consultant for the U.S. Coast Guard for the International Ice Patrol. From examinations of the plankton drifting, surface temperatures, and salinity, he drew conclusions about drifting icebergs. The hydrography knowledge he gained ultimately benefited his research. Bigelow accepted a teaching appointment at Harvard in 1921.

As secretary for the Committee on Oceanography of the NAS, Bigelow composed a report titled "On the Scope, Problems, and Economic Importance of the Oceanography, on the Present Situation in America, and on the Handicaps to Development, with Suggested Remedies" in 1929. This well-received, influential report was made public in the form of a book, *Oceanography, Its Scope, Problems, and Economic Importance* (1931). In his report, Bigelow portrayed oceanography as a youthful field and defined it in terms of three chief subdivisions:

- geological
- physical-chemical
- biological

He recommended that oceanographers be grounded in the principles of all three disciplines, for which he provided overviews summarizing the current knowledge. In portions of this report that were not published, Bigelow made specific recommendations for how best to resolve the current deficiencies in the state of oceanographic research in the United States. To substantiate the recommendations, Bigelow described the applications of oceanography and discussed the economic ramifications of improved research in the areas described. The Woods Hole Oceanographic Institution (WHOI) was established on the basis of Bigelow's report and the Rockefeller Foundation donated $2.5 million. In addition, the Scripps Institution, the University of Washington, and the Bermuda Biological Station received financial assistance.

Bigelow served as the first director of WHOI from 1930 to 1939. He made daily rounds, making

himself accessible to his scientists, and he requested that his staff perform field research at least once a year. The vessel *Atlantis* was made available for them to do so. Bigelow successfully recruited eminent biologists, chemists, and physical geologists without worrying whether or not they had already studied oceanography. He was more concerned with attracting clever, creative scientists who were willing to apply their talents to oceanography. Today, WHOI remains dedicated to research and education in the marine sciences and is the largest independent oceanographic institution in the world. Although Bigelow resigned as director of WHOI in 1939, he maintained a close association with the institute by serving as president of the trustees (1940–50) and then chairman of the board (1950–60).

In the early 1940s, Bigelow researched Georges Bank from the *Atlantis*. Fishermen depended on this region for haddock; thus scientists were exploring the cause of its long phytoplankton season compared to that of the Gulf of Maine. In collaboration with W. T. Edmonson, Bigelow published *Wind Waves at Sea, Breakers, and Surf* in 1947. The popular book summarized the physical nature of wind waves, wave dimensions and contours, and the effect of waves on small vessels, and it served as an introduction to waves for the U.S. Navy, whose use of small vessels and other amphibious craft required such detailed knowledge.

Bigelow served as editor in chief of the series *Fishes of the Western North Atlantic* (1948–64), a cooperative publication written for ichthyologists as well as general naturalists. In collaboration with William C. Schroeder, Bigelow contributed more than 40 papers on ichthyology between 1948 and 1965.

CAREER HONORS AND ACHIEVEMENTS

At Harvard, Bigelow had been appointed a lecturer (1921), associate professor of zoology (1927), professor of zoology (1931), and the Alexander Agassiz Professor of Zoology (1944). Bigelow retired as a professor emeritus from Harvard in 1950, but he continued serving on the faculty for the MCZ until his death. He had served as curator for coelenterates (1913–25), research curator (1925–27), and curator of oceanography (1927–50). When Bigelow jokingly suggested, in 1960, that the university show their appreciation for his lengthy tenure, the president of Harvard presented him with a bottle of bourbon whiskey.

Bigelow had served on several influential committees and did not shy away from administrative functions: the National Research Council Committee on Oceanography (1919–23); the National Research Council Committee on Submarine Configuration, for which he served as vice-chairman (1930–32); and the NAS Committee on Oceanography, for which he was secretary (1928–34) and chairman (1934–38).

Bigelow was the distinguished recipient of numerous medals and honors and was elected to membership of numerous academic organizations. WHOI established a chair in oceanography in his name in 1958. WHOI's board of trustees established the Henry Bryant Bigelow Award, WHOI's highest honor, for those who make significant inquiries into the phenomena of the sea. The first recipient of the medal and cash prize was Bigelow himself in 1960. He also received the Alexander Agassiz Medal of the NAS. Several universities awarded Bigelow honorary doctorate degrees during his lifetime.

Henry Bigelow died on December 11, 1967, at his home in Concord, Massachusetts. A laboratory at WHOI bears his name, as does a marine research institution in West Boothbay Harbor, Maine, which was established in 1974. In 1970 the U.S. Department of the Interior named a bay in the Gulf of Maine, located between Cape Ann and Cape Small, Bigelow Bight.

In addition to the vast amount of information he collected concerning various forms of marine life and for his comprehensive study of the Gulf of Maine, Bigelow is considered a pioneer in oceanography because of the direction in which he led the field. Before the 1930s, the field was merely an assemblage of facts and data—lists of identified fauna, maps of ocean depths at different positions, and locations and directions of currents. Bigelow encouraged oceanographers to synthesize all the information gathered and look for relationships, to embrace the different fields of biology, chemistry, and physical geology to find the connections. This effort for unification gave oceanography an ecological aim, which has persisted into the 21st century. Redfield, in his memoir written for the NAS, summarized Bigelow's contributions to oceanography, "Not only did a man emerge who had prepared himself, perhaps unwittingly, for leadership at a time when men of influence sensed that something should be done to improve the status of marine science in America, but new ideas were in the air, wafted across the ocean from a multitude of general scientific advances. Henry Bigelow, though trained in the classical tradition, was sensitive to these breezes, wise enough to grasp their implication, and bold enough to act on their meaning."

See also BIOMES, AQUATIC; ECOSYSTEMS; MARINE BIOLOGY.

FURTHER READING

Bigelow, Henry Bryant. *Memories of a Long and Active Life*. Cambridge: Cosmos Press, 1964.

———. *Oceanography: Its Scope, Problems, and Economic Importance*. Boston and New York: Houghton Mifflin, 1931.

Bigelow Laboratory for Ocean Sciences home page. Available online. URL: http://www.bigelow.org. Accessed January 17, 2008.

Redfield, Alfred C."Henry Bryant Bigelow (October 3, 1879–December 11, 1967)." In *Biographical Memoirs: National Academy of Sciences*. Vol. 48. Washington, D.C.: National Academy of Sciences, 1976.

Woods Hole Oceanographic Institution home page. Available online. URL: http://www.whoi.edu/home/. Accessed January 17, 2008.

biochemical reactions A chemical reaction occurs when substances react to form new substances with different chemical properties. Biochemical reactions are simply chemical reactions that naturally occur within or are caused by living organisms, and they typically require enzymes, biological catalysts. Most enzymes are proteins, and all enzymes are highly specific for a particular substrate. Enzymes act by increasing the rate of a biochemical reaction, but they are not altered or consumed during the reaction. The different types of biochemical reactions that occur in living systems can be grouped into six main classes: oxidation-reduction, reactions that involve the transfer of a functional group, hydrolysis reactions, ligation reactions, elimination or addition reactions, and isomerizations.

OXIDATION-REDUCTION REACTIONS

Cells obtain the energy they need to live, grow, and reproduce by oxidizing organic molecules such as carbohydrates. Chemically, to oxidize a compound means to remove one or more electrons from it. When cells burn organic molecules for energy, they are removing the electrons—the electrons that atoms of the molecule share in covalent bonds. Oxidizing or breaking down biomolecules releases the energy stored in the shared electron pairs that make up covalent linkages. Because the element oxygen is prevalent in the Earth's atmosphere and because it has a high electronegativity, oxygen often participates in the process of oxidation (hence its name), though it is not required. Any biochemical reaction involving oxidation must couple with a reduction reaction, because if something is oxidized, something else must be reduced. In other words, if something gives up an electron, something else must take it. The same number of electrons present in the reactants of a chemical reaction must be present in the products of the reaction. The atom or molecule that accepts the electron lost by oxidation becomes reduced when it accepts that electron. When a molecule accepts an electron, it often also picks up a proton (H^+) also, a process called hydrogenation because the molecule has gained a hydrogen atom (which is simply a proton combined with an electron). Hydrogenation reactions are reductions, and the converse, dehydrogenation reactions, involving the removal of a hydrogen atom, are oxidations.

The gradual oxidation of an organic molecule allows the cell to extract its energy more effectively than if all of the electrons were lost at once. (Consider a boy playing catch. If someone throws balls to him one at a time, he can catch more total balls than if someone throws him a dozen balls at once.) During cellular respiration the cell uses molecular oxygen (O_2) to oxidize organic molecules such as glucose ($C_6H_{12}O_6$) gradually, uses the energy to make adenosine triphosphate (ATP), and releases carbon dioxide (CO_2) and water (H_2O). For a single molecule of glucose, the overall process of respiration can be summarized as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \sim 30–30 \text{ ATP}$$

As O_2 oxidizes the carbon atoms of glucose, the electrons (and accompanying protons) are transferred to oxygen, which becomes reduced, forming water molecules in the process. Notice that the carbon atoms do not completely give up any electrons; they have not ionized. The carbon atoms traded covalent linkages with other equally electronegative carbon atoms in order to form covalent linkages with oxygen, which is much more electronegative than carbon; the new covalent bond is a polar covalent bond. The carbon atoms gave up more than their fair share of electrons and acquired a partially positive charge; thus this is still considered oxidation.

Enzyme names should indicate their activity, and biochemists have given enzymes a variety of names to indicate the catalysis of oxidation-reduction reactions. The names often include the term *dehydrogenase, oxidase, peroxidase, hydroxylase, reductase,* or *oxygenase*. For example, malate dehydrogenase catalyzes the transfer of two electrons from malate to NAD^+ to form oxaloacetate and NADH + H^+ during the citric acid cycle. (NADH + H^+ then carries the electrons to the electron transport chain for the synthesis of ATP.)

TRANSFER OF FUNCTIONAL GROUPS

Organic molecules often have hydrophilic side groups that increase their solubility in aqueous environments and confer unique chemical and physical characteristics on the molecule. Common side groups, called functional groups (-X), in biomolecules are hydroxyl groups (-OH), carbonyl groups (-C=O), carboxyl groups (-COOH), amino groups ($-NH_2$), phosphoryl groups ($-PO_3$), sulfhydryl groups (-SH), and alkyl groups ($-C_nH_{2n+1}$). Enzymes that catalyze the transfer of a functional group from one molecule to another

often have names that include *transferase* or a more specific term that indicates the type of group it transfers, such as transmethylase, which catalyzes the transfer of a methyl group.

$$AX + B \xrightarrow{\text{transferase}} A + BX$$

An example of an enzyme that performs a transfer is aspartate aminotransferase, also called aspartate transaminase, which moves an amino group from aspartate to α-ketoglutarate, resulting in the formation of oxaloacetate and glutamate.

HYDROLYSIS AND LIGATION REACTIONS

Hydrolysis reactions, named so because they involve the lysis or splitting of a water molecule, catalyze the breakdown of polymers by cleaving C-O, C-N, or C-C bonds (and sometimes other types). The enzyme essentially splits the water molecule and adds its components to either side of a covalent bond, breaking it and releasing two smaller molecules and energy in the process. The class of enzymes that facilitate hydrolysis reactions are the hydrolases, including enzymes such as esterases, amidases, glycosidases, peptidases, and phosphatases, named for the type of bond cleaved. For example, amylase is a glycosidase that cleaves glycosidic linkages of starch molecules to release single glucose subunits.

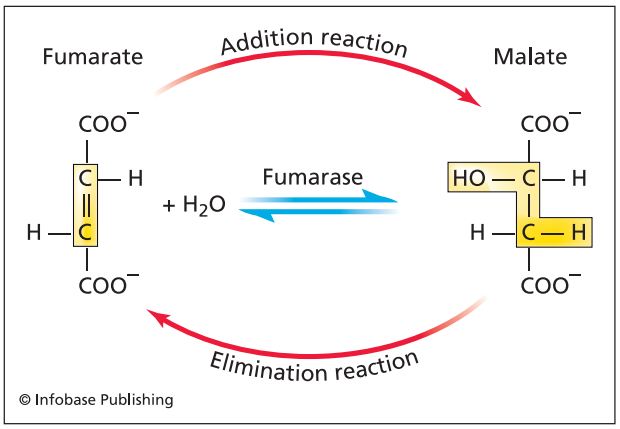
Dehydration syntheses, also called condensation reactions, are the reverse reactions of hydrolysis reactions. They join two molecules by removing a water molecule (an OH from one and an H from the other) and require energy input, usually obtained by the cleavage of an ATP molecule. Because dehydration synthesis reactions are anabolic, meaning they build large molecules from smaller ones, the common names of the enzymes that catalyze these reactions often include the term *synthetase*. As a result of confusion with enzymes that biochemists commonly called synthases (which belong to the class of enzymes called lyases and that catalyze elimination or addition reactions), newer enzyme names use the term *ligase* rather than *synthetase*. Ligases are enzymes that catalyze the formation of ester, thiol ester, or amide linkages. For example, glutamate-ammonia ligase, formerly known as glutamine synthetase, catalyzes the condensation of an ammonium ion and the amino acid glutamate to synthesize the amino acid glutamine using energy released by the hydrolysis of ATP Deoxyribonucleic acid (DNA) ligase forms phosphodiester linkages to connect adjacent nucleotides along a single strand of DNA in a duplex molecule, such as in between the Okazaki fragments formed as a result of discontinuous replication of the lagging strand during DNA synthesis.

ELIMINATION OR ADDITION REACTIONS

Elimination reactions are biochemical reactions in which two substituents are removed from a molecule, typically resulting in the formation of a double bond or a ring. The reverse addition reactions involve adding groups across a double bond. Lyases are enzymes that remove a small molecule, such as an ammonia, water, or carbon dioxide, from another molecule to form a double bond during an elimination reaction, or that catalyze the cleavage of C-C, C-O, or C-N bonds during addition reactions, converting a double bond to a single bond in the process. These reactions are often catalyzed by enzymes with names including the terms *aldolase, synthase, deaminase, hydrase, decarboxylase,* or *cyclase*. One example of such a reaction is the conversion of the four-carbon sugar fumarate to malate by the addition of water to its double bond. A step in the central metabolic pathway the citric acid cycle, this reaction is catalyzed by fumarase, which also catalyzes the reverse reaction, formation of the double bond by the expulsion of a water molecule.

ISOMERIZATIONS

Isomers are molecules that have the same number and types of atoms but different structural arrangements of those atoms. Because many biological molecular interactions are highly specific, two molecules that have the same chemical formula may have very different biochemical functions. Some isomers differ in the location where a functional group attaches; others differ in the geometric arrangement of atoms or side groups. Large biomolecules such as DNA can form loops or twists that alter its overall topology; these different forms are called topoisomers. Isomerases move around the atoms within a molecule to form different isomers. Enzymes that include the



Fumarase catalyzes the addition of water to the double bond of fumarate, forming malate, and the elimination of water from malate to form fumarate.

terms *isomerase, racemase, epimerase, mutase,* or *tautomerase* are all isomerases. A specific example of an isomerization reaction is the conversion of 1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate (BPG), catalyzed by bisphosphoglycerate mutase. In red blood cells, the molecule 2,3-bisphosphoglycerate binds hemoglobin and lowers its affinity for oxygen. This phenomenon plays an important role in regulating the transfer of oxygen from maternal to fetal circulation and in the adjustment to higher altitudes, where the partial pressure of oxygen in the atmosphere is decreased.

See also BIOCHEMISTRY; BIOENERGETICS; BIOMOLECULES; CHEMICAL BASIS OF LIFE; ENZYMES.

FURTHER READING

Berg, Jeremy M., John L. Tymoczko, and Lubert Stryer. *Biochemistry*. 6th ed. New York: W. H. Freeman, 2006.

Champe, Pamela C., Richard A. Harvey, and Denise R. Ferrier. *Lippincott's Illustrated Reviews: Biochemistry*. Philadelphia: Lippincott, Williams, & Wilkins, 2005.

Purich, Daniel L. *The Enzyme Reference: A Comprehensive Guidebook to Enzyme Nomenclature, Reactions, and Methods*. San Diego, Calif.: Academic Press, 2002.

biochemistry

Biochemistry is the study of chemical compounds and processes that occur in or are caused by living organisms. Considered a subdiscipline of both biology and chemistry, biochemistry bridges the two natural sciences. At the molecular level, all life-forms are similar because they all descended from a common ancestor. The molecules that make up the cells and tissues of living organisms, called biomolecules, are the same in prokaryotes and eukaryotes, in unicellular and multicellular organisms, and across kingdoms. Biochemistry focuses on these molecules and the biochemical reactions that form them and convert them into other substances.

OVERVIEW

Biochemistry involves the study of the structure and function of biomolecules, the major classes of which include carbohydrates, proteins, lipids, and nucleic acids, though other types that play important roles in the biochemical processes carried out by living cells, such as vitamins, are also of interest to biochemists. Most biomolecules consist of many repeated subunits called monomers. The monomeric subunits differ among the major types; carbohydrates consist of monosaccharides, proteins consist of amino acids, nucleic acids consist of nucleotides, and lipids, which are more structurally variable, contain hydrocarbon chains or fatty acids of different lengths. One characteristic all biomolecules have in common is that they are organic molecules, meaning their structure is based on the element carbon. Organic chemistry is the branch of chemistry concerned with carbon-based compounds; thus biochemists must have a strong background in organic chemistry. Though all biomolecules are organic molecules, not all organic molecules are considered biomolecules. For example, benzene, synthetic rubber, and fossil fuels such as petroleum and coal are organic compounds but do not naturally occur in living organisms so are not considered biomolecules. Biomolecules assemble to form macromolecular structures, which compose cells. For example, lipids and proteins combine to form cell membranes, nucleic acids and protein join to form chromatin, ribonucleic acid and proteins assemble into ribosomes, and DNA or ribonucleic acid (RNA) and proteins form viral particles.

Biochemists study all aspects of biomolecules including their basic structure, how they are synthesized, how they are broken down, and their function. Most chemical process that take place within living cells require special catalysts called enzymes, which are usually proteins, but some RNAs also have catalytic properties. Catalysts are substances that speed up the rate of a chemical reaction. All biochemical reactions that occur in living cells could potentially occur without the aid of a catalyst, as catalysts cannot force energetically unfavorable reactions to proceed, but in the presence of the catalyst, the reaction proceeds at 1,000 to 100,000,000 times faster. Enzymes mediate the reactions that convert biomolecules from one form to another; they join monomers, modify molecules, and digest large molecules into smaller ones. Biochemists study how enzymes work and the reactions they catalyze. The reactions are often part of a series of reactions called a metabolic pathway. Metabolism involves all of the anabolic and catabolic activities of an organism. Anabolic pathways are responsible for constructing macromolecules and require energy input, whereas catabolic pathways perform the breakdown of macromolecules into smaller molecules and release energy for use by the cell. Biochemical pathways can be linear, in which one enzyme acts on a substrate, leading to conversion of the substrate into a product, which then acts as the substrate for a second enzyme, and the product of the second reaction becomes the substrate for a third enzyme, and so on. Some biochemical pathways are cyclical, meaning that the initial substrate is regenerated after several steps. Many biochemical pathways, or at least portions of them, function in anabolism and catabolism, and many enzymes catalyze reversible reactions. The regulation of metabolic pathways is also an important topic in biochemistry.

Though only four chemical elements—carbon, hydrogen, oxygen, and nitrogen—compose more than 96 percent of the total weight of living matter, the manner in which they join to one another and their different arrangements create tremendous diversity in the structure of biomolecules. Specific interactions between different molecules play key roles in many biological processes. Biochemists study structure-function relationships at the molecular level to understand better biological phenomena such as antibody-antigen binding, receptor-ligand specificity, enzyme-substrate recognition, allosteric effects, and membrane transport channels and pumps.

HISTORY OF BIOCHEMISTRY

The origins of biochemistry date back to 1828, when the German chemist Friedrich Wöhler synthesized urea ($CO(NH_2)_2$), in vitro, from inorganic ingredients, the first biochemical synthesis. Before his success, people thought that only living organisms could synthesize organic molecules, a concept held by the vital hypothesis, which stated that the mysterious essence of life itself was necessary to synthesize the "stuff of life." The significance of Wöhler's work lay in the realization that organic molecules were simply chemical compounds. The French chemists Anselme Payen and Jean-François Persoz published their work on the isolation of an enzyme complex that they called diastase (the enzyme is now called amylase) from barley malt in the journal *Annales de Chemie et de Physique* in 1833. The Swedish chemist Jöns Jakob Berzelius, who coined the terms *protein* and *catalysis,* wrote a paper on chemical catalysis in 1835, demonstrating that malt extract efficiently digested starch in vitro. In 1860 the French chemist Louis Pasteur demonstrated that the process of fermentation, responsible for the production of alcoholic beverages and pickling, was biological rather than chemical, laying the groundwork for the investigation of other metabolic processes. In 1896 the German chemist Eduard Buchner used extracts from yeast cells to ferment sugars in vitro, supporting the finding that live cells were not required to carry out complex chemical reactions. This finding opened the door for more intensive analysis of biochemical reactions. Buchner received the 1907 Nobel Prize in chemistry for his research on fermentation. Several biochemists unraveled the basic mechanisms by which enzymes catalyze biochemical reactions at the turn of the 19th/20th century.

After Buchner debunked the vital hypothesis, metabolism became a popular research focus among chemists. By 1940 the contributions of several pioneering biochemists led to the elucidation of glycolysis, a central metabolic pathway linking the metabolism of carbohydrates, amino acids, nucleotides, and fatty acids. Because of this, glycolysis, one of the first studied, is the best understood metabolic pathway.

The discovery of the double-helical structure of DNA by James Watson and Francis Crick in 1953 initiated a revolution in the biochemistry of nucleic acids and of gene expression. Research along these lines led to the birth of a new field, molecular biology, which encompasses the processes of DNA replication, transcription (the synthesis of RNA), and translation (the synthesis of proteins). The tools and technology used to probe molecular processes, such as recombinant DNA technology and the polymerase chain reaction, have greatly advanced knowledge in biochemistry.

BIOCHEMICAL RESEARCH

Research in the field of biochemistry depends on many techniques such as chromatography, X-ray diffraction, nuclear magnetic resonance (NMR) spectroscopy, electron microscopy, radioisotope labeling, gel electrophoresis, centrifugation, and recombinant DNA technology. One major goal of biochemistry is the purification and characterization of biomolecules, especially enzymes. Biochemists often start by preparing an extract from a piece of tissue, a sample of cultured cells, or even a bodily fluid, such as blood or urine. Separation techniques such as chromatography and centrifugation generate fractions that exclude certain types of molecules on the basis of size, electrical charge, or chemical affinities. The fractions are then subjected to further purification techniques until the sample quality is sufficient for characterization, which includes the determination of size, composition, structure, and function. Molecular biological techniques and computer simulations have facilitated the study of protein structure and function. Using recombinant DNA technology, researchers can replace single amino acids and examine the resulting effect on the protein's structure and therefore function. Computer modeling allows biochemists to compare molecules with similar structures; such comparison can lead to inferences about function. Software programs that predict the overall structure of a protein given its amino acid sequence are not perfect but provide valuable information that can be reconciled with real data from techniques such as X-ray crystallography, electron microscopy, and NMR spectroscopy that reveal molecular structures.

Another aim of biochemistry is to understand bioenergetics, the energy transformations and exchanges carried out by living organisms. All organisms ultimately receive energy necessary to carry out life's processes from the Sun. Primary producers can convert radiant energy from the Sun into chemical energy via photosynthesis, and consumers obtain

Many biochemists work in laboratories, where they carry out basic research with the aim of gaining a better understanding of biomolecules and biochemical processes that occur in living organisms. *(Publiphoto/ Photo Researchers, Inc.)*

their energy by ingesting organic material either from primary producers or from other organisms that have ingested primary producers. Enzymes act as energy transforming devices by facilitating the formation of covalent bonds in organic molecules, which store chemical energy, and catalyze their breakage, releasing the stored energy. Biochemists study the flow of energy through living systems, which must follow the laws of thermodynamics, stating that energy must be conserved and that processes only occur spontaneously if the total entropy of the system increases.

Additional current avenues of exploration in biochemistry research include the nature of sex determination, regulation of developmental pathways in early embryogenesis, the effect of genes and biochemical basis for behaviors, signal transduction pathways (by which cells receive and communicate information), and apoptosis (programmed cell death). Computational biochemistry has presented opportunities for explorations using molecular graphics, three-dimensional modeling, and computer simulations of biochemical processes. Research relating metabolism to obesity has also gained support recently as a result of the increased incidence of this health concern.

APPLICATIONS

While many biochemists perform basic research with the goal of attaining a better understanding of biomolecules and biochemical processes, knowledge gained from their research can be applied to many fields. Biochemistry is applicable to medicine, as many genetic disorders result when an individual produces defective enzymes or insufficient quantities of certain enzymes. For example, glycogen storage diseases affect carbohydrate metabolism and cause symptoms including muscle weakness, poor growth, and liver problems in children. Many pharmaceuticals act by inhibiting or stimulating biochemical pathways. Understanding the mechanisms for regulating these pathways can lead to the development of novel drug therapies. In addition, biochemists help determine whether the mechanisms by which the body will break down and eliminate drugs and chemicals are safe and do not result in the production of toxic by-products. Cancer often results from the loss of regulation of cell-signaling pathways, which depend on numerous specific interactions among proteins and other biomolecules; thus many biochemists are involved in cancer research.

Many industries hire biochemists to research the biochemical aspects of the products they sell. The commercial production of numerous chemicals exploits biochemical processes carried out by microorganisms. For example, many chemical industries grow microorganisms on a large scale to produce a variety of alcohols, organic acids like citric acid, enzymes that are added to laundry detergents, and vitamins and amino acids used as food supplements. The food and wine industries also depend on the biochemical process of fermentation to produce alcoholic beverages, pickles, sausage, soy sauce, and many dairy products such as sour cream, cheeses, and buttermilk. Factories use biochemical methodologies to purify certain chemical reagents, enzymes, vitamins, pharmaceuticals, monoclonal antibodies, recombinant proteins, and acetic acid or other organic acids from cell cultures, tissue extracts, or other mixtures. Knowledge of biochemical processes helps scientists to determine evolutionary relationships, as biochemists can compare similarities in metabolic pathways and mechanisms between species. Greater degrees of differences in mechanisms or structures of the molecules involved in processes such as photosynthesis, cellular respiration, and DNA replication imply a greater evolutionary distance between species or lineages.

While biochemists have extensively structurally and functionally characterized numerous macromolecular components, none of the macromolecular assemblies per se is "living." Biochemists and other life scientists are still far away from understanding how the assembly and congregation of biomolecules into macromolecular assemblages lead to living cells and organisms.

See also BIOCHEMICAL REACTIONS; BIOENERGETICS; BIOMOLECULES; CENTRIFUGATION; CHEMICAL

BASIS OF LIFE; CHROMATOGRAPHY; ELECTROPHORESIS; ENZYMES; MOLECULAR BIOLOGY; ORGANIC CHEMISTRY, ITS RELEVANCE TO LIFE SCIENCE; RECOMBINANT DNA TECHNOLOGY; WATER, ITS BIOLOGICAL IMPORTANCE; X-RAY CRYSTALLOGRAPHY.

FURTHER READING

Berg, Jeremy M., John L. Tymoczko, and Lubert Stryer. *Biochemistry*. 6th ed. New York: W. H. Freeman, 2006.

Champe, Pamela C., Richard A. Harvey, and Denise R. Ferrier. *Lippincott's Illustrated Reviews: Biochemistry*. Philadelphia: Lippincott, Williams, & Wilkins, 2005.

Daintith, John, ed. *The Facts On File Dictionary of Biochemistry*. New York: Facts On File, 2003.

Gilbert, Hiram F. *Basic Concepts of Biochemistry: A Student's Survival Guide*. 2nd ed. New York: McGraw Hill, 2000.

Lennarz, William J., and M. Daniel Lane. *Biological Chemistry*. 4 vols. Amsterdam; Boston: Elsevier, 2004.

biodiversity

Biodiversity, or biological diversity, is the variation in living organisms and living systems within a given geographical area. Typically, this translates into the number of different species, but it also refers to genetic variation within a species, or variation within and between higher levels of biological organization such as communities and ecosystems. The United Nations Convention on Biological Diversity defines *biodiversity* as "the variability among living organisms from all sources, including, inter alia [among other things], terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species, and of ecosystems." Biodiversity is one measure of the biological health of an area; generally, a greater total number of species indicates a healthier habitat or region.

Estimations of the total number of species present on Earth today range from 2 million to 10 million, though fewer than 2 million have been catalogued. Genetic variation, differences within the genetic makeup between individuals of a species or between populations of a species, may affect fitness, the relative capacity of an organism to survive and pass on its genes to offspring. Through the natural selection of phenotypes and genotypes associated with increased fitness, new species have emerged, and others have become extinct. In the past, natural factors such as climate, geography, and interspecific interactions largely determined the degree to which certain adaptations affected an organism's fitness and thus controlled the number and types of species. The effect of human activities, amplified as the population expands, has altered the climate, landscape architecture, and distribution of species more rapidly than has ever occurred by natural means in evolutionary history. This has led to the present crisis, the sixth major extinction event in Earth's history. This human-induced mass extinction is occurring at a rate higher than that of any past mass extinction event; estimates of species lost each year range from hundreds to thousands. In addition to aesthetic, recreational, ethical, and utilitarian reasons, scientific reasons to attempt thwarting this recent severe reduction in species numbers include maintenance of ecosystem stability, prevention of human-induced environmental disasters such as drought and flooding, increased ability to recover from such human-induced and natural environmental disasters, untapped genetic resources, and potential medicinal treatments, some of which have not been discovered yet.

IMPORTANCE OF BIODIVERSITY

All species are connected to each other and to the environment in which they live through food chains, the biogeochemical cycling of nutrients, the effects of living organisms on the climate and physical geology of their habitats, and vice versa. Continued life depends on the diverse activities and interrelationships of the variety of life-forms that sustain the planet and share its resources. Society depends on other living organisms for material resources such as food, clothing, medicines, and fuel, but a reduction in biodiversity is not only a concern for those who directly harvest goods from other organisms or those who are immediately affected by the loss of a particular species or damage to one small area. All of human society depends on Earth's natural resources, including the ecosystems that maintain healthy living conditions, sustain the atmosphere, provide drinkable water, temper global climate, control erosion, naturally control pests, maintain soils for growing food, and recycle chemical nutrients. Measuring the services (such as the removal of wastes, filtration of water, or capture of carbon from the atmosphere) that an ecosystem provides is one method of gauging the health of an ecosystem or its ability to function. The productivity of an ecosystem is another. If the production of biomass, the amount of living matter, remains high, then the organisms in the community are living, metabolizing, cycling nutrients, and performing their tasks in sustaining the environment. Biologists do not know the lower limits of biodiversity necessary for the maintenance of an ecosystem's health or whether the critical factor is the total number of species present or the functional types of organisms present. The recent increase in extinction rates has turned prompt attention to these questions.

A relationship exists between biodiversity and ecosystem maintenance. An ecosystem can be described as stable if the composition of its community members remains essentially the same, with very few new species introduced and very few species disappearing from the community. Stability can also refer to the sizes of the populations within a community, that is, the number of individual members of a species in a population. One observation is that diverse ecosystems containing many different species are more stable. The greater number of species present, the greater the number of pathways through which energy and nutrients can flow through the ecosystem. If one pathway is disrupted, the energy can still flow, allowing the ecosystem to remain productive. Less biodiversity means less overlap in the functions of the species in the ecosystem and therefore a decreased ability to recover from disturbances. Predicting the effect of the loss of one species is difficult; such a loss can cause a chain reaction since all species depend on other species for resources. Ecologists have shown that in ecosystems with high biodiversity, the population sizes of individual species fluctuate a lot, but the combined productivity of the ecosystem remains high and stable, a good indicator of ecosystem health. Thus, diverse ecosystems are more productive, even though the stability of distinct populations fluctuates. One current research goal is to reveal the mechanisms by which biodiversity contributes to ecosystem stability.

Not all species are ecologically equal. Keystone species are species that play crucial roles in the normal function of an ecosystem. Their removal from an ecosystem may result in the subsequent disappearance of many other species, leading to a reduction in biodiversity. Indicator species provide useful information on the health of an ecosystem. For example, lichens, a symbiosis between fungi and a photosynthetic organism, are particularly sensitive to pollution. Rather than simply measuring quantities of chemicals present in the environment, sampling of indicator species to monitor environmental conditions provides a more accurate picture of what effects pollution may have on an ecosystem, as they reflect the integrated effects of the pollutants and the environment. Sometimes more than one species can fill a niche in an ecosystem, a factor that contributes to the stability of an ecosystem. If the population of one organism decreases, the so-called redundant organism can play the same role, preventing any negative effects on the function or productivity of the ecosystem. This concept can also be applied to diversity within a species and between ecosystems. In the case of genetic variation, the higher the diversity, the more likely that other members of the same or another population can perform similar ecological functions. Likewise, if one ecosystem were destroyed or rendered nonfunctional by a cataclysmic event, the presence of other numerous diverse ecosystems might be able to compensate for the lost one.

BIODIVERSITY THREATS AND CONSERVATION

Human pressures on ecosystems and global climate change have contributed to the serious loss in biodiversity over the past few decades. Expanding human population size and a disproportionate increased demand for natural resources have destroyed habitats, decreased the quality of the environment, and increased the number of extinct and endangered species. The purposeful and inadvertent introduction of nonnative species into new habitats also threatens many species as the nonnative species competes for food and resources and preys on or parasitizes the native species. Human activities that have altered landscapes and water bodies of different areas, contaminated the environment with pollutants, introduced alien species to ecosystems, and made hunting profitable have led to an alarming decrease in biodiversity over the past few decades.

Recognizing the decrease in biodiversity as a global problem requiring international cooperative efforts to turn around, the United Nations (UN) called a meeting in 1992 in Rio de Janeiro. The UN Conference on Environment and Development, also called the Earth Summit, addressed this and other related issues. Representatives from 172 governments met to discuss issues such as the production of toxic substances, alternative energy sources to reduce the effects of burning fossil fuels on global climate, public transportation systems to reduce air pollution, and water conservation. One result of the Earth Summit was the Convention on Biological Diversity, a treaty that outlined international strategies for preserving biodiversity.

Individual choices regarding decisions about what foods and products to buy, water usage, and energy consumption also contribute to reducing the negative impact human society has had on the environment. By decreasing one's personal contributions to pollution, the burning of fossil fuels, and hunting, an individual's actions compounded over time and collectively as a society can make a difference by reducing or eliminating the activities that lead to habitat destruction and fragmentation.

Feeding the world's 6-billion-plus population of humans has a large impact on biodiversity, and protecting biodiversity is important in food production. While some living organisms can make their own food using energy from the Sun and inorganic chemicals from the environment, humans require energy and nutrients in the form of organic compounds made by other living organisms. The food

supply is obtained from plants and animals and even fungi and algae that share the Earth's resources. The primary producers, mostly photosynthetic plants and plankton, form the foundation of food webs. Plants require fertile soil, produced as a result of the metabolic activity of diverse microorganisms that decompose organic matter and recycle the nutrients that feed future crops. Bacteria fix inorganic nitrogen from the soil into a form that plants can use, improving their growth. Birds and insects that some consider pests pollinate plants that produce fruits and vegetables that form the staples of the human diet. Though agriculture depends on cultivated species that have been bred specifically to increase yields or improve the quality of products, crop and domestic animal breeding programs still require wild relatives to maintain sufficient levels of genetic variability so they will be able to survive environmental challenges such as exposure to a new pathogen and to increase their overall vigor. Individual actions, such as purchasing organic farm products, buying locally grown products and products in season to reduce chemical treatments necessary to help the product tolerate transportation over long distances and to reduce the associated energy costs, eating more plant products to reduce the extra water and energy required to produce food products from organisms higher up in the food chain, not using plastic or Styrofoam packaging, and reducing paper and food waste, all reduce negative environmental effects and thus contribute to maintaining biodiversity.

The water supply is also connected to biodiversity. Efforts to preserve biodiversity are important in protecting water as a natural resource, and protecting water as a natural resource will help prevent a decrease in biodiversity. As the global population increases, so does the demand for freshwater. A healthy, abundant water supply depends on the actions of numerous diverse organisms including microorganisms, plants, and animals. Watersheds funnel water into lakes and streams, and wetlands absorb many metals and toxic substances that enter the water supply. Many endangered species live on or are supported by watersheds, and the watersheds and organisms that reside in them act as natural filtration systems, helping to purify the water. Landscaping near watersheds, deforestation, and damming waterways all negatively impact the water supply and contribute to the reduction in the number of aquatic species and to the function of aquatic ecosystems. Agricultural and industrial practices add chemicals to the water reserves, reducing the usable supply and requiring more energy to clean up. Individuals can help by reducing water use at home and using fewer chemicals and pollutants in household products.

Human use of fossil fuels for energy also has contributed to the decrease in biodiversity. Burning the fuels releases gases such as carbon dioxide, sulfur dioxide, and nitrogen oxide into the atmosphere, contributing to global climate change and leading to acid rain. Other pollutants released directly kill or inhibit plant growth. Coal mines and oil drilling cause dangerous chemicals and toxins to leach into the environment, destroy habitats, and kill off species.

Global climate change is among the most serious threats to biodiversity. The increased release of carbon dioxide into the air, in combination with the decrease in photosynthetic life that naturally removes it, traps heat in Earth's atmosphere. The radiant energy that is reflected off the Earth's surface cannot escape. Over the last century, the average temperature has risen about 1.3°F (0.74°C), and scientists predict the temperature may rise a few additional degrees over the next 100 years. Global warming causes melting of glaciers, and a resultant sea-level rise, which can flood wetlands, erode coastlines, affect food supplies, change migration patterns, and decrease northern forests.

Protecting biodiversity is crucial to maintaining the ecological health of the planet. The reduction in biological variation within a species, between species, and among ecosystems poses a serious threat to the ability of the biosphere to support many current life-forms, including humans. Scientists continue to explore the relationships among biodiversity, ecosystem stability, and the far-reaching impact of human activities on the environment in order to understand better how to sustain living conditions for all the life-forms that share the planet's resources.

See also CONSERVATION BIOLOGY; ENDANGERED SPECIES.

FURTHER READING

American Museum of Natural History. Center for Biodiversity and Conservation home page. Available online. URL: http://research.amnh.org/biodiversity/. Accessed January 17, 2008.

Anderson, Anthony B., and Clinton N. Jenkins. *Applying Nature's Design: Corridors as a Strategy for Biodiversity Conservation.* New York: Columbia University Press, 2006.

Evans, Kim Masters. *Endangered Species: Protecting Biodiversity.* Farmington Hills, Mich.: Thomson Gale, 2006.

Gaston, Kevin J., and John I. Spicer. *Biodiversity: An Introduction.* Malden, Mass.: Blackwell Science, 2004.

Hulot, Nicolas. *One Planet: A Celebration of Biodiversity.* New York: Abrams, 2006.

Wilson, Edward O. "Threats to Biodiversity." *Scientific American* 261, no. 3 (September 1989): 108–116.

bioenergetics Bioenergetics is the study of energy transformations and energy exchanges within and between living organisms and their environments. Life is a highly structured phenomenon, from the molecular and cellular levels to the community and ecosystem levels. The characteristic order of living systems is contrary to the natural tendency to proceed from a more ordered to a more disordered state. Maintenance of the organization necessary for life requires the constant input of energy. Thermodynamics describes the path of energy flow through living systems, and knowledge about the specific conditions of a system allows one to track the quantities of moving energy.

ENERGY IN LIVING SYSTEMS

Energy is the capacity to do work or make a change such as perform an energy transfer. Several types of work are necessary to support life's processes: synthetic, mechanical, concentration, electrical, heat, and bioluminescence. Synthetic work results in the production of new molecules that assemble into cellular structures, tissues, and whole organisms. Most energy within cells is stored in the form of chemical bonds of organic molecules, and the creation of these bonds requires the input of energy. Mechanical work includes processes such as beating cilia or moving a flagellum for locomotion. Sometimes energy exists in the form of a gradient that is used to perform work such as transporting substances across membranes. The creation of a gradient requires energy, while the dissipation of a gradient releases energy. Electrical work is a special type of work that involves a gradient of charged particles. Electrical gradients are necessary to transmit neural impulses through the nervous system and to signal muscular contraction. Homeothermic (warm-blooded) organisms use heat generated by cellular metabolism to maintain body temperature. Bioluminescence is the production of light by a living organism such as a firefly or fish that lives in the deep sea.

Cells expend energy to perform all of these types of work. The most immediate source of energy inside the cell is adenosine triphosphate (ATP), a high-energy molecule consisting of a ribose sugar attached to a nitrogenous base called adenine and three linked phosphate groups. Cells have the capacity to transform the chemical energy stored in the bonds between the phosphate groups, particularly the second and third phosphate groups, into the types of work described.

The major source of energy found in living organisms is radiation from the Sun. Phototrophs are organisms that have the ability to capture light energy from the Sun and transform it into chemical energy for cellular work. Plants, algae, and some bacteria can carry out this process called photosynthesis. Chemotrophs obtain their energy from chemical sources and can be split into two groups. Chemoorganotrophs oxidize organic compounds such as carbohydrates, fats, and proteins. Animals, protists, and fungi are chemoorganotrophs. Some microorganisms are chemolithotrophs, meaning they obtain their energy from the oxidation of inorganic compounds such as hydrogen sulfide (H_2S), thiosulfate ($S_2O_3^{2-}$), or molecular hydrogen (H_2). Organisms that are photosynthetic are also chemotrophs; the difference is that they can use organic molecules that they have synthesized by using energy obtained from sunlight rather than depend on other sources to produce them.

THERMODYNAMICS OF LIVING SYSTEMS

The energy of living systems is constantly changing forms. Thermodynamics is the study of the principles that govern this energy flow. Bioenergetics is the specialized study of thermodynamics in biology. The practical study of thermodynamics can be applied to living systems to explain energy flow and to predict the direction and extent to which chemical reactions will proceed. Thermodynamics does not, however, give any insight as to the rate of a reaction. Kinetics addresses that issue.

When considering energy flow, one must consider the system and whether it is open or closed. A closed system is separated from its environment so that no energy can enter or leave the system, whereas an open system does permit energy to enter into or be released from the system. As open systems, individual cells or organisms not only allow energy to enter, they require energy input in the form of sunlight or food to sustain life. As soon as the organism is denied a source of energy, the organization fails and the organism dies.

The state of the system refers to the variable properties, such as temperature, pressure, or volume. As long as one of these variables is held constant, one can determine useful information about the changes in energy of the system. Changes in energy usually accompany biochemical reactions, which for all practical purposes occur in relatively constant environmental conditions. Within the fraction of a second it takes for a reaction to proceed, the three variables most important to a physical chemist—temperature, pressure, and cell volume—do not change. This allows one to make predictions about the energy balances and direction of biochemical reactions.

Three laws govern energy flow through systems, but only the first two are particularly useful to a biologist. The first law of thermodynamics, the law of conservation of energy, states that the energy in the universe is constant. Simply put, energy can be transformed, but neither created nor destroyed. One can follow the energy flow by examining enthalpy,

or heat content (represented by the symbol H). The change in enthalpy, ΔH, is related to the change in internal energy (energy within the system) and the change in pressure, which is negligible. Thus, ΔH equals the change in energy. If the heat content of the products is less than the heat content of the reactants, ΔH is a negative number and the reaction is termed exothermic, meaning heat is released. If the heat content of the products is more than the heat content of the reactants, ΔH is positive, and the reaction is termed endothermic.

Consider the process of cellular respiration, during which one molecule of glucose is oxidized to carbon dioxide and water. Energy is released as the glucose molecule is oxidized; the chemical bonds between the carbon atoms are broken and carbon dioxide is formed. The reaction can be summarized as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

In this reaction, ΔH is negative, this reaction is exothermic, and heat is liberated. So, the first law of thermodynamics allows one to determine the energy changes that accompany a biochemical reaction or process.

The second law of thermodynamics, the law of thermodynamic spontaneity, states that every reaction or energy transfer increases the entropy of the universe. Entropy (represented by S) is a measure of disorder or randomness of a system. The second law allows one to predict the direction in which a biochemical reaction will proceed and the amount of energy that will be released or consumed. In order for a reaction to proceed, the change in entropy, ΔS, must be positive; in other words, entropy must increase. An energy transfer such as freezing water involves a decrease in entropy as the hydrogen bonding becomes more structured to form the three-dimensional ice crystals. However, when looking at the universe as a whole, the overall entropy still increases because reactions that are associated with an apparent decrease in entropy of the system accompany other transfers that are associated with even greater increases in entropy of the surroundings. This complicates thermodynamic calculations for biological reactions, which work together to decrease entropy locally in order to maintain the organization that supports life.

Gibbs energy (represented by the symbol G) is a more useful measure for determining whether a reaction will proceed within a specific system. The utility of G, formerly called Gibbs free energy, for biological systems with stable temperature, pressure, and volume is demonstrated by the following equation, which relates free energy to enthalpy and entropy:

$$\Delta H = \Delta G + T\Delta S$$

or, rearranged,

$$\Delta G = \Delta H - T\Delta S$$

with T being temperature in Kelvin units. Reactions characterized by a decrease in Gibbs energy will have a negative value for ΔG. These reactions are exergonic, are considered spontaneous, and can occur. Reactions with a positive value for ΔG are endergonic and cannot occur unless they are coupled to another reaction that has a large enough negative value for ΔG to ensure that when combined, the overall ΔG is still negative. The fact that a biochemical reaction has a negative value for ΔG and can occur does not mean that it will occur. Biochemical reactions require catalysts called enzymes to get them actually to take place.

EQUILIBRIUM

The change in Gibbs energy (ΔG) can be calculated if the specific reaction conditions are known. First one must examine how close the reaction is to equilibrium. At equilibrium, the forward and reverse rates of a chemical reaction are equal, and the concentrations of reactants and products remain constant. A value called the equilibrium constant (K_{eq}) can be determined by dividing the concentration of the products by the concentration of reactants. To illustrate, the equilibrium constant for the following chemical reaction

$$a\text{A} + b\text{B} \leftrightharpoons c\text{C} + d\text{D}$$

is

$$K_{eq} = \frac{[\text{C}]^c\,[\text{D}]^d}{[\text{A}]^a\,[\text{B}]^b}$$

where [A] and [B] represent the concentrations of reactants, [C] and [D] represent the concentrations of products at 25°C, and a, b, c, and d represent the coefficients of each.

The equilibrium constants for most common reversible biochemical reactions have been experimentally determined. One can predict the direction in which a reaction will proceed by plugging in the measured concentrations. If the ratio of the actual concentrations of products to reactants does not equal the known K_{eq} for that reaction, the system is not in equilibrium, and the reaction will proceed in whatever direction is necessary to reach equilibrium. If the ratio is less than K_{eq}, then the reaction will proceed to the right, and if the ratio is greater than K_{eq}, then the reaction will proceed in the reverse direction, to the left.

Calculating ΔG shows how far from equilibrium a particular system is and how much energy will be

released when the reaction proceeds toward equilibrium. The following equation is used

$$\Delta G = RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b} - RT \ln K_{eq}$$

where R is the gas constant (1.987 cal/mol-K) and T is the temperature in Kelvin (298 K). The result, ΔG, will be expressed in cal/mol and should be a negative number. This confirms that the reaction is spontaneous or thermodynamically possible but, again, does not mean that the reaction actually will occur, or, if it does, indicate the rate. The field of kinetics examines these issues, which are covered under the discussion of enzymes in this text.

See also BIOCHEMICAL REACTIONS; CELLULAR METABOLISM; CHEMICAL BASIS OF LIFE; ECOSYSTEMS; ENZYMES; PHOTOSYNTHESIS.

FURTHER READING

Chang, Raymond. *Physical Chemistry for the Biosciences*. Sausalito, Calif.: University Science Books, 2005.

Murray, Robert K., Daryl K. Granner, Peter A. Mayes, and Victor W. Rodwell. *Harper's Illustrated Biochemistry*. 26th ed. New York: McGraw Hill, 2003.

Myers, Richard. *The Basics of Chemistry*. Westport, Conn.: Greenwood Press, 2003.

Nicholls, David, and Stuart Ferguson. *Bioenergetics*. 3rd ed. Burlington, Mass.: Academic Press, 2002.

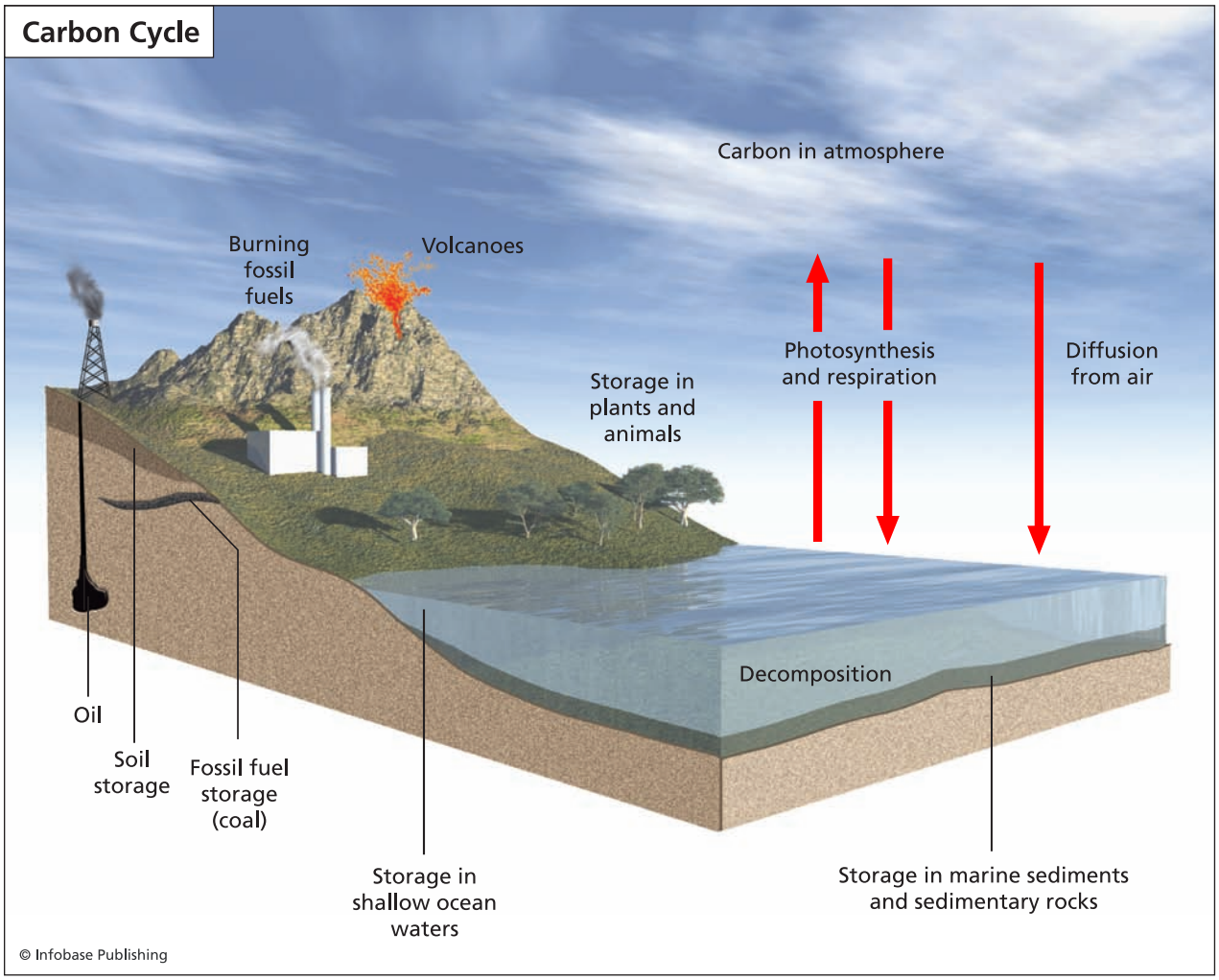
biogeochemical cycles The exchange of nutrients, chemical substances required for the normal development, maintenance, and reproduction of organisms, between organisms and the environment is an important feature of a functioning ecosystem. The process of nutrient cycling involves the use, exchange, transformation, and movement of nutrients through an ecosystem. Living organisms require a source of energy and a supply of nutrients. Whereas the Sun constantly inputs solar energy into an ecosystem, nutrients have limited availability. Not only is the quantity of a nutrient in an ecosystem important, but the form of the nutrient affects whether or not organisms can uptake and utilize it in metabolic processes. Autotrophic organisms are capable of fixing elements such as carbon or nitrogen, meaning they can incorporate the inorganic form of an element into organic molecules. Heterotrophic organisms must ingest their nutrition from preformed organic molecules. Because living organisms change the form of certain nutrients, or elements, as part of their normal metabolic processes, the organisms are called biotic components, and they play a significant role in the cycling of nutrients through ecosystems. Abiotic components of nutrient cycling include the processes that are not directly related to living organisms; for example, weathering, erosion, the formation of sedimentary rock, and other geological processes, are abiotic components. Because both biological and geochemical events participate in the movement of nutrients through ecosystems, the process is called biogeochemical cycling.

Chemical nutrients cycle at a local level as well as a global level. Gases in the atmosphere travel great distances, whereas elements in the soil usually stay within the vicinity. A reservoir is a place where a nutrient is kept in storage and serves as a supply. Two characteristics that describe reservoirs involve whether the nutrients they contain are organic or inorganic and whether the nutrients are available or unavailable. The nutrients move between reservoirs by several different processes: assimilation, photosynthesis, fossilization, burning of fossil fuels, respiration, decomposition, excretion, weathering, erosion, and formation of sedimentary rock.

Many nutrients are already tied up in living organisms, as part of cells, tissues, and body fluids. A living organism is thus considered a reservoir made up of organic materials that are available as nutrients to other organisms if the organism is eaten. As living organisms utilize the nutrients and extract energy from the organic compounds, metabolic processes produce inorganic by-products that leave the body by respiration or excretion. Organisms that have died also contain organic material that is available as nutrients to other organisms. For example, many microbes feed off detritus and leaf litter on the forest floor. Decomposition of organic material by detritovores, organisms that consume nonliving organic matter, also returns inorganic nutrients to the environment. Fossilization occurs when sediment buries dead organisms and they are transformed by physical processes into coal, oil, and peat over millions of years. These fossil fuels are composed of organic nutrients, but in a form unavailable to organisms. Burning them releases the elements into the atmosphere, soil, and water as inorganic nutrients that are available for use by organisms capable of photosynthesis or fixation and assimilation. Inorganic materials also contribute to the formation of sedimentary rock; as minerals in rocks they are unavailable, until weathering or erosion wears away the rock and they become available once again. Thus, numerous different biological and geochemical processes participate in the cycling of nutrients through ecosystems.

NUTRIENT CYCLES

Organic material, molecules that living organisms synthesize and the molecules from which they are composed, consists mostly of the elements carbon, oxygen, hydrogen, nitrogen, phosphorus, and sulfur. Though



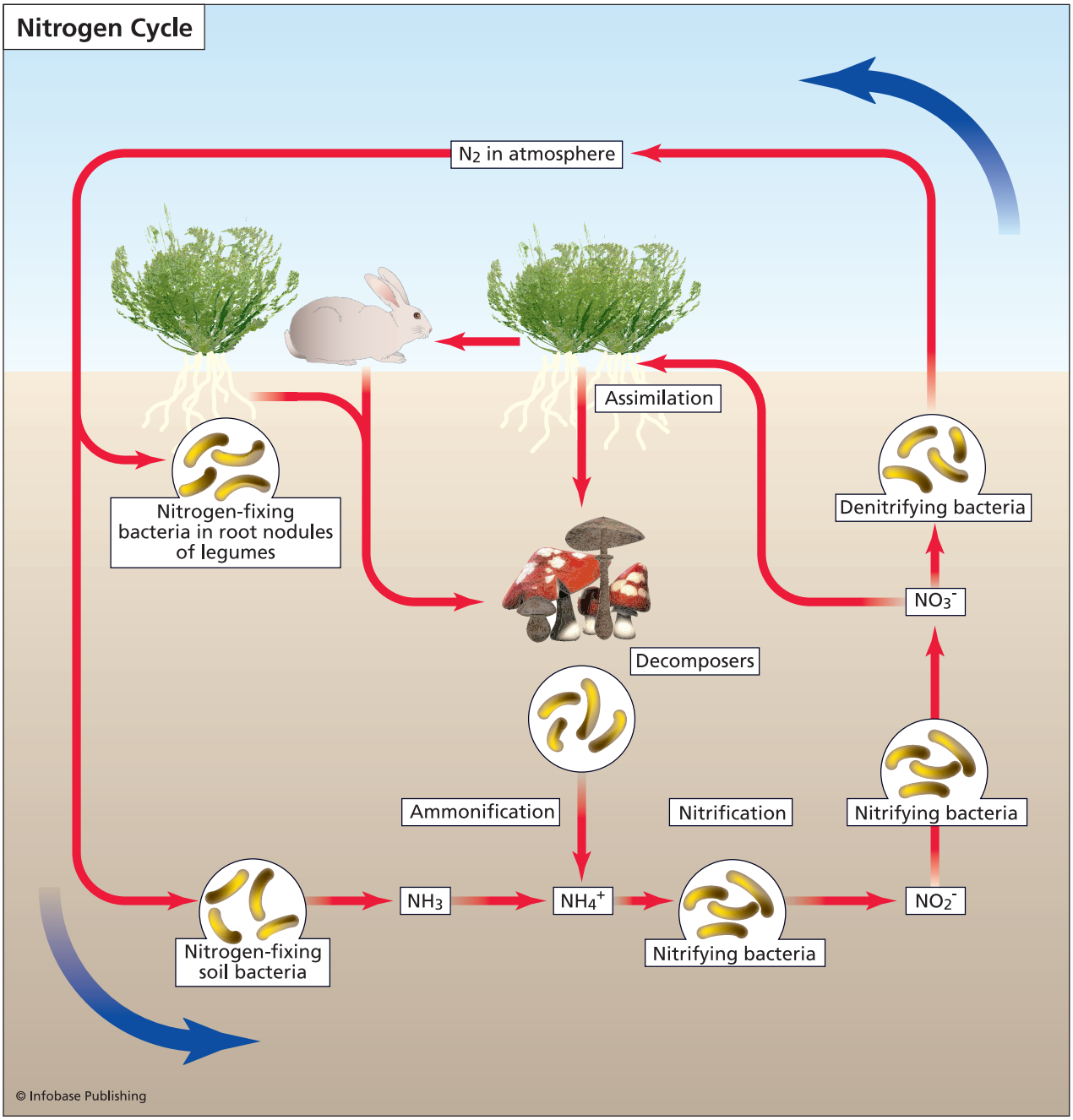
Numerous biological and geochemical processes drive carbon through the carbon cycle, which includes both inorganic and organic forms of the element. Carbon enters seawater from many sources, including the air, respiration by living things, erosion of carbon-containing rock, and combustion of fossil fuels. Carbon is removed by processes such as photosynthesis, the creation of limestone, and storage in plants and animals.

all nutrients must be recycled, these elements are the most important biologically, as they are the main components of the biomolecules proteins, nucleic acids, carbohydrates, and lipids. The continuity of life depends on the recycling of carbon, nitrogen, phosphorus, and sulfur in addition to water, since water is a major source of hydrogen and oxygen.

Carbon forms the backbone or molecular framework of all organic molecules. Carbon exists in the atmosphere as the gas carbon dioxide (CO_2), though the atmosphere consists of less than 0.1 percent CO_2. This form of carbon is only available to autotrophic organisms, organisms that are capable of incorporating inorganic carbon into organic molecules via photosynthesis or chemosynthesis. Plants, algae, and some bacteria undergo photosynthesis, during which they convert radiant energy from sunlight into chemical energy that they utilize to synthesize carbohydrates. Only specific prokaryotic species are capable of chemosynthesis, in which inorganic chemicals serve as the energy source for synthesizing organic molecules from carbon in CO_2. Primary consumers ingest the autotrophic organisms to obtain their nutrients (already in an organic form) and energy, and secondary and higher-level consumers feed off the organisms lower in the food chain to fulfill their nutritional requirements. For example, algae are autotrophic, and snails are primary consumers that eat the algae to obtain their nutrients. Some fish, such as loaches, eat the snails and are therefore considered secondary consumers. Any organism that eats the fish would be a higher-level consumer. All of these organisms carry out cellular respiration, the process by which cells break down organic chemicals into smaller components to release energy and metabolites that can be used to synthesize other biomolecules.

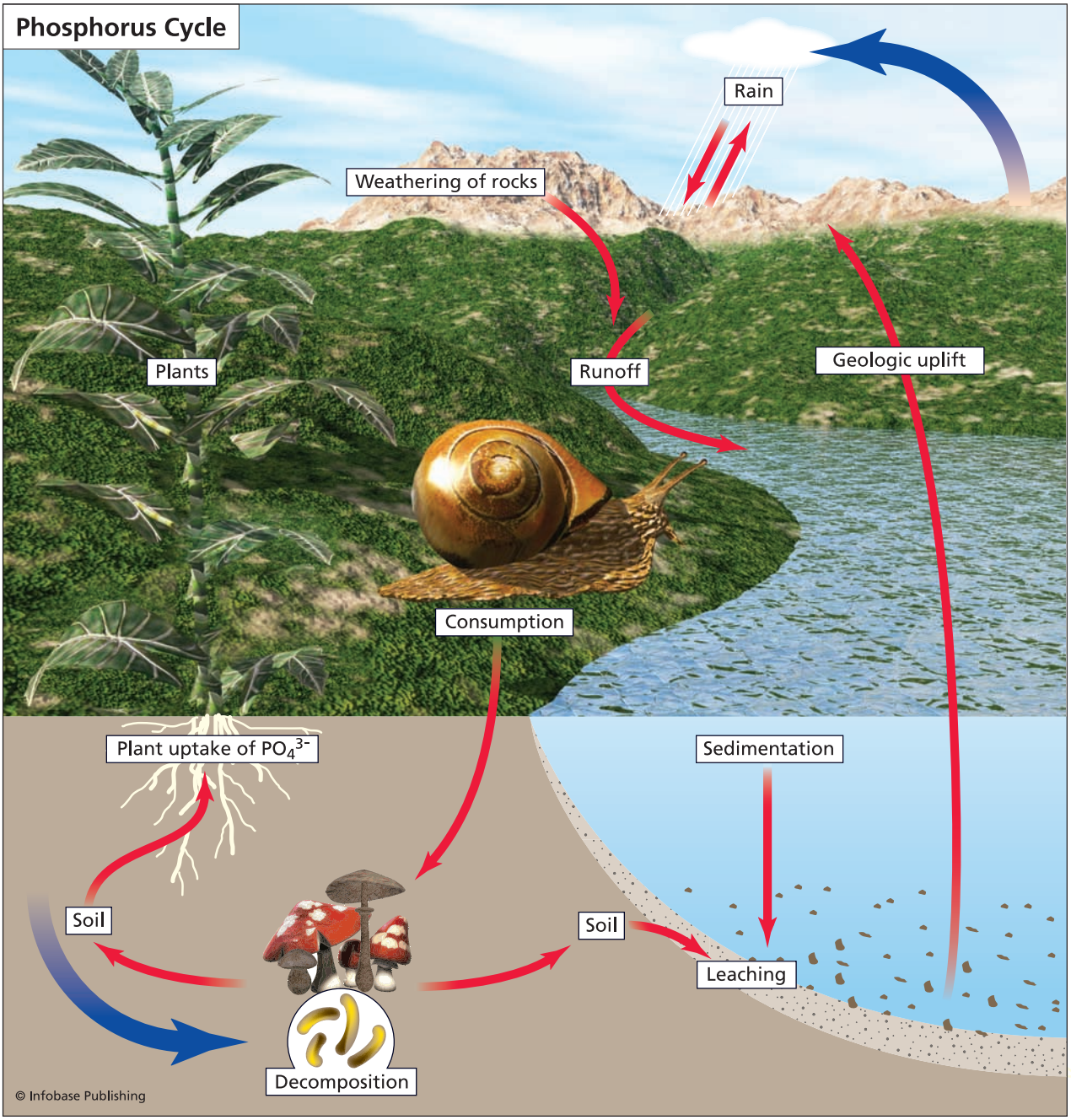
The cells use the energy to synthesize molecules of adenosine triphosphate (ATP), and they give off CO_2 as a by-product. After living organisms die, microorganisms decompose the organic material left behind as part of their normal metabolic processes, converting some of the carbon back into inorganic forms. When dissolved in water, CO_2 participates in an equilibrium reaction with bicarbonate (HCO_3^-) and carbonate (CO_3^-), which can combine with calcium and precipitate out of solution to join the largest carbon reservoir, carbonate rocks. Geological processes also convert the carbon in the remains of organisms that lived millions of years ago into fossil fuels such as coal, oil, and natural gas. Just as the biological burning of organic materials via metabolic processes releases CO_2 into the atmosphere, burning of fossil fuels and volcanic activity also release CO_2 back into the atmosphere, completing the carbon cycle.

Nitrogen is an important component of nucleic acids, both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and of amino acids, the building blocks of proteins. An insufficient quantity of



Specialized bacterial species play an important role in the cycling of nitrogen.

nitrogen in the soil often limits the growth of plants, and thus nitrogen is a major ingredient of fertilizers. The atmosphere consists of more than 78 percent nitrogen gas (N_2) and thus serves as the major reservoir of nitrogen, but this form is unavailable to most organisms. The capability of nitrogen fixation is limited to specialized species of bacteria that live in the soil, in water, and as symbionts in the roots of leguminous plants. Genera containing species that can perform nitrogen fixation include *Azotobacter, Azospirillum, Clostridium, Anabaena,* and *Nostoc.* These bacteria can break the strong triple bonds between the two nitrogen atoms in a molecule of N_2, an energetically expensive reaction that results in the reduction of nitrogen, usually to ammonia, which in the presence of water readily transforms into ammonium ions (NH_4^+). Bacteria including *Rhizobium, Bradyrhizobium,* and *Azorhizobium* infect the roots of plants such as soybeans, alfalfa, and peas, causing them to form



Phosphorus cycles through ecosystems mostly in the form of phosphate (PO_4^{3-}), which is soluble.

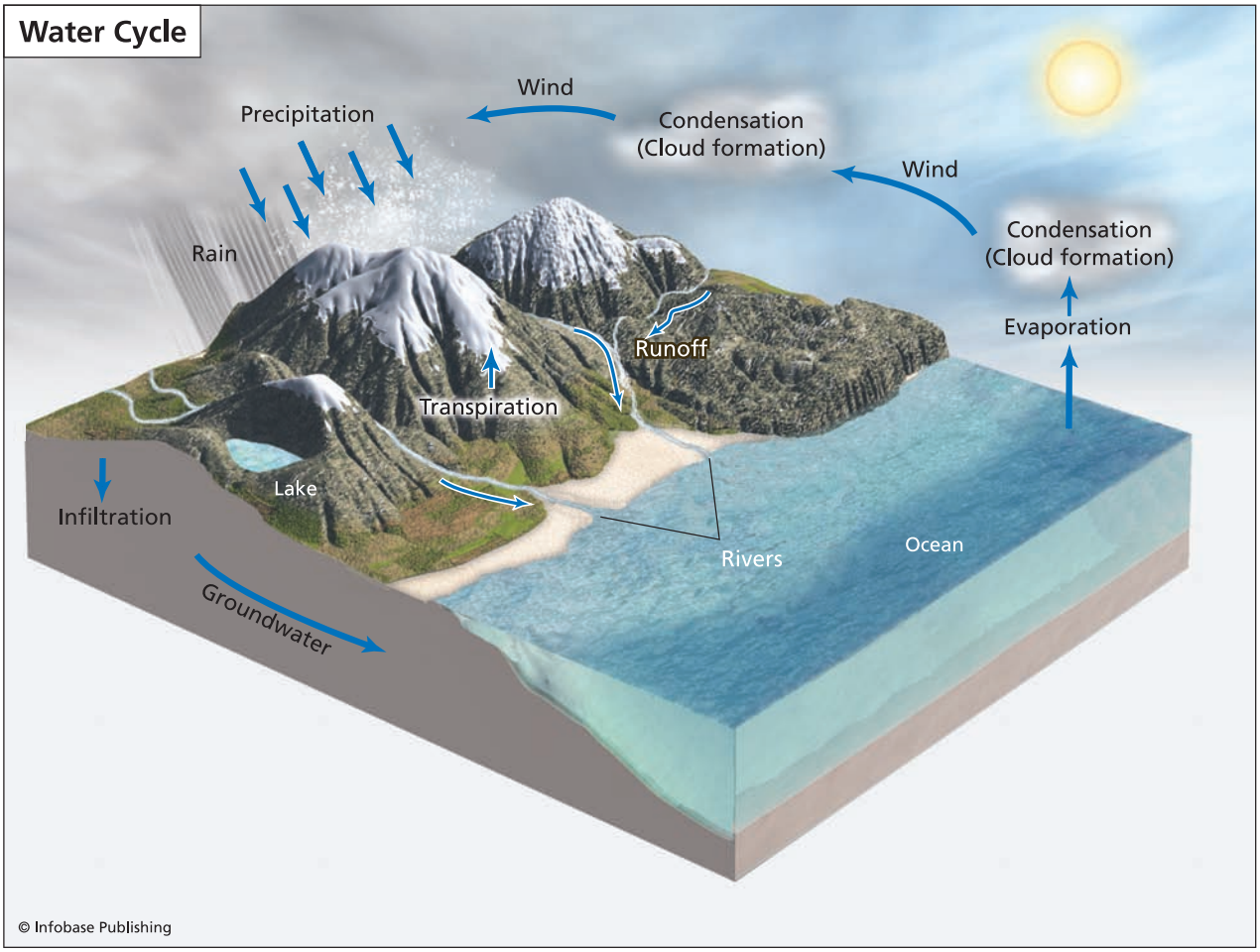
nodules. These bacteria fix nitrogen and provide the plants with a constant supply of NH_4^+. Lightning also provides sufficient energy and physical conditions to fix nitrogen. Other bacteria (*Nitrosomonas, Nitrosococcus, Nitrobacter,* and *Nitrococcus*) carry out nitrification, the oxidation of NH_4^+ to nitrite (NO_2^-) or nitrate (NO_3^-). Most plants, algae, and other organisms can readily use NO_3^- to synthesize amino acids and nucleotides for incorporation into proteins and nucleic acids, respectively. Bacteria such as *Clostridium* and *Proteus* decompose the nitrogenous organic material of detritus, releasing NH_4^+ that can either be used by some organisms or oxidized first by nitrifying bacteria and then used. The cycle is completed when nitrogen returns to the inorganic pool of atmospheric nitrogen after denitrification, during which denitrifying bacteria (certain species of *Bacillus, Pseudomonas, Spirillum,* and *Thiobacillus*) reduce nitrates in a stepwise process, creating N_2 gas.

Phosphorus is a major component of nucleic acid, which contains a backbone made of alternating sugar and phosphate moieties. ATP also contains phosphorus in the form of phosphate (PO_4^{3-}) moieties. The major reservoirs for phosphorus are mineral deposits in rocks, marine sediments, and soil. The form of phosphorus found in rocks, fluorapatite, is not soluble, but interaction with acids solubilizes and releases it from rock in a more usable form, PO_4^{3-}. Autotrophs can fix this form into organic compounds, from which heterotrophs can fulfill their phosphorus requirements by consuming the autotrophs. Fertilizers often contain phosphates to compensate for insufficient amounts of phosphate naturally present in the soil. When excess phosphate from fertilizer runoff enters streams and lakes, eutrophication can occur; algae, cyanobacteria, and other surface organisms overgrow and shut off the supply of dissolved oxygen to the rest of the water body. Aerobic microorganisms involved in decomposition of the overgrowth further contribute to the lack of oxygen available to the aquatic life, causing fish and invertebrates to die. The phosphorus continues to cycle as terrestrial organisms die and decompose, and the phosphorus enters the soil as phosphate ions and leaches into bodies of water, where it sediments and geological processes form rock. The geological uplift of marine sedimentary rock transports phosphorus to land, where weathering and erosion of the rocks release fluorapatite, completing the cycle.

Sulfur is a component of the amino acids cysteine and methioine and is therefore present in many proteins. The sulfur cycle shares many similarities with the nitrogen cycle in that specialized bacteria perform many of the transformations of different forms of sulfur. The many forms include elemental sulfur (S), hydrogen sulfide gas (H_2S), sulfate (SO_4^-), and thiosulfate (S_2O_3). SO_4^- is the form that most organisms can utilize. Members of the bacterial genera *Thiobacillus* are lithotrophic, meaning inorganic chemicals, such as sulfur compounds, fulfill their nutritional requirements. They often inhabit swamps, mud, sewage, and acidic environments that other organisms cannot inhabit because of the lack of available organic nutrients or low pH but that do contain plenty of inorganic sulfurous compounds. After other organisms incorporate the oxidized sulfur compounds in their metabolic processes, sulfur-reducing bacteria such as *Desulfovibrio* and *Desulfuromonas* regenerate reduced sulfur in the form of hydrogen sulfide (H_2S) or metal sulfides, completing the sulfur cycle. Springs or areas that contain these sulfur-reducing bacteria often smell like rotten eggs as a result of the abundance of H_2S present.

WATER CYCLE

Water is composed of two parts hydrogen and one part oxygen (H_2O); thus the hydrologic cycle, or water cycle, is responsible for the cycling of these elements. The importance of water is obvious for aquatic species, but water is just as crucial for terrestrial species, as the major constituent of all cells is water. The liquid form is most available to organisms, but some organisms can obtain water from water vapor, though not from ice, or frozen water. The oceans contain approximately 97 percent of the biosphere's water. Polar ice, then lakes and groundwater compose the remainder. Evaporation, condensation, and precipitation are the major physical processes involved in water cycling through ecosystems, and transpiration is the major biological process. Solar radiation drives the cycle by causing evaporation, the conversion of liquid water into the gaseous form, water vapor, through the input of heat energy. Approximately 90 percent of the water in the atmosphere arrived there through evaporation. As a vapor, water enters the atmosphere, where the wind can carry it to new locations. As it rises, the vapor encounters cooler air, causing it to condense into a denser liquid form. When liquid droplets become heavy enough, they fall as precipitation in the form of rain, drizzle, sleet, snow, freezing rain, or hail. All forms of precipitation transport the water back into the oceans or onto land, depending on the location. When water falls onto land, it percolates through the soil and runs off into lakes and streams, some of which empty into the oceans. Plants absorb water through roots embedded in the soil and return it to the atmosphere via transpiration, evaporative water loss, which is



The major processes that drive the water cycle, or hydrological cycle, include evaporation, transpiration, and precipitation.

responsible for roughly 10 percent of the vapor in the atmosphere.

See also BACTERIA (EUBACTERIA); BIOMOLECULES; ECOSYSTEMS; FUNGI; MICROBIOLOGY; PROTOZOA; WATER, ITS BIOLOGICAL IMPORTANCE.

FURTHER READING

Moles, Manuel C. *Ecology: Concepts and Applications*. 3rd ed. New York: McGraw Hill, 2005.

Sigee, David C. *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. Hoboken, N.J.: John Wiley & Sons, 2005.

biogeography Geography is the science of the description, distribution, and interaction of the physical, biological, and cultural features of the Earth's surface. Biogeography is a subdiscipline of geography that deals with the spatial and temporal distribution of plants and animals. The temperature, precipitation, type of soil, salinity of the soil or water, degree of Sun exposure, landscapes, and other physical features of an area influence the types of flora and fauna that live there, as do other members of the biological community. This allows for the depiction of general biological patterns on a map, such as geographical features, such as mountain, lakes, and deserts, or meteorological features, such as temperatures, isobars, and precipitation patterns. Because organisms interact with other living organisms and with their physical environment, and because these relationships influence the course of biological evolution, the fields of ecology and evolution are interconnected with biogeography, which also draws on other disciplines such as geology and climatology.

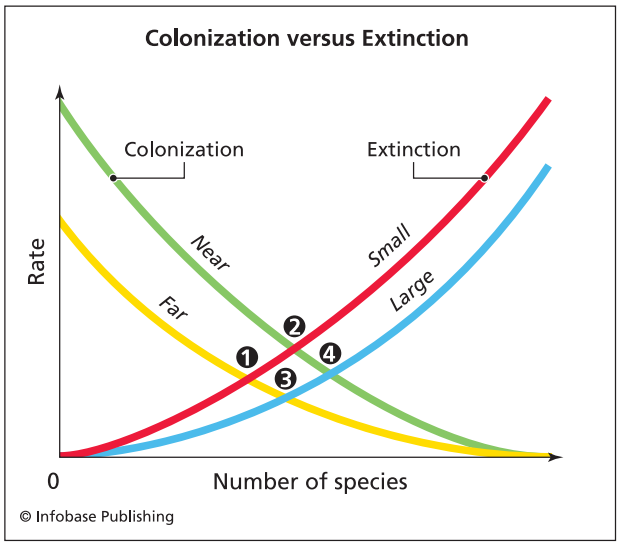
CONCEPTS OF BIOGEOGRAPHY

The Earth's biosphere contains several different biomes, ecological associations characterized by the types of vegetation present and the general climate. These biotic and abiotic factors affect the flora and fauna that typically inhabit a particular region. Dispersal also influences the distribution of a spe-

cies. Plants and animals have a variety of means for dispersal, or extending the range that they inhabit: swimming, flying, walking, water and wind currents, seeds carried in the feces of animals, spores blown by the wind, parasites on the bodies of other animals, and so on. Geographic barriers, such as mountain ranges, deserts, or oceans, can limit an organism's dispersal.

Islands provide natural isolated model systems for studying biogeographical principles, which can then be applied and examined in ecosystems that are not as clearly defined by sharp physical boundaries. The concept of a biological island is not limited to a tract of land surrounded by water, but mountain ranges, lakes, rivers, altitudes, sharp climate boundaries, and forest fragments all create isolated, inland islandlike habitats, though the degree of isolation may differ as a result of a combination of geographical or biological factors. For example, some lakes (aquatic islands) may be completely isolated, while others may receive drainage or input from streams or have streams that carry water out of them. Birds and insects can fly varied distances to find food or to mate. Some animals are fine swimmers or can travel over mountains. Some plants produce spores that the wind can blow great distances or have seeds that other animals ingest and spread to distant locations.

Biogeography explores the distribution of species in different geographic locations. Coastal dune forest ecosystems, such as this one in Riga, Latvia, develop under the shelter of the sand dunes, in sand with very little soil development, and where annual precipitation exceeds 27.5 inches (70 cm). *(AJE, 2007, used under license from Shutterstock, Inc.)*



According to the equilibrium model of island biogeography, the maximal number of species for an island is maintained by a balance of colonization and extinction rates, both of which are affected by the island size and remoteness.

One main concept of biogeography is that area and isolation affect the species richness of islands and habitat patches on continents. Biodiversity increases with increasing area and decreases with greater isolation. In other words, a smaller island located far away from other ecosystems will contain fewer species than a larger island located nearer to other islands or to shore. Species proliferate and diversify at a faster rate on bigger biological islands. The effect of isolation needs to be examined individually for each species, since dispersal mechanisms and therefore dispersal rates vary widely among species.

Another concept of biogeography is that biodiversity on islands is maintained by a balance of immigration and extinction. This equilibrium model of island biogeography shown above illustrates a relationship between the number of different species on an island and immigration rates and between the number of species on an island and extinction rates. As the number of species present increases, the rate of arrival of new species decreases. Conversely, as the number of species present increases, the rate of extinction increases. The reality is more complex than this simplified model suggests: because the size and isolation of an island affect the species richness, these factors also influence the rates of immigration and extinction. While some islands reach equilibrium in the number of different species, others do not. The species composition, however, is dynamic; this change in composition is known as species turnover.

Species richness generally increases from high and middle latitudes toward the equator. Tropical habitats such as rain forests contain many more species than habitats closer to the poles. Though this observation is well documented, the reasons for it are unknown. Several hypotheses attempt to explain this gradient in species richness, which biogeographers continue to explore. The "time since perturbation" hypothesis states that tropical habitats have been around longer and have not been perturbed frequently by the advance and retreat of glaciers; thus extinction rates are lower. Other hypotheses link the species richness to high productivity, environmental heterogeneity, lack of extreme fluctuations in weather, breadth of niches, or degree of interspecific interactions such as symbioses. The latitudinal gradient in richness could also be due to an increased area. Since species number changes as a result of extinction and speciation events, tropical environments could simply have higher speciation rates or lower extinction rates. Because of the shape of the planet Earth, tropical latitudes, located between 23.5 degrees north and south of the equator, contain more land area and water area than other latitudes. The temperature is also more uniform, giving species that prefer that temperature range a wide region across which they can spread. Larger area allows for a larger population size, which is less prone to extinction than a smaller population. The larger area could also allow for more speciation events, since there is more area over which geographic barriers may exist and lead to allopatric speciation events.

Because the Earth is dynamic, the structure of many ecosystems results from significant historical events or regional processes. Throughout the Earth's history, certain geographic areas have experienced periods of intense volcanic activity, orogeny, flooding, or glacial advancement and retreat. The conditions of a region at a particular time place unique selection pressures on the biota (all the flora and fauna) that are living there or that can colonize there afterward. For example, more species of trees inhabit temperate forests in North America and Asia than in Europe. This can be explained by the fact that temperate forests occur in areas that experienced glacial advancement and retreat during different geological periods. When glaciers advanced into these regions during the last ice age, they forced the trees to migrate farther south, where the temperatures were warmer. In Europe, mountain ranges that run east and west prevented the southward migration of trees; thus they became extinct. Asia has no such mountain barriers and eastern North America only has the Appalachians, which run north and south, so tree populations in these regions could retreat toward the equator during the ice age, only to migrate north again when global temperatures increased.

TOPICS IN BIOGEOGRAPHY

The goal of biogeography is not simply to describe biogeographic patterns, but to examine the processes that determine the distribution and diversity of organisms globally, within a continent, on a marine island, on a mountain island, or on another patch of land habitat. Biogeographers study the geological and ecological processes that affect the origin, spread, distribution, and diversification of biota inhabiting a particular region. For example, one current topic of research is the effect of global climate change on the distribution of plants and animals. As temperatures rise and rainfall patterns change, vegetation that once thrived in a particular area may suffer, and, as a result, the animal life dependent on those plants for food, shelter, or shade will migrate to find a more suitable environment. The rise in sea level resulting from the melting of glaciers and polar ice affects coastal regions, estuaries, and wetlands and the life-forms that those habitats support. Biogeographers are researching the effects that have already occurred and will use this information to try to predict future consequences. In collaboration with ecologists, conservation biologists, and environmental scientists, they can attempt to reduce the potential negative impact.

Biogeographers seek to understand how distribution patterns came about and what phenomena maintain or alter those patterns. These questions are addressed by biogeographers in conjunction with paleobiologists, paleoclimatologists, and evolutionary biologists. Studying the evolutionary history of populations in an area along with the past climates and geological features will provide insight as to the evolution and ecological interactions among species, populations, and communities. This helps biogeographers understand the natural forces that affect extinctions, migrations, and alterations of different habitats, information that can be applied to biological conservation efforts.

A biogeographer might ask why a species lives in one area but not in another. In order to answer this, he or she might examine the other flora and fauna in the two regions and consider the types and abundance of food sources or predator/prey relationships. Alternatively, the explanation could be historical. Perhaps the species previously inhabited both regions but became extinct in one of them. If so, what changed and caused the species to die out in one region but not the other? Or perhaps the species originated in one area. Did the climate change in the one area and select for adaptations that over time led to the formation of the new species? As a scien-

tist, the biogeographer must formulate a hypothesis based on observations and then test its validity.

See also BIOMES, AQUATIC; BIOMES, TERRESTRIAL; ECOLOGY; EVOLUTION, THEORY OF.

FURTHER READING

Biogeography Specialty Group of the Association of American Geographers home page. Available online. URL: http://people.cas.sc.edu/kupfer/bsg.html. Updated July 27, 2007.

International Biogeography Society home page. Available online. URL: http://www.biogeography.org. Updated December 4, 2007.

Lomolino, Mark V., Dov F. Sax, and James H. Brown, eds. *Foundations of Biogeography: Classic Papers with Commentaries*. Chicago: University of Chicago Press, 2004.

MacArthur, Robert H., and Edward O. Wilson. "An Equilibrium Theory of Insular Zoogeography." *Evolution* 17, no. 4 (1963): 373–387.

———. *The Theory of Island Biogeography*. With a new preface by Edward O. Wilson. Princeton, N.J.: Princeton University Press, 2001.

bioinformatics

The term *bioinformatics* can be defined numerous ways: as a field of research, as a collection of methods for analyzing data, or as a synonym for computational molecular biology. Broadly defined, bioinformatics is the use of computers to analyze any biological data. Here, the term *bioinformatics* will refer to the use of computers for storing, retrieving, analyzing, or predicting the composition or structure of biomolecules, specifically nucleic acids and proteins.

BACKGROUND INFORMATION

Nucleic acid includes both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), molecules made from the assembly of numerous nucleotide subunits, which each contain a sugar, a nitrogenous base, and a phosphate group. In the laboratory, biologists use and manipulate segments of nucleic acid as small as 10 nucleotides and up to thousands of nucleotides in length, though some chromosomes contain millions of nucleotides. DNA consists of four different nucleotides, which differ on the basis of the nitrogenous base they contain: adenine (A), guanine (G), cytosine (C), or thymine (T). RNA consists of four different nucleotides also, but the sugar moiety is slightly different, and the nitrogenous base uracil (U) replaces thymine in RNA. The sequence of the nucleotides in DNA indirectly encodes for the synthesis of proteins. Segments of DNA that contain information on how to make a protein are called genes; genes are located on chromosomes. During the first step in protein synthesis, the enzyme RNA polymerase uses the DNA template to make a molecule of messenger RNA (mRNA) in a process called transcription. The sequence of nucleotides on the mRNA is based on the sequence of nucleotides in the DNA; in other words, the two are said to be complementary. For every A in the DNA, RNA polymerase incorporates a U in the mRNA; for C in the DNA, it adds G to the mRNA; for G in the DNA, it adds C in the mRNA; and for T in the DNA, it adds A in the mRNA. After transcription, ribosomes scan the mRNA and assemble specific amino acids in an order based on the sequence of nucleotides in the mRNA during a process known as translation. The ribosome reads the mRNA three nucleotides at a time. Each set of three is called a codon and specifies one of 20 different amino acids. One triplet codon (AUG) indicates the start site of translation, and three other codons (UAA, UAG, and UGA) cause termination of translation to occur. The remaining codons specify a single amino acid, though in some cases, up to six different codons encode for the same amino acid, a phenomenon known as degeneracy. Although the genetic code is degenerate (redundant) in that multiple codons encode for the same amino acid, each given codon only encodes for one specific amino acid. The chain of amino acids that results from translation is called a polypeptide, and after folding into the correct conformation, or in some cases, when combined with other polypeptides, the protein is complete.

Molecular biologists have sequenced the entire genome of hundreds of viruses and organisms, including numerous bacteria, yeast, fruit flies, mice, and humans. They have isolated and identified many individual protein-coding genes from these organisms and countless others. Identification of a gene allows researchers to study the expression of that gene, determine what turns the gene on and off, the protein it encodes, the structure of that protein, and the function of that protein. Geneticists can mutate the gene, or make changes to the DNA sequence, and observe the effects of the mutation on the protein function. Genetic engineers can clone the gene into other organisms, such as bacteria, and produce large quantities of the protein, which biochemists can purify and then characterize.

ROLE OF BIOINFORMATICS

Bioinformatics facilitates the collection, storage, retrieval, and analysis of all the information gained by sequencing genomes, identifying genes, characterizing proteins, and performing other applications. Without the use of computers, researchers would not have completed the sequencing of the human genome, which contains more than 3 billion nucleotides, or possibly any other organism's genome. Since 1953,

(continues on page 87)

BIOINFORMATICS: SEQUENCE ALIGNMENT ALGORITHMS

by Jason S. Rawlings, Ph.D.
St. Jude Children's Research Hospital

Sequence alignment algorithms are tools that enable life science researchers to compare protein or deoxyribonucleic acid (DNA) sequences with one another. These are powerful tools because of the biological relationship between the information in these sequences and the resulting protein function. According to the central dogma of molecular biology, a gene's DNA sequence will determine the amino acid sequence of the corresponding protein. The amino acid sequence determines the overall three-dimensional structure of the protein, and the structure determines function of the protein. Thus, the DNA sequence of a gene contains valuable information about the function of its resulting protein. An investigator uses a sequence alignment algorithm to determine whether two or more DNA or protein sequences are similar to one another. This essay will present the different types of alignments, and how basic alignment algorithms work and give examples of how scientists use these algorithms in their research.

The true power of sequence alignment algorithms lies in their application; following are but a few examples. They can be used to retrieve the sequence of a specific gene or protein from a database, akin to searching the Internet for a specific Web page. They can also be used to identify an unknown sequence by comparing the unknown to all known sequences in a public database. They can be used to compare two sequences for overall similarity or to find just the regions within each that are similar to one another. Researchers can also determine relationships among several sequences by aligning multiple sequences to identify members of a larger family of genes or to compare proteins from different species in an evolutionary analysis.

The different types of sequence alignments all have different algorithms. Each type of alignment algorithm has its own advantages and disadvantages in terms of the accuracy of the alignment, the information it produces, and the time it takes to complete the alignment. The simplest alignment is between two sequences; these algorithms will provide a detailed, accurate analysis of the similarity of the two sequences. The time to complete the alignment is dependent on the length of the two sequences, and given the speed of today's computers, the time required is negligible.

One can also align three or more sequences together in a multiple sequence alignment. Many available programs can perform these types of alignments. The most popular are derived from the Clustal program. Multiple sequence alignments are advantageous for the amount of information they produce and for the types of downstream analyses that they allow one to perform. However, the time to complete the alignment becomes significant. In the Clustal algorithm, not only the length of each sequence is a factor, but also the number of sequences, because each sequence must first be compared to all of the others to determine the pairwise similarity of all the sequences. This requires a total of

$$\frac{X(X-1)}{2}$$

sequence comparisons, where X is the number of sequences to be aligned. Then, the multiple sequence alignment is progressively built by combining a growing number of subalignments, starting with the most similar pair of sequences. Thus, thousands of comparisons may be required for aligning a large number of sequences, making for a computationally intensive task.

Finally, one can align a single sequence against an entire database of sequences using the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information (NCBI) Web site. The user submits a sequence to the database and the BLAST program returns the sequences that are most similar. Because this process may involve comparing the input sequence to millions of sequences in the database, sacrifices in accuracy and completeness must be made in exchange for speed. Specifically, the program looks for "words" within the submitted sequence that can be matched in the database. These "words" may consist of just 10 nucleotides or amino acids (a typical gene consists of thousands of nucleotides; a typical protein consists of hundreds of amino acids). When the BLAST program finds matches in the database, it analyzes adjacent sequences for similarity, building an alignment in the process. This is repeated until either adjacent sequences are no longer similar or the entire sequence is aligned to the match found in the database. Those sequences that do not have adjacent similarity are no longer considered. The program returns to the user the sequences in the database that aligned best. In the end, only a portion of the submitted sequence may be aligned to sequences in the database.

NCBI offers several variations of the BLAST program; each is optimized for different types of input sequences, the database to be searched, and purpose of the query. Users can submit a DNA sequence in a search against a DNA database. Alternatively, a DNA sequence can be compared to a protein database; the query is translated into the corresponding amino acid sequence by the program before the search is done. Conversely, protein sequences can be compared to protein databases or compared to a DNA database that has been translated in all possible reading frames. Advanced versions of the BLAST algorithm allow for more specialized searches, such as iterative database searches or searches optimized for very short input sequences. Additionally, the user can choose to search the entire public database of sequences or limit the search to a specific genome

or subset of sequences. The BLAST algorithm is extremely versatile and fast. The time required to complete a BLAST search depends mainly on the size of the database to be searched as well as the number of other searches in the queue at the time the search is initiated. A typical nucleotide search against the entire public DNA database can be completed in seconds.

At the core, a sequence alignment algorithm must align two DNA or two protein sequences, but how do they work? A typical algorithm such as the Needleman and Wunsch algorithm (Needleman and Wunsch, 1970) uses a predefined set of rules to line up the two sequences dynamically using a point system. For DNA sequences, the rules are simple. Only one of the strands of the DNA needs to be aligned from each of the submitted sequences because of the rules governing complementary base pairing. There are four possible nucleotides at any given position in a DNA sequence: guanine (G), adenine (A), thymine (T), or cytosine (C). At each position in the alignment, the nucleotides are compared; if they match, then points are awarded, while mismatches receive a penalty. The algorithm may introduce gaps, at a great penalty, and extend those gaps for a further penalty, in order to achieve an overall alignment with the best possible score. Overhanging sequences caused either by created gaps or simply by submission of sequences of different lengths are not penalized. The example that follows illustrates how an alignment algorithm might find the best possible alignment between two sequences.

```
1:  TTCGCGAAGATTACGCGAA
2:  TTCGTTACGCGAAGGGGTA
```

Mismatches in the first alignment are bolded below.

```
1:  TTCGCGAAGATTACGCGAA
2:  TTCGTTACGCGAAGGGGTA
```

Matches: 10(10) = 100
Mismatches: 9(–5) = –45
Gaps: 0(–50) = 0
Extensions: 0(–50) = 0
Score: 100 – 45 = 55

The second alignment involves a gap that eliminates any mismatched nucleotides. The introduction of such a gap in sequence 2 leads to an overhang.

```
1: TTCGCGAAGATTACGCGAA
2: TTCG........TTACGCGAAGGGGTA
```

Matches: 13(10) = 130
Mismatches: 0(–5) = 0
Gaps: 1(–50) = –50
Extensions: 6(–3) = –18
Score: 130 – 50 – 18 = 62

The creation of a gap in the second alignment results in a better score—62 compared with 55 for the first alignment, which contained only mismatch penalties. In this example, matches are given 10 points, mismatches are penalized five points. Creating a gap incurs a 50-point penalty, with an additional penalty of three for each position in the gap. Even though creating a gap is costly, it results in an overall better alignment score in this example. Gaps incur such a large penalty because of the genetic repercussions they present. Inserting or deleting a single nucleotide in a gene sequence introduces a frameshift mutation, which will drastically alter the resulting protein sequence downstream of the mutation. The specific rules used for an alignment can be modified, providing optimization for the length of the sequences to be aligned and the overall goal of the alignment.

Protein sequence alignments work in a similar fashion; however, they present some additional challenges. There are 20 possible amino acids to consider, compared to just four for nucleotides. Also, many amino acids share similar structural and chemical properties, thus invalidating a simple match/mismatch approach. To tackle these issues, protein alignment algorithms utilize a substitution matrix. Instead of a simple match/mismatch scenario, the matrix provides a similarity score for comparing two amino acids. This score is based on several factors, including amino acid structure, chemical composition, and evolution. For example, tyrosine and phenylalanine would receive a favorable similarity score because they are structurally very similar: both contain an aromatic ring. Histidine and arginine would also receive a favorable score, even though they are structurally different, because both amino acids are basic. Since amino acid sequence is determined by the codons in DNA, certain amino acids are considered similar. For example, the codon UGU codes for cysteine, a relatively small amino acid containing a sulfur group that can uniquely influence overall protein structure via participation in disulfide bonding. A single base change, to UGG, results in tryptophan, a very bulky amino acid that does not contain a sulfur group. From analyzing many protein sequences, molecular biologists have determined that certain amino acid changes occur more frequently than others during evolution; these also receive favorable scores. As in DNA alignments, protein sequences are aligned using gap and extension penalties, with the goal of achieving the highest possible overall score.

Scientists can exploit alignment algorithms to complete complex tasks or aid in scientific research. The Human Genome Project is a prime example; in this project, scientists used DNA sequencing technology to determine the sequence of the entire human genome. While the entire genome consists of approximately 3 billion nucleotides, a typical DNA sequencer can reliably read between 700 and 1,000 nucleotides per reaction. Thus, sequencing the entire genome required the researchers to perform millions of sequencing reactions. Then, these individual sequences needed to be assembled to form the completed genome, much like putting puzzle pieces together. The strategy for assembling the entire genome relied on sequence alignment algorithms that could identify and align overlapping portions of each sequencing reaction. Both the forward and reverse strands of the genomic DNA are sequenced; the alignment program can recognize this and reverse complement the sequence reads as needed. As more sequencing reactions are added,

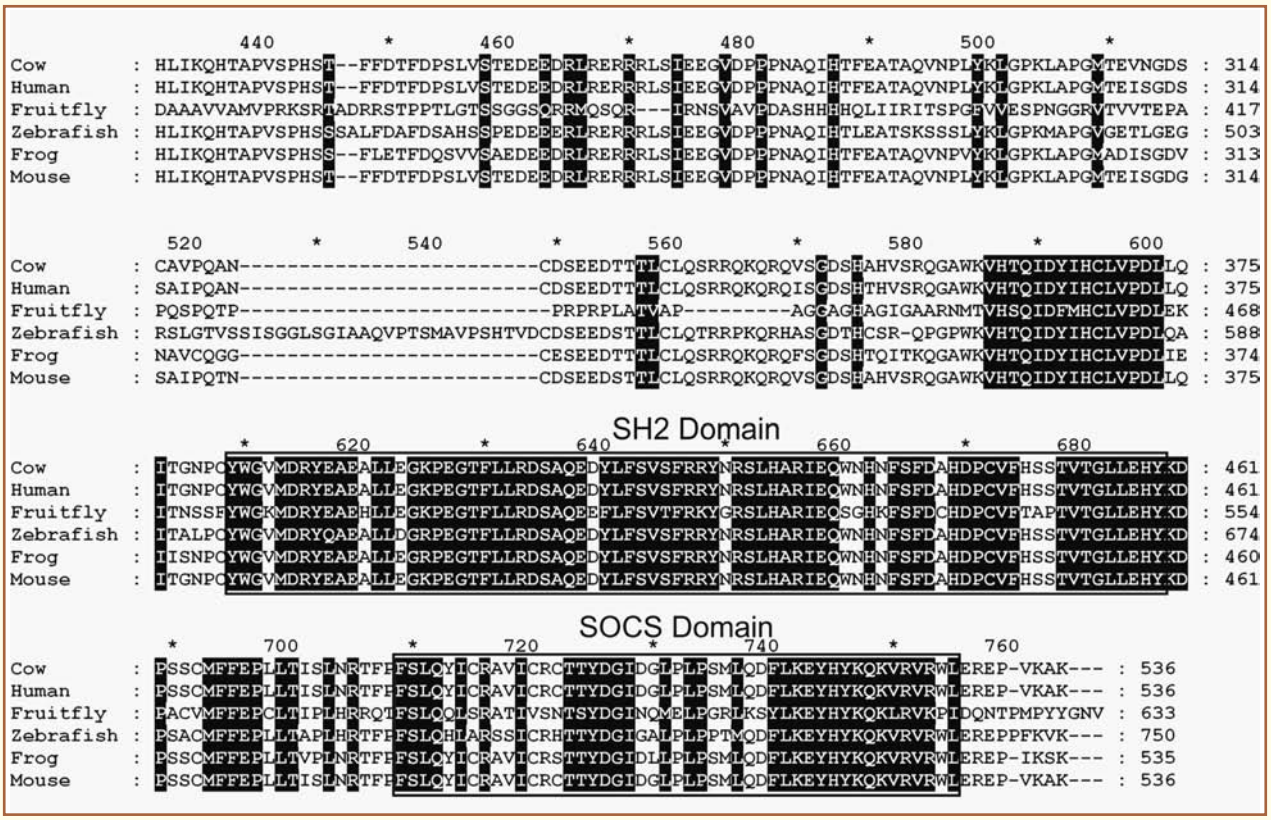
(continues)

(continued)

the resulting sequence assembly reflects a progressively larger portion of contiguous genomic sequence. Combined with genetic maps, this approach was used to construct the entire genome sequence.

Scientists can also use alignment programs to guide their research in the characterization of protein function. As an example, consider the *Drosophila melanogaster* protein, Socs36E, analyzed by a team of scientists from the University of Kentucky and published in a paper titled "Two *Drosophila* Suppressors of Cytokine Signaling (SOCS) Differentially Regulate JAK and EGFR Pathway Activities," in *BMC Cell Biology* in 2004. First, the authors submitted the protein sequence in a BLAST query to identify and retrieve similar sequences from other organisms. In mammals, there are eight suppressor of cytokine signaling (SOCS) proteins, and Socs36E was aligned to all of them using a multiple sequence alignment algorithm. From this, they discovered that *Drosophila* Socs36E is most similar to mammalian SOCS5 and used this information to retrieve the sequences of all SOCS5 orthologues from other species. (Orthologues are versions of the same gene or protein in two or more different species.) Alignment of these sequences using a multiple sequence alignment algorithm allowed for the identification of regions, or domains, of the protein sequence that have been conserved throughout evolution (see the figure titled Multiple Sequence Alignment). All SOCS proteins contain two known functional domains, a centrally located SH2 (Src homology 2) domain and a carboxy-terminal SOCS domain. From the alignment, they identified these domains in the Socs36E sequence. Further analysis using more sequences (from other proteins containing these domains) might allow for the discovery of the critical amino acid residues required for protein function. A scientist could potentially use this information to construct mutant versions of the protein by deleting or altering these amino acids, permitting detailed study of the protein's function.

Sequence alignment algorithms form the staple bioinformatics tools used by life science researchers. Although the first sequence alignment algorithms were introduced nearly 40 years ago, new alignment programs continue to emerge. Many of them focus on increasing the speed and accuracy of the algorithm, making multiple alignments consisting of many sequences feasible. Other algorithms incorporate secondary information into an alignment. For example, if the



Multiple sequence alignment of an orthologous protein from several species. Identical amino acid residues are shaded in black. The SH2 and SOCS domains are indicated. Only the carboxy-terminal portion of the protein alignment is shown. *(Jason Rawlings)*

three-dimensional structures of some of the proteins in a multiple sequence alignment have been solved, this information can be used to assist in aligning these proteins with those that do not have structural information. As scientists learn more about protein structure and function and how they relate to amino acid and DNA sequence information, these algorithms will continue to improve.

Note: The BLAST program is freely available and can be accessed online. URL: www.ncbi.nlm.nih.gov/BLAST. Accessed April 8, 2008.

FURTHER READING

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. "Basic Local Alignment Search Tool." *Journal of Molecular Biology* 215, no. 3 (October 5, 1990): 403–410.

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman. "Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs." *Nucleic Acids Research* 25, no. 17 (September 1, 1997): 3,389–3,402.

Higgins, Desmond G., and Paul M. Sharp. "CLUSTAL: A Package for Performing Multiple Sequence Alignment on a Microcomputer." *Gene* 73, no. 1 (December 15, 1988): 237–244.

Needleman, Saul B., and Christian D. Wunsch. "A General Method Applicable to the Search for Similarities in the Amino Acid Sequence of Two Proteins." *Journal of Molecular Biology* 48 (1970): 443–453.

O'Sullivan, Orla, Karsten Suhre, Chantal Abergel, Desmond G. Higgins, and Cédric Notredame. "3DCoffee: Combining Protein Sequences and Structures within Multiple Sequence Alignments." *Journal of Molecular Biology* 340, no. 2 (July 2004): 385–395.

Rawlings, Jason. S., Gabriela Rennebeck, Susan M. W. Harrison, Rongwen Xi, and Douglas A. Harrison. "Two *Drosophila* Suppressors of Cytokine Signaling (SOCS) Differentially Regulate JAK and EGFR Pathway Activities." *BMC Cell Biology* 5 (October 15, 2004): 38.

Wallace, Iain M., Gordon Blackshields, and Desmond G. Higgins. "Multiple Sequence Alignments." *Current Opinion in Structural Biology* 15, no. 3 (June 2005): 261–266.

(continued from page 83)

when James Watson and Francis Crick revealed the structure of DNA, scientists have been studying genes one at a time. Genomics, the study of entire genomes, allows scientists to study all the genes or all the mRNAs in an organism at the same time. Similarly, proteomics, the analysis of the proteins produced by an organism, allows researchers to study whole sets of proteins that may be related by function, tissue specificity, regulatory mechanisms, or other aspects. Then, a researcher can focus in on individual genes or groups of genes for more intense study. One revelation from the vast amount of sequencing information gathered over the past few decades is that the genetic code, the way DNA sequences encode for the synthesis of proteins, is universal. The basic mechanisms are the same and triplet codons generally encode for the exact same amino acids whether in an *Escherichia coli* cell or a human being. Because of this, once a gene has been identified in one species, computer programs can locate similar genes in other species whose genomes have been sequenced. If the structure of a protein has been determined, then as researchers discover proteins with similar amino acid sequences, they can immediately make inferences about the new protein's structure and function. For example, zinc fingers are a common structural motif found in proteins that bind DNA. If an unknown protein has a region that resembles a zinc finger domain, a scientist may hypothesize that the unknown protein binds DNA, a characteristic that can be easily tested in the lab. From further research of information available to the public in bioinformatics databases, the scientist might learn that proteins with zinc fingers often play a role in the regulation of gene expression and design future experiments accordingly.

The structure of a molecule determines its function; ability to predict the three-dimensional structure of a protein on the basis of its amino acid sequence, which can be determined from the DNA sequence, would be extremely valuable. Bioinformatics specialists have developed computer programs that will suggest likely secondary structures given a particular sequence and other programs that will "thread" the amino acid backbone of an unknown protein through the backbone of a protein that has the same domain. For example, one could thread an unknown protein with a zinc finger domain through the structure of a known zinc finger protein to see a possible three-dimensional structure for the unknown.

GENOME AND PROTEIN DATABASES

After completing the sequencing of a gene or of an entire genome, scientists deposit the information into a database. Examples of widely used databases include GenBank in the United States, DNA Databank of Japan (DDBJ), and the European Molecular Biology Laboratory Database (EMBL). These databases and others are freely accessible online through processing centers such as the National Center for Biotechnology Information (NCBI).

One common application of bioinformatics is to retrieve protein sequences or gather other information about a protein of interest. The Expert Protein

Analysis System (ExPASy) proteomics server is one of the most popular servers for protein information and analysis. The Web site contains links to several databases including the UniProt Knowledgebase. One can begin by entering a type of protein or a protein name, such as adenosine triphosphatase (ATPase), an enzyme that breaks apart molecules of ATP to release energy. The server will return information such as a biochemical description, references in the scientific literature related to the protein, and the actual amino acid sequence of the protein. Depending on the type of search, the server may return the sequences of several related proteins. In order to use the amino acid sequence for further analysis, the sequence must be in the FASTA format, which is simply a specific way to type out the sequence so bioinformatics tools can recognize and read the information. All of the retrieved information can be downloaded and saved to a personal computer for future use.

Because protein structure determines its function, the amino acid sequences are usually well conserved between organisms. Two proteins do not need to have identical amino acid sequences to carry out the same function. When amino acid sequences differ, the nature of the amino acids is often conserved. For example, one small hydrophobic amino acid may substitute for another hydrophobic amino acid and not affect the ability of the protein to function. The site of the mutation also matters. Regions of a protein that do not play an active role in the protein's function are more likely to tolerate mutations than regions that are crucial to the protein's function. To illustrate, consider a screwdriver. Whereas the width and thickness of the end of a screwdriver that fits into the depressions in the head of a screw are critical in the screwdriver's ability to rotate the screw, the shape or structure of the handle grip is not as important; it may or may not contain ridges, can be different lengths, or may be made of wood or plastic and still function effectively. The DNA sequence that encodes the protein will be even less conserved between organisms than the amino acid sequence for several reasons. Some parts of a gene do not code for amino acids—regulatory regions, untranslated regions occurring before the translation start site and after the translation stop codon, and introns (the noncoding intervening sequences located within the coding region) in eukaryotic genes—and are therefore under less selection pressure. Also, many codons are redundant; thus the DNA sequence might change but the amino acid sequence does not.

One can use databases such as GenBank, EMBL, or DDBJ to retrieve the DNA sequence for a protein of interest. One simply would use a pull-down menu to select for protein and then enter the name of the protein, or the type of protein, such as HSF1, which is a protein that regulates transcription of a family of genes called heat shock genes. The returned information page will contain a listing of all the related proteins and their source organisms, such as *Saccharomyces cerevisiae* (a yeast) or *Arabidopsis* (a plant). After selecting one of the choices, more detailed information on that particular protein is provided, including the number of amino acids, links to related literature given as references in PUBMED (a literature search system used by the National Library of Medicine), information about the protein's function or subcellular location, recognizable features within the sequence such as common structural motifs or experimentally determined functions of specific regions, and finally, the actual amino acid sequence. Again, the retrieved information can be saved to a computer's hard drive, and then one can use a server like ExPASy to explore the possible structure and function and perform other analyses. To learn more about a nucleotide sequence, one selects *nucleotide* from the pull-down menu before searching. The information returned is similar to that returned for a protein query in that it includes links to related publications about the sequence or gene product as well as general information about the gene product's function. If known, recognizable features such as promoter elements, ribosome binding sites, exons and introns, and polyA sites are also indicated.

BLAST, which stands for "Basic Local Alignment Search Tool," is another NCBI server that facilitates the analysis of sequence information. A researcher can use BLAST to compare a protein or nucleotide sequence with all the other sequences inputted by scientists from around the world. If other similar sequences do exist, BLAST will also offer a report on the degree of similarity and will give rough information about differences in structure or organization between the two genes. For a more accurate and detailed comparison, one can use an alignment program such as CLUSTAL to analyze the conserved regions, examine the degree of similarity, and compare the structure and organization of the two genes. Multiple alignments consist of the simultaneous comparison of more than two sequences. Alignment allows for the identification of regions of the protein that contain amino acids crucial to the protein's function or identification of sequences that are characteristic of specific protein families or secondary structures. The information may also help a researcher predict the three-dimensional structure or the function of a protein.

Comparison of a protein sequence with other protein sequences often reveals conserved features. These regions of the protein might play an important role in the folding or stabilization of the protein,

interact with other molecules, or directly participate in the protein's function. Knowing where certain amino acids exist within the folded protein helps in predicting the function of that segment. The sequence of amino acids is referred to as the primary structure of a protein, and it determines the secondary structure the chain of amino acids assumes. Alpha helices and beta sheets are examples of common secondary structures. Random coils are regions of the polypeptide that do not form either. Servers such as PSIPRED, maintained by the Bioinformatics Unit of the University College in London, can predict the secondary structure of a protein on the basis of the amino acid sequence. Other servers, such as PredictProtein, maintained by Columbia University, offer more complex structural predictions including secondary structures, accessibility of certain regions to solvent, presence of transmembrane helices, globular regions, disulfide bonds, and more. Structural biologists deposit their three-dimensional structural coordinates, obtained by techniques such as X-ray crystallography, into the Protein Data Bank (PDB). Researchers can access this information and view the three-dimensional structures of many proteins. Comparison with sequences of their own protein of interest can reveal information about structural similarities.

See also BIOMOLECULES; BIOTECHNOLOGY; DNA SEQUENCING; GENE EXPRESSION; GENETICS; GENOMES; HUMAN GENOME PROJECT; MOLECULAR BIOLOGY.

FURTHER READING

Batiza, Ann Finney. *Bioinformatics, Genomics, and Proteomics: Getting the Big Picture*. Philadelphia: Chelsea House, 2006.

"The Expert Protein Analysis System (ExPASy) Proteomics Server." Available online. URL: http://www.expasy.org. Updated December 11, 2007.

Krane, Dan E., and Michael L. Raymer. *Fundamental Concepts of Bioinformatics*. San Francisco: Benjamin Cummings, 2003.

National Center for Biotechnology Information home page. Available online. URL: http://www.ncbi.nlm.nih.gov. Updated October 17, 2007.

biological classification

One major goal of life science is to discover and catalogue all the living organisms in the biosphere. The Greek philosopher Aristotle was the first to attempt this never-ending endeavor in the fourth century B.C.E., when he recorded descriptions of as many animals as he could, close to 500 species. He organized them into groups based on observable characteristics, such as feathers, number of legs, or presence of horns. As more species became known, scientists gave them long, descriptive names in their native languages, which hindered the passage of information to other scientists, especially those from countries having different native languages. In the 18th century the Swedish botanist Carl Linneaus introduced the system of binomial nomenclature to facilitate identification of organisms and communication among scientists about various life-forms, the numbers of which naturalists believed approached approximately 10,000. Scientists across the world quickly adopted binomial nomenclature, which is still used today. Linnaeus also proposed a hierarchical system for classifying organisms, with each level becoming more encompassing.

The name for the discipline of life science concerned with identifying, classifying, and naming the diverse life-forms is *taxonomy*. In the 250 years since Linnaeus, the number of species identified and given a latinized name has grown to more than 1.78 million. Biologists suspect that the unknown biosphere holds at least as many as 10 times that number, and some estimates approach 100 million. The job of taxonomists is enormous and consists of more than simply naming new organisms. The world-renowned biologist Edward O. Wilson, of the Harvard University Museum of Comparative Zoology, has called taxonomy "the pioneering exploration of life on a little known planet." Based on evolutionary principles, modern taxonomy is more specifically referred to as systematics, a science with the goal of revealing the evolutionary history of all the organisms on the planet. This approach has increased biologists' understanding of different organisms. The work of systematists has led to the development of the tree of life and provides the foundation for ecological studies and conservation efforts.

DATA USED FOR CLASSIFICATION

Early taxonomists relied on studies of comparative anatomy and embryology, as morphological characteristics were easily observable and the current preferred basis for classification had not yet been proposed. Most naturalists believed species were static, created by a supernatural being in their existing forms. In the middle of the 19th century, the British naturalist Charles Darwin proposed the theory of evolution by means of natural selection, suggesting that new species emerged by descent with modification. Ever since Darwin's ideas gained widespread acceptance, systematists have attempted to arrange organisms phylogenetically, that is, according to their evolutionary histories. Today, biologists still rely on morphological and behavioral characteristics but also have molecular information by which they formulate classification decisions. For example, molecular methods such as nucleic acid and protein sequencing provide much information regarding the

evolutionary history of a species or group of species. While molecular approaches reveal information about the relatedness of living species, the fossil record provides information on how those relationships came to be.

A fossil can be either a remnant of an organism that lived in the past or evidence that an organism lived in the past. Fossils form when an organism or part of an organism becomes buried in sediment and either mineralizes or leaves impressions that are preserved in the Earth's crust. Remnants include structures, such as bones, teeth, or shells, that were once part of the organism but have become mineralized. Other types of fossils include impressions, molds, or casts of the original organism or its parts. Impressions form when the shape and texture of the organism are preserved in the sediment that settles around the remains. The remains themselves may decompose, but the indentations left in the surrounding sediment are preserved as it turns into rock. If an empty space remains after the organic matter decomposes, the structure left behind is called a mold. If minerals that crystallize fill the empty space after the organic matter decays, the structure left behind is called a cast. Trace fossils, such as hardened excrement or indentations such as footprints or burrows, also provide useful details. Fossils not only provide morphological information, such as an organism's size, approximate weight, and number and types of external structures, but also indicate the geological period during which the organisms lived. The material within a layer of sediment is all derived from the same period, and the layer below it is older. Thus one can follow the appearance or disappearance of different life-forms through studying the changes in successive layers of sediment.

Anatomical information, both from fossil evidence and from present-day organisms, helps biologists reconstruct phylogenies. Generally, organisms that are closely related share similar structures. For example, the phylum Vertebrata is defined by the common presence of a backbone, but members of the phylum Vertebrata can exhibit very diverse overall morphologies. Consider the appearance of a representative organism from three classes of vertebrates: a human is a mammal, a sparrow is a bird, and a bullfrog is an amphibian. The forelimbs of these animals all look different, but a closer examination of the arrangement of bones within the forelimbs will reveal that all these animals have a humerus, a radius, and an ulna. Developmentally, these bones are all derived from the same embryonic structures. Despite the very different outward appearances of the forelimbs, structures such as these are said to be homologous, meaning they are similar as a result of shared ancestry. Homologous structures in different species are derived from one structure in a common ancestor.

Gross morphological information can be misleading. Combining the structural information with molecular data will reveal a more complete picture. Sometimes organisms look similar but are not closely related. For example, bats and dragonflies both have wings used for flight. Bats, however, are mammals and are more closely related to other nonwinged organisms such as humans and fish than they are to any insect. In this case, the wings are called analogous structures, meaning they are similar as a result of convergent evolution rather than of shared ancestry. Wings for flight evolved separately in insects and in bats. Molecular data can reveal such misconceptions that result from organisms' adapting to similar conditions in the same way.

Biochemical data in the form of sequence information from deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins enable biologists to determine phylogenies even when ancestral species are extinct or fossil evidence is not available for examination. Regions of DNA that encode for functional gene products are highly conserved compared to those that do not encode for functional gene products. Even within a gene that encodes a protein, differences in the nucleic acid and even the amino acid sequence of the protein exist; they are degeneracy in the genetic code and the fact that some amino acids are simply space fillers within a polypeptide, meaning they do not play an active role in the function of the protein. Because of this, comparison of sequences between organisms provides information about their evolutionary relatedness. The concept of homology can be applied to molecular sequences just as it is applied to anatomical structures. When organisms share a high proportion of sequences within a gene or region of DNA, one may conclude that the sequences or genes are homologous, meaning those regions of DNA are derived from the same original sequence belonging to a common ancestor. Organisms will have more sequence information in common with organisms that share a recent common ancestor compared to organisms with more distant common ancestry. Taken alone, molecular sequence information only reveals relative relationships (i.e., these two organisms have more sequence information in common, thus are more closely related than two others who share less homology). The same is true for chronological information. Events can only be ordered relative to one another if fossil evidence from different geological ages is available for similar organisms. If it is, the degrees of difference in sequence can serve as a molecular yardstick.

Biochemical evidence has helped biologists understand the evolutionary relationships among plants, algae, and bacteria that are capable of photosynthesis, the process by which organisms convert

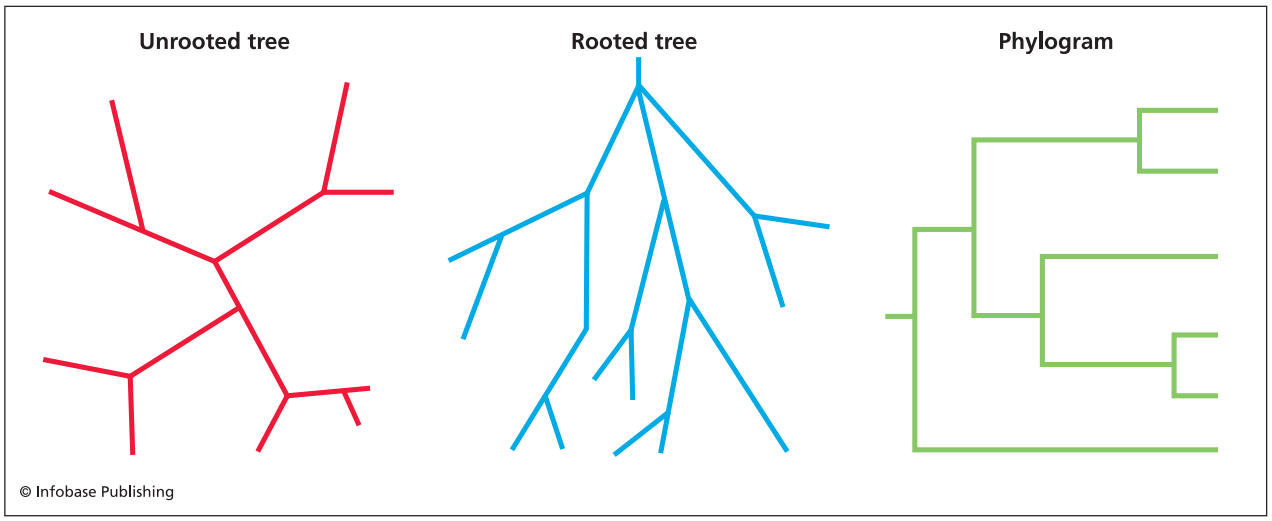
radiant energy from the Sun into chemical energy in the form of carbohydrates. While all of these groups of organisms are capable of photosynthesis, they have different types of pigments that absorb the light energy, with the groups of organisms that are more closely related sharing the same kinds. In another example, scientists have examined the genetic divergence in hemoglobin genes to elucidate the phylogeny of hominids, including humans, apes, chimpanzees, gorillas, and orangutans.

Genomics has facilitated classification efforts through the construction of genome maps that can be used to distinguish species. Complete genome sequences are available for hundreds of species from a variety of taxonomic groups, and advancements in biotechnology and bioinformatics have increased the pace at which biologists complete the sequencing of new genomes. The availability of information via the Internet eases communication of raw data and its analysis. High-quality photographs and databases are readily available for comparison with identified organisms within and between phylogenetic groups. Because of currently available methods and new technology, many biologists believe that with an effort like that put into the Human Genome Project, it is not unrealistic to think that the undiscovered biosphere could be completely explored within a few decades—if the degree of funding and the number of personnel currently performing active research toward this goal increase.

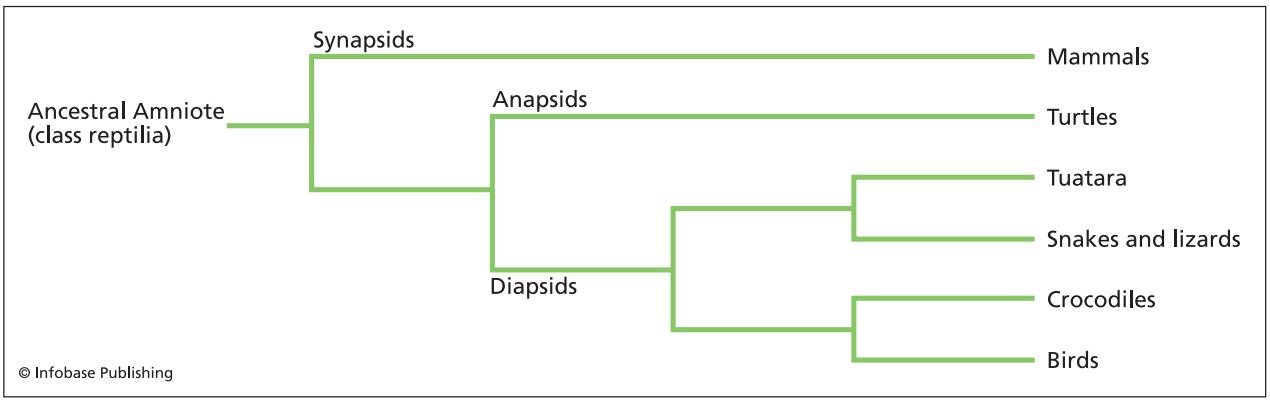
PHYLOGENETIC TREES

Taxonomists today have much more information by which they can classify organisms. Whereas 250 years ago Linnaeus resorted to similarities in basic appearance to classify plants and animals, now taxonomists group organisms on the basis of phylogenies. The resulting hierarchical classification system therefore also reveals their evolutionary relationships to other groups of organisms. Phylogenetic trees conveniently depict these evolutionary implications by branching. Branch points, or nodes, represent common ancestors that diverged by splitting and developing into two new separate lineages. The deeper the common branch points for two groups of organisms, the more distant their most recent common ancestor is. Trees may have scaled or nonscaled branches. The length of scaled branches represents the time or degree of evolutionary change. Trees may be rooted or not; unrooted trees do not reveal the position of the common ancestor, only patterns of relatedness, and many different rooted trees may be derived from a single unrooted tree. Because classification is hierarchical, each branch point leads to groups that are more exclusive. Groups of organisms are called monophyletic if they consist only of an evolutionary ancestor and all of its descendents.

Cladograms are branched depictions that represent shared characteristics. Their patterns may or may not reflect the true evolutionary history; if the shared characteristics are homologous, then they do. The term *clade* refers to a group of organisms that consists of all the organisms on all the stems derived from a single branch point. Cladistics is the mathematical method of grouping organisms into clades based on statistical data on the degree of relatedness of different species. Computers have facilitated cladistic analyses.



Phylogenetic trees are branched diagrams that depict evolutionary relationships. Rooted trees indicate the position of a shared ancestor, whereas unrooted trees do not. Phylograms have scaled branches that reflect the degree of evolutionary change.



This phylogenetic tree of the class Reptilia shows that birds are more closely related to crocodiles than other reptiles, and that mammals are derived from the same common ancestor as the ancestor that gave rise to anapsid and diapsid reptilian lineages.

Systematists consider monophyletic groups the only valid form of taxonomic category (also called a taxon), as they have a sound theoretical basis and lead to combinations of groups that naturally share a greater number of characteristics. The node that defines a taxon may be deep or shallow, reflecting more encompassing and more exclusive categories. For example, one can refer to the clade of amniotes; or, within that, the smaller clade of diapsid reptiles; or, even more specific, the crocodilian clade. A paraphyletic group of organisms contains an ancestor and only some of its descendants. For example, a grouping that consisted of the ancestral amniote, turtles, tuataras, snakes, lizards, and crocodiles but did not include mammals or birds would be paraphyletic. If the group contained mammals, turtles, tuataras, snakes, lizards, crocodiles, and birds, but not their common ancestor, the group would be polyphyletic.

The National Science Foundation supports an effort called Assembling the Tree of Life (ATOL), the goal of which is to outline the history of organismal evolution by describing the relationships among the estimated 1.7 million species described to date. The simple notion of such a tree serves as a reminder that all life on Earth is connected through the passage of genetic information. The development of such a comprehensive tree of life would organize all biological knowledge and provide a framework for future investigations.

HIERARCHICAL ORGANIZATION AND MODERN CLASSIFICATION

In order to identify new species, biologists must study organisms exhaustively and compare them to descriptions of previously identified organisms to determine whether an organism has already been identified. The scientist begins by grouping it with other organisms that exhibit the same general characteristics, then focuses on more distinguishing characteristics until reaching the most defined taxonomic group, the species, a group of reproductively isolated groups of organisms.

The separation of a species is predominantly natural: that is, species are principally defined by a biological mechanism, the ability to reproduce with one another. The higher levels of classification are man-made. The category above species is genus. In modern scientific literature, organisms are referred to by Latin names for their genus and species, as well as a third designation, a letter representing the person who named the organism. In order of increasing generality, additional levels of classification include families, orders, classes, phyla, kingdoms, and domains. Some of the categories are divided further into subcategories. For example, human beings belong to the following taxonomic categories: domain of Eukarya, kingdom of Animalia, phylum of Chordata (subphylum of Vertebrata), class of Mammalia, order of Primata (suborder of Anthropoidea), family of Hominoidea, genus of *Homo,* and species of *sapiens*. In short, humans are referred to scientifically as *Homo sapiens* L. (the *L* stands for Linnaeus, who first named humans).

Many species have common names that translate differently in different languages, but the scientific Latin names are universal. By using these names in scientific communication about different species, scientists can be sure they are referring to the same organisms. In this manner, they can build on the research of others and advance knowledge in their fields more quickly and efficiently.

In 1735 Linnaeus published *Systema naturae* (System of nature), a treatise that firmly established his scientific reputation and went through 13 editions. In it, Linnaeus strove to arrange the entire natural world into three kingdoms: *Regnum ani-*

male (the animal kingdom), *Regnum vegetabile* (the plant kingdom), and *Regnum lappideum* (the mineral kingdom), which consisted of nonliving matter such as rocks and minerals. He included fungi with plants and categorized different microorganisms, which had been discovered and described by the Dutch draper Antoni van Leeuwenhoek in the 17th century, as either plants or animals. Despite some obvious problems, most scientists did not question Linnaeus's separation of life into two main kingdoms. A century later, the German biologist Ernst Haeckel proposed a third living kingdom consisting of single-celled organisms, Protista, further divided into several phyla including algae, protozoa, and monerans, which he noted did not contain nuclei. The monerans included bacteria and blue-green algae, a prokaryotic organism that today is called cyanobacteria. Haeckel also was the first to construct a phylogenetic tree based on clades.

The American biologist Herbert F. Copeland divided Haeckel's monerans into two kingdoms: Monera, which consisted of all the prokaryotic organisms, and Protoctista, which consisted of the algae and protozoans. In 1969 the American ecologist Robert Whittaker instituted the five-kingdom system that became widely used and that clearly distinguished fungi from plants. Whittaker's five kingdoms were Monera, Protoctista (or more simply, Protista), Fungi, Plantae, and Animalia.

Though biologists still refer to Whittaker's five-kingdom system, the most modern widely accepted classification system, proposed by Carl Woese in 1990, recognizes that the prokaryotic organisms traditionally placed in the kingdom Monera include organisms from two distinct lineages. By studying and comparing the ribosomal RNA genes of various life-forms, Woese concluded that sufficient molecular evidence existed to warrant the proposal of three domains of life: Archaea, Bacteria, and Eukarya. Archaea and Bacteria both consist of prokaryotic organisms, but the two groups are as distinct from each other as they are from eukaryotic organisms. Woese believed the differences were so significant that the simple addition of a sixth kingdom was not sufficient to convey the extent of the differences.

Molecular evidence has also eliminated the kingdom Protoctista as a valid taxon, though many biologists still refer to it for convenience when discussing single-celled eukaryotic organisms other than yeasts. Single-celled eukaryotes are now found scattered throughout the eukaryotic kingdoms. A few decades ago, all of eukaryotes were included in one of four kingdoms: Protoctista, Fungi, Plantae, and Animalia. Taxonomists now believe as many as 21 kingdoms may be required. For example, whereas biologists once grouped seaweeds with algae within Protoctista, they now have separated brown seaweeds (kelp) and red seaweeds into distinct clades, and many botanists consider green seaweeds as plants. Various slime molds, formerly associated at different times with animals, plants, protozoa, and fungi, now have their own kingdoms. While many more eukaryotic kingdoms than prokaryotic kingdoms exist, this does not reflect the presumed relative numbers of existing species belonging to each domain, but rather a greater effort to identify organisms within the eukaryotic domain and particularly those in taxonomic groups including humans. Though biologists have created many eukaryotic kingdoms, one must remember that the eukaryotic organisms all are closely related, having shared a common ancestor slightly more than 1 billion years ago.

The following outline summarizes one modern classification scheme. The three domains each contain several kingdoms.

1. Bacteria
    a. Proteobacteria (purple bacteria)
    b. Planctomyces and Chlamydiae
    c. Spirochaetes
    d. Bacteroides, Flavobacteria, and relatives
    e. Green Sulfur Bacteria
    f. Gram-Positive Bacteria with high G-C
    g. Gram-Positive Bacteria with low G-C
    h. Cyanobacteria
    i. Green Nonsulfur Bacteria
    j. Thermotogales
    k. Hydrogenobacter/Aquifex

2. Archaea
    a. Euryarchaeota
    b. Crenarchaeota
    c. Karyarchaeota

3. Eukarya
    a. Diplomonads
    b. Microsporida (sporozoa)
    c. Parabasalids
    d. Myxomycota (plasmodial slime molds)
    e. Euglenozoa
    f. Naegleria
    g. Entamoeba
    h. Acrasiomycota (cellular slime molds)
    i. Rhodophyta (red seaweed)
    j. Ciliata
    k. Dinoflagellata
    l. Apicomplexa
    m. Labyrinthulids (slime nets)
    n. Oomycota
    o. Xanthophyta

p. Chrysophyta
q. Phaeophyta (brown seaweed)
r. Diatoms
s. Plantae
t. Fungi
u. Animalia (metazoa)

See also ALGAE; ARCHAEA; BACTERIA (EUBACTERIA); BIOINFORMATICS; CUVIER, GEORGES, BARON; EUKARYA; EVOLUTION, THEORY OF; FUNGI; GENOMES; HAECKEL, ERNST; HISTORY OF LIFE; INVERTEBRATES; LINNAEUS, CARL; PLANT DIVERSITY; PROTOZOA; SLIME MOLDS; VERTEBRATES; WOESE, CARL.

FURTHER READING

Hodkinson, Trevor R., and John A. N. Parnell, eds. *Reconstructing the Tree of Life: Taxonomy and Systematics of Species Rich Taxa*. Boca Raton, Fla.: CRC Press, 2007.

Maddison, D. R., and K.-S. Schulz, eds. "The Tree of Life Web Project, 1996–2006." Available online. URL: http://tolweb.org/tree/. Accessed January 22, 2008.

Nielsen, Claus. *Animal Evolution: Interrelationships of the Living Phyla*. 2nd ed. New York: Oxford University Press, 2001.

Tudge, Colin. *The Variety of Life: A Survey and a Celebration of all the Creatures That Have Ever Lived*. New York: Oxford University Press, 2000.

biological membranes

A cell is defined by the cell membrane, also called the plasma membrane or cytoplasmic membrane, the structure that separates the contents of the cell from the external environment. The main functions of the cell membrane are to contain the cytoplasm and to restrict the passage of substances into and out of the cell. Because membranes have a high lipid content, small uncharged particles can diffuse through membranes, but ions and larger molecules such as proteins or nucleic acid cannot traverse membranes without assistance. The nuclei of eukaryotic cells are bound by a double membrane that extends outward into the cytoplasm, forming a network that encloses the endoplasmic reticulum and Golgi apparatus, structures that are involved in the synthesis, modification, and transport of macromolecules. Other eukaryotic membrane-bound organelles include mitochondria, chloroplasts, lysosomes, and vacuoles. Some prokaryotic organisms have extensive invaginations of their cell membrane that increase the surface area across which membrane-dependent biochemical reactions such as photosynthesis and cellular respiration occur.

STRUCTURE

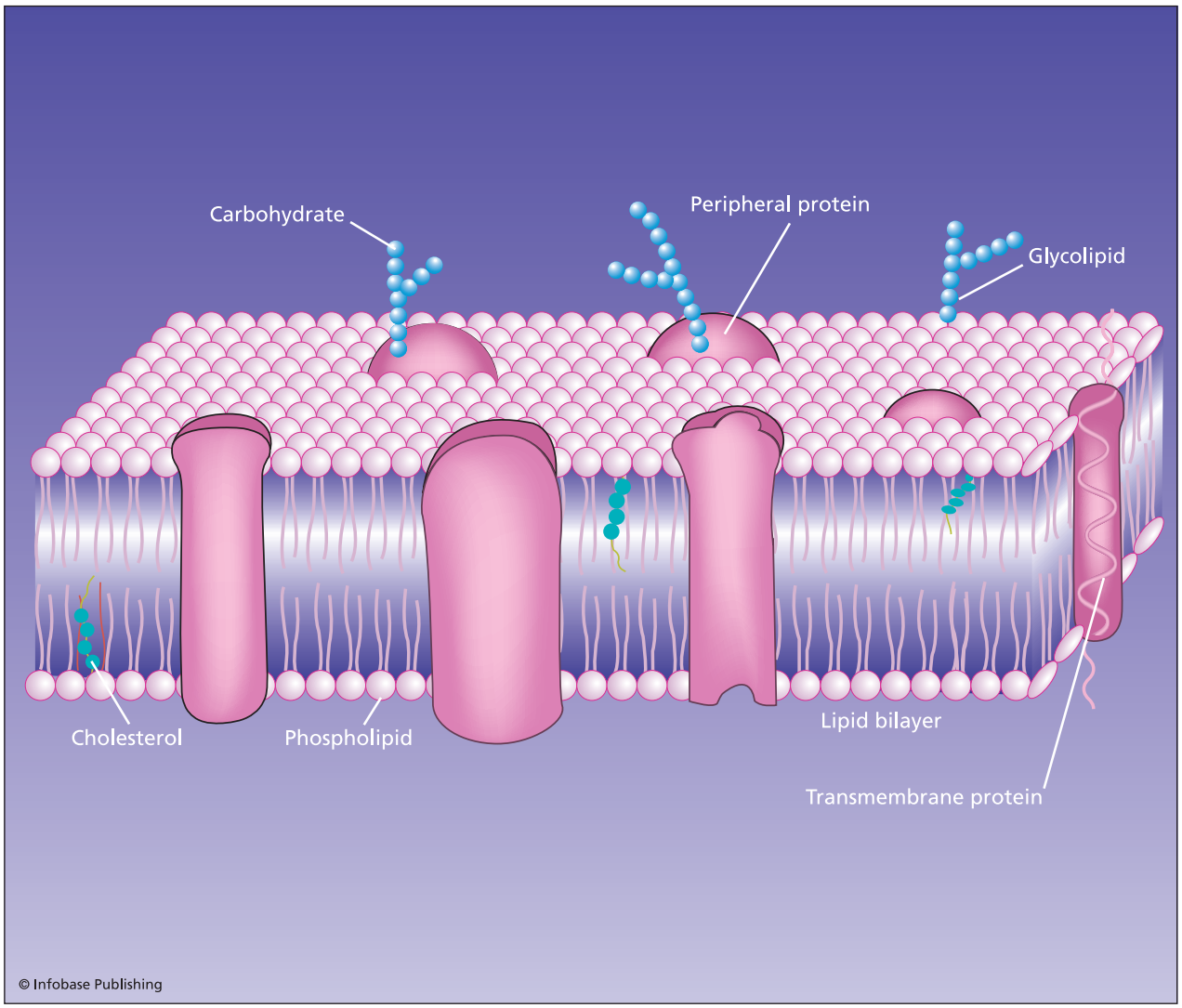
In general, biological membranes consist of a phospholipid bilayer with numerous embedded proteins. Phospholipids contain a polar head and two nonpolar fatty acid tails. In a cell membrane, the phospholipid molecules arrange themselves so the nonpolar tails align with one another and the polar heads align with one another. Because the cytoplasm of the cell and, in most cases, the external environment is aqueous, the aligned phospholipids form a bilayer, with the nonpolar regions hidden from the water and the polar heads exposed to the external environment and to the cytoplasm of the cell. This phospholipid bilayer is selectively permeable, meaning it allows some substances but not others to pass through it. Small molecules such as water or nonpolar molecules that can dissolve in lipids can pass through the membrane. Charged particles or larger molecules such as proteins, carbohydrates, and nucleic acids cannot move through the nonpolar region; thus the membrane serves as an obstacle for these substances.

Though a phospholipid bilayer forms the basis of the cell membrane, proteins make up about 50 percent or more of the total composition. Some of the membrane proteins completely span the bilayer and have ends protruding into the cytoplasm and the cell's exterior. Carbohydrate side chains linked to the membrane proteins often extend from the protein, providing additional specificity for molecular recognition. Other membrane proteins such as some enzymes are only embedded in the interior layer. Either way, the membrane proteins are held in place by hydrophobic (nonpolar) interactions between the protein and the nonpolar center of the bilayer. Proteins are simply chains of amino acids, and the amino acids can be either hydrophilic (having a strong affinity for water) or hydrophobic (lacking an affinity for water). Membrane proteins often have at least one long hydrophobic stretch that helps position it in the bilayer. The many different types of membrane proteins function as receptors for hormones or other chemical signals, as enzymes, and as transport proteins.

According to the fluid mosaic model of membrane structure, the phospholipids and the membrane proteins can diffuse laterally throughout the bilayer. This is similar to moving through a crowded subway station. As one person moves, the people he approaches slide over just enough so he can pass through, then they close the distance as soon as he bypasses them. The model is called *fluid* because of the free movement of molecules within the membrane, and *mosaic* because of the occasional globular proteins that decorate the exterior surface.

TRANSPORT ACROSS MEMBRANES

Different mechanisms exist for moving substances through biological membranes. As was already stated, despite their polarity, water molecules can diffuse through membranes by a process termed osmosis. In

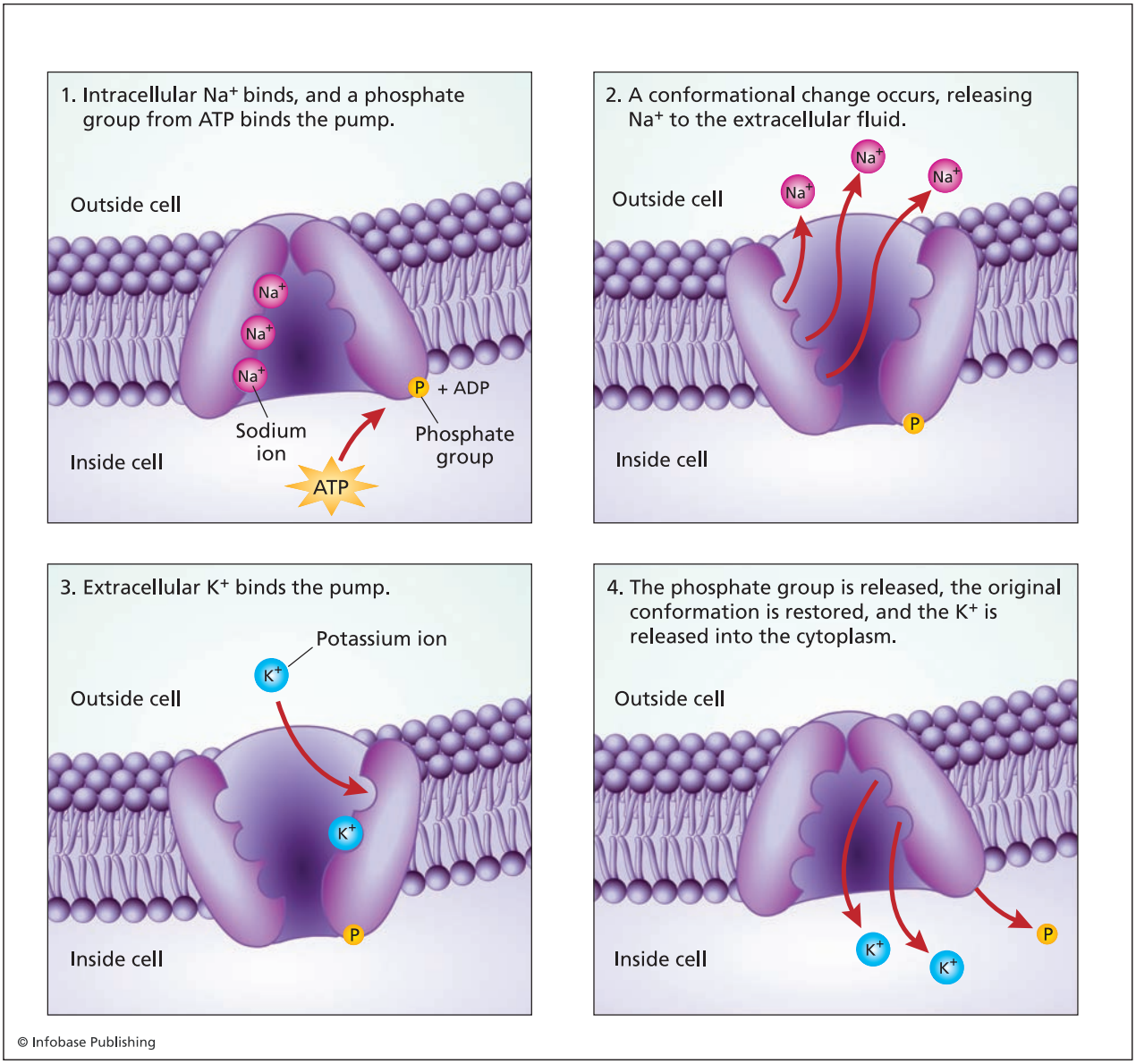


Cell membranes consist of a phospholipid bilayer with numerous embedded proteins.

the presence of solute, water molecules will surround the solute molecules to dissolve them, effectively lowering the availability of free water molecules. Because of this, the water will move from an area with a low solute concentration to an area with a higher solute concentration without additional energy input. Cells in an isotonic environment, a situation in which the solute concentration inside the cell equals the solute concentration outside the cell, will have equal rates of water movement into and out of the cell. In a hypotonic environment, the solute concentration outside the cell is lower than in the cell's cytoplasm. In this situation, water will diffuse into the cell, causing it to swell. Cell walls prevent the lysis or bursting open of cells that live in hypotonic environments by providing structural rigidity. Other adaptations for survival in hypotonic environments include mechanisms for transporting dissolved solutes out of the cell or contractile vacuoles, structures that collect and remove excess water from the cytoplasm.

The concentration of solute is higher outside than inside the cell in a hypertonic environment. Water will move out of the cell, causing it to dehydrate and shrivel up.

Other small uncharged molecules can also freely diffuse through biological membranes. Nonpolar substances such as steroid hormones are soluble in lipids, thus can dissolve in the nonpolar portion of the bilayer to pass through. Nonpolar substances such as the gases molecular oxygen (O_2) and carbon dioxide (CO_2) are small enough to slip between the lipids of the bilayer. Even polar substances that are small enough, such as ethanol or urea, can diffuse through membranes. Many lipid-soluble drugs are designed specifically so they can easily traverse membranes to penetrate cells.



The sodium-potassium pump maintains a higher concentration of potassium inside the cell and a higher concentration of sodium outside the cell.

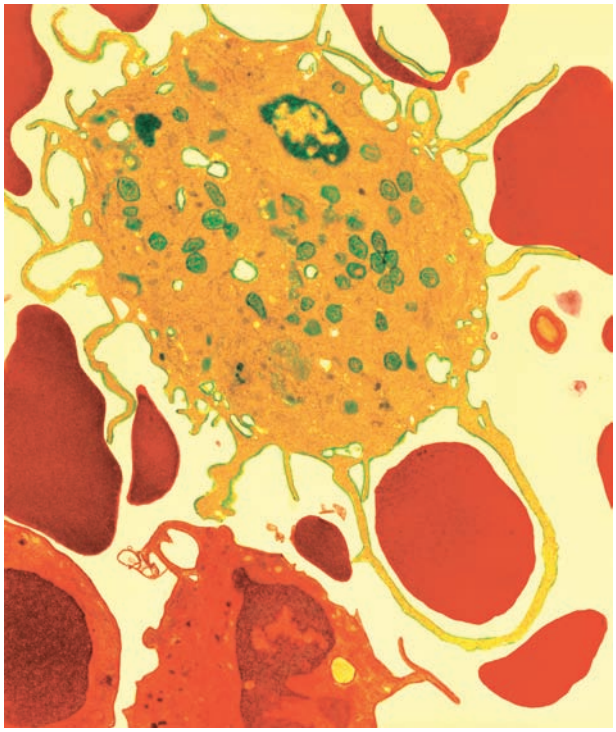
Most substances cannot diffuse through biological membranes because they either are too large or are charged and therefore cannot penetrate the hydrophobic interior of the phospholipid bilayer. Specialized proteins span the membrane to facilitate the transport of these substances. When transport proteins, also called carrier proteins, are required to move a solute across a membrane even though the solute is moving down its concentration gradient, the process is called facilitated diffusion. Because physical laws are still controlling the movement, no additional energy is required to power the transport. The transport proteins are specific for the molecules they translocate, and they can transport the solute in either direction depending on the concentration gradient. The solute first binds the carrier protein on the side of the membrane with the higher concentration. The binding causes a conformational change, a slight change in the shape of the carrier protein that exposes the solute to the other side of the membrane where the concentration is lower, and the carrier protein releases the solute. Amino acids and sugars are examples of molecules that move across membranes by facilitated diffusion.

Other mechanisms exist to transport solutes from areas of lower to higher concentration. Because this sort of transport requires the expenditure of energy, it is called active transport. If solutes could

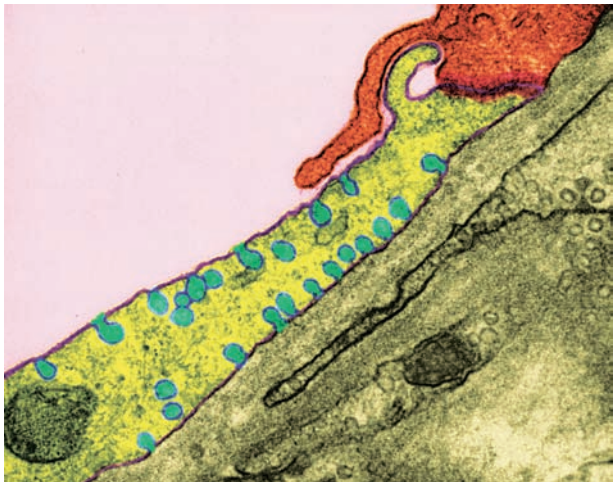
only move through biological membranes down their concentration gradients, the internal environment of the cell and of all the cellular organelles would be identical to that of the extracellular fluids. The work performed by the cell requires unique conditions for a variety of tasks, and active transport makes those conditions possible.

Living cells have voltages across their membranes, meaning the electrical charge inside the cytoplasm differs from the charge of the extracellular fluid. This unequal distribution of charges is called a membrane potential, and it is a form of potential energy. More anions (negatively charged ions) are present inside the cell, giving it a more negative charge than outside the cell. In combination with the chemical concentration gradient of the ions, the electrical gradient can be used by the cell to perform work, such as transmitting a neural impulse. Cells also employ membrane potentials to perform work of cellular respiration and photosynthesis.

The sodium-potassium pump demonstrates a specific example of active transport. The concentration of sodium ions (Na^+) is lower inside the cell than outside the cell, and the concentration of potassium ions (K^+) is higher inside the cell than outside the cell. The binding of intracellular Na^+ to the pump protein stimulates the phosphorylation of the protein by adenosine triphosphate (ATP). A resulting conformational change releases the Na^+ to the extracellular fluid, and then K^+ binds the protein. The phosphate group is released from the pump, restoring the original conformation, which releases the K^+ to the cytoplasm and prepares the protein for binding new Na^+ ions. In this manner, three Na^+ ions are exchanged for two K^+ ions for each ATP that is hydrolyzed.



The macrophage in this transmission electron micrograph is engulfing a red blood cell by phagocytosis. Note the membrane of the macrophage has completely encircled the red blood cell. *(NIBSC/Photo Researchers, Inc.)*



This capillary endothelial cell is drawing substances into the cell by pinocytosis, a specialized form of endocytosis. Pinocytotic vesicles are shown in blue. *(Copyright Dennis Kunkel Microscopy, Inc.)*

Cotransport is a specialized form of active transport that involves the coupling of a solute that moves down its concentration gradient with the transport of another solute against its concentration gradient. Often, the gradient used to power this type of transport is derived from a proton gradient. The hydrolysis of ATP drives the transport of hydrogen ions (H^+) across a membrane, creating an electrochemical gradient. As the H^+ flows back through the membrane down its gradient, amino acids, sugars, or other nutrients "piggyback" through the membrane carrier protein.

Solutes that are too large to move through carrier proteins are transported through biological membranes by the complementary processes of endocytosis and exocytosis. In endocytosis, the cell membrane reaches out and around, forming an invagination that entraps the substances contained in the enclosed fluid. This forms a membrane-bound vesicle inside the cytoplasm that can fuse with lysosomes, organelles that contain digestive enzymes that break down the substances taken into the cell. Phagocytosis is the endocytosis of solid particles such as debris from a blood clot or a bacterial cell. In receptor-mediated endocytosis, receptors on the surface of a cell bind specific particles, and the cell folds inward, forming a sac around

the attached particles. Pinocytosis is the uptake of droplets of extracellular fluid by endocytosis. In exocytosis, substances are packaged into vesicles that fuse with the cell membrane and are released to the cell's exterior. Secreted proteins are transported out of the cell by exocytosis. Membranes are also generated and membrane proteins are moved to the surface by this mechanism.

See also BIOMOLECULES; CELL BIOLOGY; CELL COMMUNICATION; EUKARYOTIC CELLS; PHYSIOLOGY; PROKARYOTIC CELLS.

FURTHER READING

Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell.* 5th ed. New York: Garland Science, 2007.

Campbell, Neil A., Jane B. Reece, Lisa A. Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, and Robert B. Jackson. *Biology.* 8th ed. San Francisco, Calif.: Pearson Benjamin Cummings, 2008.

Sztul, Elizabeth. "Cell membranes." In AccessScience@ McGraw-Hill. Available online. URL: http://www.accessscience.com, DOI 10.1036/1097-8542.116500. Accessed January 14, 2008.

biological weapons Biological weapons are biological agents, including pathogenic organisms or toxins produced by an organism, that are used for the purpose of inflicting harm or incapacitating others. Ideal biological weapons are highly infective, potent, and easy to deliver and have available vaccines directed against them. A major disadvantage of biological weapons is the possibility of backfire, since containment is difficult once a contagious disease has begun spreading. Biological weapons are usually easy to produce in mass quantities but difficult to deliver effectively to the enemy. Aerosols are the most common form of delivery. The aerosol must be either inhaled or ingested and usually must incubate for a few days before inducing symptoms.

The use of biological weapons against an enemy for hostile purposes or during armed conflict is called biological warfare. In 1972 the Biological and Toxin Weapons Convention made it illegal to develop, produce, stockpile, or acquire biological weapons. As of June 2000, more than 144 countries recognized by the United Nations, including the United States, the United Kingdom, and the Russian Federation, had signed the treaty. Military analysts agree that since biological warfare does not immediately incapacitate an enemy, other weaponry is more effective to prevent an immediate threat. Bioterrorism is another serious concern, since biological weaponry requires minimal expertise, economic outlay, and equipment. The threat of bioterrorism, the use of biological weapons as a means of terrorizing or coercion, has gained more recognition since the events following the attacks on the United States of America on September 11, 2001. Less than one month later, mail containing spores from the bacterial pathogen anthrax caused 22 confirmed cases of anthrax and five deaths. Fortunately, effective defenses are available against most biological weapons. As soon as the biological agent was identified as anthrax, people who might have been exposed were treated with the antibiotic ciprofloxacin, a precaution that may have prevented additional cases.

The Centers for Disease Control and Prevention (CDC) lists approximately 27 biological agents that could serve as potential biological weapons. The list includes bacteria, viruses, toxins, and other biological products. The CDC categorizes the agents into three categories (A, B, or C) based on the ease with which they spread and the severity of illnesses they cause.

The most serious threats, those belonging to category A, include the pathogens responsible for anthrax, smallpox, plague, viral hemorrhagic fevers, botulism, and tularemia. Anthrax is caused by the bacterium *Bacillus anthracis*. Characteristics that make it ideal as a biological weapon include its ability to form resistant spores that can easily be spread in the form of an aerosol, its high infectivity, and the availability of a preventative vaccine. The manifestation of anthrax depends on the means by which someone is exposed. Inhalation of anthrax spores is the likely mode of transmission if it is used as a biological weapon. Inhalation anthrax causes coldlike symptoms to occur within a week, but treatment is most effective post exposure prior to the establishment of infection. The mortality rate is 50 to 90 percent. Smallpox is caused by a very contagious variola virus and also has a high mortality rate (30 percent), and it can be prevented by vaccination. Because of the success of a worldwide vaccination effort during the middle of the 20th century, smallpox has been eradicated. The last documented U.S. case occurred in 1949, and the last known naturally occurring case worldwide was in Somalia in 1977. The only known specimens of this virus exist at high-security labs in the U.S. CDC and in the former Soviet State Research Center for Virology and Biotechnology. Routine vaccination for the virus that causes smallpox ended in 1972 since it is no longer present in the population; that means the general population is vulnerable to the use of variola virus as an agent for bioterrorism. The plague is caused by the bacterium *Yersinia pestis* and is normally transmitted by bites from fleas carried on rodents. Infection can lead to either the bubonic or the pneumonic form of the plague,

but only the pneumonic form can be transmitted from person to person, and an aerosol containing *Yersinia* would cause the pneumonic form. Treatment with antibiotics can cure pneumonic plague if administered within 24 hours of symptom development. Viral hemorrhagic fevers (VHFs) cause profuse bleeding from the mucous membranes and include viral diseases such as Ebola, Marburg, and Lassa fever. They can be extremely lethal, and, with a few exceptions, there is no established cure or drug treatment for VHFs. Fatality rates range from approximately 50 to 90 percent, a factor that limits the spread of these diseases. Botulism results from the ingestion or inhalation of a neurotoxin produced by the bacterium *Clostridium botulinum*. The toxin can cause muscle paralysis, including paralysis of the muscles involved in breathing, a condition that can be fatal without mechanical assistance. If antitoxin is administered early, the symptoms of botulism can be reduced. Most patients do recover within weeks or months. Tularemia is also caused by a bacterium, *Francisella tularensis*. Though nonfatal, a tularemia infection causes severe weight loss, fever, headaches, weakness, and sometimes pneumonia. *Francisella tularensis* is not transmitted from person to person but if used as a weapon would probably be spread by aerosol. One can contract the disease by inhaling or ingesting as few as 10 to 50 bacterial cells. Antibiotics can treat tularemia.

Other toxins that pose a threat include ricin and staphylococcal enterotoxin B, both of which belong to category B. Ricin is a poison that can be made from the waste of ground-up castor beans of *Ricinus communis,* a plant whose oil is used by many industries, including in paints, textiles, and cosmetics. The toxin is very stable and can be produced in the form of a powder or a mist or dissolved in a small quantity of liquid, making it easy to deliver. Even in small doses, ricin is potent; it works by inhibiting cells from synthesizing proteins. Symptoms vary depending on whether the poison is ingested or inhaled, and there is no antidote. In 2003 and 2004 mail containing ricin powder was sent to the White House and the U.S. Senate, but security officials intercepted the letters before any harm was done. Staphylococcal enterotoxin B (SEB) is a toxin produced by *Staphylococcus aureus* and a common cause of food poisoning. As a biological weapon, SEB would be aerosolized and then inhaled by victims. Symptoms after ingesting SEB include nausea, vomiting, cramps, and diarrhea, but if it is inhaled, symptoms would include sudden high fever, chills, headache, muscle aches, and a dry cough. Another class B agent is *Coxiella burnetii,* a bacterium that causes Q fever, a disease characterized by headaches, fever, malaise, coughing, and pneumonia. The mortality rate for Q fever is low, but the incidence would probably be high if it were used as a biological weapon.

Other forms of weaponry (besides firearms, missiles, bombs, and other artillery) that are related to biological weapons are chemical weapons, nuclear weapons, and radiological weapons. Chemical weapons include substances such as inflammatory or combustible mixtures, smokes, or gases that can irritate, burn, incapacitate, poison, or asphyxiate. After inhalation or absorption through the skin, chemical weapons can take effect immediately. Nuclear weapons include those whose destructive power is derived from uncontrolled nuclear reactions. Atomic bombs derive their force from chain nuclear fission reactions, in which neutrons are injected into atomic nuclei, causing them to split, releasing more neutrons, which split additional nuclei, and so on. Another type of nuclear weapon is the hydrogen bomb, which obtains its incredible amount of energy from nuclear fusion, the forced fusion of multiple nuclei to form a single, heavier nucleus. Radiological weapons disperse radioactive material through the use of conventional explosives, by fire, or otherwise by dilution. Radioactive material spontaneously emits dangerous energetic particles as their atomic nuclei disintegrate and can cause radiation sickness, the symptoms of which include tiredness, nausea, vomiting, loss of teeth and hair, and damage to the bone marrow, which can result in a decrease in red and white blood cells.

See also BACTERIA (EUBACTERIA); BIOMOLECULES; INFECTIOUS DISEASES; RADIOACTIVITY.

FURTHER READING

Judson, Karen. *Chemical and Biological Warfare*. Tarryton, N.Y.: Benchmark Books, 2004.

Langford, R. Everett. *Introduction to Weapons of Mass Destruction: Radiological, Chemical, and Biological.* Hoboken, N.J.: John Wiley & Sons, 2004.

Naff, Clay Farris, ed. *Biological Weapons*. Farmington Hills, Mich.: Greenhaven Press, 2006.

Zubay, Geoffrey. *Agents of Bioterrorism: Pathogens and Their Weaponization*. New York: Columbia University Press, 2005.

biology Biology is the branch of natural science that deals with living organisms. The study of life addresses many varied questions: What is life? How do new species originate, and how do they reproduce? What do all life-forms have in common, and how do they differ? How do particular environments affect a species, and how do species interact with one another? The quantity of information that biology encompasses is enormous, but the information can

(continues on page 102)

STRUCTURE-FUNCTION RELATIONSHIPS IN LIFE SCIENCE

by Lisa M. Dorn
University of California at Davis

There exists an interaction so fundamental to the natural world that it can be found at every level of organization, from atoms to molecules, organs, individuals, populations, communities, biomes, and even global climate patterns. This interaction is the intricate relationship that inevitably exists between structure and function. Structure is the way in which something is organized or put together, while function is the activity, intended or otherwise, performed by that something. These two share a reciprocal relationship—it does not make much sense to use a screwdriver to hammer in a nail; nor would it make much sense to design a hammering device that resembled a screwdriver. In the first case, structure dictates the function of an object. When skipping stones across a lake, one looks for the flattest stone because the aerodynamic qualities of its flat shape enable the stone to skip effectively across the lake's surface. A round stone would simply fall to the bottom. Conversely, the intended function of an object will often shape its structure. For example, the structures of airplane and helicopter wings differ because each functions for a different type of flight. The expectation that structure will dictate function is a product of observation, the observation that flat stones are more likely to skip than round stones. In biology, however, the converse expectation, that function shapes structure, is an extension of the theory of natural selection. Natural selection suggests structures that are best suited to a function that improves the organism's fitness will survive and multiply over future generations. Examples of the interaction between structure and function abound in the natural world, at every level of organization. Understanding the role this relationship plays in the development and functioning of the natural world can help illuminate the world in a new light.

At the atomic level, the relationship between the structure and function of a carbon atom helps explain why carbon is so important to the life of our planet. One of the most important aspects of the structure of a carbon atom is that it has four bonding electrons, a fairly boring statistic until you realize just how much four bonding electrons can do. These four electrons allow carbon to form combinations of single, double, or triple bonds with an almost endless combination of atoms. Carbon can even bond to itself in very different ways, forming some of the hardest and softest minerals in the world. A diamond is pure carbon with each carbon atom bonded to four other carbons, forming a three-dimensional crystal structure. This structure makes diamond the hardest mineral in the world. Another common form of carbon is a series of two-dimensional crystals called graphite. In graphite each carbon atom is bonded to only three others, forming flat sheets of carbon that resemble chicken wire. These sheets are only loosely bonded to each other, causing them to slide across each other quite easily. This sliding is what makes graphite one of the softest minerals in the world. Because graphite, the recognizable material at the tip of a pencil, is so soft, it leaves a little of itself behind every time a pencil tip touches paper.

When carbon combines with other elements, it can form gases, crystals, or chains. Carbon gases include carbon dioxide and carbon monoxide. Calcium carbonate, the main ingredient in seashells, marble, and classroom chalk, is a carbon-based crystal. In carbon chains, each carbon atom forms a bond with the carbon atoms directly above and below it, leaving two free electrons on every carbon atom to bond with other elements. As a result, carbon forms the core of thousands of important biological and other molecules including fuels, plastics, and carbohydrates.

Remarkably, all of carbon's amazing shapes and functions can be traced back to its atomic structure of four bonding electrons. In a sense, life is an eventuality of the structure of the carbon atom—an example of the fundamental relationship between structure and function. In the carbon example, function was a direct result of structure at the atomic level. However, in biology the structure-function relationship often results when structure and function affect each other across generations, a process called natural selection.

At the molecular level, the molecule cellulose elegantly demonstrates the structure-function relationship. As a major component of plant tissue, cellulose is one of the most important biological molecules on Earth. At any given time 50 percent of all of the carbon on Earth is tied up in cellulose molecules. Cellulose is an important food source for many organisms and is the major component of paper and cotton. However, the main function of cellulose is as a plant's skeleton. Plants construct cellulose out of small, energy-rich sugar molecules called glucose. Plants can afford to use these potential energy sources as construction materials because plants are experts at turning energy from sunlight into the chemical energy plants and animals need to survive, a process called photosynthesis. When plants capture this energy, they store it in the form of glucose, which is then converted into many other molecules. Some of the glucose is stored in the form of starch, which plants and animals use for food, but much of it is bound together in long strands to make cellulose. The glucose molecules used in cellulose have a unique structure, distinct from the structure of the glucose subunits of starch. This slight structural difference causes cellulose chains to lie flat (unlike starch, which has a coiled structure). The flat shape of cellulose allows the hydrogen atoms at the edges of the glucose molecules to bind to each other, reinforcing the flat shape and creating a strong thin fiber with a structure similar to that

of graphite. All of this strength is essential for cellulose to function as plant skeletons. Without the rigid structure provided by cellulose, plants would be amorphous green puddles, and the world would not look anything like it does today.

While the strength of cellulose is important to its function in plants, it creates enormous problems for everyone else. Everybody needs energy to survive, but plants belong to a unique group of organisms called phototrophs that are capable of converting light energy into chemical energy. The rest of us must therefore rely on plants for energy. Because much of the energy originally harvested from sunlight is bound up within cellulose, other organisms (including humans) must have a means of accessing that energy. There are three main strategies for getting the energy out of cellulose: use an enzyme to break down cellulose, cooperate with an organism that has this enzyme, or eat organisms that use one of the first two strategies. An enzyme is a protein specialized for catalyzing biochemical reactions, such as breaking down other molecules. Each enzyme is responsible for working on one particular molecule. The relationship between an enzyme and its target molecule is dependent on the shape or structure of the two molecules (much like two adjacent pieces of a jigsaw puzzle). Only a few organisms—most notably several unicellular microorganisms—have developed the enzyme capable of breaking down cellulose, cellulase. Herbivores rely on cooperation with these microorganisms to obtain plant resources. The larger herbivore provides a safe habitat and food for the microorganisms while the microorganisms provide the herbivore with energy. Some herbivores, such as cows, deer, and antelope, create this habitat inside their own guts. These herbivores, collectively called ruminants, have a complex digestive system with four stomachlike chambers for food digestion. The first of these chambers houses microorganisms and serves as a large fermentation vat, much like the oak barrels used to make wine. Antelopes and other ruminants feed their microorganisms by eating grasses and other plant materials. Once the microorganisms have broken down the cellulose, the products are moved through the remaining chambers, where they are churned and eventually absorbed into the animal. Many of the microorganisms are moved through the digestive tract and digested with the cellulose products. Antelopes will use these microorganisms as a source of protein and vitamins. The structure of the antelope's gut has evolved into a digestive system that functions efficiently for retrieving energy from cellulose.

The antelope's relationship with cellulose also affects the structure of antelope populations. The same is true for other members of the ruminant group. A population is a group of members of the same species that live in the same area and reproduce with each other. The functions of populations include getting food, reproducing, and protecting individuals from predators. These three functions have a large effect on the structure of populations. The East African dikdik, a small gray-brown antelope with large eyes and a strange pointed muzzle, exemplifies this relationship. Dikdik populations are structured into monogamous pairs in small home territories, a direct result of the functions of the population. Dikdiks live in arid habitats and feed on small fruits and new plant growth. These food items are rare and do not last long, so the dikdiks are constantly moving around their home territory to find food. They also need to be experts on their home territory so that they know when and where new food is available. As a result, dikdiks prefer small home territories. Dikdik food and habitats have also caused the dikdiks to be very antisocial. Because their food is scarce, females prefer to avoid competition with other females by living alone. Males, on the other hand, do not need as much food as females (because they do not have to feed offspring), so they do not really care whether they share a home range. What they do care about is finding a mate when the time comes. But female dikdiks do not make this task easy. Females stay so far apart from each other it would take far too much energy for a male to keep running from female to female to see who is ready to mate. Instead, males hedge their bets by sticking with the same female all of the time. The female tolerates the male because he helps keep other dikdiks away from her territory. Finally, the small size of the dikdiks and their tendency to live in pairs help them remain hidden from predators. This is important because dikdiks are too small to run very fast. If they lived in herds on open plains, they might become a predator's favorite snack food. What dikdiks eat, how they find mates, and how they protect themselves from predators determine their population structure.

Populations of dikdiks are surrounded by populations of many other species: the plants they eat, their predators, and many other organisms. In any given area there are all manner of populations living together. These collections of populations living in the same area and interacting are called communities. Even communities exhibit a strong relationship between structure and function. The structure of a community refers to the number of species present and the way in which the species interact with each other. The function of communities is related to their ability to respond to change, a characteristic related to the structure of the community. Communities with complicated structures are better equipped to avoid potentially devastating changes. A complicated community structure is one in which there are many different connections among species and overlaps in species' functions (eat the same prey, etc.). Community structure is often depicted as a food web diagram. Food webs depict how each species in a community uses other species as a resource such as food or nesting material.

Some communities are susceptible to change on the basis of the presence or absence of a single species. These species are referred to as keystone species because they have a large effect on all other species in the community. Removing a random species from the community is unlikely to have a large effect,

(continues)

(continued)

but removing the keystone species can radically alter the structure and function of the community. In desert communities, kangaroo rats are a common keystone species. Kangaroo rats dig burrows into the soil to keep cool and safe from predators. By disturbing the soil they make it easier for small annual plants to establish themselves and compete with perennial grasses. When kangaroo rats are removed, the perennial grasses take over the plant community, the annual plants die out, and the birds and rodents relying on the annual plants soon die out as well. The presence or absence of kangaroo rats determines the structure of the community and, in turn, affects the community's stability (ability to function).

Communities themselves are organized into biomes. A biome consists of all communities in the world with similar climate and similar plant and animal adaptations. Alternatively, one can think of a biome as all communities with an equivalent structure and function. The structure of a biome is a combination of the physical environment with the food web of interactions among species. The function of a biome relates to the way energy moves between species in the food web. Deserts, rain forests, and grasslands are all examples of biomes. While there are many different types of deserts in the world, all deserts share a similar structure: low rainfall, large daily temperature fluctuations, not much in the way of ground cover or tree canopies, plants that store water, and small burrowing animals. The Earth is patterned in a mosaic of biomes, each with a unique structure and function. The structure of the global climate determines the location of biomes across the Earth. Climate results from wind patterns arranged in large loops of air alternating between the Earth's surface and the upper atmosphere. There are six doughnut-shaped air loops called cells circling the Earth, three in the Northern Hemisphere and three in the Southern. As the winds turn, they alternately heat and cool and move water across the planet in predictable ways. Deserts are created by dry winds picking up water from the surface of the Earth. The first and second set of cells in each hemisphere meet at 30 degrees latitude, where air descends toward the Earth. The air approaching Earth is cold and dry, so as it gets closer to the warm Earth, its temperature rises and it soaks up water from the Earth's surface. The heat causes the air to start rising again, completing the second loop of air. The combination of warm and thirsty rising air leaves the Earth with hot, dry deserts. Looking at a map of the world reveals that great deserts like the Sahara, the Arabian, the Sonoran, the Kalahari, and the Australian are all found near 30 degrees latitude. Much as the structure of the carbon atom leads to carbon's functioning as the base of our most important biological molecules, so the structure of wind loops over the surface of the Earth leads to the pattern and function of biomes across Earth's surface.

No matter what level of organization one examines, amazing examples of structure-function relationships abound. The intimate association between structure and function explains why the world functions as well as it does and why studying the living world is so exciting.

FURTHER READING

Abbot, E. Stanley. "The Causal Relations between Structure and Function in Biology." *The American Journal of Psychology* 27, no. 2 (April 1916): 245–250.

Brashares, Justin S., Theodore Garland, and Peter Arcese. "Phylogenetic Analysis of Coadaptation in Behavior, Diet, and Body Size in the African Antelope." *Behavioral Ecology* 11, no. 4 (2000): 452–463.

Jarman, P. J. "The Social-Organization of Antelope in Relation to Their Ecology." *Behaviour* 48 (1974): 215–266.

McCann, Kevin Shear. "The Diversity-Stability Debate." *Nature* 405 (May 11, 2000): 228–233.

(continued from page 99)

be categorized by several convenient mechanisms. One can begin to break the subject down on the basis of the type of living organism being studied. For example, botany is the study of plants, zoology is the study of animals, and microbiology is the study of microorganisms. Those branches are still rather large, but they can each be broken down further. They can be subdivided according to more specific types of organisms—microbiology can be split into bacteriology (the study of bacteria), mycology (the study of fungi), protozoology (the study of protozoa), and so on. Another way biological subdisciplines can be further broken down is by focusing on one physiological system or process. For example, a botanist might focus on plant reproduction or plant genetics, a microbiologist might specialize in microbial ecology, or a zoologist might research ant behavior. Many of the numerous fields of study are listed in the table Branches of Biology.

CHARACTERISTICS OF LIFE

Though only 1.4 million species have been identified, biologists believe there could be as many as 50 million different species on the Earth. Species are particular types of organisms that have similar anatomies and are capable of interbreeding. While the diversity of life-forms that have adapted to exist in practically all Earth's habitats is tremendous, all living organisms share several common properties.

- Living things must have order. According to the second law of thermodynamics, the natural tendency of things is to move toward

BRANCHES OF BIOLOGY

Branch	Is the Study of
anatomy	the structure of organisms
arachnology	spiders
bacteriology	bacteria
behavioral ecology	the ecological and evolutionary basis for animal behavior
biochemistry	chemistry applied to living systems
biogeography	the distribution of a species
biophysics	physical theories and methods applied to living systems
biopsychology	the biological basis of behavior and mental states
botany	plants
cell biology	cellular structures and their functions
ecology	the interactions between organisms and their environment
endocrinology	hormones, the endocrine system, and its associated disorders
entomology	insects
ethology	animal behavior
evolutionary biology	the origin and descent of species and their change over time
genetics	heredity and variation of organisms
herpetology	reptiles and amphibians
ichthyology	fishes
marine biology	organisms that live in the ocean
microbiology	microorganisms
molecular biology	biomolecular structures and processes such as DNA replication, transcription, and protein synthesis
mycology	fungi
ornithology	birds
phycology	algae
physiology	the functions and activities of living matter
population biology	populations of organisms, especially in terms of biodiversity, evolution, and the environment
protozoology	protozoa
reproductive biology	how organisms reproduce
sociobiology	the social organization and behavior of animals
zoology	animals

disorder, but living organisms are highly structured and must maintain this structure to carry out physiological processes associated with life. One way that all organisms exhibit structural order is that they are composed of cells. Even the simplest single-celled organisms compartmentalize certain functions to specialized areas of the cell.

- Living organisms take in energy from the environment in the form of food or light energy and transform the energy to perform work associated with life. Death results when the organism is no longer able to bring in and utilize energy successfully.
- Living things grow and develop according to patterns determined by their genetic information. Chromosomes made of deoxyribonucleic acid contain the blueprints for building and assembling new cells and for the function of those cells.
- Life-forms create new life-forms similar to them. Every individual of a species might not produce offspring, but in a species there must be a mechanism in place for reproduction.
- Organisms can detect stimuli from the external environment and respond accordingly. This could be as simple as a plant's growing toward the sunlight or as complex as a bat's using sound waves to navigate its way through a dark cave.
- Despite changes in the external environment, a living organism must maintain optimal conditions inside its own cells and body. For example, waste products of metabolism must not build up, salt concentrations must remain within tolerable levels, and body temperature must be maintained.
- Life evolves, or changes, over time. Organisms adapt to changing environments, and adaptations that result in increased survival and reproductive efficiency are passed on to offspring.

Objects or things that fulfill some but not all of the characteristics listed are not considered living. For example, fire takes in components from its environment, seems to grow and develop, changes and responds to its surroundings, and even seems to create offspring, but it does not exhibit order; nor does it have a genetic blueprint.

COMMON THEMES OF THE BIOLOGICAL SCIENCES

Several unifying themes emerge when studying the biological sciences. One is that as a science, biology is a process. New information is gained by scientific investigation. Biologists formulate testable hypotheses, tentative explanations for an observed phenomenon, then plan and carry out controlled experiments to examine the validity of the hypothesis. Theories are much broader in scope, explaining multiple phenomena and observations. Before becoming a theory, an idea must be supported by an abundance of evidence and be widely accepted by scientists in that field. Two theories that dominate biology are natural selection and the cell theory. The theory of natural selection explains that individuals or species that are best adapted to their environment have better survival and reproductive success, leading to the perpetuation of those genetic qualities that suited the organism to that environment. The cell theory states that cells are the fundamental structural and functional unit of all living things.

Another common theme of biology is that life is characterized by a hierarchy of order with each level of organization building on the previous level. At each level, structure determines function, beginning with the architecture of an atom all the way through the structure of biological communities. As components from one level join to develop a higher level of organization, new properties emerge and new functions can be carried out.

All matter, including living organisms, is made up of the chemical elements, and atoms are the smallest particles of an element that retain that element's properties. Chemical linkages called covalent bonds hold atoms together to form molecules. Living organisms are composed mostly of biomolecules, which are large, complex molecules, including proteins, carbohydrates, lipids, and nucleic acids. Biomolecules assemble to form cellular structures such as chromosomes, biological membranes, ribosomes, and other organelles, each of which has a unique function that contributes to the growth or maintenance of the cell, the smallest functional unit of life.

An organism can be unicellular, meaning a single cell of that species carries out all of the activities necessary for life, or multicellular, containing up to trillions of individual cells. In complex multicellular organisms, the cells are the functional units of a tissue, a group of cells that have a common structure and work together to perform a common function. Several tissues join to form an organ that is part of a physiological system. For example, the mouth, pharynx, esophagus, stomach, small intestine, colon, gallbladder, and pancreas are all components of the human digestive system. Alone, each performs a specific function—the stomach churns food, the pancreas secretes digestive enzymes, food is chemically digested in the small intestine, and so on. Together, all the organs work to harvest energy from food to provide the body with energy for life's work and material for building new biomolecules.

An organism, then, can comprise a single cell or multiple body systems, each of which cannot support life alone, but together with the others allows a multicellular organism to carry out all of life's necessary processes. All the individual organisms of a species that live in a defined geographic area make up a population. A community comprises all the populations of all the different species that live in the same habitat. The combination of all of the different populations in a community and the abiotic (nonliving) factors make up an ecosystem. The world's largest communities, called biomes, are characterized by specific types of vegetation and climates. Temperature, precipitation, soil types, and wind conditions all affect the types of organisms that inhabit a biome. For example, dry grasslands called savannas exist in tropical areas that have little rainfall. The prolonged dry seasons support the growth of large, scattered trees amid drought-resistant undergrowth. Because of this, large grazing herbivores inhabit savannas. Since no life has been found elsewhere in the universe, the final level of the biological hierarchy of organization is the biosphere, which is simply the entire portion of the Earth that is inhabited by living organisms.

Another unifying theme of the biological sciences is evolution, a process of change. The physical structure of the Earth has evolved over the 4.5 billion years since its formation. Fossil evidence suggests that life first appeared 1 billion years afterward, beginning with Archaea, prokaryotic organisms that could live in the harsh, acidic, hot conditions of the nascent Earth. Just as the conditions of the planet affected the type of organisms that lived on it, the presence of life in turn affected the conditions of the planet. Most notably, molecular oxygen produced by photosynthetic bacteria accumulated in the atmosphere, setting the stage for aerobic life-forms. Species changed, and the diversity of life-forms increased. Between 1.7 and 1.8 billion years ago, eukaryotic life-forms first appeared, and over the next billion years, some evolved into multicellular beings. The first soft-bodied invertebrate animals appeared, and then vertebrate animals. Algae arose, leading to the development of aquatic plants. Approximately 475 million years ago, terrestrial plants and fungi colonized the land, creating an environment that led to the evolution of terrestrial animals.

Throughout each major episode in the history of life on Earth, organisms that were particularly well adapted to a particular environment survived and passed on their characteristics to offspring by natural selection, perpetuating the genetic variations that gave a species reproductive success in that environment. Both abiotic factors, such as temperature, light, water, and nutrient availability, and biotic factors, the presence of other life-forms, affect the organisms that live in a particular environment. Organisms interact with other organisms of the same species by competing for food, territory, and mates. They also interact with other species through predator-prey relationships, symbiotic relationships, and competition for ecological niches. These interactions not only contribute to the evolution of species, but shape populations and entire communities. The resultant changes then select for variations that make an organism better adapted to the new environment.

APPLICATIONS OF BIOLOGY

New knowledge and advances in biology have impacted and continue to impact profoundly the way people live. Understanding the complex organization of the human body has led to improvements in health care and medicine. Humans now have longer life expectancies than ever before, with the average life expectancy of people in the United States reaching 77 years at the turn of the last century, compared with 49 years in 1901. Advances in microbiology have led to the decrease in the spread of infectious diseases and the development of antibiotics and vaccines to treat and prevent infections that were once life-threatening. Production in agriculture has increased as a result of selective breeding programs, genetic engineering, and a better understanding of the pests that destroy crops. Technological advances in biology have permeated forensics by helping criminal investigators to determine the time elapsed since death by the insects associated with a human corpse and to identify suspects on the basis of deoxyribonucleic acid (DNA) evidence they have left behind. Learning about Earth's history and the previous life-forms it supported can help society envision the future, to understand, anticipate, and work to correct detrimental situations such as the depletion of natural resources and extinction of species.

What humans have learned by studying life has shaped populations, affected how people treat one another, changed how humans care for themselves, impacted governmental spending, changed how wars are fought, and revealed the impact humans have had on Earth and the other species with whom humans share the planet. The study of biology has affected almost every aspect of society and will continue to do so as scientists make new and exciting discoveries.

See also AGRICULTURE; ANATOMY; BIOCHEMISTRY; BIOGEOGRAPHY; BIOINFORMATCS; BOTANY; CELL BIOLOGY; CONSERVATION BIOLOGY; ECOLOGY; ENVIRONMENTAL SCIENCE; ETHOLOGY; EUKARYOTIC CELLS; EVOLUTIONARY BIOLOGY; GENETICS; MARINE BIOLOGY; MICROBIOLOGY; MOLECULAR BIOLOGY; ORGANIC CHEMISTRY, ITS RELEVANCE TO LIFE SCIENCE; PHYSIOLOGY; PROKARYOTIC CELLS; SCIENTIFIC INVESTIGATION; SOCIOBIOLOGY; ZOOLOGY.

FURTHER READING
Campbell, Neil A., Jane B. Reece, Lisa A. Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, and Robert B. Jackson. *Biology*. 8th ed. San Francisco: Pearson Benjamin Cummings, 2008.
Lewis, Ricki, Bruce Parker, Douglas Gaffin, and Marielle Hoefnagels. *Life*. 6th ed. New York: McGraw Hill, 2007.

biomes, aquatic Many factors profoundly influence the ability of an organism to survive in a particular environment. Biotic factors, the other organisms living in the same area, and abiotic factors, the surrounding chemical and physical conditions, both affect the degree of success to which an organism can find and obtain food and reproduce and raise young and therefore affect the distribution and abundance of a particular species. The biosphere comprises all of the portions of the planet inhabited by life and can be divided into ecosystems that consist of all the organisms and the physical environment in which they live. A biome is the largest useful ecological association that occupies a large geographic region and can contain several ecosystems. Characterized by the types of life-forms that inhabit them, biomes can be largely divided into aquatic and terrestrial types.

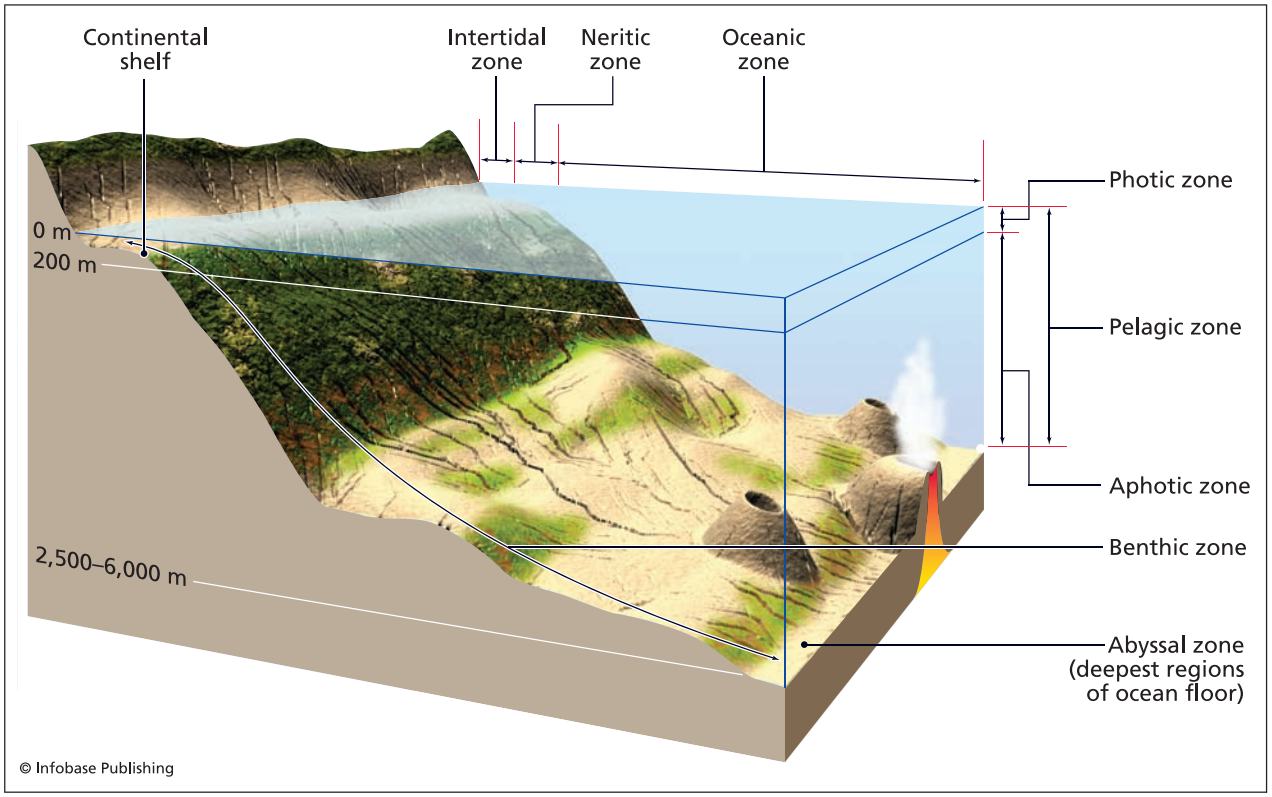
Aquatic biomes include marine and freshwater environments and cover approximately 75 percent of Earth's surface. Because the oceans consume so much area, they have a great impact on global climate and weather patterns in addition to playing a major role in the hydrological cycle. Physical factors, including light, temperature, and the movements of waters, and chemical factors, including salinity and dissolved oxygen concentration, influence the biology of aquatic biomes. Divisions of both marine and freshwater biomes into zones based on the amount of light present, the distance from the shore and depth of water, and open water versus bottom waters provide a variety of physical and chemical environments that support diverse life-forms. Marine biomes are typically defined by salt concentrations exceeding 3 percent and are classified according to physical features of the environment, such as zonation, mechanical forces of waves or currents, and range of temperature. Intertidal zones, oceanic pelagic, coral reefs, and marine benthic zones are the major marine biomes. Because estuaries occur where freshwater rivers flow into oceans, they have variable salt concentrations. Freshwater biomes typically have salt concentrations below 1 percent and include lakes and ponds, wetlands, streams, and rivers.

MARINE BIOMES

Marine habitats can be divided into zones dependent on various physical factors. Biological communities are distributed in the ocean on the basis of the adaptations particular organisms have to the conditions provided by the different zones. Because water absorbs light from the sun, the amount of light that penetrates a water body decreases with increasing depth. Organisms that depend on light must therefore reside near the surface of the water. As autotrophs, photosynthetic organisms make their own food from inorganic carbon sources, such as carbon dioxide (CO_2), using radiant energy from the Sun to incorporate the carbon into organic molecules such as carbohydrates. Organisms that undergo photosynthesis are called producers, and they form the basis of food chains. They reside in the photic zone, near the water's surface, where sufficient sunlight penetrates to support photosynthesis. The aphotic zone lies below the photic zone and is characterized by very little light. The benthic zone is the bottom of the ocean floor, where all the debris and dead organisms eventually settle. The abyssal zone is the region located at the ocean's deepest points. The pelagic zone is composed of the open waters, making it the largest, in terms of volume, of the marine biomes. The continental shelf is the submarine tract of land that surrounds a continent and leads to a steep downward slope into the deep ocean. With respect to water depth, the neritic zone encompasses the shallow waters over the continental shelf. The intertidal zone, or shoreline, is the area where land and water intersect. The waters beyond the steep slope that leads into the deep ocean make up the oceanic zone.

Intertidal Zones

As the gravitational actions of the Moon and the Sun cause displacement of the sea surface, the waters alternately submerge and recede from the shores, or intertidal zones, creating a unique sunny environment for marine life-forms. The lower boundary of a shore occurs where the seabed is exposed to the air during the lowest of tides, and the upper boundary is the area wetted during the highest tides. The climate of the geographical area and the nature of the seabed, rocky or sandy, influence the types of life that inhabit an intertidal zone. Different conditions exist even within the boundaries of an intertidal zone, as some areas are exposed to air for longer periods than others. The mechanical action of the waves on the shorelines and the often twice-daily turn of the tides help maintain relatively high nutrient levels. Intertidal zones with sandy seabeds lack a firm substrate to which seaweeds or sessile animals can attach and are completely exposed to the hot Sun and the cold wind. Animal inhabitants include sand fleas, crabs, and clams that burrow in the wet sand. These animals depend on the tides to deliver food in the form of



Physical factors such as the depth, distance from the shore, and light define different zones of marine biomes.

algae, seaweed, or dead marine creatures. Protected sandy areas such as bays or lagoons contain sea grass and algae, producers that can support a food web. Sea turtles and some birds nest in the intertidal zones of beaches. Rocky shores include both steep cliffs and gentle slopes that support the growth of marine algae, lichens, and other organisms that can withstand the harsh action of the waves and periods of no water. The demanding conditions select for organisms with structural adaptations that allow them to attach to a rocky surface and prevent desiccation. Animal life-forms that inhabit rocky shores include barnacles, limpets, marine snails, mussels, crabs, sea urchins, and starfish.

Oceanic Pelagic Zones

The oceanic pelagic zone consists of the vast open waters making up more than 70 percent of the Earth's surface that can be further divided in terms of the gradient of physical features along the depth of the water column. Ocean depths average around 13,123 feet (4,000 m) but extend to more than 32,808 feet (10,000 m) in some places. Light penetrates and the temperatures are warmer at the upper surface, but traveling deeper presents conditions characterized by cooler temperatures, less light, and higher pressures. A thermocline in which the temperature rapidly decreases with depth separates the warmer upper layer from the cooler deeper waters. Most of the sea's biomass exists in the photic zone as plankton. Phytoplankton, the photosynthetic, microscopic life that floats near the surface, serves as the basis for the pelagic food chain. Zooplankton, animallike microscopic life that floats or swims weakly, such as protozoans, krill, worms, copepods, and larvae of some invertebrates, feed on the phytoplankton, and small fish and larger invertebrates then eat the zooplankton. Nutrient levels are higher in the surface waters as a result of mixing. Moving deeper down the water column, the light gradually fades, and some organisms exhibit bioluminescence, the emission of light from living organisms. Bioluminescence serves different functions in different species: to confuse predators, to recognize other members of one's own species, to attract mates, or to attract prey. Deeper than 3,300 feet (1,000 m), the ocean is completely dark, temperatures approach freezing, and the water pressure is 100 to 1,000 times greater than at the surface. Very little food reaches these depths. To compensate for lack of vision, fish that inhabit this region of the pelagic zone often have lateral line systems, groups of cells that detect vibrations due to disturbances in the water, warning them when predators or prey are near. Creatures that live at these depths also often have an acute sense of smell. Some organisms move up and down the water column

daily. They feed near the surface, then migrate to deeper levels to hide in the darkness to be safe or to conserve energy by slowing their metabolism in the cooler temperatures. Some fish migrate across the ocean rather than up and down the water column in order to breed. Other animals that inhabit the oceanic pelagic biome include numerous fishes, squids, sea turtles, and marine mammals.

Coral Reefs

Reefs are massive skeletal structures formed by the secretions of reef-building cnidarians in warmer waters, typically greater than 68°F (20°C). Corals are the most common reef builders, though other invertebrates also secrete calcium carbonate, which precipitates to form a reef structure. Photosynthetic protists called zooxanthellae live within the coral tissues, provide nutrition, and remove carbon dioxide from the water. Because of the dependence of the corals on these photosynthetic organisms, reefs are limited to shallow waters where light can penetrate. When sediment from construction or highways clouds the waters, photosynthesis is inhibited, and the reefs suffer. Individual sessile polyps sit within a cuplike structure, from which they extend tentacles to feed on small fish, crabs, or other small animals at night, when photosynthesis does not occur. Oceanic currents continuously wash the reefs, introducing nutrients and removing waste products. Unusually strong waves, such as are present during hurricanes or tropical storms, may destroy entire reefs. The polyps of living corals form massive colonies that serve as the basis for unique ecosystems. The diversity within coral reefs exceeds that of any other aquatic ecosystem. The corals themselves are the dominant animals, but the nooks and crannies of the reef structure provide shelter for small fish and numerous invertebrates, fish and sea urchins feed off algae that grows on the coral, and shrimp or smaller fish feed off the parasites living on larger fishes. Starfish and fish such as parrot fish prey on chunks of the living coral tissue. After the corals die, their skeletons remain, and new polyps attach to and grow on the remains. Fringe reefs surround volcanic islands and

Brilliantly colored coral reefs serve as the foundations for unique ecosystems rich in biodiversity. *(National Oceanic & Atmospheric Administration/Department of Commerce)*

enclose a narrow section of water between the reef and the island. Barrier reefs result when the central island begins to sink into the ocean, forming a lagoon inside the reef, which protects the interior from the harsh oceanic swells. When the volcanic island becomes completely submerged, the resulting structure is called a coral atoll.

Marine Benthic Zone

The marine benthic zone is the region of seafloor below the neritic zone, the offshore pelagic zone, extending along the ocean bottom to the abyssal zone, the deepest, coldest regions. The physical and chemical conditions vary tremendously at different depths and distances from the shore in the benthic zone. Sediment formed from the erosion of continental rocks, the calcareous and siliceous oozes and phosphate-rich minerals secreted by marine creatures, and other minerals that have precipitated from seawater, in addition to the remains of former marine life, covers the benthic zone. Bacteria on the ocean floor decompose this and other fallen organic matter, which serves as food for unusual marine benthic creatures. Some areas of seafloor are covered by oceanic crust from recently erupted volcanoes or underwater mountain ranges. Photosynthetic organisms live in the shallow benthic regions, where sufficient sunlight penetrates, but most of the benthic zone receives no sunlight. Unique ecosystems exist near deep-sea hydrothermal vents, cracks in the ocean floor near midoceanic ridges that expel superheated mineral-rich fluids. Near these vents, communities consisting of diverse microbial and animal life thrive. Chemosynthetic prokaryotic organisms live within giant tube worms and use the reduced inorganic compounds such as hydrogen sulfide formed from the heat and sulfates as an energy source to fix carbon and serve as the primary producers for the community. Clams, mussels, copepods, crabs, shrimp, octopi, and starfish also live around hydrothermal vents.

Estuaries and Coastal Wetlands

Estuaries occur where freshwater rivers join the saltwater sea. The water in an estuary flows up the channel back into the river during high tides, and then in the reverse direction out into the sea during low tides. This water movement transports organisms, draws in nutrients, and removes wastes. Because the high salinity of the seawater makes it denser than the freshwater flowing down the river, the saltier seawater often occupies the lower level of the estuary, and the upper layer has a lower salt concentration. This creates a dynamic chemical environment that varies temporally with the coming and going of the tides and along a spatial gradient, both vertically and horizontally. The flow of waters from the river and the incoming sediment create a variety of physical structures such as channels and islands. Mangroves and salt marshes on sea-level coasts and sandy shores transition between land and sea rather than river and sea, but these coastal wetlands also create a unique chemical and physical environment for a variety of marine fish and invertebrates to inhabit. Grasses and algae dominate salt marsh vegetation, and different genera and species of mangrove trees inhabit mangrove forests, which also contain crocodiles and alligators. Fish are abundant in estuaries and salt marshes, and many marine fishes use these grounds to breed and raise young. They lay their eggs on the sediment and the larvae feed off the abundant phytoplankton and zooplankton. Waterfowl and mammals such as muskrats commonly feed and breed in these transitional biomes.

FRESHWATER BIOMES

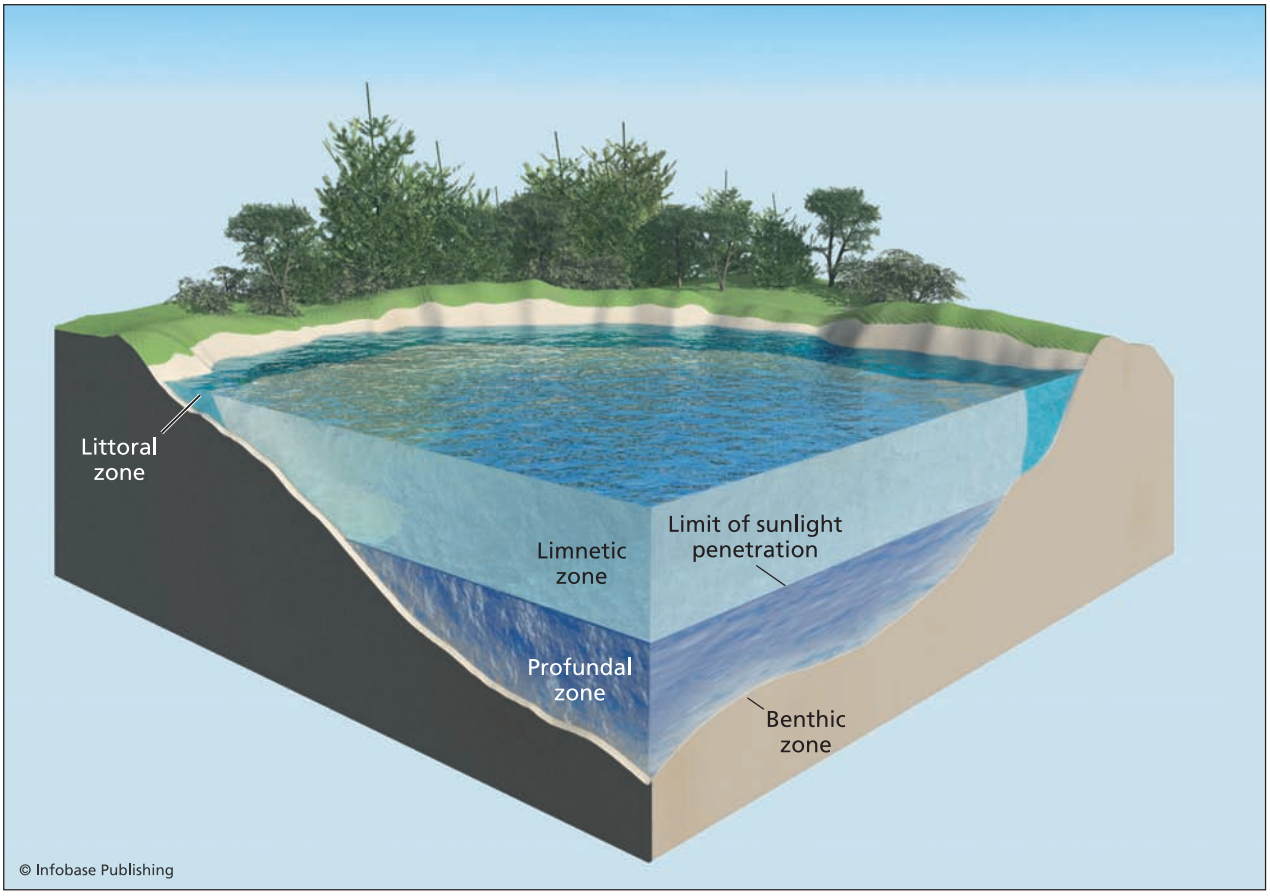
Freshwater biomes are usually found inland and are characterized by low salinity, typically below 1 percent. Life-forms that inhabit these biomes have adapted to the low-salt conditions and could not survive in marine environments. Freshwater biomes are especially important to people because they provide water used for drinking, energy, transportation, and recreation and support many occupations. The major freshwater biomes include lakes and ponds, wetlands, rivers, and streams.

Lakes and Ponds

Lakes are inland bodies of standing water. Smaller lakes are called ponds and may only cover a few square yards (a few square meters), but some lakes cover thousands of square miles (or square kilometers). Lakes and large ponds share many of the same physical features with respect to zonation as marine environments. A photic zone at the water's surface supports photosynthetic life-forms, and an aphotic zone lies deeper, where sunlight cannot penetrate. The photic zone can be further divided on the basis of the nearness of the waters to the shore of the lake; the littoral zone lies closest to shore, and the limnetic zone lies farther from the shore. The littoral zone is shallow and warm and contains rooted vegetation, phytoplankton, and sometimes floating aquatic plants. These producers support animals including snails, clams, insect larva, crustaceans, fishes, and amphibians. The vegetation and small animals support other animals such as turtles and ducks. The limnetic zone contains most of a lake's phytoplankton, which maintain the health of the lake ecosystem by providing food for other organisms and by oxygenating the water. This zone only extends to the point where light cannot penetrate. Below that point

lie the cold, open waters called the profundal zone. The lack of light limits algal or plant growth; thus oxygen content is low. The fish that live in the profundal zone depend on food produced in the limnetic or littoral zone. Snails, clams, crayfish, worms, and insect larvae live on the lake bottoms, the benthic zone.

Lakes exhibit diverse conditions with respect to temperature, salinity, oxygen concentration, and nutrient availability. Variation in the types of organisms present exists between geographic regions, from season to season within a lake, and between the zones within a lake. The biological zones of life are related to the physical structure. Thermally stratified lakes consist of three layers: the epilimnion, the metalimnion, and the hypolimnion. The epilimnion is the wind-mixed top layer that constantly exchanges gases with the atmosphere. The middle layer of the water column, the metalimnion, is also called the thermocline because of its steep temperature gradient. The depth of the thermocline changes during the day. The bottom layer is the hypolimnion. In geographic areas where the temperatures are cold enough to freeze in the winter, the hypolimnion is warmer than the water near the surface immediately underneath the ice. As the ice melts, the thermal stratification shifts. By springtime the waters may be completely mixed, but as the temperatures continue to increase, the epilimnion becomes warmer and less dense, the hypolimnion becomes colder and denser, and the thermocline becomes steeper. The lake remains stratified until fall, when the temperatures begin to decline and the epilimnion cools and becomes denser. Eventually the cold air causes the surface waters to become colder than the bottom waters. After ice forms on the surface and blocks the wind, the layers remain stratified with the hypolimnion warmer than the epilimnion until the coming of spring. Such seasonal changes in temperature within different zones of a lake cause water to mix, as does wind when the surface is not covered with ice. This mixing changes the water chemistry by moving nutrients from the bottom up to the surface waters and carrying dissolved oxygen from the surface waters to the bottom waters. In tropical climates, the surface waters remain warmer than the bottom waters, though at higher elevations the waters cool enough to cause mixing at night.



Lakes can be divided into four general zones based on depth and distance from the shoreline.

In eutrophic lakes, high primary productivity in surface waters due to high nutrient content leads to oxygen depletion as the biomass decomposes. *(Michael P. Gadomski/Photo Researchers, Inc.)*

Oligotrophic lakes have low nutrient content, high oxygen concentrations, and low biological productivity. The diversity of phytoplankton and the benthic organisms is great, and the fish that inhabit oligotrophic lakes generally require higher oxygen concentrations. Trout and whitefish are commonly found in such environments. On the other hand, eutrophic lakes have high nutrient content, low oxygen concentrations, and high biological productivity. Particularly in warm conditions, the high nutrient concentration and sunlight stimulate primary productivity in the surface waters. The quantities of phytoplankton increase, and as the microorganisms die and sink to the bottom, the action of decomposers depletes the oxygen that is present. These conditions support fish such as carp, catfish, and bowfins and invertebrates that can tolerate low oxygen concentrations and higher temperatures. Vascular aquatic plants are abundant in the littoral zone of eutrophic lakes.

Wetlands

Wetlands, including marshes and swamps, are areas of land that are covered intermittently with shallow water and that can support the growth of aquatic plants. In addition to serving many important ecological functions such as removing nutrients from the surface and groundwater, performing denitrification (the conversion of organic nitrogen back into nitrogen gas), and protecting surrounding terrestrial habitats from storms and floods, wetlands provide environmental intermediate conditions between a terrestrial and an aquatic ecosystem. Because the water that fills the basins is not aerated and the soil is saturated with water, plant species characteristic of wetlands have adaptations that allow them to survive in anaerobic soils. Called hydrophytes, such plants have thin cuticles, less rigid structures since the water supports them, flat leaves that enable flotation, small feathery roots into which water can directly diffuse, and numerous open stomata since water loss from transpiration is not a concern. Examples of hydrophytes include water lilies, buttercups, duckweed, and hornworts. The abundance of plants and algae supports many herbivorous creatures such as crustaceans, muskrats, and aquatic insect larvae, which in turn support a variety of birds. Carnivorous animals including dragonflies, otters, alligators, and owls are also common.

Rivers and Streams

Rivers and streams are running bodies of water that serve to drain landscapes. A watershed is the region drained by a stream or river. Water from rains runs off either above or below the ground, picking up dissolved materials along the way, and collects in small creeks, sometimes called the headwaters. The creeks merge into streams and then rivers that eventually lead to the sea or to large inland basins. The flow of water through rivers and streams is constantly changing as a result of precipitation events, melting snow and ice, evaporation, transpiration, sediment, discharge of water from aquifers, and many human-induced events such as irrigation, levee construction, or the draining of wetlands. Gravity moves the water from higher to lower altitudes, carrying with it sediment, nutrients, minerals, and life-forms. The concentrations of nutrients and salts change as the flow nears the mouth, where the water enters the ocean or other large water body. As in marine biomes and lakes, the degree to which light penetrates influences the type and abundance of organisms near the surface. Light can penetrate deeper in clearer streams than in rivers downstream, but clear streams are still cloudier than clear lakes because of the dynamics of river systems. The chemical composition of a river depends on the climate and the geological features of the watershed. In tropical areas where the annual rainfall is high, the soil has been washed clean of dissolved materials; thus rivers in these regions contain less salt, whereas rivers in drier areas are saltier. The oxygen content depends mostly on temperature. Colder waters carry more dissolved oxygen, and warmer waters have lower oxygen content. Because rivers have a large surface area relative to their volume, and because the water is always moving, oxygen is absorbed and mixed into the waters regularly and does not have a major impact of the types of life that inhabit the waters. The distribution of organisms along a river continuum seems to follow a general pattern. The headwaters usually contain higher amounts of oxygen but low amounts of nutrients and salts, and the bottoms of the channels are typically rocky. Fish such as trout inhabit these oxygen-rich, cold streams. The nutrient and salt concentration increase as the water travels toward its downhill destination, covering river bottoms with silt and sediment. Leaves and pieces of plants that live on the banks of the headwaters and other biomass such as insects and animal feces that are washed into streams during storms serve as the major food source for animals that live in streams. Aquatic microbes attack these food fragments, and stream invertebrates shred the organic material and feed on the fine particles of food. The types of invertebrates that inhabit streams and rivers are mostly benthic, meaning they live on the bottom in the sediments. The content of the sediments affects the composition of the invertebrate communities. Moving down the river continuum to the middle headwater streams, the width typically increases, and because the water is less shaded, algae and rooted aquatic plants are more abundant. These, in addition to the fine particulate matter carried in by the small headwater streams, feed the fish and invertebrates. The water is slightly warmer, moves more slowly, and is less oxygenated—conditions preferred by fish such as bass. Fine particulate matter and increased phytoplankton populations are the main food sources in the larger rivers, contributing to the differences in the type of fish that inhabit larger rivers compared to the headwaters.

See also BIOMES, TERRESTRIAL; BIOSPHERE; ECOLOGY; ECOSYSTEMS; HYDROTHERMAL VENTS; MARINE BIOLOGY; PHOTOSYNTHESIS.

FURTHER READING

Day, Trevor. *Lakes and Rivers*. New York: Chelsea House, 2006.

———. *Oceans*. New York: Chelsea House, 2006.

Moore, Peter D. *Wetlands*. New York: Chelsea House, 2006.

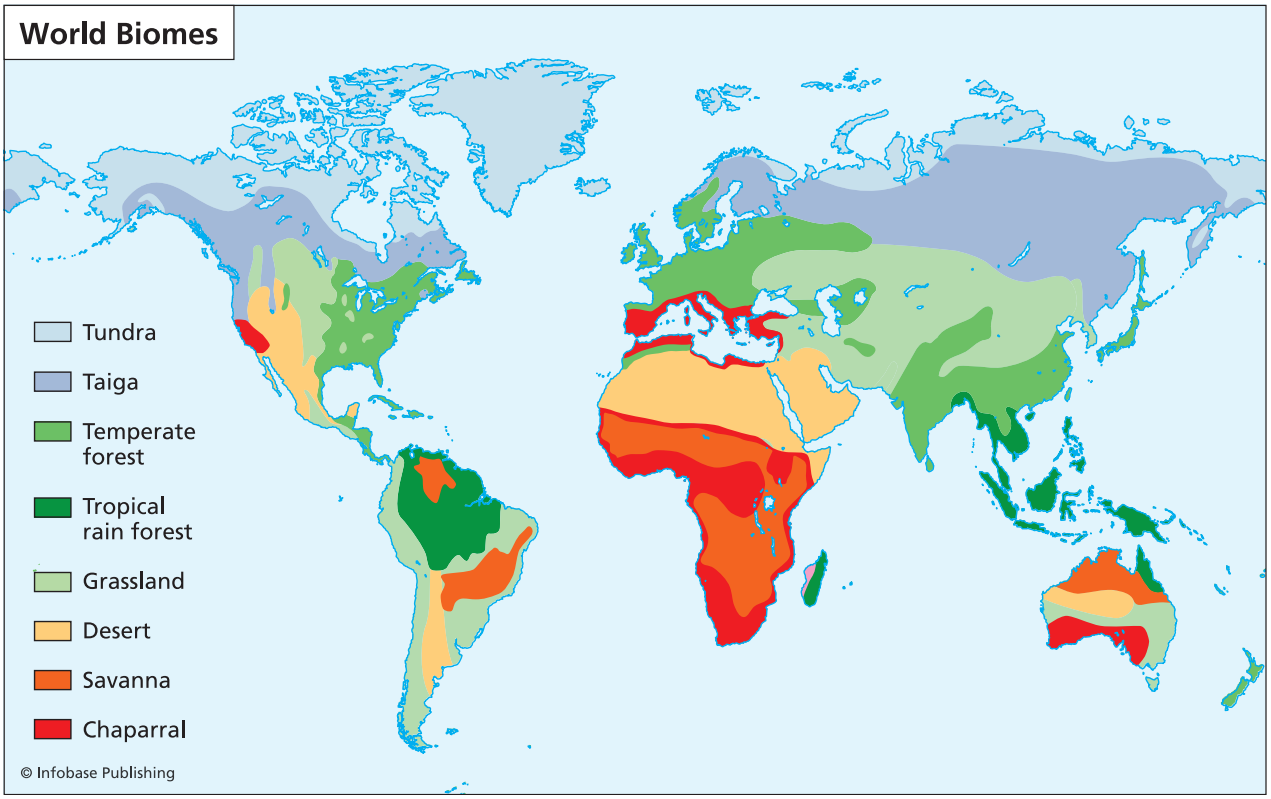
Sigee, David C. *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. Hoboken, N.J.: John Wiley & Sons, 2005.

Weigel, Marlene, and Julie L. Carnagie, ed. *Encyclopedia of Biomes*. 3 vols. Farmington Hills, Mich.: UXL, 2000.

Whitfield, Philip, Peter D. Moore, and Barry Cox. *Biomes and Habitats*. New York: Macmillan Reference USA, 2002.

Woodward, Susan L. *Biomes of Earth: Terrestrial, Aquatic, and Human-Dominated*. Westport, Conn.: Greenwood Press, 2003.

biomes, terrestrial Ecology is the study of the relationships between organisms and their environment. Environmental factors include biotic, those relating to other living organisms, and abiotic factors, those relating to nonliving components of the environment, such as physical, chemical, and geological features. A biome is a large region of characteristic ecological associations, characterized by certain types of vegetation and climatic conditions. The biosphere, the portion of the planet that contains life, consists of several major terrestrial and aquatic biomes; scientists have not reached a general consensus about the exact number of types of biomes. While different biomes contain characteristic combinations of climate and types of fauna and flora, individual species or particular environmental conditions are



Differences in climate affect the distribution of life-forms on Earth, forming characteristic communities called biomes that cover large geographic areas.

not necessarily distinguishing, and biomes overlap physically and biologically. Areas within one biome may resemble those of another type of biome, and the same biome at distant geographic locations may contain different resident species. With this in mind, major terrestrial biomes include the tropical rain forest, savanna, desert, chaparral, temperate grassland, temperate broadleaf forest, coniferous forest, tundra, and mountains.

TROPICAL RAIN FOREST

Situated in equatorial and subequatorial regions, tropical rain forests experience year-round warm weather, with temperatures averaging 77°F–84.2°F (25°C–29°C) and 78.7–157.5 inches (200–400 cm) of precipitation annually. Almost half of the precipitation is due to water released into the air by transpiration from the vegetation. Three major geographical regions host tropical rain forests: Central America in the Amazon River basin, the Zaire basin in Africa, and Indo-Malaysia. The warm, moist climate makes this biome the most diverse in the world; an estimated 1.5 million different species live in the rain forest. The different life-forms in the tropical rain forest are distributed into four main layers. The uppermost emergent layer consists of scattered trees with heights between 100 and 240 feet (30 and 73 m), requiring widespread buttresses to support them. Their bark is thin since water loss is not a concern, and their lowest branches are quite high, since at lower heights sunlight cannot penetrate through the dense vegetation that is characteristic of the tropical rain forest. The leaves are exposed to the Sun and wind, but they are small so they do not dry out. The roots of these trees do not penetrate deeply. The upper canopy, which consists of trees ranging from 60 to 130 feet (18 to 40 m), is rich in food sources and supports most of the animal life. Light can penetrate the upper portion of this layer, but not all the way to the bottom portion. Plants called epiphytes (such as orchids) grow on trees in the upper canopy where sunlight is accessible, and they derive their moisture and nutrients from the air. Leafy vines also cling to the trees for support and as a means to reach the sunlight in the upper canopy. Leaves on the upper canopy trees are sometimes oily and are shaped in a manner that allows the water to drip off them rather than collect in pools where mold can grow. Trees in the third canopy, the understory, reach approximately 60 feet (18 m) tall. This layer also consists of smaller trees, shrubs, and numerous plants that have broad, large leaves to collect as much sunlight as possible. These organisms thrive when a taller tree that has been blocking the sunlight falls down. The air does not

Dense vegetation, trees with giant buttresses, vines, and epiphytes are characteristic of tropical rain forests, such as this one in Oahu, Hawaii. *(Dhoxax, 2007, used under license from Shutterstock, Inc.)*

circulate well in the understory, light barely penetrates this far down, and the humidity remains very high. The forest floor is completely shaded; thus few plants grow there. The topsoil is nutrient poor, as the frequent rains wash away all the minerals and excess nutrients. As soon as the insects, earthworms, fungi, and other microorganisms decompose the leaf litter, the plants take up the recycled nutrients. Insects make up the largest group of animal life in tropical rain forests. Resident insects, such as butterflies and beetles, and birds often display bright colors. Mammals contain adaptations that facilitate living in trees, such as monkeys' having prehensile tails for hanging from branches. Many reptiles and amphibians also inhabit the rain forest.

SAVANNA

Savannas, or tropical grasslands, are plains that have shrubs, a few scattered trees, and drought-resistant undergrowth. Located in equatorial and subequatorial regions, with temperatures that range from 68°F to 86°F (20°C–30°C) and average 75.2°F–84.2°F (24°C–29°C). The warmth and humidity make for very wet summers, when the majority of the annual rainfall, which averages 11.8–19.7 inches (30–50 cm), occurs. Likewise, the winter is very long and dry and frequently includes several months with no rainfall. The dry conditions select for plants that have adaptations such as smaller leaves, deep roots that penetrate to the water table, and thick bark that protects against fires, which are common and help maintain this biome by controlling the density of woody plants. Grasses serve as food for large herbivorous mammals such as elephants, wildebeest, zebras, and giraffes. Carnivores such as lions and hyenas prey on the grazers. During the wet season, the vegetation is lush and the rivers flow freely, but during the dry season the plants die and the animals must wander in search of water and food.

DESERT

Occupying about one-fifth of the Earth's land, desert biomes receive less than 11.8 inches (30 cm) of precipitation per year, with an average of about 5.9 inches (15 cm) per year. Deserts generally occur in rings that circle the globe near 30° north and south latitude, and most deserts are hot, but some in the arctic are cold. The temperatures are extremely variable, with hot deserts experiencing temperatures that occasionally near 122°F (50°C) and cold deserts as low as -22°F (-30°C). Animals that live in deserts burrow to escape the heat or the cold, depending on the type of desert. The hot and dry conditions of the desert limit the vegetation that can survive there; wind and infrequent flash floods mold the mostly bare landscape. Succulent plants such as cacti and shrubs with roots that penetrate deeply are the most common forms of plant life. Adaptations include tolerance to heat and low moisture, ability to store water, and reduced leaf area to prevent water loss by transpiration. The photosynthetic mechanisms differ in a manner that reduces photorespiration, allowing the stomata to remain closed or to limit their opening to nighttime, thereby reducing water loss. Desert soil is salty, making it even harder for plants to access any available water from it, and it lacks organic matter, with the exception of underneath shrubs. Cold deserts may have some lichens, grasses, and mosses. Because of the extreme weather, many fauna are nocturnal. Snakes, lizards, scorpions, ants, beetles, rodents, and birds represent the animal life found in deserts.

CHAPARRAL

The chaparral is sometimes referred to as the Mediterranean woodland and shrubland after the location of its main area of distribution. Chaparral also occurs along midlatitude coasts of North America,

Desert flora, as shown in this Nevada yucca forest, have adaptations that help the plant tolerate long periods of drought, such as having reduced leaf surface area to prevent evaporative water loss and the ability to store water. *(John and Karen Hollingsworth/U.S. Fish and Wildlife Service)*

Australia, Chile, and South Africa. The landscape is varied—sometimes flat, sometimes rocky, or along mountainsides. Weather changes with seasons; as in the savanna, rainfall ranges between 11.8 and 19.7 inches (30 and 50 cm), but in contrast, chaparral summers are dry and the winters are rainy. Fall, winter, and spring experience temperatures around 50°F–53.6°F (10°C–12°C), but in the summer the temperature may reach 104°F (40°C) and in drought conditions fires may occur. Flora and fauna possess adaptations similar to those of desert life-forms for tolerating hot and dry conditions. Woody plants are evergreen and have small, tough leaves. The trees often have thick, fire-resistant bark, whereas the shrubs are often oily and burn easily, but to compensate, they regrow quickly. Depending on the geographical location, chaparral residents include mammals that eat twigs and buds, such as deer, wild sheep and goats, antelope, kangaroos, and hares. Carnivores such as puma and aardwolves and omnivores such as foxes, skunks, and jackals inhabit this biome, which also serves as a winter home for many migratory birds and insects.

TEMPERATE GRASSLAND

Like the savanna, temperate grasslands are biomes characterized by vast plains dominated by grasses with scattered trees growing alongside streams. Precipitation is greater than in the savanna; rainfall is seasonal and averages between 11.8 and 39.3 inches (30–100 cm) per year. As the name suggests, moderate temperatures typify this biome—20°F (-6.7°C) in the middle of winter and 70°F (21.1°C) in the middle of summer. Humid, rainy grasslands grow tall grasses and are called prairies, and drier grasslands, which experience greater temperature extremes, grow shorter grasses and are called steppes. Though no geological or biological features block the wind, the deep roots of the grasses prevent the soil from blowing away. The soils are naturally nutrient-rich and fertile from the growth and decay of deep grass root systems, though humans have taken advantage of this and converted much of this land for agricultural purposes, an act that has depleted much of the organic matter from grassland soil. In addition to grasses, common plants of temperate grasslands include flowers such as asters, coneflowers, sunflower, goldenrod, and clover. Near rivers, where sufficient water is present, trees such as cottonwoods, oaks, and willows grow. Large mammalian grazers such as horses, pronghorn, antelope, and bison inhabit temperate grasslands alongside many burrowing animals such as prairie dogs and mice. Predators include coyotes, bobcats, and wolves, and

birds include wild turkey, eagles, Canada geese, and the endangered prairie chicken.

TEMPERATE BROADLEAF FOREST

Temperate broadleaf forests, also called deciduous forests because they contain trees that shed their leaves before winter each year, occur at midlatitudes in the Northern Hemisphere, as well as in southern Australia and New Zealand. Precipitation in this biome averages 27.6–78.7 inches (70–200 cm) annually, and temperatures range from around 32°F (0°C) in the winter up to 86°F (30°C) in the summer. The soil on the forest floor is usually fertile and contains a lot of organic matter. Because of this, humans have cultivated much of the land that formerly supported these forests in their natural form; many of the temperate forests present today were planted by humans. Biodiversity is not as high as in tropical rain forests, but biomass is. Temperate forests contain several layers or zones, with the highest including tall trees like the common lime, birch, maple, oak, and beech. Smaller trees and saplings grow under the highest canopy, followed by shrubs and ferns, and then herbs. The forest floor contains lichens, club mosses, true mosses, mushrooms, and microorganisms, which play an important role in breaking down old wood and organic matter and in recycling the nutrients within the forest ecosystem. The forest provides shelter, shade, and protection for many birds, insects, and other animals such as squirrels, chipmunks, mice, deer, bears, raccoons, foxes, frogs, salamanders, and snakes. In the winter, some of the animals migrate south, and others hibernate.

CONIFEROUS FOREST

The coniferous forest, also called the taiga, is the largest terrestrial biome. It is located north of the temperate forest, south of the tundra, and extending across northern North America and Eurasia, and its dominant plants are conifers. The Russian taiga plays an important ecological role as a carbon sink, helping to reduce global climate change by incorporating carbon from carbon dioxide into organic material at a rate faster than it emits carbon dioxide back into the atmosphere. Annual precipitation averages about 15.7 to 39.3 inches (40–100 cm), but some coastal coniferous forests receive up to 118 inches (300 cm)—these are considered temperate rain forests. The winter temperatures of the taiga are harsh, dipping as low as -94°F (-70°C) in Siberia. Summertime highs can approach 86°F (30°C), but summers are short—the growing season only lasts about 130 days. In the northern forests of North America, the ocean moderates the temperatures, extending the length of the growing season there. Evergreen trees such as pine, fir, hemlock, and spruce have waxy needlelike leaves and cones as reproductive structures. The trees themselves have a cone shape to prevent snow from accumulating on them and weighing down the flexible branches. Because the leaves do not fall off the trees, they are ready to photosynthesize as soon as the weather permits the growing season to begin. The trees are tall and thin and have thick bark to resist occasional fires during droughts. Deciduous trees such as larches, birches, and aspens also grow in the taiga. Logging companies are destroying many of the trees of coniferous forests, though conservation efforts are attempting to limit this process. The soil is nutrient-poor and acidic, conditions that limit the plant diversity, though shrubs, herbs, and mosses can tolerate the taiga, as do lichens. Berry-bearing shrubs are an important food source for many animal taiga residents. Animal life includes herbivores such as hares, squirrels, voles, deer, beavers, caribou, buffalo, and moose and predators such as Siberian tigers, fox, lynx, wolverines, and bobcats. Black bears eat whatever they can find, including berries, tubers, insects, small mammals, and fish. Birds such as wood warblers and woodpeckers that migrate to the taiga to breed keep the insect population under control. Seed-eating birds such as finches and sparrows and predatory birds such as hawks and owls live in the taiga year-round.

TUNDRA

Many animals, such as caribou, that spend winters in the taiga spend part of the year in the tundra, the biome that covers the northernmost lands of the Arctic Circle. The tundra is typically cold and dry. With the Arctic Ocean nearby to moderate the climate, the temperatures are not as extreme as in the taiga, averaging -22°F (-30°C) in the winter and about 50°F (10°C) in the summer when the sunlight shines on the tundra 24 hours a day, but the growing season is short. Because precipitation only reaches about six to 10 inches (15.2–25.4 cm) a year, plant life is mostly herbaceous, or nonwoody, and includes lichens, mosses, grasses, herbs, low-growing shrubs, some flowers, and very few birch trees in southern areas of the tundra. As the taiga does, the tundra serves as a carbon sink, taking in more carbon from the atmosphere than it releases. The cold temperatures inhibit decomposition by microorganisms; thus organic matter accumulates in this superficial layer of soil that freezes and thaws each year. When it thaws, decomposition occurs. One can observe cracks on the Earth's surface from an aerial view, just as cracks form on concrete from repeated freezing and thawing. Below the uppermost layer of soil, the ground remains frozen. Water and plant roots cannot penetrate through this layer called permafrost, which may extend from 10 inches

Tundra flora typically lack woody tissue and only persist for one growing season; tundra fauna include large grazing animals such as the caribou shown here. *(U.S. Fish and Wildlife Service)*

(25.4 cm) to three feet (30.5 cm) down, so when the surface snow melts in the summer, temporary lakes form. A global increase in temperatures has melted some of the permafrost, increasing the rate of decomposition of the dead plant matter in the upper layer of soil and releasing more carbon back into the atmosphere, which contributes to global warming—forming a vicious, self-feeding cycle. Resident animal species include large mammalian grazers such as musk oxen, and others such as caribou (also called reindeer) migrate to the tundra during the short summers. Polar bears, wolves, wolverines, foxes, and snowy owls prey on these creatures, and on smaller squirrels, rabbits, lemmings, fish, seals, and birds like harlequin duck sandpipers and plovers that migrate from the taiga to nest in the tundra and feed off insects.

MOUNTAINTOPS

The Antarctic is too cold to support life-forms similar to those of the Arctic tundra. The climate on mountaintops, however, does resemble that of the tundra and is sometimes referred to as the alpine tundra or the alpine biome. Vegetation and soil conditions similar to the Arctic tundra's are found at these altitudes of about 10,000 feet (3,048 m). The growing season is short, and the winters are long and cold. Two main differences are that alpine soil can drain well, and the mountain life-forms must deal with high exposures to ultraviolet radiation from the Sun. Because the atmosphere is less dense, the partial pressures of carbon dioxide, which plants need for photosynthesis, and oxygen, which animals need for respiration, are low; thus plants and animals have special adaptations to compensate. Moving from the top of a mountain down toward its base, one may encounter several distinct ecological associations unique to the particular altitude.

See also BIOMES, AQUATIC; BIOSPHERE; ECOLOGY; ECOSYSTEMS; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED; PLANT FORM AND FUNCTION.

FURTHER READING

Allaby, Michael. *Deserts*. New York: Chelsea House, 2006.

———. *Grasslands*. New York: Chelsea House, 2006.

———. *Temperate Forests*. New York: Chelsea House, 2006.

Day, Trevor. *Lakes and Rivers*. New York: Chelsea House, 2006.

———. *Taiga*. New York: Chelsea House, 2006.

Moore, Peter D. *Tundra*. New York: Chelsea House, 2006.

Weigel, Marlene, and Julie L. Carnagie, eds. *Encyclopedia of Biomes*. 3 vols. Farmington Hills, Mich.: UXL, 2000.

Whitfield, Philip, Peter D. Moore, and Barry Cox. *Biomes and Habitats*. New York: Macmillan Reference USA, 2002.

Woodward, Susan L. *Biomes of Earth: Terrestrial, Aquatic, and Human-Dominated*. Westport, Conn.: Greenwood Press, 2003.

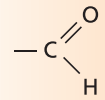
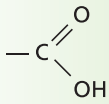
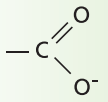
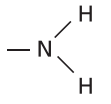
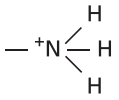
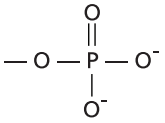
biomolecules Organic molecules that compose cells are known as biomolecules. Often called macromolecules, most biomolecules are rather large and are composed of numerous monomers, repeating subunits covalently linked to form polymers. The joining of monomers is usually accompanied by the loss of a water molecule in what is called a condensation reaction. One monomer contributes a hydrogen atom to the water molecule that is removed and the other, a hydroxyl group. Conversely, biomolecules can be broken down into monomeric subunits by hydrolysis reactions wherein a covalent linkage is broken by the addition of one hydrogen atom from a water molecule to one monomer and a hydroxyl group attaching to the adjacent monomer. Cells break down polymers into monomeric subunits and then reassemble them to create the biomolecules they need. There are four main classes: carbohydrates, proteins, nucleic acids, and lipids. Despite the fact that each of these types of biomolecules mostly consists of only four major elements (carbon, hydrogen, nitrogen, and oxygen) and two minor elements (sulfur and phosphorus), they exhibit a remarkable diversity of structure and function.

CARBON

The element carbon is the major component of all biological molecules. Because it has a valence of 4, it tends neither to gain nor to lose electrons to complete its outer shell. Instead, a carbon atom participates in strong covalent linkages with other carbon atoms (C), oxygen (O), nitrogen (N), and hydrogen (H) to form a variety of large and complex macromolecules. Double and triple bonds can also form between carbon atoms. Hydrocarbon chains form the basis of organic biomolecules, vary in length, and can be straight, circular, or branched. Because carbon-hydrogen bonds are nonpolar, however, simple hydrocarbons are not common in living organisms, which consist of cells that contain 70–95 percent water.

FUNCTIONAL GROUPS

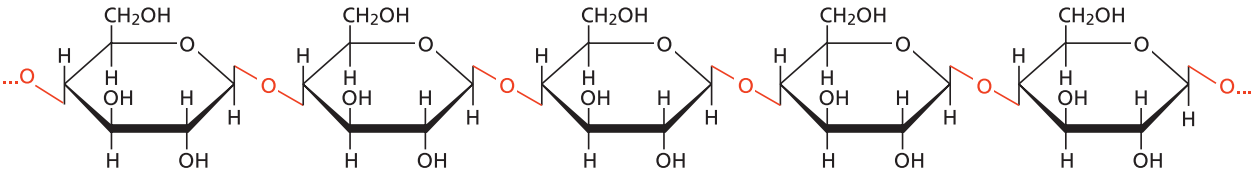
The addition of hydrophilic side groups to the nonpolar hydrocarbon skeletons acts to increase the solubility of biomolecules in an aqueous environment, such as inside a cell or body tissue. In combination with the basic structure of the carbon skeleton of an organic compound, the side chains attached to the carbon atoms help determine that molecule's chemical and physical properties. Side chains that commonly participate in the chemical reactions of a molecule are called functional groups. Six play a crucial role in biochemistry: hydroxyl groups, carbonyl groups, carboxyl groups, amino groups, sulfhydryl groups, and phosphate groups.

Functional Group	Formula	Compound Name
Hydroxyl	— OH	Alcohols
Carbonyl		Aldehydes
		Ketones
Carboxyl	 *nonionized* *or*  *ionized*	Carboxylic acids
Amino	 *nonionized* *or*  *ionized*	Amines
Sulfhydryl	— SH	Thiols
Phosphate		Organic phosphates

The functional groups attached to an organic compound contribute to that molecule's chemical and physical properties.

CARBOHYDRATES

Carbohydrates include sugars and related compounds that have the general formula $(CH_2O)_n$, with n usually being 3, 4, 5, or 6. For example, the molecular



Cellulose consists of thousands of glucose molecules linked in an unbranched chain.

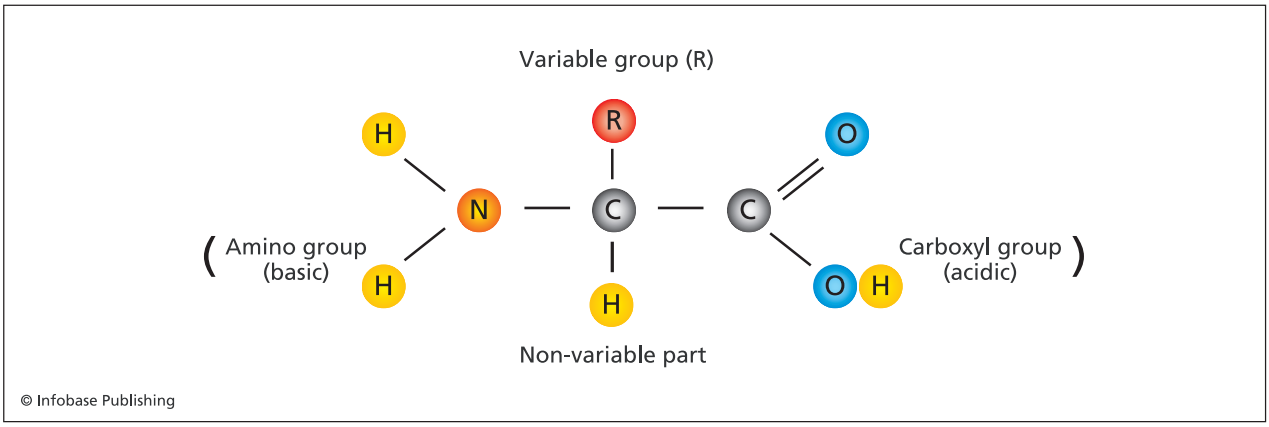
formula for glucose is $C_6H_{12}O_6$. Also known as simple carbohydrates, monosaccharides such as glucose, fructose, and galactose contain a single aldehyde or ketone group. They can be drawn as open chain structures but in aqueous solutions exist as closed five- or six-carbon ringed structures. Disaccharides form by a condensation reaction between two monosaccharides, forming a special type of bond called a glycosidic linkage. Sucrose, common table sugar, consists of a molecule of glucose linked to a molecule of fructose. Maltose and lactose are two other common disaccharides formed from two glucose molecules or a glucose and a galactose molecule, respectively. When between three and 50 subunits join together, the resulting molecule is called an oligosaccharide. The term *polysaccharide* refers to much larger molecules that contain hundreds or thousands of monosaccharides that are often cross-linked and branched to form more complex structures such as starch, glycogen, or cellulose.

Glucose serves as the main source of cellular fuel, and other carbohydrates are often converted into glucose when energy is needed. When catabolized by aerobic respiration, a single molecule of glucose yields up to 38 molecules of adenosine triphosphate (ATP). Glucose is also the primary sugar dissolved in blood that circulates throughout the body of animals. Plants store surplus energy in the form of starch, a helical glucose polymer consisting of hundreds to thousands of subunits. During digestion, animals hydrolyze, or break, starch into individual glucose subunits that can immediately be utilized for energy or stored in the form of glycogen, a highly branched polymer of glucose subunits. Other carbohydrates perform structural roles. For example, plant cell walls are made primarily of cellulose, unbranched chains consisting of thousands of covalently linked glucose molecules. The configuration of the glucose monomers in cellulose differs from that of those in starch, causing the linkages to differ slightly. Because of this, separate enzymes are required to digest starch and cellulose, and many animals including humans do not synthesize the latter. Undigested cellulose, or fiber, is still an important part of a healthy diet. Another polysaccharide that performs a structural role is chitin, the major component of arthropod skeletons and fungal cell walls.

PROTEINS

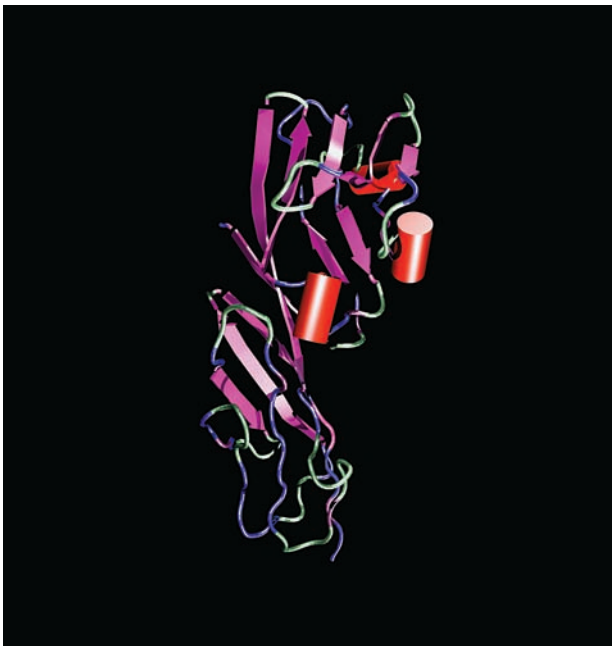
Proteins are the main macromolecular constituent of cells. Over half of a human body is made of protein. Proteins consist of one or more polypeptides that are constructed by linking 20 different amino acids in specific sequences. All amino acids consist of a central carbon bound to four side groups: a hydrogen atom (–H), an amino group ($–NH_2$), a carboxyl group (–COOH), and a variable side chain (denoted by R). At a neutral pH, the amino group and the carboxyl group are ionized to $–NH_3^+$ and $–COO^-$. The chemical composition of the variable group is what gives an amino acid its unique properties. The acidic amino acids include aspartic acid and glutamic acid; the basic amino acids include lysine, arginine, and histidine; amino acids with uncharged polar side chains include asparagine, glutamine, serine, threonine, and tyrosine; and the nonpolar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, glycine, and cysteine. Amide linkages called peptide bonds form by condensation reactions between amino acids to synthesize polypeptide chains.

After the synthesis of a polypeptide chain, a protein must fold into its final, or native, configuration before it is considered active, or ready to perform its main function, which usually involves the specific interaction with another molecule. The order or sequence of amino acids in a chain is referred to as the protein's primary structure. The secondary structure that a protein assumes depends on hydrogen bonds between hydrogen atoms of the amino groups and oxygen atoms of the carbonyl groups of different amino acids. The alpha-helix and the beta-pleated sheet are two common secondary structures. An alpha-helix resembles an old-fashioned telephone cord. The beta-pleated sheets involve two or more regions of the polypeptide chain lying parallel to each other and have a zigzagged appearance as if one had taken a sheet of paper and folded it back and forth at



Amino acids all have a central carbon with four side groups: a hydrogen atom, an amino group, a carboxyl group, and a variable R group.

regular intervals, then opened it slightly. At the tertiary level of protein folding, the polypeptide chains adopt their three-dimensional conformations that are stabilized by a combination of hydrophobic interactions, hydrogen bonds, and ionic interactions. Disulfide bridges, covalent linkages between the sulfhydryl groups of two cysteines, also reinforce a protein's tertiary conformation. Some proteins consist of more than one polypeptide chain. In these cases, the final quaternary structure results when one or more polypeptide chains join and aggregate in a purposeful arrangement with one another. Most proteins have one of two general shapes, either fibrous or globular. Fibrous proteins include actin and myosin, components of muscle fibers. Globular proteins such as hemoglobin have a roughly spherical form.



The final three-dimensional structure of a protein includes a combination of secondary structures arranged in a unique conformation. In this depiction of a CD4 receptor protein molecule, the alpha-helices are shown as cylinders and the beta-pleated sheets are shown as flat ribbons with arrowheads on the ends. *(Dr. Tim Evans/Photo Researchers, Inc.)*

Heating or treatment with certain chemicals denatures proteins, or destroys their conformation by disrupting the chemical bonds and interactions formed between atoms of the molecules. Because the structure of a protein is crucial to its ability to function properly, denaturation results in the loss of function and is sometimes used to destroy cell populations such as in the process of boiling water potentially contaminated with bacteria or other microorganisms.

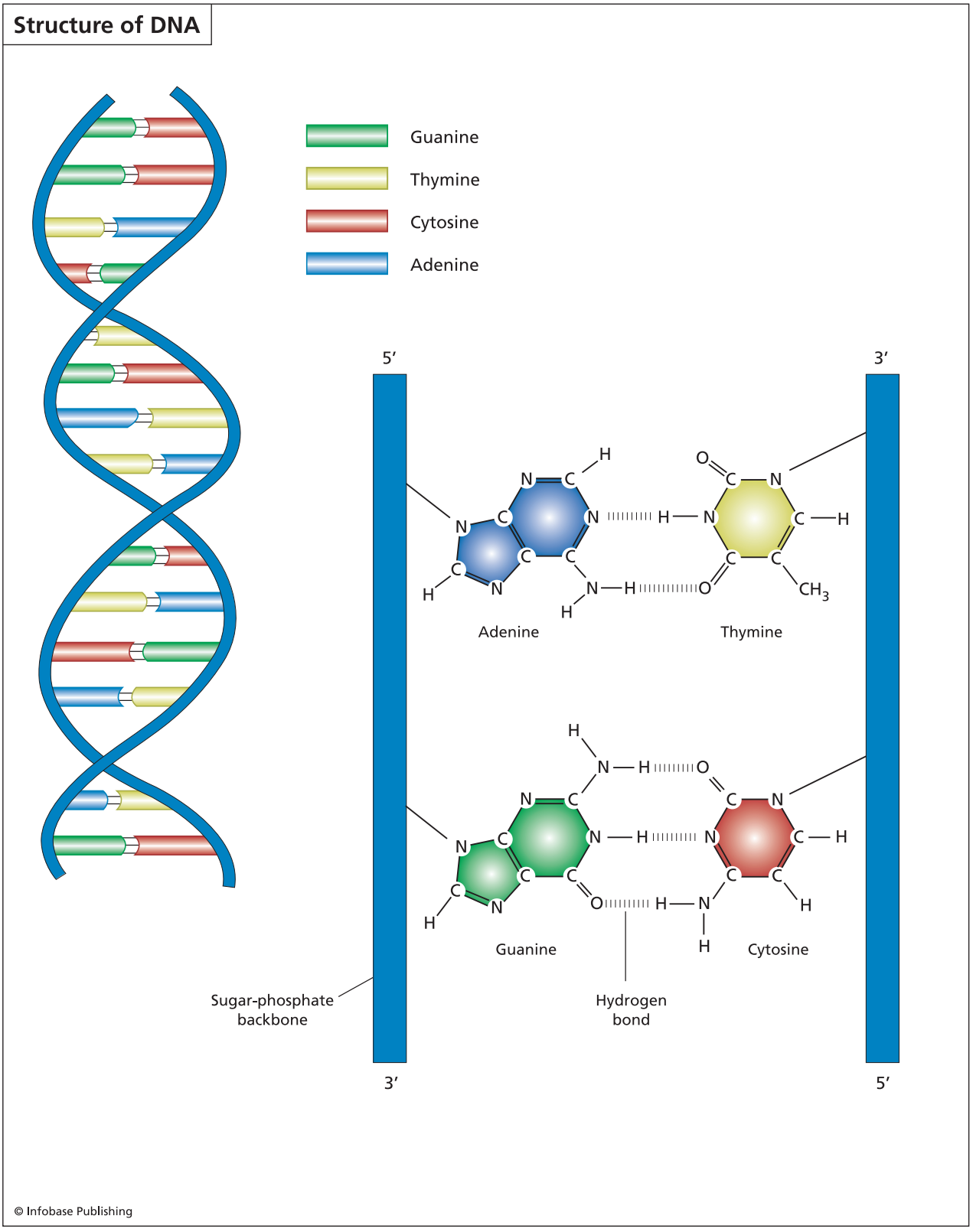
The enormous architectural diversity of proteins allows them to serve many diverse functions. Some act as structural building blocks for cells or tissues such as that found in skin, ligaments, and muscles. Many play enzymatic roles, and others such as insulin are hormones involved in communication between cells. Receptors located on the surface of cell membranes and antibodies, a component of specific immunity, are proteins. The movement of substances within a cell, across biological membranes, and for cellular motility is also accomplished by proteins.

NUCLEIC ACIDS

Nucleic acids are composed of chains of nucleotides bonded together by phosphodiester linkages. Nucleotides consist of three components: a nitrogenous base, a five-carbon sugar, and a phosphate group. There are two main types of nucleic acid, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

One major structural difference between DNA and RNA is the sugar molecule they contain. DNA is a polymer of deoxyribonucleotides, which, as the name implies, have deoxyribose sugar molecules.

RNA contains ribose sugar molecules that differ in that they have an extra oxygen atom attached to the 2' carbon. A nucleoside is formed by the linkage of a nitrogenous base to the pentose sugar. The bases are divided into two general categories, purines and pyrimidines. The purines consist of two fused



The two nucleotide chains of DNA are linked by specific hydrogen bonds that form between adenine and thymine, and between cytosine and guanine.

rings and include either adenine or guanine. The pyrimidines have a single ring and include cytosine, thymine, and uracil. In general, thymine only exists in DNA, and uracil is only in RNA. The addition of a phosphate group to the 5' carbon atom of the sugar in a nucleoside results in a nucleoside monophosphate, also known as a nucleotide. A condensation reaction between the phosphate group of one nucleotide and the 3' hydroxyl group of another creates a phosphodiester linkage. Several of these linked in succession form the sugar phosphate backbone of a single nucleotide chain. In DNA, two chains are linked by hydrogen bonds that form between specific base pairs, creating a double-stranded molecule. Adenine always pairs with thymine, and cytosine always pairs with guanine, giving the strands the characteristic of complementarity. The two strands are antiparallel to one another, meaning they run in opposite directions. If the phosphate group attached to the 5' sugar carbon of a nucleotide points upward, on the other strand the phosphate group of the complementary nucleotide will be pointing downward, and the hydroxyl group attached to the 3' sugar carbon will be pointing upward. The regular pairing of a purine with a pyrimidine between complementary chains maintains a consistent width along the length of the double-stranded molecule. The two polymers twist around one another at a repeat length of 10 nucleotides, giving it an overall helical shape.

DNA carries the genetic information in living organisms. Genes are simply segments of a linear DNA molecule that encode for a specific protein or RNA molecule. Inside cells, DNA exists as part of chromosomes that duplicate before the cell divides to ensure that each daughter cell receives all the genetic information. RNA performs several roles during the process of protein synthesis. RNA is also a structural component of ribosomes, and the ribonucleotide adenosine triphosphate (ATP) is the main molecule used by cells when they expend energy.

LIPIDS

Lipids are biomolecules that are insoluble in water but soluble in nonpolar organic solvents. Unlike other biological macromolecules, lipids are not polymers. This diverse class of biomolecules includes triglycerides (commonly called fats), phospholipids, and steroids.

The major class of lipids, triglycerides, contains both fats and oils. Fats are solid at room temperature, whereas oils are liquid. Triglycerides consist of one molecule of glycerol, a chain of three carbons with attached hydroxyl groups, to which three fatty acid chains are attached by ester linkages. Fatty acids have carboxyl groups with long unbranched hydrocarbon tails that give fats their hydrophobicity. Examples include stearic acid (CH_3 $(CH_2)_{16}COOH$), which is found in beef tallow; butyric acid ($CH_3(CH_2)_2COOH$), which is found in butter; and oleic acid (CH_3 $(CH_2)_7CH{=}CH(CH_2)_7COOH$), which is found in olive oil. A fatty acid is said to be saturated if it contains only single bonds and the maximal possible number of hydrogen atoms. Monounsaturated fatty acids have a single double bond. Polyunsaturated fatty acids have more than one double bond per molecule, and therefore a lower melting point, making them liquid at room temperature. Most animal fats are saturated, whereas fats from fish and plants are unsaturated and are referred to as oils.

Phospholipids are structurally similar to triglycerides, except they have a phosphoric acid and an amino alcohol group in place of the third fatty acid. Because the end containing phosphorus is soluble in water but the other end is nonpolar, phospholipids make good emulsifiers, substances that mix with both lipids and water. When placed in an aqueous solution, phospholipids will aggregate to form a micelle, a spherical structure in which the hydrophobic tails all point inward and the hydrophilic heads face outward. In cell membranes, phospholipids form bilayers with the hydrophilic heads facing toward the cell's exterior and the cytoplasm while the hydrophobic tails point inward toward one another, to the exclusion of water molecules.

Steroids share a unique structure consisting of three six-carbon rings fused with one five-carbon ring. Different functional groups are attached to the fused ring structure to give a variety of steroids including cholesterol and the substances synthesized from it including bile salts, the sex hormones, and vitamin D.

Lipids serve a variety of functions in living organisms. Fats are a major source of stored energy that can be utilized when glycogen levels are low, they are the major component of biological membranes, they form a waxy coating on plants that protects against water loss, and they play hormonal roles.

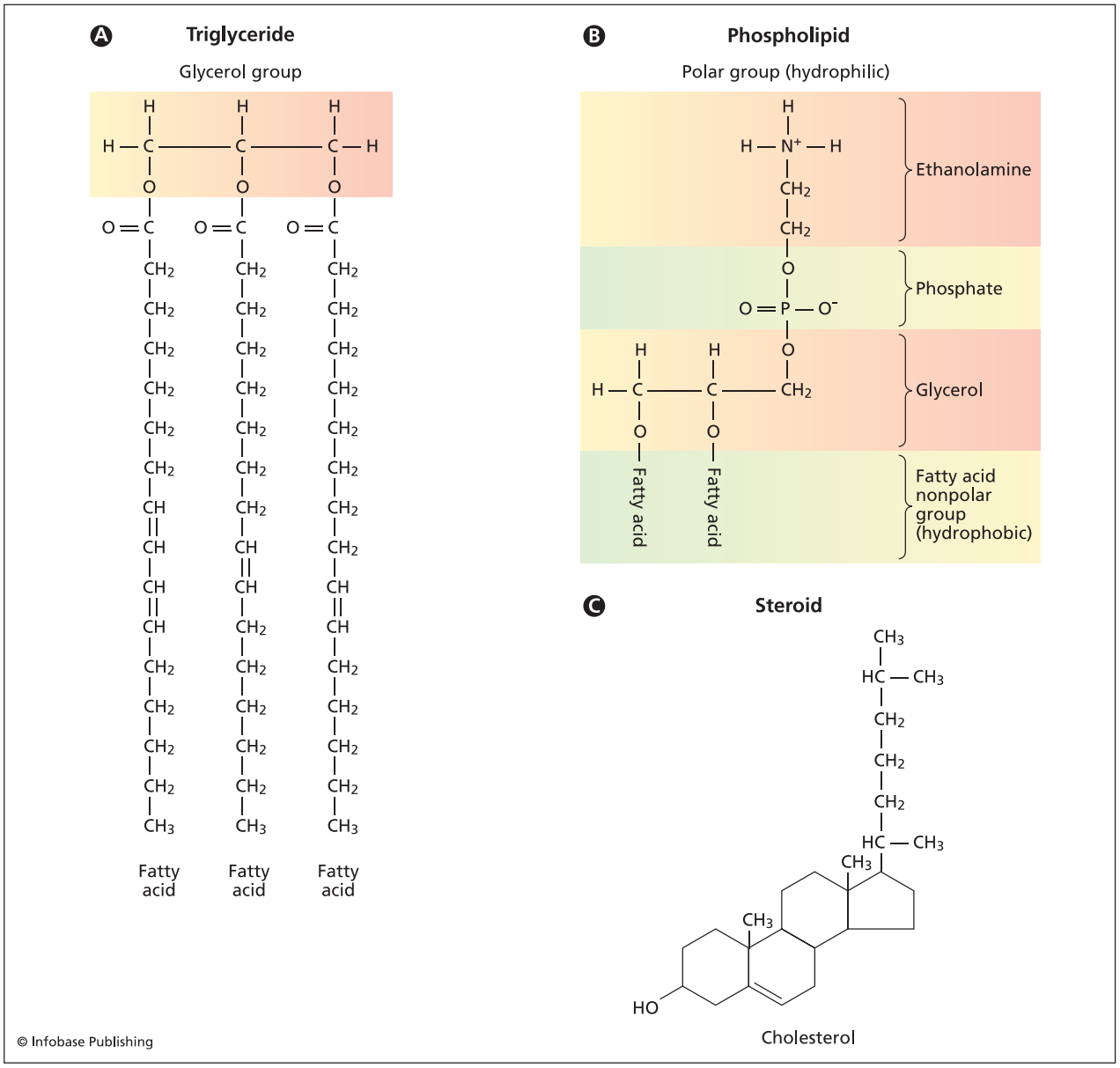
See also BIOCHEMICAL REACTIONS; BIOCHEMISTRY; CELLULAR METABOLISM; CHEMICAL BASIS OF LIFE; DEOXYRIBONUCLEIC ACID (DNA); GENE EXPRESSION; NUTRITION; ORGANIC CHEMISTRY, ITS RELEVANCE TO LIFE SCIENCE.

FURTHER READING

Champe, Pamela C., Richard A. Harvey, and Denise R. Ferrier. *Lippincott's Illustrated Reviews: Biochemistry*. Philadelphia: Lippincott, Williams, & Wilkins, 2005.

Chang, Raymond. *Physical Chemistry for the Biosciences*. Sausalito, Calif.: University Science Books, 2005.

Daintith, John, ed. *The Facts On File Dictionary of Biochemistry*. New York: Facts On File, 2003.



The structures of lipids vary tremendously. A) Triglycerides consist of a glycerol molecule, to which three fatty acids are attached. B) Phospholipids differ from triglycerides in that the third carbon of the glycerol backbone is linked to a phosphoric acid linked to an amino alcohol group. C) Steroids such as cholesterol have a fused-four-ring structure.

Karukstis, Kerry K., and Gerald R. Hecke. *Chemistry Connections: The Chemical Basis of Everyday Phenomena*. Boston: Academic Press, 2003.

Lennarz, William J., and M. Daniel Lane. *Biological Chemistry*. 4 vols. Amsterdam; Boston: Elsevier, 2004.

Murray, Robert K., Daryl K. Granner, Peter A. Mayes, and Victor W. Rodwell. *Harper's Illustrated Biochemistry*. 26th ed. New York: McGraw Hill, 2003.

Widmaier, Eric P. *The Stuff of Life: Profiles of the Molecules That Made Us Tick*. New York: Times Books, 2002.

bioremediation Bioremediation is the use of biological processes to remedy or resolve an environmental problem such as to clean up an oil spill or contaminated groundwater. Microorganisms including bacteria, fungi, and protists have diverse metabolic abilities and naturally utilize a variety of substances to fulfill their energy and nutritional needs. Chemicals and other substances that harm the environment or are toxic to some life-forms serve as food for others. Though microorganisms present in soil and water have been actively decomposing and recycling human waste since the beginning of civilization, the

first formal use of bioremediation to treat synthetic compounds and spills was in 1975, when a group of scientists observed that growth of hydrocarbon-degrading bacteria was enhanced by the addition of nutrients to the soil. Increasing the metabolism of the bacteria increased the rate at which the hydrocarbons were consumed. Since then the use of bioremediation has been improved and expanded to remove metals, acidic waters produced by coal mining, toxins, and other synthetic organic compounds, in addition to cleaning up hydrocarbons from oil spills.

Bioremediation is a specific application of biodegradation, the biological breakdown of organic substances. Something that is biodegradable can be broken down by microorganisms that decompose the material into smaller components that feed into either biogeochemical cycles or into smaller molecules that other organisms can take in for nutrition. Because carbon makes up a significant mass of organic compounds, decomposition of natural and human-made organic compounds results in the conversion of much of the carbon into carbon dioxide, which is liberated. The microorganism also incorporates some of the carbon into its own biomass. Bioremediation is the specific use of microorganisms to biodegrade harmful pollutants such as petroleum products, benzene, and toluene, into innocuous products. The bacteria *Pseudomonas* and *Bacillus* and fungi that break down toxins have been most useful, but plants are also used in bioremediation. Some plants can uptake and concentrate toxic metals from diluted samples in soil or groundwater.

In situ bioremediation (ISB) is carried out at the site of contamination and can be used to treat contaminated soils, aquifers, lakes, or other bodies of water. Natural bioremediation refers to the simplest form of bioremediation used today. Microorganisms that are already present in the environment are left to perform the task without any assistance, although a worker usually monitors the levels of the contaminant to ensure the microbes are removing it. One must also make sure the contaminant does not leak away or is not washed away before being metabolized. In many cases, assistance is needed to biodegrade or to remove the pollutant more quickly. Two different strategies can accomplish this: biostimulation or bioaugmentation. The addition of chemicals or nutrients to accelerate the process of bioremediation is known as biostimulation. As the bacterial growth rate increases, the organisms metabolize the polluting substance at a faster rate. This method is more common, but, as natural bioremediation does, biostimulation depends on the previous existence of pollution-eating microorganisms at the contaminated site. Optimizing the conditions with respect to the concentrations of other nutrients such as phosphates, nitrogen-containing compounds, and oxygen simply encourages the growth of the native pollutant-eating microbes. Hydrogen peroxide, which decomposes to oxygen and water and is more soluble in water than oxygen gas, is often added to meet the high demand for oxygen when the metabolizing bacterial population is large. When using ISB to treat contaminated water, one difficulty is that water movement carries the nutrients away. Because of this, ISB works better for spills on beaches or soil than on open bodies of water. Another method involves seeding the polluted environment with microorganisms known to metabolize the specific contaminating compound. This method, called bioaugmentation, may involve the introduction of a naturally occurring bacterial strain or a genetically engineered strain. In the United States, the Toxic Substances Control Act gives the Environmental Protection Agency the authority to regulate the release into the environment of microorganisms that have been genetically engineered for the purpose of breaking down chemical pollutants.

Despite pumping air, supplying nutrients, or adding microbes to a contaminated site, in some situations, the optimal conditions for the microorganisms to grow and multiply cannot be achieved underground. In these cases, it is appropriate to dig up the contaminated soil and place it in containers called bioreactors. The advantage of bioreactors is that the soil can be heated to speed up metabolism, the concentration of different nutrients can be controlled, the soil can be mixed or aerated efficiently, or anaerobic conditions can be created, and they can be used to treat solid, liquid, or gaseous contaminants. To treat groundwaters, wells are drilled and the groundwater is pumped into tanks, where nutrients and air are mixed into the water to stimulate microbial growth, and then the treated water is returned into the ground. Alternatively, the nutrients and air can be added to the wells.

In addition to being inexpensive, the advantage of bioremediation is its use of natural biodegradation processes; thus it does not require the introduction of any additional dangerous chemicals into the environment. The pollutant is converted to harmless gases and water. With ISB, the contaminated soil or water does not need to be removed and taken to a different location for cleanup, so costs are significantly lower. A disadvantage is that the microorganisms might not be able to tolerate the conditions where the contamination exists. Other chemicals toxic to the pollution-eating microorganisms might be present at the site or the concentrations might be too high. Another disadvantage is the length of time required to bioremediate a site—months or even years depending on the type of pollutant and the conditions of the contaminated site.

Petroleum hydrocarbons were the first type of compounds targeted by bioremediation. Anaerobic bacteria, bacteria that do not use oxygen as an electron acceptor in cellular respiration, have proved efficient at metabolizing other substances, such as chlorinated hydrocarbons, that scientists previously believed were recalcitrant to bioremediation. Since they undergo anaerobic metabolism, oxygen does not need to be supplied. The development of bioremediation techniques for the removal of metals, gasoline additives, and chemicals generated during the synthesis of explosives has also progressed. Researchers have identified microorganisms that can reduce metals to less toxic valence states. Microbiologists continue to seek new bacterial strains that utilize potential pollutants as growth substrates. To isolate them, they collect samples of soil or other environmental samples and culture the microorganisms that are present in media containing mineral salts and the desired growth substrate, in other words, the contaminant to be biodegraded. By adding the desired substrate but not adding other organic compounds, the medium selects for organisms that are capable of using that specific substrate to meet all of their metabolic needs.

Cometabolism refers to the situation when an organism does chemically transform a substance but does not use it as an energy source or for synthesis of other molecules. When a microbe has one enzyme that has a broad specificity and binds and transforms a substrate, but its other enzymes that act at a later stage in the same metabolic pathway do not recognize the transformed compound as a substrate, then the by-product accumulates unless another bacterial species is present and can metabolize it further. For example, several types of bacteria can metabolize aromatic hydrocarbons. Environmental contaminants such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) are chlorinated aromatic hydrocarbons that the bacteria begin to metabolize but cannot carry the process through to completion, so chlorinated by-products accumulate. Some bacteria that can utilize biphenyl cometabolize PCBs to chlorobenzoates. Though the bacteria have enzymes that can further metabolize benzoate, the enzymes do not recognize chlorobenzoates, the product of PCB metabolism. Other types of bacteria can metabolize the chlorobenzoate, however, so they perform the next step in the breakdown process. Cometabolism is a slow process but can be accelerated somewhat by the addition of other organic compounds to encourage growth. Scientists are learning that many synthetic organic compounds like DDT that were previously thought to be nonbiodegradable, or recalcitrant to bioremediation, can be biodegraded by microbial consortia, an association of several microorganisms that cooperate to complete the task. Different types of bacteria perform sequential steps in biodegradation of the compound. Though this is good news, the process is complicated by the fact that some of these bacteria are aerobic and others are anaerobic. Using genetic engineering techniques, researchers are trying to create strains of bacteria that contain and express the genes necessary to carry out the entire metabolic pathway to completion within one organism. Researchers have also identified bacteria that can remove the radioactive element uranium from contaminated groundwater and convert it into insoluble uraninite.

Landfarming has been useful in getting rid of petroleum refinery wastes. In this method, a farmer spreads the waste products over a field and allows indigenous oil microbes to metabolize the pollutants. Initial studies suggest that fields used for landfarming will still grow crops, but the quality of the crop product with respect to nutritional and chemical content is still being investigated.

See also ECOLOGY; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED; ENVIRONMENTAL SCIENCE.

FURTHER READING

A Citizen's Guide to Bioremediation. Washington, D.C.: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, 2001. Available online. URL: http://www.clu-in.org/download/citizens/bioremediation.pdf. Accessed January 17, 2008.

Crawford, Ronald L., and Don L. Crawford, ed. *Bioremediation: Principles and Applications.* New York: Cambridge University Press, 2005.

Lovely, Derek R. "Cleaning Up with Genomics: Applying Molecular Biology to Bioremediation." *Nature Reviews/Microbiology* 1 (October 2003): 36–44.

Mulligan, Catherine N. *Environmental Biotreatment: Technologies for Air, Water, Soil, and Wastes.* Rockville, Md.: Government Institutes, 2002.

Singh, Ajay, and Owen P. Ward, eds. *Applied Bioremediation and Phytoremediation.* New York: Springer, 2004.

U.S. Environmental Protection Agency, Technology Innovation Program. "Hazardous Cleanup Information." Available online. URL: http://www.clu-in.org/. Accessed January 17, 2008.

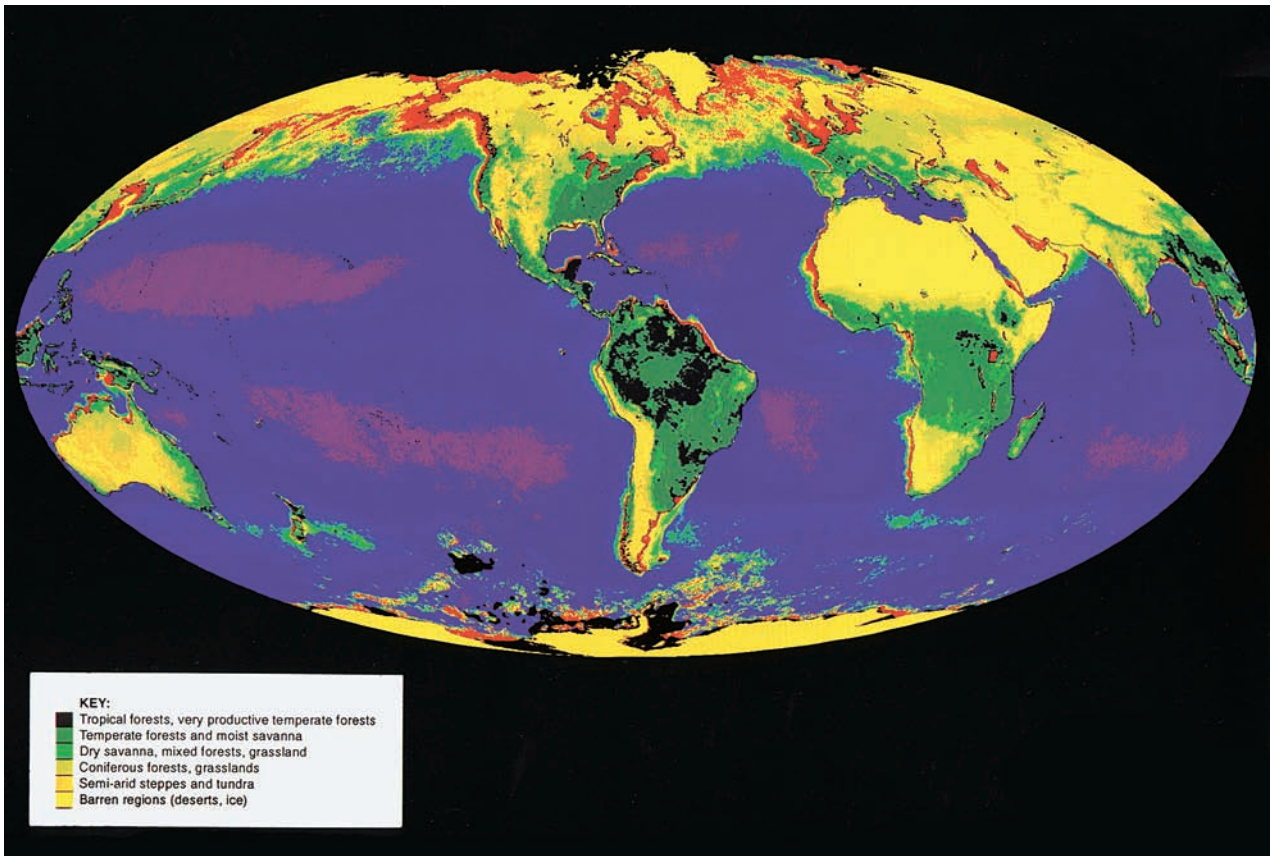
biosphere The biosphere is the zone of the Earth that contains life and consists of the lower part of the atmosphere (air), the hydrosphere (water), and part of the lithosphere (rock and soil). All organisms live within the biosphere, which spans about 13 miles (21 km), or 1/600 the diameter of Earth. Most life exists within a much narrower band close to the surface of the land or oceans, where sunlight and water

are readily available. The biosphere can be divided into smaller units called ecosystems, comprising all the living organisms and the physical environment within a defined area. A decaying log on the forest floor and all the life-forms that live in or on it provide an example of an ecosystem, as is the Sahara in northern Africa. The biosphere can be considered the largest, most inclusive ecosystem on the Earth. Within the biosphere, all of the life-forms on the planet and the many varied environments interact with and affect one another.

STRUCTURE OF THE BIOSPHERE

The biosphere encompasses parts of the atmosphere, the hydrosphere, and the lithosphere. The boundaries of the biosphere are not well defined. At one extreme, winds can carry dormant microbial spores higher up in the atmosphere than can support active, metabolizing life-forms. At the other extreme, scientists have discovered microbial life in the subsurface continental sedimentary basins of North America at depths of about two miles (near 3.2 km), and isolated thermophilic prokaryotes from 3.3 miles (5.3 km) in a borehole drilled in granite gneiss below the country of Sweden. Life also exists near hydrothermal vents on the floor of the deep ocean, found at an average depth of 1.33 miles (2.1 km) and with temperatures reaching 750°F (400°C). Despite these extreme examples, the majority of life exists in the more moderate conditions of the upper 330 feet (100 m) of the lithosphere and hydrosphere. Geographic areas within this crowded region do exist that cannot support much life and are practically barren.

The atmosphere is the blanket of air that surrounds Earth. The major constituents are 78 percent nitrogen (N_2) and 21 percent oxygen (O_2). Carbon dioxide (CO_2) is an important constituent, though its concentration is only about 385 parts per million. Life in the biosphere helps maintain the chemical composition of the atmosphere, which is in a highly oxidizing state. The amount of water (H_2O) vapor is variable and forms clouds in the troposphere, the lowest layer of atmosphere, which contains about 80 percent of the atmospheric mass and extends five to nine miles (8–14.5 km) from Earth's surface. The troposphere is responsible for most of the weather that is observed from the ground and is the only layer of atmosphere known to support life. Birds and insects live in the troposphere.



This satellite photograph shows differences in vegetation throughout the biosphere; dense vegetation is represented by purple and green, sparse vegetation by various shades of brown. *(NASA)*

The lithosphere is the solid, rocky crust consisting of inorganic minerals covering the entire planet. Most terrestrial organisms live between 10 feet (3 m) underneath and 100 feet (30 m) above Earth's surface.

The hydrosphere comprises all the water on the Earth including the oceans, lakes, rivers, ice sheets and glaciers, and moisture present in the air as vapor. The oceans contain 97 percent of Earth's water, and most oceanic life inhabits the upper 656 feet (200 m).

Although all of the components of the biosphere can be clearly defined, they are not necessarily separated physically. Most habitats contain components of all three, as the air, water, and earth all contribute essential ingredients to life. For example, in a rocky coastal biome, the rocks and dislodged sediment represent part of the lithosphere, the water and moisture sprayed into the air represents the hydrosphere, and the oxygen and other gases mixed into the water by the aerating action of the crashing waves represent the atmosphere.

ABIOTIC FACTORS AFFECTING THE BIOSPHERE

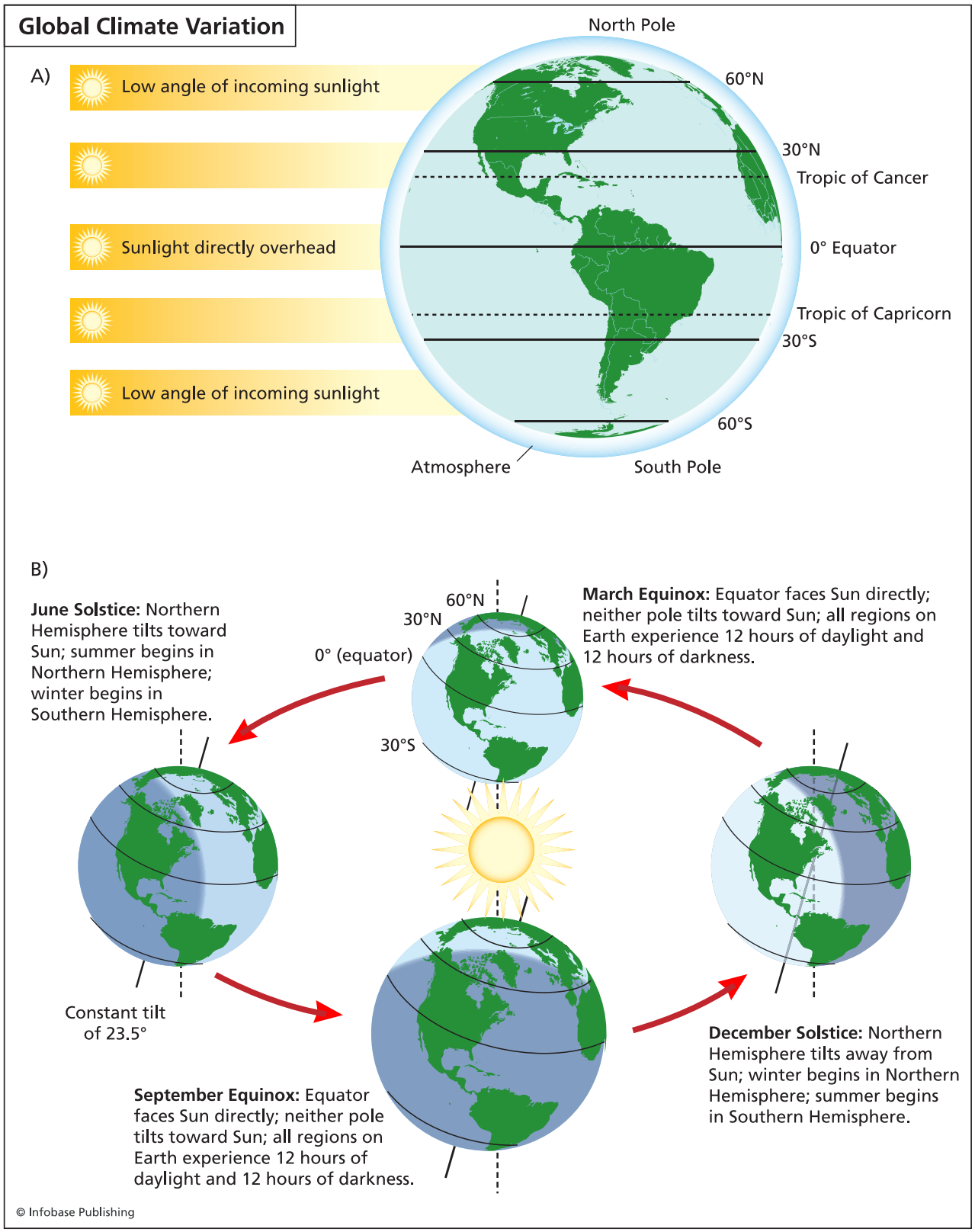
Abiotic factors are nonliving elements that influence whether life can exist, and, if so, what type of life-forms may exist in a particular region of the biosphere. Climate is the major abiotic factor affecting life in the biosphere. The distance from the Earth to the Sun is small enough to warm the entire planet but great enough so it is not too hot. Even slight variations in this distance affect the climate of a geographic region, as demonstrated by the seasonal climate changes due to the Earth's tilt toward or away from the Sun. Other influential abiotic factors include daily weather, erosion, earthquakes, and chemical reactions that occur to the landscape and in the atmosphere. Geological, chemical, and biological processes contribute to the cycling of elements necessary for life, mainly carbon, nitrogen, oxygen, and phosphorus. Climate and weather drive water through the hydrologic cycle, upon which all life depends.

The shape of the planet impacts the biosphere because it affects the path sunlight, the biosphere's ultimate energy source, must take to reach Earth's surface. At the equator, the sunlight hits the Earth's surface at a right angle, delivering the most intense radiation to equatorial areas. As a result of this great amount of energy input, biomes that occur in this region, such as tropical rain forests, are highly productive. The farther away one moves from the equator and toward either pole, the lower the angle of the incoming radiation; thus the sunlight is less direct: in other words, more radiation has already been absorbed by the time it reaches Earth's surface. The intensity of light that reaches the surface also varies seasonally as a result of Earth's tilt. The planet tilts 23.5° relative to its axis, causing the Northern Hemisphere to experience summer while the Southern Hemisphere experiences winter, and vice versa. The regions of the planet that lie between 23.5° north and 23.5° south latitude, known as the Tropics, receive the most sunlight year-round, and the least seasonal variation, since these regions are always closest to the Sun. Because light intensity is seasonal, so are many life cycles. Some animals only mate or breed in the spring, some animals hibernate in the winter, and plant life often becomes dormant before the winter.

Because solar radiation is most intense near the equator, more water evaporates in those areas. The warm moist air rises and then falls as precipitation. Some of the air, now dry, absorbs moisture from the Earth's surface as it moves away from the equator. At latitudes of about 60°, the air becomes moist enough to drop more rain, then is dry again over the poles. The rotation of the planet about its axis as the air flows near its surfaces also creates predictable wind patterns. These winds are named for the location where they originate; for example, the westerlies flow from the west to the east in temperate zones, and the northeast trade winds blow from the northeast. Wind shapes landscapes and amplifies temperature effects; thus organisms living in windy areas of the biosphere must be able to tolerate increased heat and water loss.

Geological features such as mountain ranges or nearby large bodies of water create regional or local effects on climate. Mountains affect sunlight, temperature, and rainfall on an area. For example, in the Northern Hemisphere, south-facing slopes receive more sunlight than north-facing slopes, causing different types of vegetation to grow on different sides of a mountain range. Mountains also direct air upward, where it cools and can cause rainfall in an area. Nearness to water bodies affects life not only by providing water, but by affecting local climate. Because water has a high heat capacity, it can absorb large amounts of heat energy from the Sun, with minimal effects on its temperature. This aspect of the hydrosphere moderates the climate of nearby terrestrial environments, keeping the temperatures lower than in regions farther inland. After the Sun sets and the water cools, the water body releases warmth into the surrounding air, preventing the nighttime temperatures from dipping very low.

The incoming solar radiation warms the Earth's surface; matter on Earth's surface absorbs some of it and reflects some of it back into the atmosphere as heat. Greenhouse gases in the stratosphere trap this heat within Earth's atmosphere. Some of the Sun's warmth serves to evaporate water from the oceans and Earth's surface. Once the water vapor rises into



Climate varies globally. A) Because Earth is curved, sunlight that reaches Earth's surface at higher latitudes has penetrated a greater length of atmosphere, thus is more diffuse than sunlight hitting the Earth's surface near the equator. B) Earth's tilt causes the regions near the equator to receive more sunlight annually than regions nearer to the poles.

the atmosphere, winds carry it over the continents, and it falls as precipitation.

The amount of moisture present influences the types of life-forms that can inhabit an area. The simple presence of water is not sufficient; it must be available to organisms. Even marine organisms must have mechanisms for preventing water loss due to osmosis because of the hypersaline conditions, and freshwater organisms must have adaptations that prevent their cells from swelling and bursting as a result of water diffusing into them. All terrestrial life must resist desiccation or dehydration, and organisms living in dry areas must have special adaptations for enduring long periods of drought or for storing water.

The composition of the soil also affects the biosphere. The mineral composition and acidity or alkalinity may limit the type of vegetation that can grow. The physical nature of the landscape is also important, and processes such as erosion and weathering can shape and mold terrestrial and aquatic habitats. The chemical composition of the atmosphere and dissolved gases in bodies of water allows organisms suited for certain environments to respire in the conditions of that specific environment.

See also BIOMES, AQUATIC; BIOMES, TERRESTRIAL; ECOLOGY; ECOSYSTEMS.

biotechnology The term *biotechnology* is sometimes used interchangeably with *genetic engineering* or *recombinant DNA technology,* but the three have distinct meanings. Biotechnology is simply the practical application of knowledge from biological science, and it often refers to the use of living organisms or materials they generate to make commercial products or accomplish specific useful tasks. The products may be pharmaceuticals, cloned mice, or a genetically altered crop of corn. Examples of tasks facilitated by biotechnological advances include bioremediation, the use of microorganisms to clean up an oil spill, and deoxyribonucleic acid (DNA) fingerprinting to identify a suspect in a crime. Genetic engineering is the artificial manipulation of the genome of an organism, a task that is accomplished using tools of recombinant DNA technology. Biotechnology is more encompassing and includes the application of biological knowledge, biological processes, or organisms that may or may not be genetically modified in order to accomplish a task or goal. The benefits of biotechnology to basic research are numerous. New techniques and methods have led to the rapid advancement of knowledge in a variety of diverse fields including cell and molecular biology, developmental biology, applied and environmental microbiology, evolutionary biology, infectious disease, human health and medicine, genomics, and microbiology. This article discusses several applications of biotechnology to commercial industries, human health, and agriculture.

People employed knowledge of life sciences for their own benefit long before the term *biotechnology* was coined. One of the oldest applications of biotechnology is fermentation, the process used to make alcoholic beverages such as wine and soured foods such as pickles and buttermilk. Until the mid-1800s, when the French chemist Louis Pasteur demonstrated that microorganisms called yeast were responsible, people thought fermentation was a chemical rather than biological process.

Another traditional use of biotechnology is plant tissue culture, the propagation of plants under sterile conditions. By growing seeds or plants on specialized media, a horticulturist can generate clones of a plant, better control the environmental conditions, and nurture plants that are particularly tricky to grow successfully. Creating clones, identical genetic copies, of plants is easier than creating other organisms because many plant cells are totipotent, meaning they have the ability to develop into a complete new organism under the right conditions. If plant cells have been transformed, genetically engineered to exhibit a particular characteristic, tissue culture allows for the totipotent transformed cells to develop into a whole plant.

Microorganisms perform a variety of beneficial biotechnological services. Certain types of bacteria can be utilized to extract minerals from ores in a biotechnological process called biomining. Traditional methodology involves digging the ores from the earth, mechanically crushing them, and then subjecting the ores to harsh chemicals or physical treatments. Bioprocessing uses bacteria such as *Thiobacillus ferrooxidans* that oxidize inorganic compounds, such as copper sulfides, and in the process produce acid and ferric ions that leach out the copper. *Thiobacillus* is endogenous to the environments that are mined for copper, and the simple addition of sulfuric acid will encourage their growth and increase their metabolism. Two factors that slow the process are the generation of heat and the presence of heavy metals that are toxic to the bacteria. Using bacterial strains that are thermophilic, meaning strains that not only tolerate but prefer higher temperatures, will help solve this problem. Finding genes that help some microorganisms resist harm caused by heavy metals and cloning them into bacteria used in biomining will also improve efficiency.

Bacteria with unusual metabolic capabilities can also be used to digest organic matter into methane

and carbon dioxide gases. Biotechnology companies are researching how to take advantage of this by creating financially profitable bioenergy plants. Sewage and wastewater treatment facilities also employ microorganisms to digest organic material before chemical treatments. Bioremediation efforts utilize microorganisms to break down oil, chlorinated solvents, petroleum products, or other hazardous pollutants into inert substances.

Forensic investigations have come to depend on biotechnology. DNA fingerprinting is a method for identifying an individual on the basis of his or her unique DNA. An investigator obtains a sample of a person's DNA from cheek cells, body fluids such as blood or semen, or hair. Biotechnology is used to analyze the sample, taking advantage of the less than a fraction of 1 percent of the differences in DNA sequences between individuals. The resulting DNA fingerprint can be compared with that of related individuals to determine familial relationships or to identify a suspect in a crime. Methods similar to those used for DNA fingerprinting can be used to diagnose certain genetic disorders, which are caused by mutations or alterations in a person's DNA that result in loss of function of a certain gene.

Biotechnology also affects the manner in which governments fight wars or terrorists incite fear in people. Biological weapons employ microorganisms or substances they produce to cause harm. For example, anthrax is a disease caused by *Bacillus anthracis,* a bacterium that forms endospores. These structures are tough, and easily dispersed, and when they make their way inside a host, they convert back into metabolically active, growing bacterial cells that secrete toxins that cause illness and potentially kill the host. In 2001 anthrax was intentionally spread through the U.S. mail, causing the death of five people and illness in 22 others.

As plant tissue culture does, some modern uses of biotechnology involve culturing or manipulating living animal cells in vitro. Assisted reproductive technology (ART) encompasses the different methods and technology during which both eggs and sperm are handled in order to help a couple conceive. In vitro fertilization involves the removal of eggs from a woman's body, combining the eggs with sperm to achieve fertilization, the subsequent culture of the zygote and embryo, and the return of the early embryo into the woman's body for gestation. The success of ART is due to the decades of research on human anatomy, reproductive physiology, hormone structure and function, cell growth and division, and early embryonic development in addition to the perfection of skills, laboratory techniques, and specialized equipment.

Stem cell technology is another branch of biotechnology that has received much recent attention. As a multicellular organism develops, cells become specialized to perform unique functions in a process called differentiation. For example, an adipose cell functions to store fat, and a muscle cell functions in contraction. After differentiation, a cell cannot switch roles; in other words, an adipose cell cannot turn into a muscle cell. Stem cells are cells that are capable of self-renewal and that have the ability to develop into a number of different cell types, on the basis of signals they receive from other cells or environmental cues. Biologists study stem cells to learn about the processes of development and differentiation, in hopes of developing technology for replacing tissues such as injured spinal cord nerves, brain tissue destroyed by strokes or degenerative diseases such as Parkinson's, or cardiac tissue damaged during a heart attack. Stem cell technology also offers hope for a cure for diabetes and treatment for leukemia. Other potential uses of stem cells include preclinical drug testing, screening chemicals for potential toxicity, and developing gene therapy methods.

Another medical use of biotechnology involves tissue and organ transplantation. In order to be successfully transplanted, a donor organ must share certain molecular characteristics with the recipient's tissues and organs. Because the donor organ must meet these specific criteria, many patients die while waiting for organs that are compatible. Medical researchers have learned to grow some types of human tissues in vitro, including skin, ligaments, and blood cells. A patient's own skin can be cloned, ensuring a perfect molecular match, by removing a small piece, separating the cells, and placing them in a medium designed to nurture them and induce them to multiply. The cloned skin can then be grafted onto the patient. Because current technology limits the number of cell divisions to about 20, only small pieces of skin can be created in this manner. Researchers are also trying to learn how to stimulate stem cells to differentiate into specialized tissue types and someday whole organs for transplantation purposes.

The creation of organs from cells in vitro falls in the realm of bioengineering. One recent success of this application of biotechnology is brain-driven prostheses. Computerized limbs debuted in 2001, and in 2005 scientists created the first robotic arm controlled by thoughts alone.

The Human Genome Project utilized advances in DNA sequencing technology to determine the sequence of the 3 billion nucleotide pairs that make up the human genome. Researchers are currently using bioinformatics, computer technology to analyze and store biological data, to assign functions to the more than 20,000 genes believed to encode proteins. This information will assist medical scientists

in determining the underlying cause of numerous genetic disorders, aid in their diagnosis, and lead to potential treatments. One novel approach to treating genetic disorders is through the introduction of normal copies of the gene to tissues that are expressing mutant, nonfunctional protein. This strategy, called gene therapy, is still in development.

Epidemiologists utilize biotechnology to follow the spread of infectious diseases such as avian influenza, the bird flu. Viruses mutate rapidly, resulting in the creation of new strains. By examining the genetic composition of viruses causing different outbreaks, epidemiologists can determine the origin and follow the transmission of a particular strain. Biotechnology also helps physicians diagnose different infectious diseases, thus expediting initiation of the proper treatment. Antibodies, proteins naturally made by the immune system that specifically recognize other proteins, are also used in home pregnancy tests.

Some biotech companies produce antibiotics, hormones, enzymes, or chemicals that are useful to humans by using microorganisms. In some cases the microorganisms have been genetically modified to synthesize the product, but in other cases the microorganisms naturally make the valuable substance. The companies grow the microorganisms in large quantities and extract and purify the substance for market. Plants and animals can also be used to generate proteins or other useful products. Pharming is a technology that combines agriculture and biotechnology. Scientists genetically modify plants or animals so they produce useful proteins and secrete them into their milk, blood, or eggs. Examples of pharming include genetically modified cows that make human serum albumin, sheep that produce blood clotting agents and anticoagulants, and corn that synthesizes a type of plastic that is biodegradable.

The genetic engineering of crops can increase their yields and make them more resistant to drought, extreme temperatures, or pests. Other purposes for genetically modifying food crops include improving the nutritional value by increasing the content of certain vitamins. Pigs have been genetically altered to produce healthier unsaturated fats. Foods may also be engineered for use as edible vaccines, eliminating the need for injections.

Biotechnology has also led to the cloning of several types of animals. While the success of cloning mammals has stimulated debate and controversy about the ethics of cloning, it has also opened doors for many possible applications. Farmers could clone cattle that produce large quantities of high-quality meat, dairy cows that yield more milk, and sheep that generate fine wool. Horse breeders could clone moneymaking thoroughbreds. Conservationists could clone animals in danger of becoming extinct. During manipulation of the early embryos, scientists can insert genes that will make a cloned animal's organs more suitable for transplantation or genes for biopharming.

The numerous applications of biotechnology have changed lives, saved lives, saved ecosystems, solved crimes, solved genomes, changed genomes, improved crops, and done more. Newly gained knowledge will lead to additional successes, rapidly advancing biotechnology beyond society's ability to adjust. Members of society need to educate themselves about the latest scientific developments in order to make wise decisions about the smartest way to use the available biotechnology.

See also BIOINFORMATICS; CLONING OF DNA; CLONING OF ORGANISMS; DEOXYRIBONUCLEIC ACID (DNA); DNA FINGERPRINTING; DNA SEQUENCING; FORENSIC BIOLOGY; GENE THERAPY; GENETIC ENGINEERING; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

Biotechnology Australia. "Biotechnology Online." Available online. URL: http://www.biotechnologyonline.gov.au/. Accessed January 23, 2008.

Council for Biotechnology Information home page. Available online. URL: http://www.whybiotech.com/. Accessed January 23, 2008.

Grace, Eric S. *Biotechnology Unzipped: Promises and Realities*. Rev. 2nd ed. Washington, D.C.: Joseph Henry Press, 2006.

Spangenburg, Ray, and Kit Moser. *Genetic Engineering*. New York: Benchmark Books, 2004.

Steinberg, Mark L., and Sharon D. Cosloy. *The Facts On File Dictionary of Biotechnology and Genetic Engineering*. 3rd ed. New York: Facts On File, 2006.

Walker, Sharon. *Biotechnology Demystified: A Self-Teaching Guide*. New York: McGraw Hill, 2007.

botany Botany is the scientific study of plants, organisms that are photosynthetic, are multicellular, have cell walls made of cellulose, and typically lack locomotor abilities or sensory and nervous organs. Historically fungi and algae were included in the domain of botany as well, though these organisms belong to different kingdoms. Through photosynthesis, plants carry out vital processes necessary for sustaining the conditions that make the biosphere habitable by other life-forms: they manufacture organic molecules that many other organisms ingest as food, they produce molecular oxygen (O_2) that many organisms require for cellular respiration, and they remove carbon dioxide (CO_2) from the air. Plants also provide many practical products such as fibers for cloth, fuel for energy, and wood for construction. Because of these essential ecological and

utilitarian functions that plants provide, botany is a very important discipline of the life sciences.

WHY STUDY BOTANY?

Early interest in plants stemmed from the practical products they provided: food, wood for fuel and construction, fibers for cloth, and medically useful substances. After the scientific revolution, which ended roughly around the beginning of the 18th century, natural philosophers became interested in plants for the sake of science itself. Clearly living organisms, but so different from animal life, plants were mysterious. Scientists began to explore their structure, physiology, evolutionary history, and means of reproduction. In the early 1770s an English chemist named Joseph Priestley burned a candle in a jar that also contained a sprig of mint and found that plants changed the composition of air. After a while, the air within the jar could no longer support the burning of the candle, but 27 days later, he was able to relight the candle in the jar. The mint had altered the composition of the air, allowing it to support the burning of the candle once again. Priestley also placed a live mouse in a closed jar, and it died. When he placed a mouse in a jar with a plant, the mouse survived. In 1779 the Dutch-born British physician and plant physiologist Jan Ingenhousz expounded on Priestley's experiments and showed that light was necessary for the plant to perform the process that changed the air composition. These early experiments led to the eventual description of photosynthesis, the mechanism by which organisms use light energy to make organic compounds from CO_2, creating O_2 as a by-product.

Photosynthetic organisms have the ability to capture light energy from the Sun, convert it into chemical energy, and then utilize the energy to fix carbon, or to incorporate inorganic carbon from CO_2 into organic food molecules like carbohydrates. Organisms that have the ability to manufacture food by photosynthesis are called producers, and they form the foundation of food webs in all ecosystems. Living organisms that do not have the ability to fix carbon must ingest producers, material made by producers, or other organisms that have fed on producers. Thus all life-forms that are not producers are ultimately dependent on them for food. Though plants may be the most familiar producers on the planet, algae and some prokaryotic organisms can also produce their own food using either sunlight or inorganic chemicals as a source of energy. The plant products wheat, rice, corn, millet, and sorghum supply about 70 percent of all the food energy and about 90 percent of the protein consumed by the world's population. Fruits and vegetables are also plant products, and foodstuffs such as grasses that are not directly consumed by people are necessary to feed cattle or other livestock from which meat and dairy products are obtained. Thus, agriculture depends on knowledge of plants to feed the world.

Photosynthesis is not only important in that all life on Earth depends on the food molecules it supplies, but also in that oxygenic photosynthesis (the type carried out by plants, algae, and most bacteria) removes CO_2 from the atmosphere and releases O_2 as a by-product. (This explains the change in the composition of the air in Priestley's experiments.) Most life-forms, including humans, require oxygen to extract energy from food molecules, and until photosynthesis released enough oxygen into the early atmosphere, the only life-forms on Earth were anaerobic. A complete understanding of photosynthesis is therefore crucial to understanding how plants help sustain the current conditions of the biosphere.

Studying plants is also important as they are responsible for supplying the majority of energy for society. The burning of fossil fuels satisfies more than 85 percent of energy demands to power activities such as transportation and the generation of electricity. Coal, natural gas, and petroleum-based products are produced by natural geological and chemical processes acting on the organic material produced by former life, mostly plants. Most fossil fuels originated during the Carboniferous, the geological period that occurred from approximately 354 to 290 million years ago, from the remains of the abundant forests and vegetation growing in and near swampy lowlands and microorganisms that accumulated at the bottom of water bodies. After these life-forms died, natural chemical and geological processes buried their remains and subjected them to extreme pressures and temperatures over millions of years, eventually converting them into the fossil fuels. Because it took hundreds of million of years to create the fossil fuels currently burned for energy, they are considered a nonrenewable resource. Biomass, the organic material produced mainly by plants that does not go directly into food or consumer products, can be converted to biofuels and is considered a renewable source of energy. The world population fills approximately 7 percent of its energy needs with biomass.

Botany is relevant to the manufacture of many practical and commercially valuable products. Cloth is manufactured from cellulose fibers obtained from plant cell walls. Flexible fibers such as those from cotton or flax plants are used to make clothing, and stronger, tougher fibers are used to make upholstery, ropes, or other materials. Even some synthetic fibers like rayon and acetate are produced by treating cellulose with different chemicals. Wood used as lumber for making furniture and as a raw material for making paper is also taken from plants. Many beverages

are derived from plants. Coffee, tea, and cocoa are made by steeping plant products in hot water, and juices are obtained by crushing and straining fruits and vegetables. For thousands of years people have used plants for a variety of medicinal purposes ranging from treating nausea to dulling pain. Researchers continue to seek new potential pharmaceuticals from plant products for treating diseases such as cancer, Alzheimer's disease, and cardiovascular diseases. In addition to these few specific uses, botanical species provide society with numerous other goods. The industries that manufacture these products all require an in-depth knowledge of botany to produce high-quality products efficiently, cheaply, and safely.

Botanical knowledge is also crucial for maintaining a healthy environment. Many botanists have devoted their careers to researching aspects of environmental concerns that involve plants. Understanding the effect that human population growth and the accompanying industrial expansion have on plants could help in preventing severe long-term ecological consequences. Plants may also become part of the solution. For example, during photosynthesis plants remove atmospheric CO_2, a gas that exacerbates the natural greenhouse effect and contributes to global warming. Plants also perform many ecosystem services necessary for maintaining vital functioning ecosystems such as recycling nutrients. Continued research in plant physiology and ecology may lead to solutions to problems such as global warming and chemical pollution or at least reduce the negative consequences.

SUBDISCIPLINES

Plants provide diverse products and perform many functions within ecosystems. Because of this, in addition to the fact that one can examine plants at any level of biological organization, the field of botany encompasses numerous, far-reaching subdisciplines. Plant anatomy is concerned with the structure of plants, which provides useful information about evolutionary relationships and about past climates. For example, analysis of the size and shape of fossil leaves yields information about the mean temperature during a specific period of history. Plant physiology focuses on plant function, including how plants obtain their nutrition, how they distribute water and nutrients throughout their bodies, how they reproduce, and how they develop and grow. Plant anatomy and physiology are often studied in concert since the structure of a plant is closely related to its function, a theme common to all living organisms. To illustrate this connection, consider that some desert plants grow very long roots to help them find moisture deep down in the water table, if present, and others have few or no leaves to reduce water loss from transpiration. Biochemical properties, such as the chemical processes involved in photosynthesis, are also linked to the unique structure and function of a particular plant.

Plant ecology is the study of how plants interact with other members of the community in which they live as well as their physical environment. In addition to providing ecosystem services related to the events of photosynthesis (carbon fixation, O_2 release, and CO_2 removal), plants perform numerous other diverse functions for ecosystems. Many plants live in symbiotic relationships with other organisms; for example, nitrogen-fixing bacteria may reside in and on plant root systems, and insects that feed off plant nectar, in turn, pollinate organisms farther away than the wind could carry pollen grains. Tall vegetation provides cool, shady areas for other organisms and offers protection by hiding them. Root systems of trees slow the rate of erosion of sloped areas. Transpiration, the loss of excess water through the leaves, helps maintain the humidity of tropical rain forests. Even after dying, plants continue to serve an important role in their community. The leaf litter on a forest floor provides nourishment for saprobes (organisms that obtain nourishment from dead or decaying organic matter), insulates the ground, prevents moisture from escaping the ground, supplies materials for nest building, fertilizes the upper layer of soil, and provides a habitat for many arthropods, worms, fungi, protists, and bacteria. A tree stump or fallen branch can be home to hundreds of other species by providing a variety of microhabitats. In addition to examining the ecological functions such as the listed examples, a plant ecologist may study the impact of human activities on the plants in a particular environment, or how the introduction of a nonnative plant to a new habitat affects the native species.

Plant taxonomy, or plant systematics, involves the naming and classification of plants. Historically, plant classification depended on easily observable but artificial characteristics such as the number or length of stamens. Modern classification schemes depend on evolutionary relationships.

Economic botany is the study of the relationship between people and plants. As already mentioned, people depend on many plant products to fulfill the basic needs of food, shelter, and clothing, but also to obtain other commercially valuable and useful products such as herbs and spices, dyes, beverages, and medicines. Examples of medicines extracted from plants or made from plant products include digoxin from foxgloves used to treat congestive heart failure and arrhythmia, the anticancer drug taxol from Pacific yew trees, quinine from the bark of the South American cinchona tree used to treat malaria, and

the decongestant pseudoephedrine from ephedra species such as Ma Huang. Another aspect of economic botany is horticulture, growing fruits, vegetables, flowers, and ornamental plants. Horticulture is considered both a science and an art, depending on one's purpose—food production or aesthetics. Substances from plants are not always beneficial, and economic botany covers these also. Poison ivy, poison oak, and poison sumac produce urushiol, which causes many people to develop itchy rashes. Because people depend on the ecological functions of plants, economic botanists are also helpful in conservation efforts and in development of strategies for the sustainable use of plant resources. Many common plants can cause severe problems if ingested: raw rhubarb leaves can cause convulsions, coma, and death; daffodil, hyacinth, and narcissus bulbs cause nausea, vomiting, and diarrhea; and mistletoe berries can be fatal.

Other subdisciplines of botany include bryology, the study of mosses and liverworts, and pteridology, the study of ferns and related plants. Paleobotany is the study of fossil plants, and palynology is the study of pollen and spores, both modern and fossilized.

BRIEF HISTORY OF BOTANY

Throughout history, humans have been interested in plants for food and for the medicinal products they provide. The ancient Greek philosopher Theophrastus is considered the founder of botany. He authored two manuscripts in approximately 300 B.C.E., *De causis plantarum* (About the reasons for vegetable growth) and *De historia plantarum* (A history of plants). For 1,500 years the focus of botany was related to issues of farming and gardening. One of the first botanical references leading to the development of botany as an empirical science was *De plantis libri* (Book of plants), written by the Italian botanist Andrea Cesalpino in 1583. Cesalpino classified plants into three main groups—trees, shrubs and herbs, and seedless plants—which he further divided on the basis of structures of the fruits. The Swiss naturalist Gaspard Bauhin published the first significant work that attempted to describe all of the approximately 6,000 known species in 1623, titled *Pinax theatric botanici* (Illustrated exposition of plants). In the 18th century, the Swedish botanist Carl Linneaus went further by classifying all the known plants according to the structures of their reproductive organs and introduced binomial nomenclature, a system for naming plants that facilitated communication about different species among scientists. Linnaeus published numerous works; two of the most influential were *Systema naturae* (System of nature), initially published in 1735, and *Species plantarum* (Plant species) in 1753.

Microscopy allowed scientists to examine plant morphology at the cellular level, a procedure that led to a greater understanding of plant physiology, including the means by which plants reproduce. The British scientist Robert Hooke had first visualized cells while examining cork under the microscope in the late 17th century, and the English physician and botanist Nehemiah Grew and the Italian physician and biologist Marcello Malpighi founded the field of plant anatomy with their microscopic studies of plants. A better understanding of plant anatomy led the way to plant physiology, pioneered by the English botanist and clergyman Stephen Hales, who investigated the movement of water throughout plants, transpiration, plant respiration, and other topics in the early 18th century. A few decades later, Priestley and Ingenhousz contributed to the knowledge of the process of photosynthesis. The French botanist Charles-François Mirbel observed that each plant cell was enclosed by a cell membrane in 1809, and by 1830, most of the cellular structures of plants and the main plant tissues had been described.

The 19th century saw the emergence of cell biology, genetics, and evolutionary biology, all of which greatly influenced the development of botany. Famous botanists from this time whose work strongly influenced all life sciences included Matthias Schleiden, a German botanist who is considered a cofounder of the cell theory, and the Swiss botanist Karl Wilhelm von Nägeli, who classified tissues and proposed a mechanism by which plant cells form. The Scottish botanist Robert Brown distinguished between gymnosperms and angiosperms and discovered the cell nucleus in 1840, and in 1875 the German plant cell biologist Eduard Strasburger elucidated the process of nuclear division in plants. During the 20th century, many advances and discoveries further developed botanical science: the process of energy transfer during photosynthesis, the identification and the action of plant hormones, the ecological roles of plants, knowledge of genetics to improve agriculture and horticulture, causative agents of many plant diseases, and the biochemistry and metabolism of plants.

See also Calvin, Melvin; environmental concerns, human-induced; Ingenhousz, Jan; Linnaeus, Carl; photosynthesis; plant diversity; plant form and function; Priestley, Joseph; Schleiden, Matthias.

FURTHER READING

Botanical Society of American home page. Available online. URL: www.botany.org. Accessed January 18, 2008.

Levetin, Estelle. *Plants and Society*. 4th ed. New York: McGraw Hill, 2006.

Stern, Kingsley R., James Bidlack, and Shelley Jansky. *Introductory Plant Biology*, 11th ed. New York: McGraw Hill, 2008.

Boveri, Theodor (1862–1915) German *Cytologist* Theodor Boveri was the first to describe chromosomes as independent entities and to establish their continuity throughout the cell cycle. His observations of the cell divisions that immediately followed fertilization in sea urchin embryos elucidated the function of centrosomes in cell division and demonstrated that individual chromosomes contributed different hereditary features to the offspring. He also studied different aspects of early development and showed that the egg and sperm were equivalent with respect to hereditary input to the embryo.

PERSONAL LIFE

Theodor Boveri was born in Bamberg, Germany, on October 12, 1862. He had one older brother and two younger brothers. He attended the Realgymnasium in Nuremberg from 1875 to 1881 and entered the University of Munich. Though he had a talent for painting and playing the piano, he planned to study history and philosophy. After only one semester, he switched to natural science. He became an assistant to Carl von Kupffer at the Anatomical Institute in Munich and conducted his dissertation studies on the structure of nerve fibers.

After receiving his doctorate in 1885, he accepted a fellowship, the Lamont-Stipendium, and moved to the Zoological Institute in Munich. The institute had a new director, the German zoologist Richard Hertwig, who established his reputation in the 1870s by describing the process of fertilization as the fusion of a sperm cell within the membrane of an egg cell. In Munich, Boveri obtained his habilitation (an academic achievement following a doctorate) in zoology and comparative anatomy in 1887. Hertwig took on Boveri as his assistant from 1891 to 1893; during that time Boveri became interested in cell biology.

In 1893 Boveri became a professor of zoology and comparative anatomy at the University of Würzburg and director of the Zoological Institute. In 1897 he married one of his Ph.D. students, Marcella O'Grady, an American biologist, with whom he had one daughter, Margaret.

Boveri suffered from depression and mental illness. His first episode occurred in 1890, initiated by the news that his father had financial difficulties and his mother was ill. Boveri became severely depressed and could not work for several months. For the remainder of his life he suffered recurrences of neurasthenia, a psychological disorder characterized by lack of motivation, extreme tiredness, and low self-esteem. His general health was also poor, and around 1912 he experienced an illness that caused slight paralysis on one side. The outbreak of World War I distressed him further, and he died in 1915 at the young age of 53.

CHROMOSOMAL RESEARCH

Boveri's major researches began in 1885, after arriving at the Zoological Institute in Munich. By the time Boveri began his studies, biologists had learned much about cell division and chromosomes, but no one had firmly established that chromosomes carry the genetic material or explained how they are reproduced and pass on to daughter cells. Hertwig had already described the process of zygote formation by fusion of the egg from the mother with a sperm from the father. The nucleus of the fertilized egg divided numerous times to form the nuclei of all the body cells; therefore all body cells contained nuclear matter from both parents, and the substance of this matter was likely to be the hereditary material. The German anatomist Walther Flemming had described the movement and distribution of chromosomes during mitosis in 1882. While studying chromosomes in roundworm eggs, the Belgian embryologist and cytologist Edouard van Beneden determined that the number of chromosomes is constant within a species, the number is halved when eggs and sperm are formed, and fertilization restores the normal number of chromosomes found in body cells. Today the process of nuclear division that results in half the number of chromosomes is called meiosis, and it is well characterized.

These studies all contributed to the foundation for Boveri's research on chromosomes in the roundworm *Ascaris megalocephala*. He published a series of brilliant papers in 1886–90 laying the foundation for and demonstrating the physical basis of several observed phenomena. Boveri began by simply describing the process of egg maturation up to the point of fertilization, including the formation of polar bodies, small cells that remain unfertilized but contain "leftover" chromosomes.

He next established the individuality of chromosomes, a property retained during cellular division. The chromosomes are only visible under the microscope during mitosis, when they are condensed and thickened enough to be seen. Biologists were therefore uncertain whether they were present at other times. If chromosomes did play such an important role as carrying the genetic material, which provided continuity during the cell cycle and from generation to generation, then one would expect the chromosomes to be permanent organelles. Boveri had observed finger-shaped lobes in the *Ascaris* nuclei during early cleavage. Using these as landmarks, he

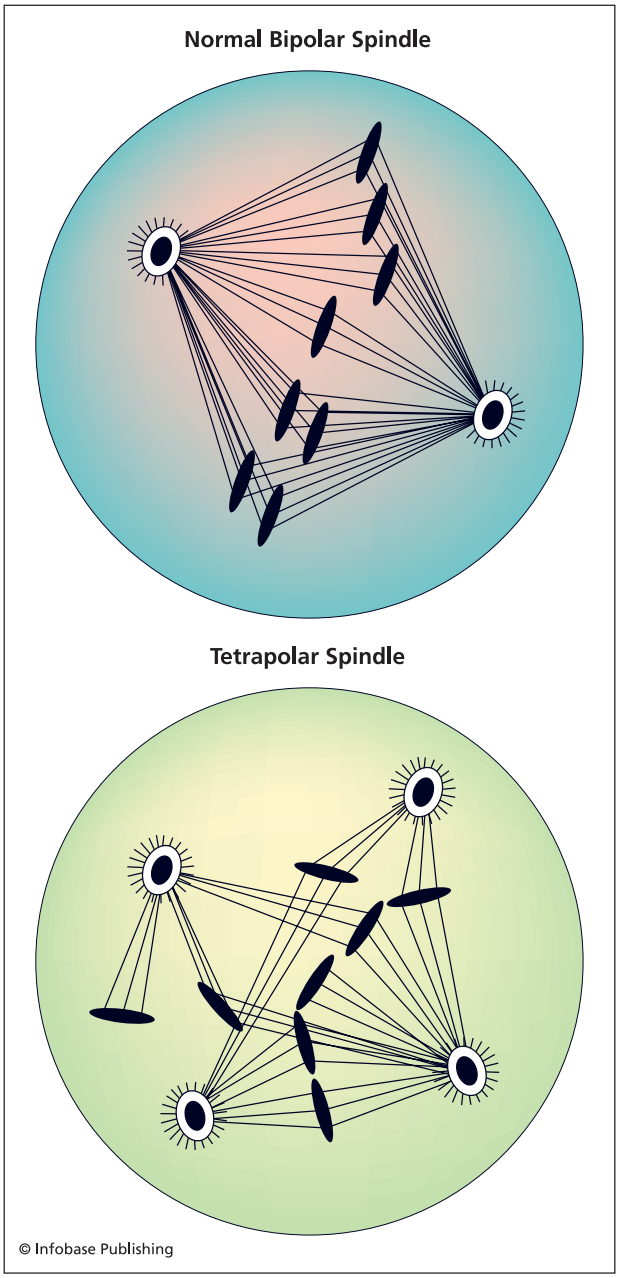
collected morphological evidence demonstrating that chromosomes were continuously present; they simply condensed during mitosis and dispersed between mitotic events. He went further to propose that the chromosomes were independent units and that they were organized, stable structures. The establishment of these concepts was necessary before chromosomes could be considered carriers of the genetic information, the main tenet of the developing chromosome theory of inheritance.

As an extension to the studies demonstrating the individuality of chromosomes, Boveri confirmed the equal contribution of the egg and the sperm to the chromosomal composition of the fused nucleus of the zygote, as proposed by van Beneden. Shaking unfertilized sea urchin eggs resulted in nucleated and nonnucleated fragments. Fertilization of both types could result in normal development, and occasionally nonfertilized, nucleated fragments developed. The fact that both the maternal and the paternal nucleus could direct normal development demonstrated their equivalence. These studies also provided strong evidence that the chromosomes are the nuclear factors of heredity.

GENETICALLY DIFFERENT CHROMOSOMES

In 1889 Boveri had found that during normal fertilization the egg cell engulfs a centrosome from the midpiece of the sperm in addition to the sperm head. While observing the development of *Ascaris* eggs, he occasionally came across tetrasters, eggs containing four rather than the normal two mitotic poles from which microtubules of the spindle apparatus grow. These structures formed when an egg engulfed two sperm midpieces. From the arrangement of the chromosomes and the four poles, he concluded that the resulting four nuclei would contain different chromosomal elements. For example, in one such tetrafoil cleavage he observed that two nuclei would only receive one chromosome, the third nucleus would receive four, and the fourth would receive two.

Normally one sperm cell fertilizes one egg cell, forming a diploid zygote that divides to form two cells that subsequently divide to form an embryo with four total cells. Boveri demonstrated that the centrosome is the organizing center for cell division. After fertilization, it divides, and each half nucleates the growth of microtubules and serves as a pole competing for chromosomes in the nucleus of each of the resulting cells. Each chromosome duplicates to form two connected equal copies that a normal bipolar spindle apparatus divides into two equal sets. Boveri also determined that a normal bipolar spindle resulted from the fusion of two half-spindles growing from the two different division centers that meet at a duplicated chromosome. All of this laid the groundwork for Boveri's next major discovery—that chromosomes are genetically different.



Theodor Boveri examined normal (bipolar) and multipolar cleavages, such as the imminent tetrafoil cleavage shown here, which resulted in unequal distributions of chromosomes. These studies allowed Boveri to draw conclusions regarding the role of chromosomes in inheritance.

Scientists did not know whether each chromosome contained all the hereditary material or each chromosome contained different portions of the complete genetic complement such that the entire set of hereditary elements was divided among the individual chromosomes. Boveri was aware that in

sea urchin eggs one could artificially produce the tetrafoil cleavages that he previously examined in *Ascaris* by performing artificial fertilization in the presence of excess sperm, conditions that encouraged the double fertilization of one egg with two sperm. A single round of cell division in the dispermic zygotes produced a four-cell embryo. (Both of the sperm cells carry a centrosome that divides to produce two centrosomes for a total of four mitotic poles.) The dispermic embryos seemed to develop normally until gastrulation, when they died. Boveri ingeniously figured out that he could exploit these induced tetrafoil cleavages in fertilized sea urchin eggs to examine the effect of the composition of chromosomes and distribution of the cytoplasm on inheritance and development. The number of cells after division corresponds to the number of poles, and cleavage planes form midway between the poles. Equal distribution of the content within only two sets of chromosomes into four cells is therefore impossible. Because of this, tetrafoil cleavage results in cells with abnormal numbers of chromosomes. Examination of the development of these cells would give information about the composition of the individual chromosomes. If the chromosomes carried different genetic properties, then as the cells with abnormal numbers developed, defects would develop in portions of the embryo that arose from the abnormal cells.

In 1895 the American cytogeneticist Thomas Hunt Morgan had observed that shaking dispermic eggs after insemination caused trefoils (resulting in three cells) to form instead of tetrafoils. The agitation probably disturbed one of the centrosomes. Because the chromosomes would only divide three ways rather than four, trefoils result in nuclei with a correct chromosomal distribution more often than tetrafoils. Boveri hypothesized that if each chromosome contained all the genetic material, then development should proceed normally, but if each chromosome only contained a portion of the genetic material, that is, each chromosome were qualitatively different, then each cell would need a complete set of equally distributed chromosomes in order to develop normally. Probability predicted that approximately 11 percent of triasters would develop normally. Boveri performed the experiment and raised the cells of the tetrafoils and trefoils in isolation after separating them by treatment with calcium-free seawater. The data showed that trefoils developed normally with a higher frequency than tetrafoils—about 11 percent (as expected) in comparison with only 1 percent of tetraster eggs that developed normally. Comparing the abnormal chromosomal distributions with the developmental abnormalities, he concluded that not only was the total number of chromosomes important, but so was the mix of chromosomes. Around the same time in 1902, Walter S. Sutton, a cytologist from Columbia University in New York, published his findings from studies of grasshopper chromosomes that suggested chromosomes were individual units, they occurred in pairs, each parent contributed one member of a pair to the offspring, and the paired chromosomes separated during nuclear division in gamete formation. Together, Sutton and Boveri's work demonstrated that chromosomes are the physical basis of the laws of inheritance as described by Gregor Mendel.

ROLE OF CYTOPLASM IN INHERITANCE AND DEVELOPMENT

Though nuclei clearly played the major role in inheritance, Boveri was also interested in the role, if any, of the cytoplasm of the egg in inheritance. Though the egg and sperm contributed equally to the formation of the nucleus, the egg contributed almost all of the cytoplasm to the zygote. Boveri knew that differential distribution of the chromosomes affected the normal development of the tetrafoils and trefoils, but the possibility existed that differential distribution of the cytoplasmic contents also played a role. Dispermic embryos turned out to be useful for examining this scenario. Boveri separated the cells of normal four-cell embryos by placing them in calcium-free seawater and observed that the development of all four proceeded normally and equally to produce quadruplets; thus all of the cellular contents (with respect to cytoplasm and chromosomes) must have been distributed equally. The cytoplasmic material was also evenly distributed for trefoil and tetrafoil cleavages, and, when isolated, their cells usually developed normally until the blastula stage, when most of them died. The rest died during the gastrula stage. In general, cells from isolated trefoils seemed to proceed normally until later stages of development than cells from tetrafoils, but this must have been due to higher frequencies of normal chromosomal distribution, as mentioned previously. These experiments suggested that differential cytoplasm probably does not play a role in the abnormal development of trefoils and tetrafoils.

In another study examining the potential role of cytoplasm in inheritance, Boveri attempted artificial fertilization of nonnucleated fragments of eggs from one species of sea urchin with sperm from another species. The two species displayed different larval skeleton shapes, making it easy to determine whether the sperm nucleus was responsible for the inherited traits. Some of the resultant larvae resembled the species of the mother and some resembled the species of the father. Since no original nuclei were supposedly present, the observation that some larvae resembled the father's species reinforced the importance of the

nucleus in inheritance, but the fact that some resembled the mother suggested the cytoplasm played a role in inheritance. Unfortunately, Boveri had technical difficulties repeating the experiment and later retracted his published findings. He concluded that the larvae resembling the mother must have resulted from the persistence of some maternal chromosomes in the egg fragment that cooperated with chromosomes supplied by the sperm to allow development to proceed. Though technical limitations at the time prevented him from exploring this further, other researchers later reached a similar conclusion. However, Boveri remained open-minded about the possibility that cytoplasmic factors did play a role in inheritance.

Boveri was more successful in demonstrating that the cytoplasm affected development. During embryogenesis in *Ascaris,* a process called chromatin diminution eliminates certain chromosomes or parts of certain chromosomes from some cells that form somatic tissues. He showed that positioning of the nuclei during the first cleavage of dispermic eggs affected whether or not diminution occurred. Thus, components of the cytoplasm must interact with the nucleus in directing this process.

TUMOR RESEARCH

In 1914 Boveri published a paper on the origin of malignant tumors, suggesting that tumors resulted from abnormal numbers of chromosomes, a condition called aneuploidy. In 1890 the German cytologist David Hansemann had reported that chromosomes failed to separate properly in the cells from human skin cancers. This led to daughter cells with abnormal numbers of chromosomes. Decades later, Boveri proposed that individual cells with incorrectly combined sets of chromosomes could develop into tumors; thus tumors could originate from any condition that gives rise to aneuploid cells, including both unbalanced division of the chromosomes during cell division or from divisions involving more than the normal two centrosomes. Boveri was one of the first scientists to propose that tumors resulted from irregularities at the cellular level. Today cancer researchers continue to observe the chromosomal chaos in cancer cells—whereas human cells normally have 46, cancer cells may have fewer or even hundreds of chromosomes. This phenomenon of aneuploidy in cancer cells probably is a result of the uncontrolled cell divisions that follow transformation rather than a cause of tumor formation.

At the time of his death on October 15, 1915, the linear order of genes along chromosomes had not yet been established by Morgan and his coworkers. Several decades passed before the extent and significance of Boveri's discoveries were appreciated. His scientific publications were all in German and few English translations are available, but his name is frequently mentioned as a pioneer in genetics. His work merged the fields of cytology, genetics, and embryology and provided the foundation for the interpretation of genetic phenomena at the chromosomal and cellular levels and led to a better understanding of the cycle of cell division.

See also CELLULAR REPRODUCTION; CHROMOSOMES; GENETICS.

FURTHER READING

Baltzer, Fritz. "Theodor Boveri." *Science* 144 (May 15, 1964): 809–815.

Moritz, Karl B., and Helmut W. Sauer. "Boveri's Contributions to Developmental Biology—a Challenge for Today." *International Journal of Developmental Biology* 40 (1996): 27–47.

Oppenheimer, Jane. "Theodor Boveri." In *Dictionary of Scientific Biography*. Vol. 2. Edited by Charles Coulston Gillispie. New York: Scribner, 1,970–1,976.

Sander Klaus. "Theodor Boveri on Cytoplasmic Organization in the Sea Urchin Egg." *Roux's Archives of Developmental Biology* 202: 129–131.

Brock, Thomas (1926–) American *Bacteriologist* Thomas Brock is a bacteriologist who discovered bacterial life thriving at temperatures higher than scientists previously imagined any life-form could exist. His discovery of prokaryotic organisms growing in hot springs at Yellowstone National Park led to the proposal of a whole new domain of life, the Archaea.

BECOMING A MICROBIOLOGIST

Thomas D. Brock was born in Cleveland, Ohio, on September 10, 1926, to Thomas Carter Brock, a power engineer, and Helen Sophia Ringwald, a former nurse. When he was age 15 the family moved to Chillicothe, and within a few months his father passed away. Although the older Thomas had only received an eighth-grade education, he encouraged his son in educational pursuits such as backyard chemistry experiments and building electrical equipment. After graduating from high school in Chillicothe, Brock entered an electrical program in the U.S. Navy. In 1946, after travels to Chicago and Alaska, he enrolled at Ohio State University (OSU) on the G.I. bill. His initial plan was to become a writer, but he switched his major to botany and graduated with honors in 1949. OSU offered Brock a graduate assistantship, and he performed research in mycology (the study of fungi) for a master's and a doctoral degree. By the time he earned a Ph.D. in 1952, Brock had developed an interest in ecology, in particular, microbial ecology.

After graduating, however, postdoctoral positions and academic jobs in general were scarce. The Ohio Agricultural Experiment Station in Wooster, Ohio, hired Brock as a summer research associate to work on soil fungi, a position that prepared him for a position in the Antibiotics Research Department at Upjohn, a pharmaceutical company. He moved to Kalamazoo, Michigan, with his new wife, Louise.

Brock only had two microbiology courses at OSU, but while working for Upjohn, he learned a lot about microorganisms, especially bacteria, since most antibiotics treat bacterial infections. In his free time he also researched a local railroad and taught himself German, a skill that would be useful later in his career. Antibiotics research became boring after five years, and he left Upjohn to join the Biology Department at Western Reserve University (now Case Western University), in his hometown of Cleveland. Part of his job responsibility was teaching general bacteriology, nursing microbiology, mycology, medical microbiology, and advanced microbiology. Having learned enough microbiology to begin feeling like a "real microbiologist," but realizing that the teaching load was too heavy to develop the research program he desired, Brock gave up his assistant professorship and became a postdoc for L. O. Krampitz in the Department of Microbiology at the medical school. With a project on the M protein of group A *Streptococci,* this position provided the opportunity to learn biochemistry, immunology, and a bit of clinical microbiology.

During the summers of 1958 and 1959, Brock vacationed in northern Ontario on Lake Memesagamesing, where he learned boating, fishing, and other aquatic activities. Meanwhile, Brock put his German skills to good use by translating many scientific papers of historical microbiological significance. He included these in a book about the history of microbiology, *Milestones in Microbiology,* the first edition of which was published in 1961.

MICROBIAL ECOLOGY

After only one year of postdoctoral studies, Brock accepted a job as an assistant professor of bacteriology at Indiana University, in Bloomington, in 1960. His teaching responsibilities and research initially focused on medical microbiology, but Brock began thinking about microbial ecology again.

In summer 1963 Brock made plans to study marine microbiology at the Friday Harbor Laboratories of the University of Washington. He focused his research on *Leucothrix mucor,* a widespread marine microorganism, and found that this filamentous organism formed knots under certain culture conditions. Brock was the first to observe these sorts of structures. Not only did this research lead to a cover story in the journal *Science* and a feature article in the *New York Times* in 1964, but it also marked the beginning of his research on microbial ecology of sulfur hot springs.

Thiothrix, an organism related to *Leucothrix,* lives in sulfur springs. This led Brock to visit Yellowstone National Park, in Montana, in 1964. The developments of microorganisms present in the runoff channels of the Yellowstone hot springs surprised him, and he collected some samples. At the same time, he was working on authoring a text, *Principles of Microbial Ecology* (1966), and his research on ecosystems for the book stimulated his view of the springs as steady-state ecosystems. During the following summer, while vacationing with his wife, Brock planned to examine the chlorophyll levels (in cyanobacteria) in the thermal gradient of the outflow channels. He observed pink, gelatinous, stringy masses of biological material at high temperatures (131°F–140°F or 55°C–60°C). The sample contained large amounts of protein but no chlorophyll, leading to his conclusion that the material was bacterial. On the basis of his findings, he wrote a proposal and received a grant from the National Science Foundation to fund further research on microorganisms in the Yellowstone hot springs. He established a temporary laboratory facility at West Yellowstone and set to work.

DISCOVERY OF *THERMUS AQUATICUS*

Initial studies concentrated on photosynthesis measurements in the thermal mats of cyanobacteria. As part of an undergraduate honor's thesis project, one of Brock's students, Hudson Freeze, attempted to culture the pink bacteria from one of the springs. Though that attempt was unsuccessful, they did grow yellowish bacteria at 158°F (70°C). They named it *Thermus aquaticus* and found similar strains from several other sources.

Over the next two years, Freeze worked on determining the deoxyribonucleic acid (DNA) base composition and measuring the growth rates of *T. aquaticus* at different temperatures. With the assistance of his technician Pat Holleman, Brock worked on the taxonomy of the hyperthermophilic microorganism. He had observed the pink bacterium growing in hot springs with temperatures of 176°F (80°C) but suspected they might grow at even higher temperatures. He placed a string in the source pool, Octopus Spring, which had a temperature greater than 194°F (90°C), and observed pink growth on it. Photomicrographs taken from slides immersed and incubated for one day in the spring appeared in a *Science* paper published in 1967, "Life at High Temperatures." Brock and some of his graduate students also immersed slides in boiling pools, with temperatures

at or greater than 198°F (92°C). Because of the high altitudes, water boils at lower temperatures in Yellowstone than at sea level. Amazingly, they discovered bacteria actively growing and dividing at boiling temperatures as well.

The article received much attention, and Brock soon found himself traveling across the globe to search for hyperthermophiles in geothermal regions in Italy, Iceland, New Zealand, Japan, Central America, and the Caribbean. He found bacteria in boiling springs of neutral or alkaline pH at all locations, even at the lower altitudes, where the water boiled at 212°F (100°C).

IMPACT OF THE DISCOVERY OF HYPERTHERMOPHILES

The discovery in 1977 of hydrothermal vents deep in the ocean broadened the scope of thermophilic bacterial research. These fissures in the ocean floor spew superheated, mineral-rich fluid. Temperatures can reach 750°F (400°C) where the water shoots out of the vent. Hydrothermal vents support diverse communities of organisms, with thermophilic bacteria forming the bottom of the food chain. As chemoautotrophs, they synthesize organic compounds using reduced inorganic iron and sulfur compounds as their energy source. The discovery of the hydothermal vent communities gave insight into the earliest life-forms on Earth, as the conditions produced by the vents resemble the presumed conditions of the early Earth. Thus the interest in hyperthermophiles increased.

In 1983 Kary Mullis, a biochemist from California, conceived of the idea of the polymerase chain reaction (PCR). This molecular biological technique functions to generate large quantities of a specific segment of deoxyribonucleic acid. PCR involves repeated cycles of replication, interrupted by brief periods of heating, to denature double-stranded DNA. The enzyme DNA polymerase, like most protein enzymes, is sensitive to heat. At high temperatures the enzyme denatures and loses functionality. The power of PCR lies in its ability to amplify DNA exponentially: each cycle can theoretically double the amount of DNA already present. But the heating step of each cycle destroyed most DNA polymerases, requiring the researcher to add new enzyme after each cycle. The fact that *T. aquaticus* grew at such high temperatures meant it possessed a thermostable DNA polymerase. Such an enzyme would mean the cycles could run repeatedly without the need for adding fresh enzyme. After biochemists isolated and purified the enzyme, called Taq (for *T. aquaticus*) polymerase, the popularity of PCR took off, and Kary Mullis won the Nobel Prize in chemistry in 1993 for his invention of PCR. *Science* magazine created a new award in 1989, "The Molecule of the Year," and named Taq polymerase the first awardee. Today all modern molecular biology laboratories employ PCR as an integral part of DNA-based research.

Carl Woese, a professor of microbiology at the University of Illinois at Urbana-Champaign, proposed a new biological classification system that includes a taxonomic level higher than kingdom, that of domain. He suggested that all life-forms belong to one of three domains: Eubacteria, now simply called Bacteria; Eukarya, including all eukaryotic organisms; and another prokaryotic category, Archaebacteria, now simply called Archaea. Woese's phylogenetic research examining ribosomal ribonucleic acid from bacteria and archaeans suggested members of the two domains were sufficiently different to warrant two distinct domains. Woese placed many of the organisms Brock discovered in Yellowstone in the domain Archaea. The fact that Brock had already researched and characterized these microorganisms facilitated Woese's analysis.

WISCONSIN, BOOKS, AND LAKES

In 1971 Brock joined the Department of Bacteriology at the University of Wisconsin-Madison as the E. B. Fred Professor of Natural Sciences. He had married Katherine Middleton earlier that year. After the move to Wisconsin, Brock maintained his productivity. His Yellowstone research continued for five more years, and he branched out into investigating the bacterial genera *Sulfolobus* and *Chloroflexus*. *Sulfolobus* species belong to Archaea and grow optimally at a pH of 2 or 3 and temperatures around 167°F–176°F (75°C–80°C). *Chloroflexus* species are photosynthetic thermophiles belonging to the group of green, nonsulfur bacteria.

The Wisconsin lakes attracted Brock, whose interest in ecology led to a desire to learn more about limnology, the study of freshwater lakes. His major course of investigation during the late 1970s was the study of cyanobacterial populations of Lake Mendota, though he also studied other lakes. In 1985 he published the book *A Eutrophic Lake: Lake Mendota, Wisconsin.*

In addition to *Principles of Microbial Ecology* and *Milestones in Microbiology,* Brock has published numerous scientific papers and articles concerning microbiology written for a more general audience. During 1967–69 Brock spent his evenings and weekends working on another college textbook, *Biology of Microorganisms,* originally written for nonmajors, but its newer editions are intended for undergraduate microbiology majors. The fifth edition, published in 1986, became the first full-color microbiology textbook on the market. The latest edition, the 11th, titled *Brock Biology of Microorganisms* (2006), was written by Michael Madigan and John Martinko.

Brock wrote the first full-length English biography of the German physician and microbiologist Robert Koch. This successful book, *Robert Koch: A Life in Bacteriology and Medicine,* was published in 1988. In 1989 Brock published an updated and revised version of *Milestones in Microbiology,* and in 1990 he published a history of bacterial genetics, *The Emergence of Bacterial Genetics.* According to Brock's own account, the University of Wisconsin did not view his historical research and publications as valid research, and Brock was pressured to retire.

Brock's discovery of life at high temperatures impacted the life sciences in many ways. Before the 1970s biologists thought the upper temperatures at which life could exist approached 163.4°F–170.6°F (73°C–77°C), but Brock showed that some bacteria thrived in boiling springs at temperatures greater than 212°F (100°C). His research on extreme thermophiles led to better understanding of ancient life-forms and eventually to the proposal of a whole new domain of life, Archaea, consisting of many prokaryotic organisms that display other unique extreme abilities. Some survive in acidic environments, some require high salt concentrations, and some produce methane as a by-product of their metabolism—giving new meaning to the limits of biological diversity.

See also ARCHAEA; BACTERIA (EUBACTERIA); HYDROTHERMAL VENTS; MICROBIOLOGY; POLYMERASE CHAIN REACTION; PROKARYOTIC CELLS.

FURTHER READING

Brock, Thomas D. "Knots in *Leucothrix mucor*." *Science* 144 (1964): 870–872.

———. "Life at High Temperatures." *Science* 158, no. 3804 (1967): 1,012–1,019.

———. "Life at High Temperatures." *Science* 230, no. 4722 (1985): 132–138.

———. *Milestones in Microbiology 1546–1940*. Washington, D.C.: American Society for Microbiology Press, 1999.

———. "The Road to Yellowstone—and Beyond." *Annual Reviews of Microbiology* 49 (1995): 1–29.

———. "The Value of Basic Research: Discovery of *Thermus aquaticus* and Other Extreme Thermophiles." *Genetics* 146 (August 1997): 1,207–1,210.

Buffon, Georges-Louis Leclerc, comte de (1707–1788) French *Naturalist*

Georges-Louis Buffon was a prominent 18th-century naturalist, who authored a mammoth 44-volume treatise, *Histoire naturelle* (*Natural History*), which summarized everything that scientists knew about the natural world at the time. His observation that organisms living in similar but distant environments were distinct became one of the founding principles of the field of biogeography. Buffon's work influenced other renowned biologists for generations, including the famous English evolutionary biologist Charles Darwin.

Georges-Louis Leclerc was born on September 7, 1707, in Montbard, France. His father, Benjamin-François Leclerc, married Anne-Christine Marlin, who had rich relatives, making them members of the French aristocracy. George-Louis had four younger siblings.

In 1717 Georges-Louis entered the Collège des Jésuites in Dijon. From 1723 to 1726 he studied law, and then he traveled around, studied medicine and botany, and finally settled back in France in 1732. His mother had died, and he inherited her family's fortune and the title comte de Buffon. From 1734 on, he was known simply as Monsieur de Buffon.

After he engaged in some engineering and mathematical pursuits, the French Royal Academy of Sciences admitted Buffon as an assistant member of the section on mechanics in 1734. He translated two well-known works into French: *Vegetable Staticks,* by Stephen Hales, and *The Methods of Fluxions and Infinite Series,* by Sir Isaac Newton. Buffon began his botanical researches in the 1730s, and after becoming an associate member in 1739 he transferred his appointment to the botanical section. That same year he also became keeper of the Jardin du Roi (the royal botanical garden). Under Buffon's direction, the garden doubled in area, enlarged its buildings, increased its collections, and developed into a major scientific research center.

At the request of the minister of the navy, Jean-Frédéric Phélypeaux, comte de Maurepas, Buffon began cataloging the royal natural history collections that eventually formed the basis of the Musée National d'Histoire Naturelle (the [French] National Museum of Natural History), which was formally founded in 1793. This project developed into the ambitious endeavor that resulted in the comprehensive work *Histoire naturelle, générale et particulière* (*Natural History, General and Particular,* 1749–1804). Though Buffon believed that humans could not hope to understand nature completely, his writings attempted to convey all that could be understood with respect to natural history, geology, and anthropology. *Histoire naturelle* was translated into many languages and distributed worldwide. Of the 50 proposed volumes, Buffon was only able to complete 36 before his death.

At the time, the widely accepted explanation for the diversity of life was the biblical account stating that a supernatural divine being created all life-forms in their existing forms approximately 6,000 years ago. Buffon bravely suggested that species changed and that many species had died out. After observing that different geographical regions had unique flora and fauna, he concluded that life originated in one central region, and then species either grew more

complex or degenerated into the existing forms. As species spread out, some could survive in the particular conditions of a specific habitat, while others died out in that area. The well-known evolutionary biologist Charles Darwin later praised Buffon for his emergent conception of species evolution.

The first 15 volumes appeared in 1749–67 and covered topics such as the history and theory of Earth, the formation of the planets, some general biology, development and reproduction, the natural history of humans, and the natural history of animals. The next seven (1774–89) supplemented the first 15. The rest of the work consisted of nine volumes on birds (1770–83) and five on minerals (1783–88). The remaining eight volumes on reptiles, fishes, and cetaceans were written by comte de Lacépède after Buffon died. One section of the fifth supplementary volume was particularly famous. Published in 1778, *Les epoques des la nature* (Epochs of nature), described his theory of the Earth and attempted to merge geology and biology. His theory is summarized as follows: the planet originated from a piece of the Sun that broke off; some solidification occurred as the primitive Earth cooled and vapors and other substances condensed to form a vast ocean covering the surface; marine organisms appeared; sediment formed from physical and chemical action in the ocean; water burst through barriers in the ground that led to underground caverns, leading to a lowering of the water level; volcanoes, earthquakes, and the force of the waters formed geological features; animals emerged; continents separated; and finally humans dominated Earth. Buffon's chronology of the planet's history suggested that Earth was much older than the biblical estimate of 6,000 years. Experiments he performed on cooling rates of globes made of different materials and of different sizes led him to propose 75,000 years as the age of Earth, but sedimentation observations led him to consider an age of around 3 million years. The Catholic Church condemned his books for these statements.

In *Histoire des animaux,* Buffon discussed animal nutrition, development, and reproduction. Biologists did not know how new organisms came to be. Many thought that miniature beings existed in the gametes (the sperm or the egg), a notion termed preformation, and then development consisted of growth and unfolding of parts on the preformed beings. Though preformation was a commonly accepted theory, Buffon rejected it, for one reason, because it did not explain heredity. Also, nobody had yet observed an egg from an animal that gave birth to living offspring (as opposed to an egg that hatches after being laid). His more complex theory of reproduction involved the ingestion of nutritive matter that subsequently took on characteristics of each internal organ, then passed these on to the embryo during development.

Buffon noted that animals did exhibit a wide variety of physiologies that corresponded to their habitats, and that different forms of animals were spread around the world. The species that inhabited different geographic locations were distinct even when the climates of the regions were similar. This observation, which came to be known as Buffon's law, was one of the founding principles of the field of biogeography. Regarding the concept of species, Buffon subscribed to the belief that a species was defined reproductively by the ability of two members successfully to create fertile offspring as a result of mating. More encompassing groups were arbitrary, he claimed, and invented for the convenience of classification. This insight resembled more modern thinking, as biologists over the last 250 years have been gradually reclassifying organisms on the basis of phylogenetic histories rather than seemingly random but conveniently observed traits.

While Buffon did assert that humans are subject to the same scientific analyses and natural processes as other animals, he did believe that they are superior. He reasoned that human beings became superior because they are social animals and that the development of language as necessitated by their social characteristics led to the development of the ability to reason, which set humans apart from other animals. This intellect allowed human beings to dominate all climates and environments, whereas animals were limited in this capacity. Buffon also considered that humans and apes shared a common ancestor.

In addition to *Histoire naturelle,* Buffon presented a variety of topics to the Academy of Sciences, including works on astronomy, mathematics, physics, forestry, physiology, and pyrotechnics. These contributions are preserved in the academy's *Memoirs* (1737–52).

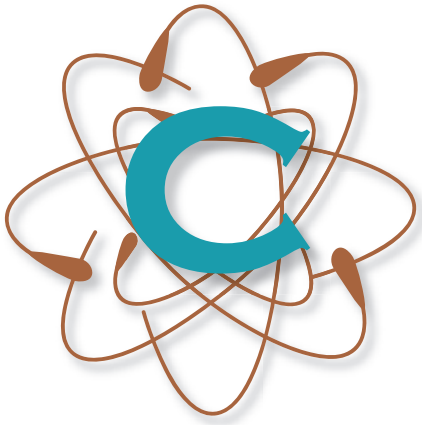
Scientists around the world respected Buffon, and many academic organizations elected him to membership: the French Royal Academy of Sciences, the French Academy, the Royal Society of London, and the academies of Berlin and St. Petersburg.

In 1752 Buffon married Françoise de Saint-Belin-Malain, and they had one son together. Buffon died on April 16, 1788, in Paris. In addition to his historical scientific treatise on the natural world, his bravery in contradicting the popular beliefs regarding the age of the Earth and the immutability of species paved the way for future naturalists to explore scientific truths about the natural world.

See also Darwin, Charles.

FURTHER READING

Roger, Jacques. *Buffon: A Life in Natural History.* Translated by Sarah Lucille Bonnefoi. Ithaca, N.Y.: Cornell University Press, 1997.



Calvin, Melvin (1911–1997) American *Chemist*

Melvin Calvin was a chemist at the University of California at Berkeley who unraveled the details of photosynthesis, a biochemical process whose importance to life science cannot be overstated. All life-forms require energy in the form of organic molecules such as carbohydrates that they oxidize during cellular respiration. Heterotrophic organisms must take in these organic molecules through the ingestion of other living organisms or organic matter made by other living organisms. Autotrophic organisms, also called primary producers, can synthesize their own organic molecules if provided a source of inorganic carbon, such as carbon dioxide (CO_2), and an alternate source of energy. Many autotrophs are photoautotrophs, meaning they obtain their energy from sunlight. Plants, algae, and certain types of bacteria have specialized cellular structures and biochemical pathways that allow them to use this radiant energy to build carbohydrates. A few types of prokaryotic organisms can use energy stored in reduced inorganic molecules, such as hydrogen sulfide, in order to make organic molecules. Such organisms form the foundation of biological communities found near hydrothermal vents on the deep ocean floor. The overwhelming majority of ecosystems depend on photosynthetic primary producers as the basis of their food chains. For his research on the assimilation of carbon dioxide into organic compounds during photosynthesis, Calvin received the Nobel Prize in chemistry for 1961.

CHILDHOOD AND EDUCATION

Melvin Calvin was born on April 8, 1911, in St. Paul, Minnesota. His father had immigrated to the United States from what is now Lithuania and worked as a cigar maker, an auto mechanic, and then a grocer. His mother was a seamstress who emigrated from the Georgian region of Russia. Melvin; his younger sister, Sandra; and his parents moved to Detroit before Melvin entered high school. He became interested in chemistry after learning about the periodic table of the elements and recognizing the beauty in its organized and explanatory nature. While working in a grocery store as a high school student, he realized the importance of chemistry in everyday life, as it was related to the composition of foods, to the ink on the printed labels of cans, and to everything else that surrounded him.

Calvin enrolled at the Michigan College of Mining and Technology (now Michigan Technological University) in 1927. For financial reasons, he took some time off after his sophomore year and gained practical chemistry experience working as a quality control analyst for a brass factory. After receiving his bachelor of science degree in chemistry in 1931, he studied the electron affinity of halogens for his doctoral thesis under the guidance of George Glockler at the University of Minnesota. After receiving a Ph.D. in chemistry in 1935, he researched the catalytic behavior of coordinated metal compounds as a postdoctoral fellow in the laboratory of Michael Polanyi at the University of Manchester, in England. During this time Calvin became interested in metalloporphyrins, large organic molecules composed of four rings with a metal atom in the center. Calvin examined the activation of molecular hydrogen (H_2) by porphyrins with and without metal present. His later work would involve the oxidation-reduction properties of another well-known porphyrin—chlorophyll, a pigment that absorbs sunlight for use in photosynthesis.

Gilbert Lewis, an American physical chemist whose name is associated with dot structures used to depict chemical bonds, hired Calvin as an instructor at the University of California at Berkeley (UCB) in 1937. Calvin was the first non-UCB graduate the Chemistry Department had hired in 25 years. Within 10 years he became a full professor of chemistry and also served as professor of molecular biology in 1963–80. He was director of the Laboratory of Chemical Biodynamics and associate director of Lawrence Berkeley Laboratory from 1967 to 1980.

DELINEATING PHOTOSYNTHESIS

Calvin's early research at Berkeley focused on hydrogen activation and hydrogenation reactions. He also assisted Lewis in writing a review on the color of organic molecules, a characteristic related to electron excitation. This endeavor helped prepare him for his future work on photosynthesis, as did coauthoring with Gerry Branch *The Theory of Organic Chemistry* (1941), a textbook credited with launching the field of theoretical organic chemistry in the United States. After the war, Ernest O. Lawrence, director of the Radiation Laboratory at Berkeley that is now named in his honor, suggested to Calvin that he find a useful application of carbon 14, as the laboratory had an available supply of this radioisotope after the war ended. Calvin proposed an interdisciplinary research endeavor for a bioorganic chemistry group that would draw on knowledge from biology, physics, and chemistry. Calvin's team used the radioactive carbon 14 isotope to follow the pathway of carbon during photosynthesis, the process by which photosynthetic organisms convert carbon dioxide (CO_2) and water (H_2O) into carbohydrates $(CH_2O)_n$ and oxygen (O_2) using energy obtained from sunlight. Using the unicellular green alga *Chlorella pyrenoidosa,* Calvin and his colleagues exposed the cells to CO_2 that contained radioactive carbon 14, killed the cells, made extracts from the cell contents, and then analyzed the molecules that contained carbon 14 using paper chromatography. After identifying the labeled components, Calvin was able to puzzle together the steps of the pathway through which the carbon traveled. In this simple, yet elegant manner, Calvin delineated the biochemical process of photosynthesis.

$$CO_2 + H_2O \xrightarrow{\text{light}} (CH_2O)_n + O_2$$

Calvin and others published 23 papers and two books (and numerous others later) directly related to the research performed during the period 1946–56 on the path of carbon in photosynthesis. One surprising finding of Calvin's research was that the CO_2 is initially assimilated into a familiar organic compound, phosphoglycerate, rather than being reduced, as scientists assumed. Scientists believed that photoexcited chlorophyll led to the transfer of hydrogen atoms from water to the CO_2, forming formaldehyde, a two-carbon sugar that could polymerize to form larger sugars, releasing oxygen in the process. In reality, the carbon atom from CO_2 combines with something else to form phosphoglycerate, which is subsequently reduced, and the light energy from the Sun is used to regenerate cofactors used in the assimilation, rather than in the initial reduction, as scientists thought occurred. Calvin outlined all the intermediate products that are involved in the regeneration of the assimilation products.

The 1940 discovery of the isotope carbon 14 by Samuel Ruben and Martin Kamen, also at the University of California at Berkeley, provided Calvin the tool he would need to trace the steps leading to the synthesis of sugar from CO_2 during the dark reactions. The following year, Kamen and Ruben showed that the O_2 released during photosynthesis was derived from water, not from CO_2, as scientists assumed.

THE EXPERIMENTAL PROCESS

When Calvin began his studies, biochemists knew a few things regarding photosynthesis. The generation of O_2 was a separate physical and chemical event from the reduction of carbon dioxide. The term *light reactions* referred to the reactions that occurred during the illumination of the chloroplasts, including the production of oxygen gas. Scientists named the reactions that resulted in the reduction of CO_2 the *dark reactions,* though they may occur in the presence or absence of light.

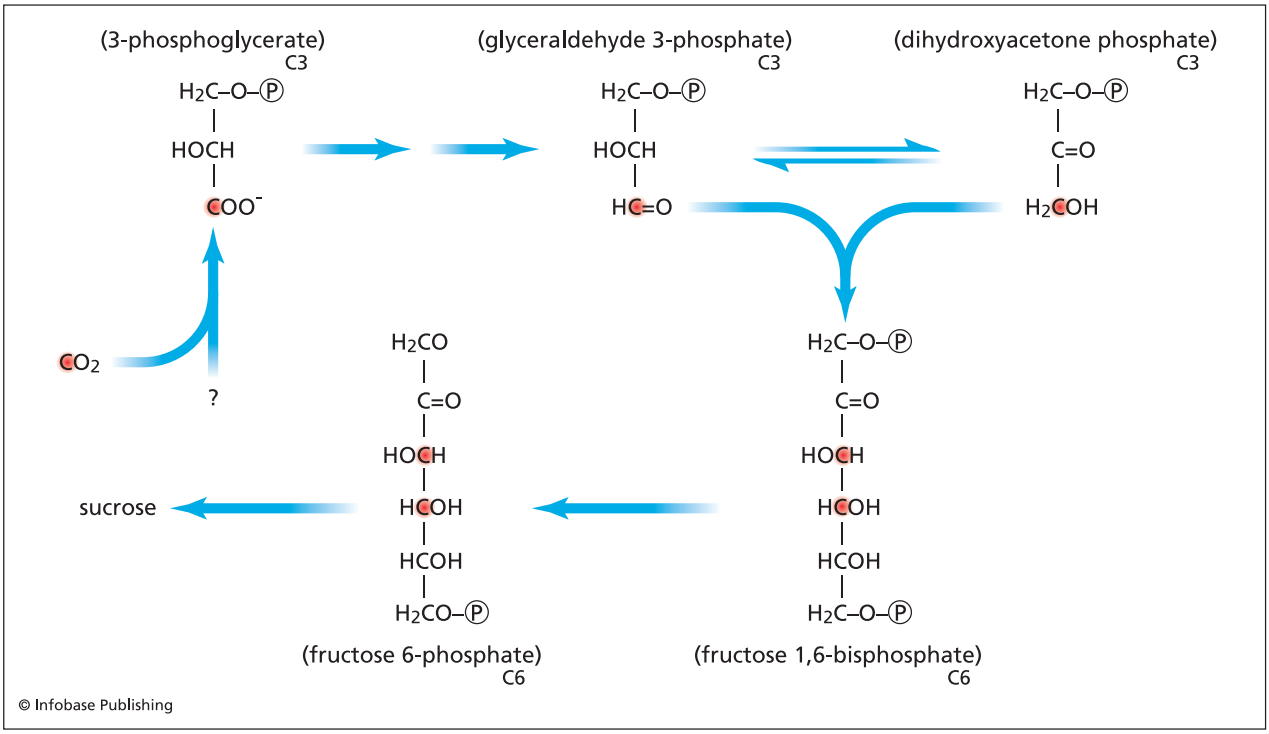
In one of his earliest experiments, Calvin illuminated some plants and stored them in the absence of CO_2 to let the plants synthesize plenty of the high-energy intermediate compounds. When they reintroduced CO_2 to the plants, even in the absence of light, the plants were able to incorporate radiolabeled CO_2. This initial result confirmed that CO_2 incorporation was part of the so-called dark reactions.

Calvin switched to using the green alga *Chlorella,* so they could grow large quantities quickly and reproducibly in a continuous culture. An apparatus called a lollipop because it was shaped like a flat circle held a suspension of the algal cells, and carbon 14–labeled CO_2 was streamed through the suspension during exposure. Lights on each side of the clear lollipop provided illumination. Dropping the suspension into alcohol stopped the enzymatic reactions and killed the cells. After extracting the organic compounds, they used ion exchange chromatography to separate the extract components. Because anion exchange columns (chromatography columns that bind negatively charged ions) bound the radioactive

material tightly, they concluded that the initial products were acidic. The strength of the ionic interaction suggested that the early compounds included phosphate esters rather than ordinary carboxylic acids (molecules containing $-COO^-$), which would have eluted easily. Additional tests suggested that the compound was phosphoglycerate, a compound already known to have a role in glycolysis, the breakdown of the sugar glucose.

Calvin and various students and collaborators spent the next 10 years trying to identify all the compounds containing carbon 14. A key technique in their procedure was two-dimensional paper chromatography. After extracting the radiolabeled organic compounds, they allowed the sample to diffuse in one direction through the paper filter. Then they turned the paper 90 degrees and allowed the compounds to diffuse in a second direction. Compounds with different sizes and properties diffused at different rates, resulting in a spotted pattern on the filter paper, with each spot representing one specific organic compound. After exposing X-ray film to the filter paper and developing it, they could line up the black spots with areas on the paper that contained the carbon 14–containing compounds. The number of spots, their positions on the filter paper, and their relative radiolabel intensities contained all the information Calvin needed to follow the pathway of carbon during photosynthesis except the identity of each black spot on the chromatogram. Identifying the compounds turned out to be a complex task. Sometimes fluorescence or ultraviolet absorption of the paper itself was informative; other times, they had to elute the material from the paper, perform chemical manipulations, and run another chromatography experiment to gain information about the compound's identity. They found that within 30 minutes the cells had incorporated the radiolabeled carbon in several carbohydrates, amino acids, and numerous intermediates. The labeled compounds included alanine, aspartic acid, citric acid, glutamic acid, glycine, malic acid, phosphoenolpyruvic acid, phosphoglyceric acid, serine, sucrose, triose phosphate, uridine diphosphoglucose, sugar phosphates, and sugar diphosphates.

By exposing *Chlorella* to radioactive CO_2 for shorter periods, they figured out which compounds were produced early after incorporation and which were produced later on. By briefly increasing the span of time between the CO_2 exposure and dropping the algal suspension into alcohol, they obtained different relative intensities of radioactivity in the compounds, reflecting the sequential reactions in which the carbon 14–labeled compounds participated. Exposure for only a fraction of a second led to one distinctly dominant radiolabeled compound—phosphoglycerate, the first product of photosynthesis. The subsequent appearance of hexose phosphates suggested



Melvin Calvin and his colleagues systematically determined the steps that occurred between the incorporation of the carbon 14 isotope (represented in red) from carbon dioxide and the formation of a hexose phosphate.

that two phosphoglycerate molecules combined to form a hexose phosphate.

Next they chemically dismantled the radioactive phosphoglycerate to see which one of its three carbon atoms was the radioisotope. By doing the same for the hexose phosphate, they were able to follow the path of the carbon 14 from phosphoglycerate through the stepwise formation of the hexose phosphates and ultimately into sucrose.

The fact that phosphoglycerate contained three carbons suggested that the single carbon atom from CO_2 combined with a two-carbon intermediate; thus their next step was to identify that presumed two-carbon compound. This turned out to be more complicated than expected, as no two-carbon intermediate existed. To build up the levels of the unknown precursor molecule, they restricted the CO_2. While searching for a potential carbon acceptor, rather than increased levels of a two-carbon substance, they found large quantities of five-carbon (pentose) and seven-carbon (heptose) sugars. The relationship of these compounds was not immediately clear. In order to determine the sequential order in which these molecules participated in the photosynthetic carbon pathway, they identified which carbon atoms of the various sugars were radiolabeled and the relative percentage of each that was labeled. Five-carbon sugars were labeled at one end of the molecule, while the six- and seven-carbon sugars had labels at the central carbons. They concluded that the labeled CO_2 combined with the five-carbon sugar ribulose 1,5-bisphosphate to form a six-carbon sugar that subsequently split into two three-carbon phosphoglycerate molecules. From these studies they were also able to determine the pathways that explained the origin of the ribulose 5-phosphate, which could be converted to ribulose 1,5-bisphosphate by the transfer of a phosphate group from an adenosine triphosphate (ATP) molecule. Transferring a two-carbon fragment from the seven-carbon fragment sedoheptulose to glyceraldehyde would result in two five-carbon compounds, both of which can be converted to ribulose 5-phosphate in a single step.

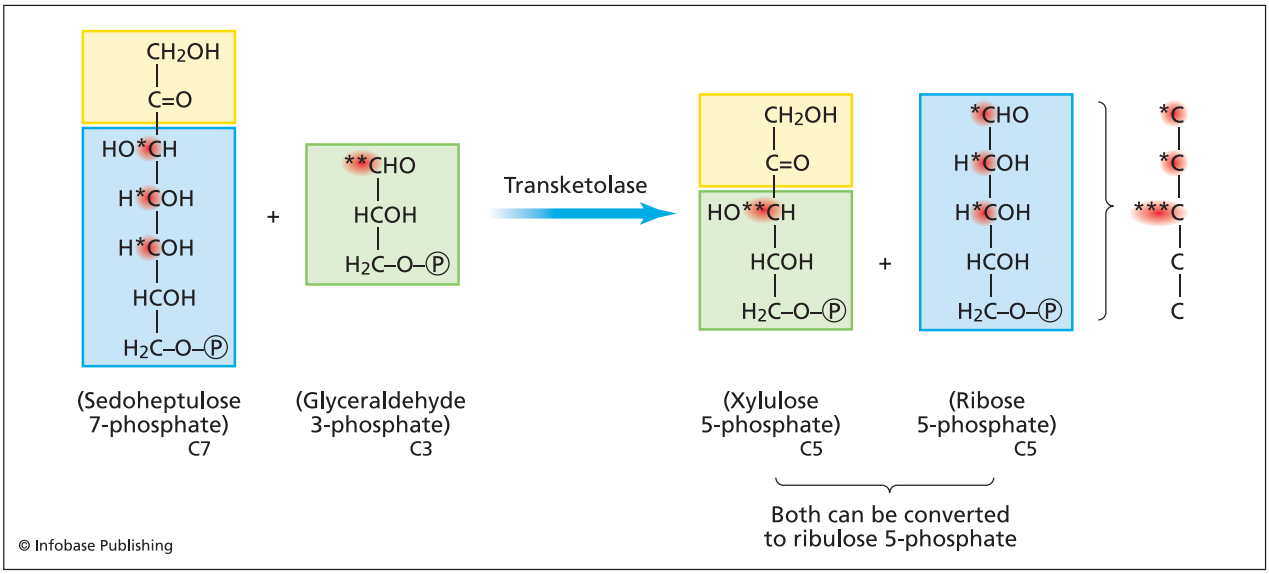
C_7 (sedoheptulose 7-phosphate) + C_3 (glyceraldehyde 3-phosphate) → C_5 (ribose 5-phosphate) + C_5 (xylulose 5-phosphate)

They explained the origin of the seven-carbon sedoheptulose by the joining of a three-carbon derivative of glyceraldehyde 3-phosphate with a four-carbon compound.

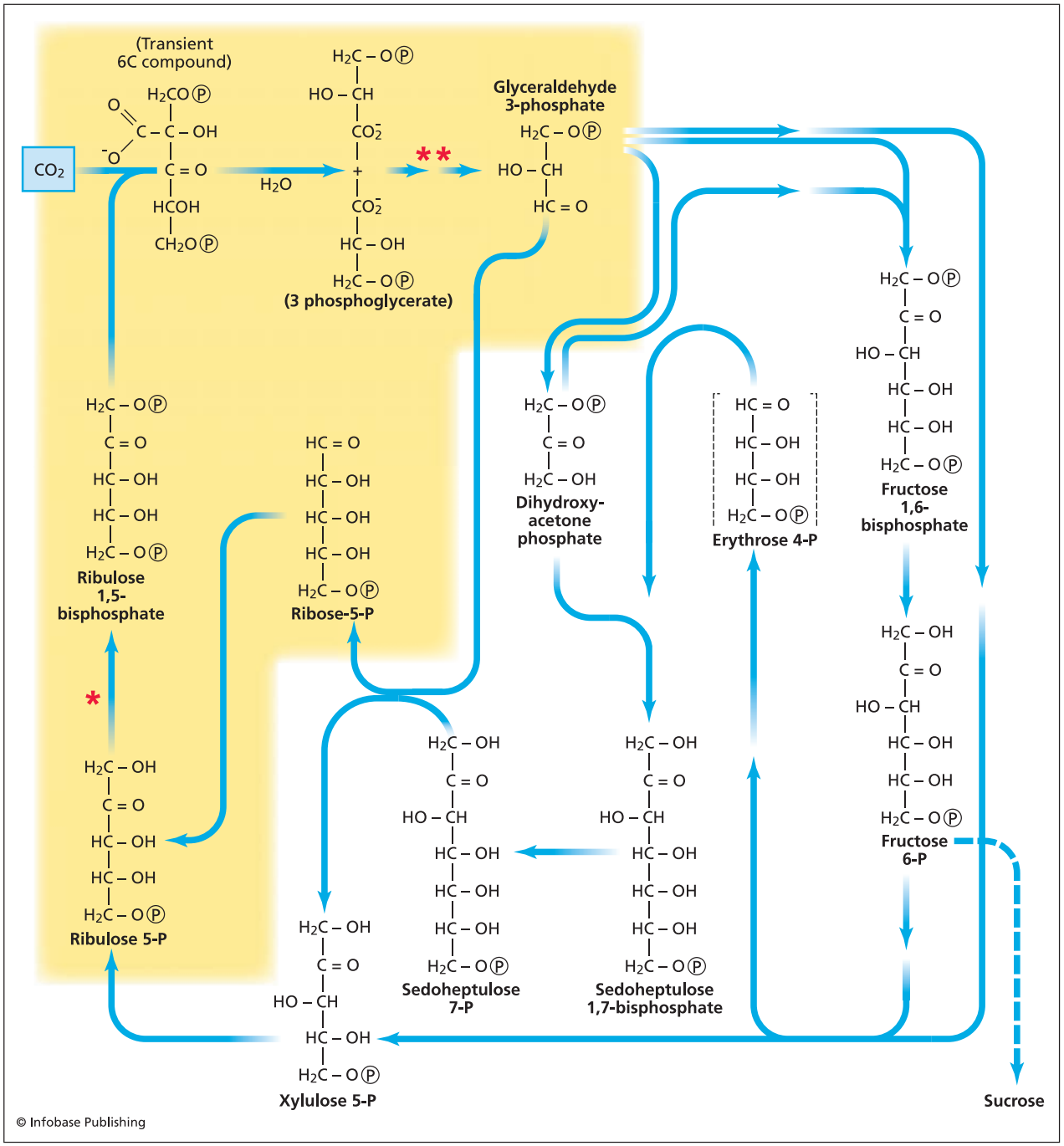
C_4 (erythrose 4-phosphate) + C_3 (dihydroxyacetone phosphate) → C_7 (sedoheptulose 1,7-bisphosphate)

This leads to the next question: where did the four-carbon compound come from? Calvin explained the origin of the four-carbon compound with a reaction that simultaneously explained the seemingly unbalanced radioisotope intensities of the different carbons in the pentose phosphates.

C_6 (fructose 6-phosphate) + C_3 (glyceraldehyde 3-phosphate) → C_4 (erythrose 4-phosphate) + C_5 (xylulose 5-phosphate)



A reaction involving sedoheptulose and glyceraldehyde explained the asymmetric radiolabeling scheme observed in ribulose 1,5-bisphosphate. The red asterisks indicate the relative intensities of the radiolabel.



The first product of photosynthesis is 3-phosphoglycerate. The remainder of the cyclical photosynthetic carbon pathway serves to regenerate ribulose 1,5-bisphosphate to allow continued incorporation of carbon from CO_2. The red asterisks indicate the steps in the pathway that require high-energy compounds formed during the light reactions of photosynthesis. The highlighted (yellow) portion of the diagram indicates the reactions that textbooks typically depict when summarizing the Calvin cycle.

After identifying the sources of all the radiolabeled sugars revealed by their chromatography experiments, Calvin was able to puzzle them together in a sequence by following the position of the carbon 14 label. The result was a cyclical pathway driven by the energy ultimately obtained from sunlight. Each complete turn of the pathway fixed an additional carbon atom and regenerated the ribulose 1,5-bisphosphate. When they turned off the light source, glyceraldehyde 3-phosphate continued to accumulate, but sucrose did not. From this Calvin concluded that the conversion of glyceraldehyde 3-phosphate into sucrose requires

high-energy compounds (ATP [adenosine triphosphate] and NADPH [nicotinamide adenine dinucleotide phosphate]) generated during the light reactions. The CO_2 combined with ribulose 1,5-bisphosphate during the dark reactions. The regeneration of ribulose 1,5-bisphosphate also requires high-energy compounds made during the light reactions.

The Royal Swedish Academy of Sciences awarded Calvin the 1961 Nobel Prize in chemistry for this body of work.

OTHER RESEARCH, AWARDS, AND SERVICE

Calvin contributed his expertise in bioorganic compounds to many other questions related to life science. He performed research on the chemical evolution of life by simulating presumed conditions of the early Earth in order to see what types of organic molecules could form. He also examined ancient rocks for molecular fossils, the presence of organic compounds that might support the existence of life. Applying similar methods, he examined meteorites in search of evidence of extraterrestrial life. He found none. Using knowledge and skills that he developed while studying photosynthesis, he sought plants capable of producing hydrocarbons that could be used as an alternative, renewable energy resource and attempted to create artificial chloroplasts for capturing solar energy.

In addition to the Nobel Prize, Calvin received the Davy Medal of the Royal Society in 1964, the Priestley Medal of the American Chemical Society in 1978, and the National Medal of Science in 1989. He belonged to the National Academy of Sciences, the American Academy of Arts and Sciences, the Royal Society of London, and more. He served as chairman for the Pacific Division of the American Chemical Society (1951), president of the national American Chemical Society (1971), and president of the American Society of Plant Physiology (1963–64). Numerous national panels and committees sought Calvin's expertise, including the Joint Committee on Applied Radioactivity (now the Atomic Energy Agency), the President's Science Advisory Committee for Presidents John F. Kennedy and Lyndon Johnson, and the Energy Research Advisory Board. He consulted for and then joined the board of directors of the Dow Chemical Company in 1963 and served until 1981, when his age forced him to retire. Several universities awarded Calvin honorary doctorate of science degrees. In 1997 Calvin's alma mater, Michigan Technological University, established the Melvin Calvin Nobel Laureate Lecture in his honor. The building that Calvin helped design to replace the old Radiation Research Laboratory at Berkeley was renamed after him when he formally retired in 1980.

In 1942 Calvin married the former Genevieve Jemtegaard, a juvenile probation officer whom he met through a friend. She later assisted Calvin in his research and is a coauthor with him on several scientific papers. The couple had two daughters, Elin and Karole, and one son, Noel. Calvin died of a heart attack on January 8, 1997.

Today the carbon pathway of photosynthesis is referred to as the Calvin cycle. Melvin Calvin's work was revolutionary not only in that it explained the mechanism that supports almost all life on Earth, but also in that it demonstrated how to use radioisotopes to trace carbon through a biochemical pathway. Since carbon forms the backbone of all organic molecules, this pioneering technique led to an explosion in biochemical research and a much greater understanding of cellular metabolism.

See also BIOMOLECULES; CHROMATOGRAPHY; PHOTOSYNTHESIS.

FURTHER READING

Calvin, Melvin. *Following the Trail of Light: A Scientific Odyssey*. Washington, D.C.: American Chemical Society, 1992.

———. "The Path of Carbon in Photosynthesis." *Science* 135, no. 3507 (March 16, 1962): 879–889.

Calvin, Melvin, and Andrew A. Benson. "The Path of Carbon in Photosynthesis." *Science* 107 (1948): 476–480.

The Nobel Foundation. "The Nobel Prize in Chemistry 1961." Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1961/index.html. Accessed January 23, 2008.

Seaborg, Glenn T., and Andrew A. Benson. "Melvin Calvin (April 8, 1911–January 8, 1997)." In *Biographical Memoirs: National Academy of Sciences*. Vol. 75. Washington, D.C.: National Academy Press, 1998.

cancer, the biology of Cancer develops when cells lose the ability to control their proliferation. Several checkpoints throughout the cell cycle monitor the cell's size, damage to deoxyribonucleic acid (DNA), and readiness to proceed through mitosis. If the conditions are not met, progression through the cell cycle will be arrested until the unfinished steps are completed. The immune system has mechanisms for recognizing and destroying abnormal cells, such as those that are precancerous, but sometimes precancerous cells escape recognition and continue to grow and divide, producing more abnormal cells. Cancer is considered a genetic disease, meaning mutations in certain genes lead to cancer. Usually several mutations must accumulate before cancer develops.

One characteristic of cancer cells is their lack of differentiation. During development and matura-

tion, cells undergo a process called differentiation, in which they become specialized to perform a particular function dependent on the tissue type. Tissues are composed of conglomerations of cells that perform a common function in the body. The following are four main tissue types:

- connective tissue
- muscle tissue
- nervous tissue
- epithelial tissue

Connective tissue functions to bind other tissues together and to provide support. Some connective tissues are fibrous, such as tendons and ligaments, and others are fluid, such as blood and lymph. Muscular tissue functions to move the body and its parts. Nervous tissue composes the brain and spinal cord and is specialized for receiving stimuli and transmitting neural impulses between the brain and spinal cord and the rest of the body. Epithelial tissue forms a protective cover for the body and lines body cavities. Many specialized epithelial cells also secrete substances, absorb nutrients, and play a role in excretion of waste products. Cancer cells lose their specialized function and no longer contribute to the tissue's function.

To the trained observer, cancer cells look different from normal cells under the microscope. They have larger nuclei that sometimes contain an abnormal number of chromosomes, and their cytoskeleton is disorganized, making the cell shape appear irregular. Normal cells stop growing after completing a limited number of cell cycles, but cancer cells continue to grow and divide indefinitely. This conversion is called transformation, and the transformed cells are termed immortal. Whereas normal cells stop growing once they have spread out and completely covered the bottom surface of a culture dish, cancer cells pile up when grown in culture. In healthy cells, the presence of chemical signals called growth factors either stimulates cell growth or inhibits it, depending on the conditions. Cancer cells do not require the presence of growth factors to undergo cell division; nor do they respond to instructions to stop dividing.

Cells arise from other cells. When a so-called parent cell grows large enough, it undergoes mitosis and cytokinesis, a series of highly regulated events including the duplication and partitioning of a cell's genetic material and cytoplasmic contents, resulting in the production of two identical daughter cells. Those daughter cells each grow and eventually divide into two more daughter cells, and so on. Several complex mechanisms regulate the cycling of a cell through this progression of events. One checkpoint within the cycle ensures that any mutations in the DNA are repaired prior to the synthesis of new DNA. The development of cancer begins with a single cell containing a mutation that interferes with the regulation of cell division. If the mutation is not repaired and the cell escapes destruction by the immune system, the daughter cells will inherit the same mutation, and all the cell descendants will also divide uncontrollably. Without properly functioning control mechanisms, the cell ignores the checkpoints as it moves through the cell cycle, increasing the chance that new mutations will become permanent. New mutations can promote the development of a tumor, or neoplasm, a mass of tissue that has lost its original physiological function and arises from uncontrolled cell division. In order for cancerous cells to continue growing and dividing, particularly once they pile up to form dense masses of cells, they need nourishment. Angiogenesis is the formation of new blood vessels that penetrate into the tumor to supply all the cells with necessary nutrients and oxygen. Benign tumors do not have the ability to invade tissues and remain localized. Additional mutations can give the tumor cells the ability to invade underlying tissues, a condition called malignancy. If malignant tumors invade lymphatic or blood circulation, their cells can metastasize, or travel to distant body parts, and initiate the growth of secondary tumors throughout the body.

CAUSES OF CANCER

Scientists have identified several genes associated with the development of cancer. These genes fall into two main groups: oncogenes are genes that cause cancer, and tumor suppressor genes are genes that stop cancerous growth. Protooncogenes are genes that encode proteins that stimulate progression through the cell cycle and prevent apoptosis, the programmed self-destruction of abnormal cells. Apoptosis is a normal physiological process designed to prevent mutated or damaged cells from dividing. The mutation of protooncogenes can transform them into oncogenes, genes that convert normal cells into cancerous cells. For example, many protooncogenes encode growth factors, chemical signals that normally regulate mitosis and cellular differentiation. Other types of protooncogenes include those encoding proteins that are involved in signal transduction pathways such as enzymes called kinases, membrane receptors that bind hormones or other chemical signals, and transcription factors that bind to DNA and activate genes.

Tumor-suppressor genes encode proteins that normally function to inhibit the cell cycle or to promote apoptosis. Mutations in these genes can result in the loss of this control measure and lead to cancer. The molecule p53 is a tumor-suppressor protein that halts the cell cycle and promotes the repair of

damaged DNA. The p53 protein can also stimulate apoptosis. Some protooncogenes interfere with p53 function. If p53 loses its ability to inhibit the cell from progressing through the cell cycle, either because its gene is mutated or because some other malfunctioning protein inactivates it, the cell can become cancerous. This gene is associated with more than half of all cancers.

Some types of cancer run in families. Two different genes associated with breast cancer, *BRCA1* and *BRCA2,* encode tumor-suppressor proteins. Humans have two copies (called alleles) of every gene, one from the father and one from the mother. If only one allele of the breast cancer gene is mutated, the normal protein encoded by the other allele will compensate. Cancer develops when someone has two faulty versions. If a child inherits a mutated form of one of the breast cancer genes from either parent, the risk for cancer increases because only one more mutation must occur. The tumor-suppressor protein encoded by the *RB* gene is linked with retinoblastoma, a cancer that originates in the retina. As with the *BRCA* genes, both copies of the *RB* gene must be mutated for cancer to develop. A third type of cancer for which someone can inherit a predisposition is thyroid cancer. Only one copy of the associated gene, *RET,* needs to be present for someone to have an increased risk for this type of cancer.

Exposure to environmental agents such as chemical mutagens found in tobacco smoke, benzene, asbestos, pesticides, and herbicides also increases the risk of cancer. Mutagens are agents that cause mutations, and carcinogens are agents that induce unregulated cell division. If the right combination of genes is affected, mutagenesis can result in carcinogenesis. Some chemical agents induce mutations by substituting organic chemical groups for hydrogen atoms on DNA. Other chemicals, such as polycyclic hydrocarbons found in tobacco, are not carcinogenic until they are inside the body cells, where natural biochemical processes alter them, making them highly reactive and able to cause breaks or mutations in DNA. Phorbol esters are not mutagenic but cause cancer by activating a biochemical pathway that encourages cell division.

Viruses cause cancer by carrying oncogenes into cells, inhibiting tumor-suppressor genes, or inhibiting the function of tumor-suppressor proteins. Research has demonstrated associations of several viruses with certain types of cancers: hepatitis B and C cause liver cancer; Epstein-Barr virus causes non-Hodgkin's lymphoma and nasopharyngeal cancer; human immunodeficiency virus (HIV) is linked with Kaposi's sarcoma and non-Hodgkin's lymphoma; human papillomavirus can cause cancer of the cervix, vulva, and penis; and human T-cell leukemia virus (HTLV-1) is associated with adult hairy cell leukemia and lymphoma.

Radiation from ultraviolet light (as from the sun), X-rays, radon gas, and radioactive isotopes such as uranium 235 has been shown to cause cancer. Ultraviolet radiation induces the formation of covalent linkages between adjacent nucleotides that, if left unrepaired before the next round of DNA synthesis, can lead to permanent mutations. The other three types of radiation listed are ionizing radiation because they promote the formation of very reactive substances that can directly damage the DNA or other biomolecules.

TYPES AND TREATMENTS

The type of tissue cells from which a tumor originates defines the cancer. Malignant neoplasms that arise from connective tissue, bone, cartilage, or muscle are called sarcomas. Epithelial cells and tissues, such as the skin, mucosal membranes, or glandular tissues, give rise to carcinomas. Leukemia, cancer of the blood, is characterized by a marked increase in the number of white blood cells, and lymphoma is cancer of the lymph tissue. People often refer to carcinomas by the location in the body where the cancer originated. For example, breast cancers are carcinomas derived from epithelial cells of the ducts or the lobes of the breast tissues. Colon cancers are carcinomas that originate in the innermost lining of the wall of the large intestine.

Different types of cancer progress at different rates and respond best to different types of treatment. The size and location of a tumor and whether or not it has spread also help a physician determine the best course of treatment, and sometimes a combination of methods is used. Such methods include surgery, chemotherapy, radiation therapy, hormone therapy, and biologic therapy. The goal of surgery is to remove the tumor. Chemotherapy involves potent pharmaceuticals that aim to destroy the cancer cells while causing minimal negative side effects. To target the cancer cells, the drugs exploit the fact that cancer cells are rapidly growing, synthesizing DNA, and dividing, whereas normal cells are mostly quiescent. A few mechanisms of action for anticancer drugs include binding the enzyme that synthesizes DNA (DNA polymerase), preventing DNA synthesis by mimicking nucleotides, and preventing the formation of microtubules of the spindle apparatus. A disadvantage of chemotherapy is that some healthy body cells also actively grow and divide, and these cells suffer damage from the treatment. Affected cells include the bone marrow, resulting in anemia and a weakened ability to fight infection; the lining of the gastrointestinal tract, resulting in nausea, vomiting, and diarrhea; the hair follicles,

resulting in hair loss; and the kidneys, resulting in the buildup of toxins in the blood due to kidney failure. Radiation therapy involves using ionizing radiation to destroy the tumor site with minimal injury to healthy tissues. The ionizing radiation probably kills the cells by breaking chemical linkages in DNA and other biomolecules. Cancers that depend on certain hormones for growth respond to hormone therapy. The goal of this type of treatment is to stop the body from producing the necessary hormone or to prevent it from acting on the cancer cells. Biologic therapy, also called immunotherapy, aims to stimulate the immune system to fight and destroy the cancer cells. Interleukins and interferon are examples of agents used in biologic therapy. The immune system naturally produces these substances, but the doses administered are higher than natural levels.

See also ANIMAL FORM; CELL BIOLOGY; CELLULAR REPRODUCTION; GENE EXPRESSION.

FURTHER READING

American Cancer Society homepage. Available online. URL: http://www.cancer.org. Accessed January 14, 2008.

Hammar, Samuel P. "Cancer (Medicine)." In AccessScience@ McGraw-Hill. Available online. URL: http://www.accessscience.com, DOI 10.1036/1097-8542.105800. Accessed January 14, 2008.

Keinsmith, Lewis J. *Principles of Cancer Biology*. San Francisco: Benjamin Cummings, 2006.

National Cancer Institute homepage. National Institutes of Health. Available online. URL: http://www.cancer.gov. Accessed January 14, 2008.

Panno, Joseph. *Cancer: The Role of Genes, Lifestyle, and Environment*. New York: Facts On File, 2004.

Carson, Rachel (1907–1964) American *Conservation Biologist*

Rachel Carson was a marine biologist and writer who launched the environmental movement after writing one of the most influential books of all time, *Silent Spring,* which warned readers about the irreversible damage caused by the indiscriminate use of pesticides. Carson had a talent for making scientific information accessible to general readers, and this book educated and alarmed the public into taking action.

CHILDHOOD AND EDUCATION

Rachel Louise Carson was born on May 27, 1907, in Springdale, Pennsylvania. She grew up on a 65-acre farm, spending time with her older brother and sister listening to the songbirds, strolling through the apple orchard, and exploring nature. Rachel attended the local school but did not have many childhood friends. She spent most of her free time reading or with her mother, with whom she shared an especially close relationship throughout her life.

Rachel especially enjoyed reading books about nature and animals by writers such as Ernest Thompson Seton and Henry Williamson. In 1918 *St. Nicholas,* a literary magazine that published works of young authors, accepted her story "A Battle in the Clouds," which told of a young pilot's struggle after being shot by German gunfire. Two others were accepted over the next year. Though at the time most children only attended public school until the 10th grade, Rachel's parents agreed that she should continue her schooling, so they enrolled her at Parnassus High School, just across the Allegheny River. After graduation in 1925, Rachel enrolled at the Pennsylvania College for Women (PCW, now Chatham College). Because she enjoyed writing, she decided to major in English.

Rachel's family struggled to pay for her college even though she received some aid in the form of a scholarship. After an initial adjustment to being away from home, she participated in extracurricular activities including basketball, field hockey, the student newspaper, and the literary magazine. During the summertime, Rachel tutored students to earn money. Though she was an English major, in order to fulfill general education requirements, as a sophomore, Rachel enrolled in a biology course that changed her life. She was captivated by learning about nature and the plants and animals that she had admired and that had given her pleasure since childhood. After taking several more biology courses, Rachel changed her major to biology and graduated with high honors in 1929.

Carson spent the summer following graduation as an intern at the Marine Biological Laboratory at Woods Hole, Massachusetts, where she saw the sea for the first time. Examining tissue specimens from ocean organisms under the microscope and studying the nervous system of turtles, she yearned to learn more about marine biology. That fall she entered Johns Hopkins University on a full tuition scholarship to study marine zoology. To help support her family, Carson worked as a laboratory assistant and taught a lower-level zoology course at Johns Hopkins and other courses at the University of Maryland in College Park, all the while working on her master's thesis, titled "The Development of the Pronephros during the Embryonic and Early Larval Life of the Catfish." The pronephros is a temporary kidney that only functions in catfish embryos for 11 days before being replaced by a permanent kidney. After obtaining a master's degree in 1932, she continued teaching at Johns Hopkins and the University of Maryland. She began the doctoral program at Johns Hopkins,

Rachel Carson's book *Silent Spring* warned the public about the dangers of pesticide overuse. *(U.S. Fish and Wildlife Service/NOAA)*

but in 1935 her father died, leaving her financially responsible for her mother.

JOINS THE BUREAU OF FISHERIES/FISH AND WILDLIFE SERVICE

Carson contacted Elmer Higgins, whom she had met at Woods Hole in 1929. He was the head of the division of scientific inquiry at the Bureau of Fisheries in Washington, D.C., and in charge of writing a series of radio scripts for a program called "Romance under the Waters." He invited Carson to help him with this task, and her entertaining and informative scripts exceeded his expectations. In 1936 she took the civil service test required by all government employees, and after she obtained the highest score, the bureau hired her as a junior aquatic biologist. When the radio broadcasts were to be published as a booklet, Carson's boss asked her to compose a general introduction. She did, but her boss felt it was too literary for the government booklet, and he suggested she submit it to The *Atlantic Monthly*. After rewriting the introduction, she eventually submitted her piece, "Undersea," which was accepted for publication. She enjoyed writing very much and upon receiving such positive feedback, she began writing natural history articles for the *Baltimore Sunday Sun Magazine*.

BECOMES BEST-SELLING AUTHOR

Upon reading "Undersea," an editor at the Simon and Schuster publishing company invited Carson to expand her article into an entire book about the ocean and marine life. Carson loved biology and she loved writing. The idea of not having to choose between two exclusive careers thrilled her. Though still working at the bureau, she accepted the proposal and went to work.

Carson was a perfectionist, and that trait made writing *Under the Sea-Wind* a major task. She wanted to instruct her readers about sea life and keep them interested, so she wrote from the animals' perspective. She researched, wrote, and rewrote, carefully choosing every word. The book was divided into three parts: life on the shore, life in the open sea, and life at the sea bottom. Though the book was nonfiction, she wrote it as a story about seabirds and marine animals, naming them (on the basis of their scientific names) and giving them human characteristics. She described their habits and daily activities—a pair of sanderlings named Silverbar and Blackfoot sought sand bugs and crabs in between waves rolling onto the shore, a mackerel named Scomber escaped prey and haul nets, and an eel named Anguilla journeyed out to sea. Her efforts were rewarded when the book was published in 1941, and reviewers praised her ability to make science understandable for the average reader. The timing of the book's release was unfortunate, however, since Japan attacked Pearl Harbor in December 1941, and the United States entered World War II. People were too preoccupied to read a fantastical book about sea life.

Carson became discouraged and felt that writing books was not worth the time it took. She now was supporting her mother and her two nieces since her sister had died, and she was dealing with her own health problems including appendicitis and shingles. In 1940 the Bureau of Fisheries had merged with the U.S. Biological Survey to form the U.S. Fish and Wildlife Service (FWS). At work, Carson contributed to the war effort by writing conservation bulletins to educate people about fish and encouraging them to consume fish as an alternative source of protein in times of scarcity. She also continued submitting brief nature articles to popular magazines. By 1949 Carson was promoted to chief editor for all the FWS publications, and she moved to Silver Spring, Maryland, her home base for the remainder of her life.

Though she remembered the feeling of frustration from the poor sales of her first book, after working on a series of FWS pamphlets, Conservation in Action, about the nation's wildlife refuges, she found herself yearning to write another book, a biography of the ocean for the average reader. She was awarded a Eugene F. Saxton Memorial Fellowship

so she could afford to take time off from her job at the FWS to immerse herself fully in her research. She started interviewing oceanographers, reading technical papers, and converting scientific jargon into understandable prose.

Her goal was to depict the wonders of the ocean while melding the fields of marine biology and physical oceanography. She began by summarizing the origin of the planet Earth and its oceans and progressed into the history of the ancient Earth and the emergence of the first life-forms. She profiled the field of marine geology, described the seasonal changes in the sea, and outlined the divisions of life within the different oceanic zones. The book contained a simplified summary of relatively new information regarding tides, waves, and currents that was gathered during the war. The text emphasized the importance of all life-forms in the oceans, from the microscopic protozoa to the larger fish, introducing to readers ecology, the concept of food chains, and the interdependence of organisms within a biological community on one another. She also pointed out the potential economic wealth in the forms of minerals and petroleum found within the sea. While showing why all nature was worth preserving, she was priming her readers for the acceptance of the ideas she would later propose in her masterpiece, *Silent Spring*.

The *Yale Review, Science Digest, Nature,* and the *New Yorker* all published portions of her manuscript before it was released. *The Sea around Us* was published by Oxford University Press in 1951, jumped onto the *New York Times* best-seller list within two weeks, and remained there for a record 86 weeks. (It was number one for 39 weeks.) This instantly successful book received the National Book Award for nonfiction in 1952 and was voted Outstanding Book of the Year in the *New York Times* Christmas Poll. Carson received the John Burroughs Medal for writing a natural history book of outstanding literary quality. PCW and Oberlin College awarded Carson honorary doctorate degrees, and the National Academy of Arts and Letters elected her to membership. *Under the Sea-Wind* was rereleased and hit the best-seller list this time. Royalties from both of her books permitted her to resign from her government job in 1952 so she could concentrate on writing full-time. She bought land in West Southport, Maine, and built a summer cottage with a breathtaking view of the ocean. There she spent summers with her mother and nieces, wading in the waters and looking for marine life.

Having published a book that explored the lives of sea creatures and another on the physical aspects of the oceans, she began toying with the idea of writing a field guide for animals that lived on the Atlantic shores of America. She had applied for and received a fellowship from the Guggenheim Foundation so she could take time off again from FWS but ended up returning a portion of the money, since sales of her other books now allowed her to quit her job. She struggled for a few years on how to arrange the manual and finally decided to organize it by ecosystem. One section was devoted to life on the rocky shores of New England, another to life on the sandy beaches of the mid-Atlantic, and the third to life in the coral reefs and mangroves of the South. Titled *The Edge of the Sea* and published in 1955, her third book was also a best-seller. The National Council of Women of the United States named it Outstanding Book of the Year, and the American Association for University Women gave Carson an Achievement Award.

The following year Carson wrote an article providing suggestions for how to teach children to appreciate nature. The article, "Help Your Child to Wonder," was published in *Woman's Home Companion* (1956). This text was adapted into a book, *The Sense of Wonder,* published in 1965, after Carson's death.

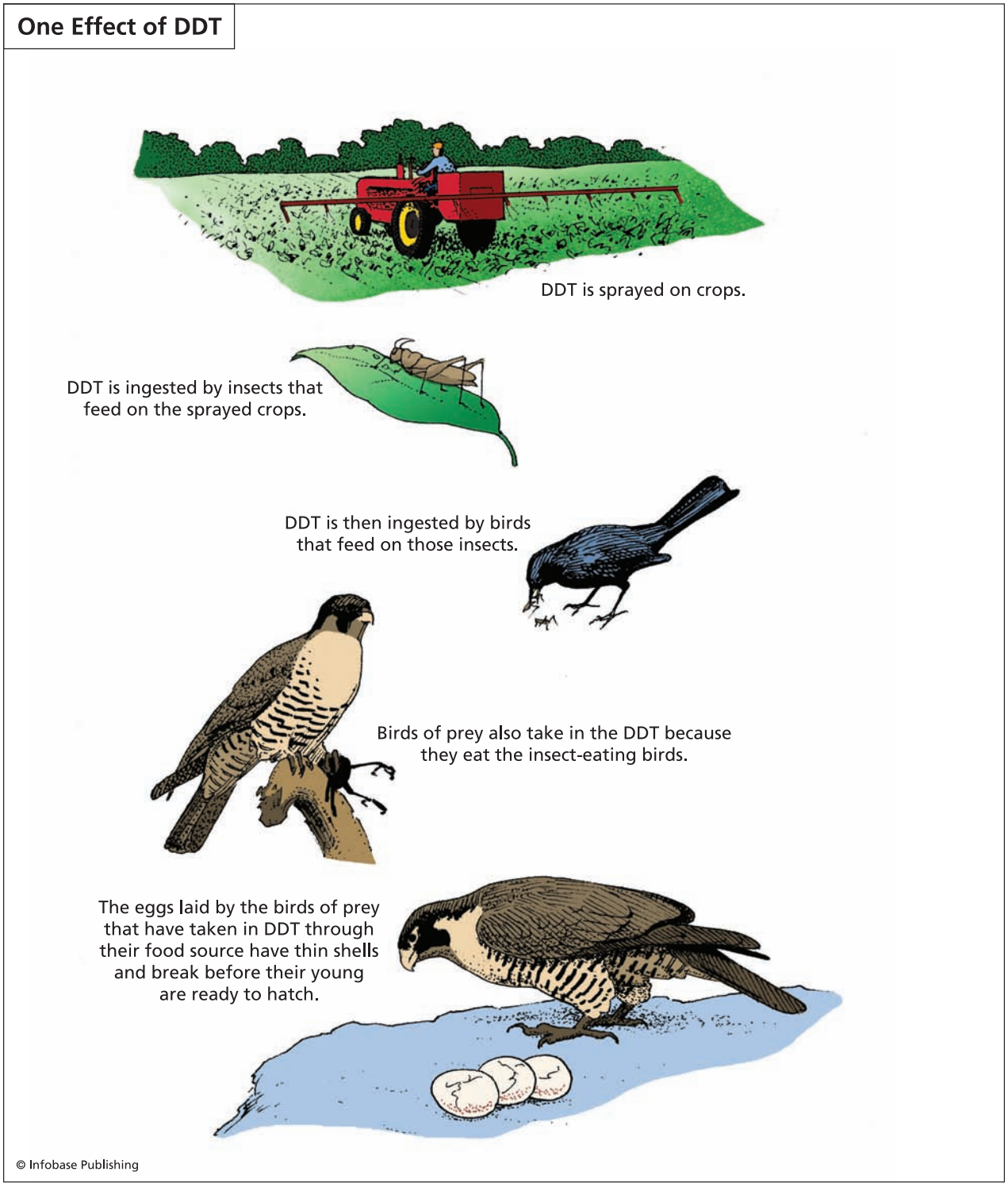
WRITES INFLUENTIAL BOOK *SILENT SPRING*

Though Carson never married, she developed several close relationships with women, from whom she received support, encouragement, and advice. Much of her correspondence with these women is preserved. One friend, Olga Owens Huckins, mailed Carson a copy of a letter to the editor of the *Boston Herald* complaining about the mosquito control program in Massachusetts. She wrote that in her backyard bird sanctuary, she had discovered as many as 14 lifeless songbirds that she believed died from pesticide spraying. The lifeless birds had their claws clutched to their chests and their bills gaping open as if they had died in agony. Carson viewed this dreadful scenario as a call for action.

For more than a decade, companies had been synthesizing a chemical called dichlorodiphenyltrichloroethane, a chlorinated hydrocarbon more commonly known as DDT. The Swiss chemist Paul Hermann Müller was awarded the Nobel Prize in physiology or medicine in 1948 for his discovery of DDT as a contact poison against insects. DDT was found to be effective against many types of annoying insect pests, including flies, lice, gnats, beetles, and mosquitoes. After studies showed it was safe for human use at insecticidal doses, it was used to combat typhus and malaria during World War II by killing the insects that transmitted the diseases. At the time, people said Müller was lucky to have discovered a substance so beneficial to medicine while researching it as an insecticide for moths. Slowly, however, scientific reports began to find its use was not as safe as initially believed. Carson had been

following these reports since the 1940s, when she first encountered them while working at the FWS.

The American Association of Economic Entomologists published an article concerning DDT's potentially damaging side effects in 1944. The article pointed out that DDT killed not only pests but also insects that were beneficial to humankind: for example, insects that feed on crop-destroying pests were killed, as were insects that play an important role in plant pollination. Carson dug into the scientific literature and explored the effects on her own, learning that many fertilized bird eggs never



As pollutants such as DDT move up through the food chain, their concentration increases.

developed or hatched and others hatched malformed animals. The bird population was steadily declining, and Carson believed this was due to the spraying of DDT. She searched for a magazine willing to publish an article about the dangers of DDT, but despite the fact that Carson was a best-selling author, the magazines were all afraid to publish something so controversial.

Carson decided to write another book, one that pointed out the dangers of this chemical that industries were hailing as the miracle solution to all pests. In a letter to her editor at Houghton Mifflin, she pledged to expose the dangers of supposedly safe pesticides and to provide substantial scientific evidence. This job would require familiarity with the scientific method and scientific literature, and knowledge of cell biology, physiology, ecology, agronomy, organic chemistry, and biochemistry. Though she was recognized as a writer, she was trained as a scientist. Carson convinced her editor that she was up to this task, and she began accumulating evidence.

At first, the government and scientific establishment readily supplied Carson with answers to he requests for information. After her reason for gathering the evidence became known, however, her requests were often blocked or left unanswered. She examined congressional testimony and interviewed countless medical and agricultural experts. Her motivation was fueled in 1957, when residents of Long Island lost a suit against the state of New York to stop the spraying of DDT for gypsy moths.

Her book, *Silent Spring,* opened with an imaginary scenario of a picture-perfect town in middle America. Suddenly, the farm animals all became sick, crops suffered, and birds and chirping insects were no longer heard, all as a result of pesticide and chemical fertilizer overuse. She exposed the truth about the poisonous effects of lingering pesticides and herbicides (chemicals that kill weeds) in the soil and water. Carson did not deny the major benefit of pesticide use in agriculture—namely, the increase in crop yield and therefore an increased food supply. She simply asked, "At what cost?" She wanted the use of most pesticides to be evaluated carefully and controlled accordingly, but she passionately believed that DDT had to be banned altogether. Carson squashed the argument that chemicals lingering in the soil do not directly affect humans by explaining that the dangerous chemicals accumulate in all creatures, beginning with the tiny organisms at the bottom of the food chain, such as plankton and small fish, and working their way up to humans. After entering the food chain, the DDT, which cannot dissolve in water, accumulates in the fatty tissues. Those organisms at the top of the food chain were at the greatest risk, since the organisms they ate contained increasingly concentrated amounts of the stored residues, and as a result, they could develop cancer and have shortened life expectancies. Birds were particularly susceptible; DDT interferes with their calcium levels, leading to weakened egg shells. Humans, as an integral part of the Earth's ecosystem, were not immune. Carson demonstrated DDT toxicity to living cells and said that DDT spraying programs were pointless since the insects developed resistance anyhow. None of this information was brand new, but all the previous reports were hidden in scientific journals and focused on one small aspect of the problem. She made this information accessible and meaningful by presenting a clear, complete overview in plain language.

Not only were the reports Carson uncovered appalling, the years she spent performing her extensive research were difficult personally. Her mother and her niece had both passed away, and at age 50, she adopted her five-year-old grandnephew, who required more attention than Carson's work permitted. In addition, she was diagnosed with breast cancer and was undergoing radiation treatment. In January 1962, she finally submitted the completed manuscript for *Silent Spring* to Houghton Mifflin, complete with 55 pages of references supporting her claims.

Silent Spring reached number one on the best-seller list within two weeks. The release of her critical book infuriated the billion-dollar chemical and agricultural industries, which were at risk of losing money. Unable to retaliate by discounting the overwhelming scientific evidence presented against pesticide use, they resorted to personal attacks, calling Carson a hysterical woman and assaulting her credentials. But the public bought her book, read it, and took her message to heart. They wrote to their elected representatives in Congress and to government agencies in protest. President John F. Kennedy set up a special panel of the President's Science Advisory Committee to evaluate the positive and negative effects of pesticide use. By May 1963, Rachel Carson was vindicated. She testified to Congress, imploring them to develop new policies to protect the environment. The committee recommended eliminating the use of persistent toxic pesticides. As a result of Carson's educating the public and the government on this scientific matter, within one year, more than 40 bills were passed through state legislatures regulating the use of pesticides. Within 10 years, the federal government followed suit. The effects of her discoveries reverberated around the world. In 1972 the use of DDT was banned in the United States.

CARSON'S IMPACT

Rachel Carson succumbed to cancer on April 14, 1964, in Silver Spring, Maryland. She had received

numerous awards and recognition prior to her passing, including the Conservationist of the Year Award from the National Wildlife Federation, the National Audubon Society Medal, and the Schweitzer Medal of the Animal Welfare Institute. In 1969 the Department of the Interior changed the name of the Coastal Maine Refuge to the Rachel Carson National Wildlife Refuge. In 1980 she was posthumously awarded the Presidential Medal of Freedom by Jimmy Carter. Carson's greatest reward, however, was knowing her work resulted in the salvation of countless voiceless animals and in turn, of humankind.

Not only did Rachel Carson educate the general public regarding marine science and effect change in the way pesticides were used, but she also pioneered movements in environmental conservation and ecology. Ecology is the study of the relationships between organisms and the environment; both affect one another, and ecologists aim to understand how. One avenue of investigation for modern ecologists is a direct consequence of Carson's speculation on the effect of human activities on the environment. Though her content was scientific, Carson's poetic style of writing appealed to general audiences, making it an effective means to educate the public about technical matters. She called attention to the irony that though the goal of pesticide use was to benefit humans, it actually harmed biological communities, including humans. Though the idea of conservation had been around for almost a century, the public as a whole did not take action to preserve nature until Carson revealed and explained how current practices caused contamination of the food supply with substances that caused cancer and genetic damage and could lead to the extinction of species. She scared the world into caring.

In 1965 friends of Carson founded a nonprofit organization, the Rachel Carson Council, to educate the public about chemical contaminants and alternatives. On April 22, 1970, Americans celebrated the first Earth Day, now recognized annually as a means to protest practices damaging to the Earth and to contribute positively to protecting our environment.

See also ECOLOGY; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED; ENVIRONMENTAL PROTECTION AGENCY; ENVIRONMENTAL SCIENCE.

FURTHER READING

Byrnes, Patricia. *Environmental Pioneers*. Minneapolis: Oliver Press, 1998.

Carson, Rachel. *Silent Spring*. Boston: Houghton Mifflin, 1962.

Lear, Linda. *Rachel Carson: Witness for Nature*. New York: Henry Holt, 1997.

———. "The Life and Legacy of Rachel Carson." Available online. URL: http://www.rachelcarson.org/. Accessed January 14, 2008.

Cech, Thomas (1947–) American *Molecular Biologist*

Among molecular biologists, the name Thomas Cech is permanently linked with ribozymes, the term given to ribonucleic acid (RNA) molecules that catalyze biological reactions, a task that scientists previously believed only proteins were capable of performing. His discovery of catalytic RNA earned him the 1989 Nobel Prize in chemistry, shared with Sidney Altman. Their groundbreaking research demonstrated that RNA played a more critical role in cellular processes than previously imagined. Since 2000 Cech has served as president of the Howard Hughes Medical Institute (HHMI), the largest private supporter of biomedical research.

BECOMES A BIOCHEMIST

Born on December 8, 1947, in Chicago, Illinois, Thomas grew up in Iowa City with his father, who was a physician; his mother, who was a homemaker; and his sister and brother. In elementary school he showed an interest in geology and claims to have knocked on the doors of professors at the University of Iowa to ask about meteorites and fossils as a young teenager.

In 1966 he enrolled at Grinnell College, where he met his future wife, Carol. Cech was drawn to physical chemistry and performed undergraduate research at the Argonne National Laboratory and at Lawrence Berkeley Laboratory. After graduation in 1970, Cech entered the University of California at Berkeley as a graduate student in chemistry; there he researched chromosome structure and function in the laboratory of John Hearst. Thomas and Carol moved to Cambridge, Massachusetts, in 1975. He performed postdoctoral research at the Massachusetts Institute of Technology under the direction of Mary Lou Pardue.

NOBEL PRIZE–WINNING RIBOSOMAL RNA STUDIES

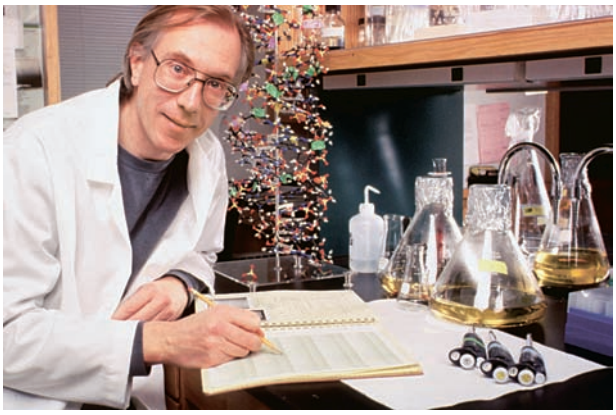
The Department of Chemistry and Biochemistry at the University of Colorado (CU) at Boulder hired Cech in 1978. Though as a doctoral student and postdoctoral fellow he studied chromosomal structure and organization in the mouse genome, when he set up his own lab at CU, he switched to using the single-celled, ciliated freshwater protozoan *Tetrahymena thermophila,* planning to look at a single gene. *Tetrahymena* has two nuclei, a micronucleus that functions in reproduction and a macronucleus that contains the expressed genome. Not only is the macronucleus transcriptionally active, but it contains numerous copies of each gene, including about 10,000 copies of the large ribosomal RNA (rRNA) gene. These copies exist extrachromosomally in the nucleoli as small deoxyribonucleic acid (DNA) molecules that resemble minichromosomes in that they

have their own telomeres. The gene encodes a large rRNA precursor that is posttranscriptionally processed to become the 17S, 5.8S, and 26S rRNAs. The portion of the gene that encodes the 26S rRNA contains an intron, an intervening noncoding segment that must be removed to create mature, functional 26S rRNA. Having a high copy number and being extrachromosomal made the large rRNA gene an attractive candidate for structural and functional analysis, since these features would facilitate the isolation of the complete gene and all of its associated proteins. One of Cech's first goals was to examine the proteins that regulated the transcription of this gene.

In vitro transcription of isolated nuclei in the presence of α-amanitin, a toxin that inhibits transcription of messenger RNA (mRNA) and transfer RNA (tRNA), yielded several rRNA products. One product of about 9S accumulated posttranscriptionally, and sequencing performed by his student Art Zaug showed that it was the intervening sequence within the 26S rRNA. Excited to have achieved such clean splicing of the intron in the in vitro transcription system, Cech decided to attempt isolation of the splicing enzyme. The notion of introns was still new, having been discovered in 1977 independently by Phillip Sharp at the Massachusetts Institute of Technology and Richard Roberts at Cold Spring Harbor Laboratory, who shared the 1993 Nobel Prize in physiology or medicine for their finding. Cech believed the splicing enzyme must be present in high concentration in the *Tetrahymena* nuclei since so many copies of the gene were transcribed and processed.

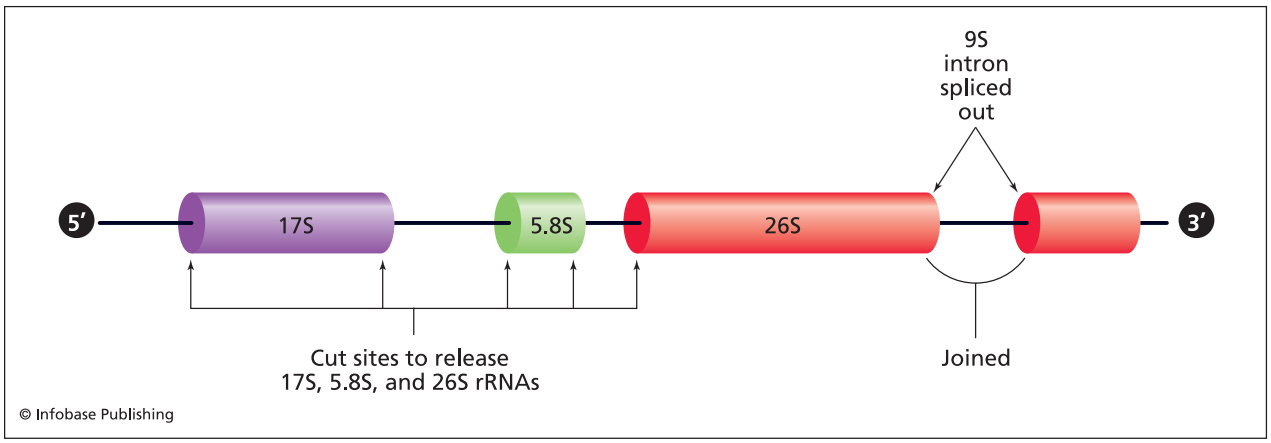
To set about isolating the enzyme, Zaug first isolated the unspliced precursor RNA to use as a substrate. They set up two groups of tubes with conditions similar to their in vitro transcription conditions and added substrate RNA to both groups. To the experimental group they added nuclear extracts, and to the other group they did not, planning for that group to serve as the negative control. With no nuclear extract added, they thought no enzyme would be present, and no splicing would occur. Surprisingly, splicing occurred in both sets of tubes.

At the time, scientists believed the sole functions of RNA were to serve as an intermediate for carrying genetic information from the DNA to the protein building machinery and to assist in protein synthesis. Proteins performed the crucial functions of catalyzing biochemical reactions (enzymes) and controlling gene expression by serving as regulatory proteins. So Cech assumed Zaug had made a mistake when setting up the experiment. After numerous repeated attempts using precursor RNA that had been treated in a manner that denatures proteins and destroys their ability to function, such as adding detergent and phenol, digesting with proteinases, and boiling, they obtained the same result. Cech then suspected that the precursor RNA had already been spliced before they set up the in vitro splicing reactions. While examining this possibility, Cech noticed that the spliced segment contained an extra guanosine residue that was not present in the original transcript. Other experiments showed that while the other three nucleotides were not necessary for the splicing to occur, small quantities of guanosine triphosphate (GTP) were necessary to release the intron.



Thomas Cech discovered that RNA had catalytic abilities while he was studying splicing of an intron from an rRNA precursor molecule from the single-celled protozoan *Tetrahymena.* *(University of Colorado)*

Cech secretly performed an experiment in which he added radiolabeled GTP to unlabeled purified precursor rRNA to see whether it was being added during excision. He did not want his students or colleagues to think he was so naïve as to believe that a covalent bond could form between the GTP and the RNA without a protein enzyme present to catalyze the reaction. The results clearly demonstrated what Cech himself was resistant to accept, that in the absence of protein, the RNA catalyzed its own cleavage and, in the process, added a guanine to the removed segment. Though they had numerous negative results suggesting no protein was involved, in order to obtain a result that would positively demonstrate RNA was responsible, Cech and his lab group used brand new recombinant DNA technology. By making the precursor RNA in vitro from recombinant DNA and using purified RNA polymerase, they eliminated the possibility that a small amount of protein remained tightly bound during RNA extraction and purification from the *Tetrahymena* nuclei. In 1981 and 1982 Cech published several papers that broke a paradigm in life science. RNA could function as an enzyme; they named such RNAs *ribozymes*. The precursor RNA catalyzed the removal of its own



A single precursor RNA transcript encodes three rRNAs in *Tetrahymena.*

intron through breaking one bond and reforming another.

Over the next few years Cech and his research group unraveled the mechanism by which RNA catalyzed its own splicing. The first step was the addition of the "extra" guanosine to the phosphorus atom of the 5' splice site by a transesterification reaction. Then the newly formed 3' hydroxyl attacked the 3' splice site in a second transesterification.

Enzymologists argued that self-splicing was different from catalysis because true enzymes did not change during the course of the reaction, but by removing part of itself, the precursor RNA was changed. Cech and Zaug responded by removing some of the precursor RNA's nucleotides and showing that it could then function as a multiple turnover catalyst. Though Cech's results seemed at first unbelievable, other groups soon had similar results. In 1983 Sidney Altman at Yale University published data showing that a stable RNA molecule had all the classic characteristics of an enzyme. The catalytic activity of RNase P, which cleaves precursor transfer RNA molecules, cleaved RNA in the absence of any protein. Cech and Altman shared the Nobel Prize in chemistry in 1989 "for their discovery of catalytic properties of RNA."

HOWARD HUGHES MEDICAL INSTITUTE

In 1988 Cech became an HHMI investigator. HHMI is the largest private supporter of basic biomedical research, providing financial resources for researchers at U.S. universities and other research organizations. With $781 million in disbursements for 2006, HHMI is second only to the federal government in dollars spent on scientific research. In January 2000, Cech became the president of the HHMI. With an endowment near $17.5 billion, HHMI supports six major areas of research: cell biology, genetics, immunology, neuroscience, computational biology, and structural biology. As president, Cech has addressed ethical issues such as stem cell research, cloning, and the relationship between academic scientists and biopharmaceutical companies. Two of Cech's main priorities at HHMI have been bioinformatics and bioethics. Cech has announced his plans to step down as president of HHMI; Robert Tjian, a molecular biologist from the University of California at Berkeley, will assume the office on April 1, 2009.

Structural biologists in Cech's lab have made significant progress toward determining the structure of the *Tetrahymena* ribozyme in order to gain further insight into the mechanism by which the active site catalyzes splicing. Others in the Cech lab are trying to obtain atomic-resolution structures of group I introns, a common type of intron found in mRNA, rRNA, and tRNA that share the same folding pattern. Another current focus of Cech's laboratory are telomeres, the structures at the ends of chromosomes. Scientists believe that maintenance of these structures is related to cellular senescence and therefore important in aging and cancer research. Telomerase, an RNA-containing enzyme, prevents the deterioration of telomeres caused by shrinking during successive rounds of replication. The Cech lab has cloned the gene for the catalytic center of the telomerase enzyme and found that it shares motifs in common with reverse transcriptase, an enzyme that synthesizes double-stranded DNA from RNA and that was thought to be limited to retroviruses. Studying the telomerase reverse transcriptase (TERT) will help scientists better understand how telomeres replicate themselves.

In addition to his Nobel Prize, Cech has received the National Medal of Science (1995), the Heineken Prize from the Royal Netherlands Academy of Arts and Sciences (1988), the Award in Molecular Biology from the National Academy of Sciences (1987), and the Pfizer Award in Enzyme Chemistry from the American Chemical Society (1985). Most recently,

In 2007 the Chemical Heritage Foundation awarded him the Othmer Medal. The National Academy of Sciences elected Cech to membership in 1987, and the American Academy of Arts and Sciences in 1988. Cech also holds an appointment as a professor of biochemistry, biophysics, and genetics at the University of Colorado Health Sciences Center in Denver.

Cech and his wife, Carol, have two daughters. Allison was born in 1982, and Jennifer was born in 1986.

See also ALTMAN, SIDNEY; BIOMOLECULES; GENE EXPRESSION.

FURTHER READING

Cech, Thomas R. "RNA as an Enzyme." *Scientific American* 255, no. 5 (November 1986): 64–75.

Cech, Thomas R., Arthur J. Zaug, and Paula J. Grabowski. "In Vitro Splicing of the Ribosomal RNA Precursor of Tetrahymena: Involvement of a Guanosine Nucleotide in the Excision of the Intervening Sequence." *Cell* 27, no. 3 (1981): 487–496.

Doudna, Jennifer A., and Thomas R. Cech. "The Chemical Repertoire of Natural Ribozymes." *Nature* 418, no. 6894 (July 11, 2002): 222–228.

Jacobs, Steven A., Elaine R. Podell, and Thomas R. Cech. "Crystal Structure of the Essential N-Terminal Domain of Telomerase Reverse Transcriptase." *Nature Structural and Molecular Biology* 13, no. 3 (March 2006): 218–225.

Kruger, Kelly, Paula J. Grabowski, Arthur J. Zaug, Julie Sands, Daniel E. Gottschling, and Thomas R. Cech. "Self-Splicing RNA: Autoexcision and Autocyclization of the Ribosomal RNA Intervening Sequence in *Tetrahymena*." *Cell* 31, no. 1 (1982): 147–157.

Lundmark, Cathy. "More Roles for RNA." *Bioscience* 54, no. 12 (December 2004): 1,162.

The Nobel Foundation. "The Nobel Prize in Chemistry 1989." Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1989/index.html. Accessed January 23, 2008.

cell biology As the name suggests, cell biology is the subdiscipline of biology that explores life at the cellular level, specifically, the activities, functions, and properties of cells. All life has a cellular basis, and the cell is the smallest fundamental unit of life. Below the cellular level, only lifeless molecules and ions are present; thus an appreciation for cell biology is important to all aspects of life science. In order to answer the question "What is life?" scientists have resorted to reductionism, examining the components of living systems to understand how they work together. Cells differ in size, shape, composition, and structure. While in some cases a single cell makes up an entire organism that carries out all of life's processes, in other cases, a cell is one of billions or trillions that make up a single organism. Cells may be specialized to carry out unique functions within a multicellular being, but all cells have in common a few fundamental properties. Cell biology encompasses all that cells have in common, and the differences between individual types. By studying different types of cells and making comparisons, biologists can better define life and its limitations. Over the last few decades, the convergence of cell biology, biochemistry, genetics, and molecular biology has advanced knowledge in all those fields synergistically.

BRIEF HISTORY OF CELL BIOLOGY

Although some individual cells are visible to the naked eye, the observation of most cells requires the assistance of a microscope to magnify them. Cell size is most often expressed in terms of micrometers; one micrometer (1 µm) equals one-millionth of a meter (10^{-6} m), the equivalent of 3.94×10^{-5} inch. Prokaryotic cells are typically a few micrometers in diameter or length, whereas plant or animal cells average 20–30 µm. Subcellular structures, sometimes called organelles, are measured in nanometers (nm); one nanometer equals one-billionth of a meter (10^{-9} m), or one-thousandth of a micometer. Because of the size of typical cells, the discipline of cell biology lagged behind the invention of the compound microscope.

A microscope is an optical instrument used to magnify an image of an object. Near the end of the 16th century, in Holland, a man named Zacharias Jansenn made a living grinding lenses for eyeglasses. He discovered that when he put two lenses together, the effect was much greater than using a single lens alone. Compound microscopes have two or more lenses in succession that enlarge the image of an object, resulting in a greater total magnification. Common compound microscopes today achieve magnifications of approximately 1,000 times, putting most cells in the observable range.

Two 17th-century scientists who paved the way for cell biologists were Robert Hooke and Antoni van Leeuwenhoek. Hooke was an accomplished British scientist who served as the curator of experiments and secretary for the Royal Society of London. In 1665 he published a best-selling book titled *Micrographia,* which contained drawings of specimens and descriptions of objects viewed under a microscope, including insects, bird feathers, sponges, and more. Biologists credit Hooke for the discovery and naming of cells. The boxlike pores or perforations he observed in thinly sliced cork reminded him of the cells or rooms of a monastery. At the time, Hooke did not realize the connection between life and these structures, which he named *cells,* which were actually the remains of cell wall tissue from

dead plant cells. Leeuwenhoek was a Dutch shop owner who sold clothes and buttons. He used ground glass lenses to examine carefully the quality of cloth sold in his shop. As was common at the time, he ground his own lenses, and he became remarkably skilled at this process. Though he had no training in science, his interest in it, his ability to create lenses of high quality, and his keen observational skills led to a decades-long communication with the Royal Society about his microscopic observations. He first examined rainwater under his microscope and was shocked to see numerous tiny creatures, which he called animalcules. Leeuwenhoek had discovered microorganisms, and for the remainder of his life he observed different microbiological specimens including peppercorn and hay infusions, scrapings from his teeth, bodily fluids and excrement, and other objects such as hair and insect parts. He kept very detailed descriptions of his observations in the form of correspondence with the Royal Society, and though his prose, grammar, and style were not scientific, the information contained within his letters was invaluable and led to the founding of microbiology. His letters are the first record of living organisms composed of a single cell.

By 1831 the resolving power of microscopes had improved sufficiently for the English botanist Robert Brown to recognize the ubiquity of a structure that he named the nucleus from his microscopic examinations of germ cells of orchids. Leeuwenhoek had observed these round structures but never elaborated on their importance, whereas Brown determined they were crucial to the process of fertilization. In 1838 the German botanist Matthias Schleiden concluded that all plant tissues were composed of cells and that new plants developed from single cells. The following year, the German physiologist Theodor Schwann concluded that all animal tissues were composed of cells. Independently, Schleiden and Schwann formulated the basis of the cell theory, purporting that all organisms are composed of one or more cells and that the cell is the basic structural unit of life. In 1858 the German pathologist Rudolf Virchow added another tenet to cell theory, stating that all cells originate in preexisting cells. The cell theory has both defined and shaped biology to the same degree that the atomic theory has influenced chemistry.

Modern cell theory is founded on the same principles as originally proposed by Schleiden and Schwann and supported by Virchow. In summary, the theory states the following:

- Cells are the fundamental structural and functional unit of life.
- All organisms consist of one or more cells (viruses are not considered cells).
- All cells arise from preexisting cells (with the exception of the first cells formed when life originated).
- All cells are composed of the same basic substances.
- Metabolism is a cellular function.

As the use of dyes and the development of staining techniques improved the level of detail seen when using the microscope, scientists learned more about the structures within the cell.

Meanwhile, in the late 1800s, the French chemist Louis Pasteur tied living organisms to what are now referred to as biochemical processes when he demonstrated that living organisms carried out fermentation, the chemical conversion of sugars to alcohols. Later biochemists performed biochemical reactions outside living organisms using extracts, which facilitated the understanding of cellular metabolism. In the 1920s and 1930s German biochemists, including Gustav Embden, Otto Meyerhof, Otto Warburg, and Hans Krebs, delineated pathways that further explained cell function. Gycolysis and the Krebs cycle explained how cells extracted energy from organic molecules and how cells synthesized many of their building blocks. During the same 50-year period, other scientists were pioneering the field of genetics. The Austrian monk Gregor Mendel established the basic laws of inheritance in the late 1800s. Soon afterward Johann Friedrich Miescher discovered nucleic acid, and then Walther Flemming discovered chromatin, which he noted separated into thread-like strands during cell division. Wilhelm Roux and August Weismann proposed the germ plasm theory of inheritance, suggesting that chromosomes carried the genetic information in sperm and egg cells to the next generation. (Other aspects of the germ plasm theory were incorrect, such as the claim that only germ cells contained all the hereditary information, and that somatic [nongametic] cells only contained certain portions relevant to their specific function.) Today biologists know that all normal nucleated cells contain all the deoxyribonucleic acid (DNA) of an organism, but different somatic cell types express different portions of the genome. Walter Sutton and Theodor Boveri proposed the chromosomal theory of heredity, stating that the behavior of chromosomes during meiotic division explained Mendel's laws of inheritance.

The invention of electron microscopy in 1932 led to many new breakthroughs in cell biology. For the first time, biologists could directly observe details of subcellular organelles and even distinguish shapes of macromolecules. In place of light, as is used in light microscopy, a beam of electrons illuminates the specimen. Since electrons have wavelengths much

shorter than wavelengths of photons of visible light, the resolution is much greater, allowing the observer to distinguish between objects 0.1–0.2 nm apart, approximately 1,000-fold greater than the resolution of light microscopes.

By the middle of the 1900s, biologists had a decent appreciation for cells, how energy flowed in and out of them, and how they reproduced. Cell biologists, molecular biologists, and biochemists continued to work out the details of these processes. Oswald Avery, Colin MacLeod, and Maclyn McCarty demonstrated that DNA mediated genetic transformation in bacteria, and then Alfred Hershey and Martha Chase showed that DNA was the carrier of genetic information in bacteriophage. In 1953 James Watson and Francis Crick solved the structure of DNA, a discovery that led to a rapid explosion of knowledge in the molecular life sciences.

SUBDISCIPLINES AND CURRENT RESEARCH IN CELL BIOLOGY

Cell biology encompasses a variety of research and often overlaps with the subdisciplines of molecular biology and biochemistry. Microbiologists, botanists, or zoologists may study cell biology from the perspective of their organismal specialty. One can also approach cell biology from an evolutionary standpoint, or the reverse—describe the origin and evolution of life at the cellular level.

Anatomy at the cellular level is just as important as it is at the organismal level. A biologist needs to explore the biological system as a whole first, then examine the individual components, and ultimately determine how a component contributes to the working system. Beginning at the cellular level, a scientist might observe and describe the function of a liver cell, specifically the fact that the hormone glucagon stimulates the catabolism of glycogen into glucose. How does this happen? One must be familiar with the structural characteristics of the cell and its membrane to answer this question. The hormone never enters the cell, but it causes the release of glucose from the cell into the external environment, where in the body it would find its way to blood circulation. First, glucagon is a protein, and structure and composition of the membrane that surrounds the cell do not permit the movement of proteins across this barrier to the cell's interior. Proteins are too large, and even if the molecule were smaller, glucagon is soluble in aqueous solutions, so it could not make its way through the lipid bilayer of the membrane. Cells have receptors that are embedded in the membranes and extend into the cell's interior, the cytoplasm, and to the exterior. The exterior portion contacts the hormone, which initiates a conformational change in the transmembrane receptor, an event that activates a nearby enzyme to produce another molecule, which stimulates a cascade of events that occur within the cell and ultimately leads to the synthesis of glucose. As with glucagon, glucose cannot simply diffuse through the cell membrane but can be transported across by a carrier protein, another type of structure embedded in the cell membrane. The receptor protein embedded in the membrane functions in communication between the external environment and the interior of the cell, and the structure embedded in the membrane functions in the transport of molecules across the membrane. One can only understand the cell's response to glucagon after knowing how the cell membrane is constructed as well as the structures embedded within it. Thus an understanding of cellular physiology requires a prior appreciation of cell anatomy.

The preceding example relates to one current hot topic in cell biology, the subject of cell signaling and communication between cells. Signaling includes the recognition of a stimulus at the exterior portion of the cell membrane and the transfer of that message to the cytoplasm of the cell. One hallmark of life is the ability to perceive and respond to changes in the external environment, and this often involves the communication of information across a cell membrane. In multicellular organisms, the external environment includes everything that is happening in other parts of the body. An appropriate response often involves the coordination of multiple events. For example, consider two seemingly simple steps in the digestion of a meal by a mammal. As food reaches the animal's stomach, the nervous and endocrine systems begin instructing the stomach and small intestine to secrete digestive enzymes to break down the food. At the same time, the smooth muscles surrounding the stomach and small intestine must alternately contract and relax to move the food through the digestive tract. What would happen if the small intestine secreted digestive enzymes but never underwent peristalsis to move the food through the tract? Or what if the food moved through the digestive tract without ever being catabolized? In either case, the animal would not efficiently meet its nutritional needs from that meal. Cell signaling is necessary for the secretory cells and the muscle cells to work together to achieve a goal.

Cell biologists have also found cell signaling to be important in regulation of the cell cycle. When cells lose the ability to control their own division, they may become cancerous. Cancer research is another major topic of cell biological research. In addition to mechanisms of cell signaling, cancer researchers study other aspects of mitosis and cytokinesis in order to find new ways to treat cancer. Any type of drug that interferes with cell growth, division, DNA

replication, or formation and function of the spindle apparatus for moving chromosomes could be a candidate for cancer therapy.

Differentiation and development are also popular research topics among cell biologists. During development of a multicellular organism, cells become differentiated, or specialized, to perform a particular function for the organism. Once a cell completes differentiation, it maintains its general character, meaning it cannot change into another type of cell. Stem cells are cells that have not yet fully differentiated and have the ability to continue dividing indefinitely. In an adult organism, certain types of stem cells are responsible for renewing tissue that has been damaged or that has a limited life span. Embryonic stem cells are unique in that they have the ability to develop into any type of specialized cell. By studying both stem cells and differentiated cells, cell biologists can look for similarities and differences. Embryonic stem cell research will help cell biologists understand the natural processes that occur during development. This knowledge has the potential to cure patients with serious diseases and conditions, such as diabetes, Parkinson's disease, and spinal cord injuries.

METHODS IN CELL BIOLOGICAL RESEARCH

Cell biologists utilize a collection of methods and techniques that allow them to explore the structure and function of cells. A few standard methods used in modern cell biology laboratories are microscopy, cell fractionation, and cell and tissue culture. Many experiments also rely on biochemical and molecular biological methods, such as protein separation and detection techniques, enzyme assays, and recombinant DNA technology, including DNA cloning and mutation analysis.

The utility of microscopy in cell biology research has been invaluable. Structural analysis typically precedes functional studies at any level of biological organization. Cells are too small to observe with the naked eye; different types of microscopy allow biologists to observe various aspects of cellular anatomy. Treatment with dyes or specialized staining techniques may highlight subcellular structures or allow a biologist to examine the intracellular location of certain biomolecules or cellular structure. For example, Gram staining is a technique that distinguishes between types of prokaryotic cells on the basis of the composition of the cellular envelope, a characteristic that is important in identification of microorganisms and that has medical diagnostic significance. Another staining protocol, Masson's trichrome, helps histologists (scientists who study tissues) distinguish cells from surrounding connective tissue. The incorporation of fluorescent labels linked to antibodies designed to recognize and bind specific proteins highlights areas within a cell where the protein of interest is found. For example, if an antibody recognizes histone proteins that compact DNA into chromatin, the chromosomes will fluoresce a certain color, depending on the type of label. If the antibody specifically recognizes receptors on the surface of the endoplasmic reticulum (ER), the membrane of the ER will fluoresce. Specialized methods used to prepare specimens for electron microscopy are designed to preserve or accentuate certain subcellular structures. For example, freeze-fracture replication is a procedure in which small pieces of tissue are rapidly frozen, placed within a vacuum chamber, and then split apart using the edge of a sharp knife. As the fracture plane spreads from the point of contact, bilayered membranes separate, leaving behind impressions and elevations where integral membrane proteins existed. After coating the exposed fractured plane with a heavy metal, one can observe the specimen using an electron microscope and study the topography. Transmission electron microscopy is best for examining internal structures of cells, and scanning electron microscopy is best for examining the surface structure of three-dimensional specimens.

Just as biologists can gain a better understanding of multicellular organisms by studying the structure and function of isolated tissues and cells, cell biologists can learn more about the structure and function of whole cells by studying their subcellular structures in isolation. Cell fractionation is the separation of different organelles from cells. Most often the starting material is a tissue sample, which the researcher homogenizes in a buffered solution by chopping, grinding, subjecting to repeated cycles of freezing and thawing, or exposing to hypotonic conditions. The homogenate may need to be filtered, depending on the type of tissue. Differential centrifugation then separates the different organelles according to their different densities. Generally, centrifugation of the cell homogenate at increasing force causes the nuclei to pellet out of solution first, followed by the mitochondria and lysosomes, and finally microsomes (vesicles formed from the endoplasmic reticulum and the Golgi apparatus). The fractions containing different organelles can then be used for further analysis.

The capability of studying cells in isolation allows cell biologists to examine the effects of certain physical conditions or of certain chemical substances on cell function. Whole cells can be removed from living organisms and grown in vitro in the laboratory under optimized conditions. Certain cell types are more tolerant of this than others and can grow for longer periods. Scientists have discovered a few cell lines that are immortal: in other words, they can grow and multiply in vitro indefinitely. By studying cells from tumors that have acquired this characteristic,

scientists have learned much about what controls the normal process of cell division and what goes wrong in transformed cells, cells that have lost the ability to regulate this process. Treatment of cultured cells with carcinogenic chemicals, ultraviolet radiation, or tumor viruses has resulted in their transformation, leading to the creation of new cell lines for use in cellular, biochemical, or medical research.

See also CELL COMMUNICATION; CELL CULTURE; CELLULAR METABOLISM; CELLULAR REPRODUCTION; CENTRIFUGATION; CHROMATOGRAPHY; CHROMOSOMES; DUVE, CHRISTIAN DE; EUKARYOTIC CELLS; HOOKE, ROBERT; LEEUWENHOEK, ANTONI VAN; MICROSCOPY; PROKARYOTIC CELLS; RECOMBINANT DNA TECHNOLOGY; SCHLEIDEN, MATTHIAS; SCHWANN, THEODOR; VIRCHOW, RUDOLF.

FURTHER READING

American Society for Cell Biology (corporate author), Joseph G. Gall and J. Richard McIntosh, eds. *Landmark Papers in Cell Biology: Selected Research Articles Celebrating Forty Years of The American Society for Cell Biology*. Woodbury, N.Y.: Cold Spring Harbor Laboratory Press, 2000.

Bechtel, William. *Discovering Cell Mechanisms: The Creation of Modern Cell Biology*. New York: Cambridge University Press, 2006.

Hine, Robert, ed. *The Facts On File Dictionary of Cell and Molecular Biology*. New York: Facts On File, 2003.

Ling, Gilbert N. *Life at the Cell and Below-Cell Level: A Hidden History of a Fundamental Revolution in Biology*. New York: Pacific Press, 2001.

Rothman, Stephen. *Lessons from the Living Cell: The Limits of Reductionism*. New York: McGraw Hill, 2002.

cell communication

One of the hallmarks of life is the ability to respond to the environment. As the basic unit of life, a cell must have a means for transmitting information from the outside of the cell to the inside of the cell. In multicellular organisms, the cells that make up different tissues and organs must communicate with other tissues and organs to coordinate the life processes necessary for growth, survival, and reproduction. Information is carried from the inside of a cell to the outside (and vice versa) through its membrane via molecular signals. Cells secrete chemicals that bind to specific receptors on other cells and initiate a series of intracellular reactions. The process of signal transduction involves pathways that are stimulated by the specific binding of a small molecule called a ligand to a receptor and that result in a variety of cellular responses.

Just as people can communicate by having a face-to-face conversation or over long distances through the use of telephones or e-mail, cellular communication also can occur over a range of distances. Short-distance communication occurs when a cell secretes chemicals such as growth factors into the extracellular fluid, affecting the neighboring cells. This is called paracrine signaling. Transmission of neural impulses across chemical synapses is another example of cell communication over short distances. A neuron releases chemicals called neurotransmitters into a space called a synapse between the neuron and another cell. The neurotransmitter binds specific receptors on the second cell and initiates a response in that cell. The cells of many tissues are connected to their neighbors by gap junctions, where the membranes of the adjoining cells are separated by a narrow gap, across which channels connect the cytoplasms of the two cells. Gap junctions allow solutes such as ions and water-soluble organic molecules in the cytoplasm to diffuse freely between the two cells. Sometimes cells communicate directly by the binding of a molecule exposed at the surface of one cell to a molecule on the surface of an adjacent cell.

Hormones work over greater distances. In animals, hormonal signaling is a function of the endocrine system. Cells of endocrine glands produce and secrete hormones into blood circulation, through which they travel all over the body until they reach their target cells, cells that have receptors that specifically recognize and bind the hormone. Plant hormones can travel through vessels, through cells, and through the air as a gas.

COMMON CELL-SIGNALING MECHANISMS

Many different molecules work together through several different complex mechanisms and pathways to communicate information between cells. Though different pathways use different molecular intermediates, they share several common features. Some common characteristics include the binding of a ligand to a specific receptor on a cell surface, the use of G proteins as molecular switches for biochemical pathways, second messengers to amplify signals and stimulate different intracellular activities, phosphorylation as a mechanism for activating proteins, and biochemical cascades. These factors work together to generate a variety of responses.

Communication between cells involves chemicals called ligands, small signal molecules that specifically bind to receptor molecules on a cell's surface. Ligands are usually water-soluble and therefore cannot diffuse through cell membranes. Though a ligand may contact numerous types of cells throughout the body or in the local extracellular medium, only cells that express the specific receptors will be affected. A single cell may have up to 100,000 receptors on its surface, though one-tenth of that is more common. The number and combination of receptors are

dynamic and change as a cell grows or develops. The receptors are mostly glycoproteins that are embedded in the phospholipid bilayer and span the entire width of the membrane so portions of the receptor are in contact with both the extracellular environment and the cell's interior. Ligand binding induces a conformational change in the receptor glycoprotein that is transmitted through the membrane and initiates a specific response inside the cell.

Cell-signaling pathways often employ guanosine triphosphate (GTP) binding proteins (G proteins) as switches to turn pathways on or off. G proteins can bind either GTP or guanosine diphosphate (GDP). When GDP is bound, the G protein is inactive, and when GTP is bound, the G protein is active and can interact with other proteins to alter their activity. The G protein cannot bind other proteins when the GTP has been hydrolyzed to GDP; the switch is turned off. G proteins are also called heterotrimeric G proteins, because they consist of three different subunits. Another class of G proteins, referred to as small monomeric G proteins, also bind guanine nucleotides but function in signaling mediated by a different type of receptor.

Second messengers play a role in relaying the information sent to the cell membrane by a signal molecule to the cell's interior. These small, nonprotein, water-soluble molecules or ions can diffuse through the cell rapidly, to carry information throughout the cytoplasm. They amplify the original signal and mount a complex coordinated response that results in a change in cellular activity. Cyclic adenosine monophosphate (cAMP) and calcium ions (Ca^{2+}) are common second messengers.

Proteins often carry the information in a cell-signaling pathway. Usually, the proteins are already present in the cytoplasm of the cell but are inactive. Phosphorylation, the covalent addition of one or more phosphate groups, is a common means of changing the activity of a protein. Protein kinases are enzymes that catalyze the transfer of a phosphate group from an adenosine triphosphate (ATP) molecule to a protein, either stimulating or inhibiting that protein. The kinase activity may be incorporated in the receptor itself, but more commonly kinase exists as a separate enzyme that is either associated with the membrane or floats freely in the cytoplasm. Phosphatases, enzymes that remove phosphate groups, work in conjunction with kinases to regulate the activity of cellular proteins. Phosphorylation affects several types of proteins including different enzymes, channel proteins, ribosomal proteins, regulatory proteins, and receptors.

CELL SURFACE RECEPTORS

Three main classes of membrane receptors include G protein–linked receptors, tyrosine-kinase receptors, and ion-channel receptors. G protein–linked receptors play a variety of roles and are present across kingdoms. They are important in development, disease, and sensation, and many pharmaceuticals act by influencing G protein pathways. As the name suggests, G protein–linked receptors work in conjunction with G proteins. The binding of a signal molecule to a G protein–linked receptor causes a conformational change that activates the receptor, which in turn activates the G protein by exchanging the bound GDP with a GTP. Once activated, the G protein then transfers the signal to an intracellular target, either another nearby enzyme or an ion channel. At the same time, the G protein hydrolyzes the GTP to GDP and an inorganic phosphate and dissociates from the enzyme. The G protein will remain inactive unless another signal is received by the G protein–linked receptor.

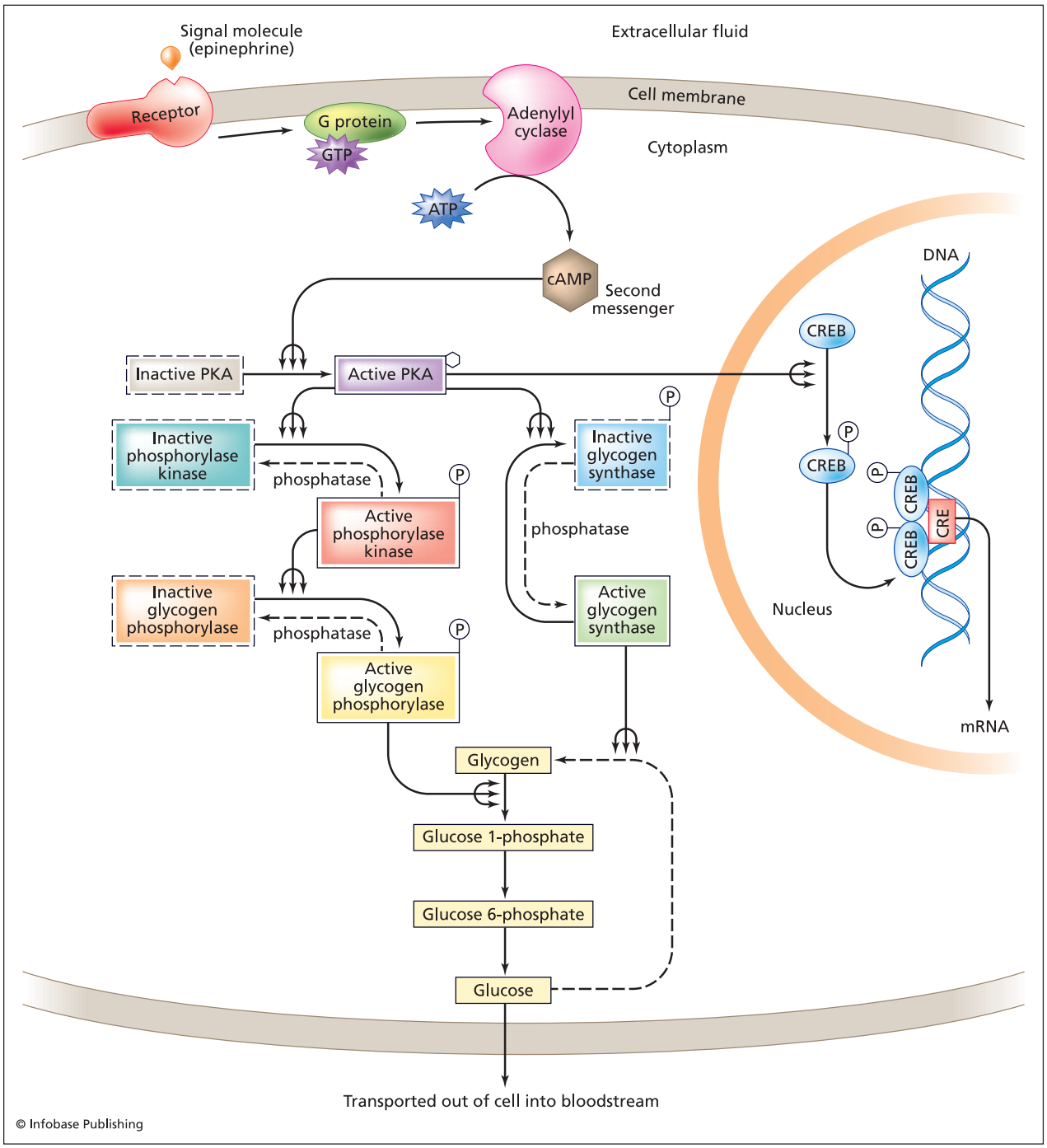
Several G protein–linked receptors have been cloned and their structures determined. A common structural motif involves a single long polypeptide chain that loops back and forth to cross the membrane seven times. The amino terminus of the polypeptide extends into the extracellular fluid and the carboxyl terminus protrudes into the cytoplasm, with three loops occurring on each side of the membrane. The last exterior loop usually binds to the signal molecule, and the last interior loop interacts with the G proteins.

Another class of receptors, the tyrosine-kinase receptors, are responsible for mediating cell responses to growth factors, signaling molecules that exert a positive effect on cell growth or differentiation. The cytoplasmic portion of these receptors has an enzymatic activity that transfers a phosphate group from a molecule of ATP to the amino acid tyrosine on a protein. In contrast to the G protein–linked receptors, tyrosine-kinase receptor proteins only span the cell membrane once. When bound by growth factors at the exterior sites, two receptor monomers aggregate to form a dimer. Each monomer then transfers a phosphate group from an ATP to a tyrosine residue on the cytoplasmic side of the other receptor protein. When phosphorylated, the receptor protein is activated to bind certain cytoplasmic proteins, causing conformational changes that result in the initiation of several signal transduction pathways.

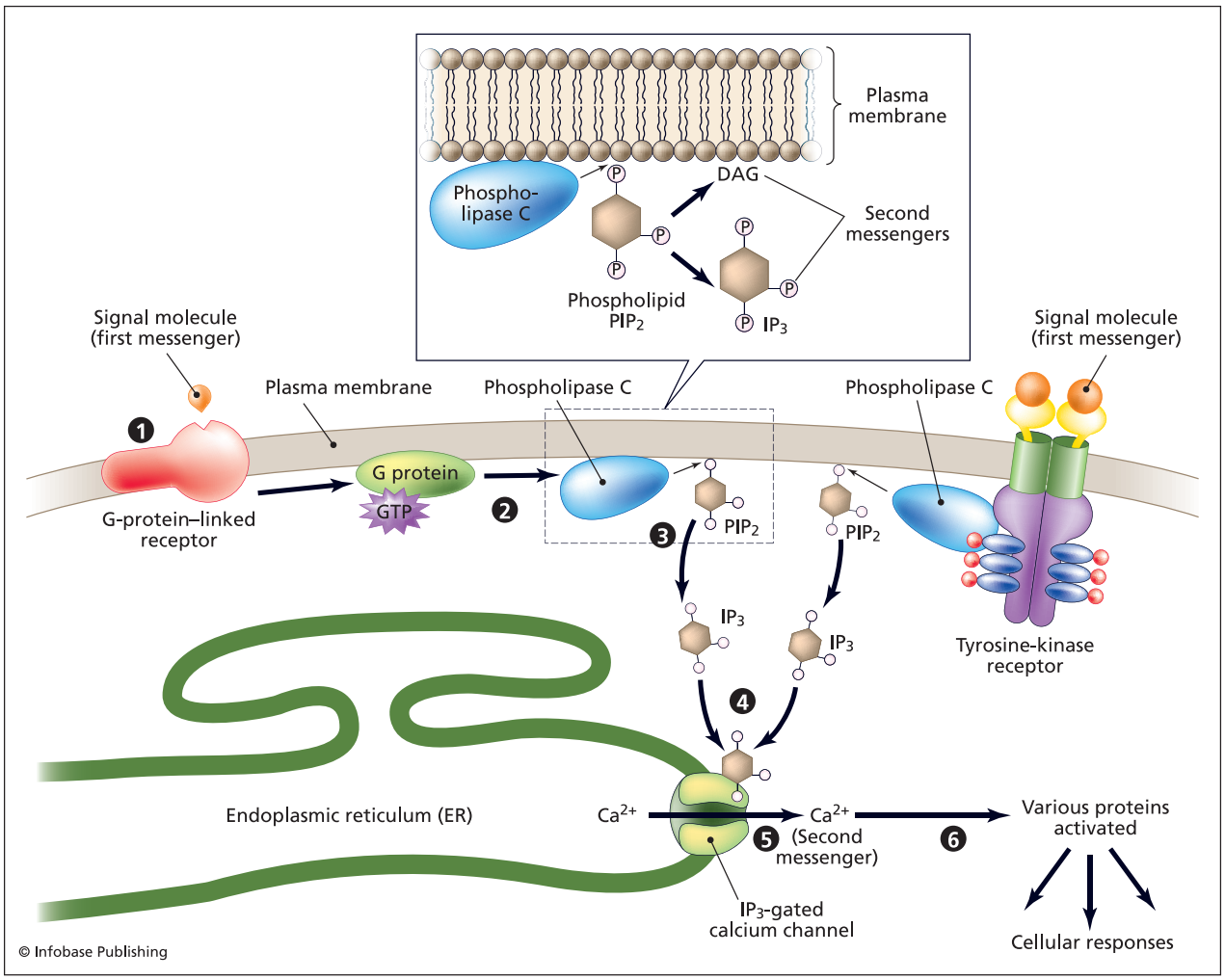
Receptors that transport ions through the membrane are called ligand-gated ion channels. Ligand binding can open the channels or close them. The channels are specific for the ions they transport, such as sodium or calcium ions. These sorts of receptors play an important role in the transmission of nervous impulses between cells at chemical synapses.

SIGNAL TRANSDUCTION PATHWAYS

Binding of a ligand to its membrane receptor initiates a complex set of reactions that result in altered activity of the target cell. The ultimate goal or change in the cell's activity could be the expression of a new gene product, alteration in the rate of cell division, secretion of a hormone, or turning on a specific metabolic pathway while turning off another. The final result depends on the type of cell and the signal that reaches it, but before any change in activity can occur, the signal must be converted from the form of a signal molecule to a response by the cell, a process called signal transduction. The signal molecule itself remains on the outside of the cell, and the receptor remains embedded in the membrane, but the information is relayed to



The second messenger cAMP mediates the mobilization of glucose stores through a series of reactions activated by PKA.



Inositol triphosphate (IP_3) and calcium (Ca^{2+}) often function as second messengers in signal transduction pathways.

proteins in the cytoplasm by various intracellular signal transduction pathways.

One example of an intracellular pathway is the cAMP-mediated pathway for glucose mobilization. In times of high stress, the body needs to increase the amount of available glucose in anticipation of muscular activity. The adrenal medulla secretes the hormone epinephrine, and the pancreas secretes glucagon. These hormones bind to their receptors on the surface of liver cells where glycogen is stored. The membrane-associated enzyme adenylyl cyclase converts ATP to cAMP in response to the binding of a signal molecule to its receptor. The cAMP molecules diffuse into the cytoplasm, where they bind a site on the enzyme protein kinase A (PKA). PKA consists of two catalytic subunits and two regulatory subunits. The binding of cAMP to the regulatory subunits causes them to dissociate from the catalytic subunits, activating the enzyme. PKA can then add phosphate groups to serine residues on its target proteins, phosphorylase kinase and glycogen synthase. The first enzyme, phosphorylase kinase, catalyzes the phosphorylation of another enzyme, glycogen phosphorylase, which breaks down glycogen into glucose 1-phosphate moieties. Another enzyme converts glucose 1-phosphate into glucose 6-phosphate, and yet another converts that into glucose. Glycogen synthase, the other enzyme phosphorylated by PKA, is inhibited by phosphorylation and cannot perform its normal function of converting glucose to glycogen for storage. Because the original hormone binding at the cell surface can activate hundreds of adenylyl cyclase enzymes, leading to the production of numerous molecules of the second messenger cAMP, the signal is amplified. Also, each enzyme can catalyze numerous reactions, further amplifying the original signal.

The signal relayed by the second messenger cAMP extends beyond the cytoplasm. The activated PKA not only phosphorylates cytoplasmic enzymes, but

travels to the nucleus, where it phosphorylates a transcription factor called CRE-binding protein (CREB). This transcription factor binds to genes that contain a specific sequence, the cAMP response element (CRE). Binding of CREB turns on the CRE-containing genes, whose products influence processes such as cell proliferation and differentiation. While PKA mediates most of cAMP's effects, cAMP can also directly bind to and open some ion channels, such as in sensory neurons involved in the sense of smell.

Other enzymes called protein phosphatases rapidly reverse the action of protein kinases. Whereas kinases add phosphate groups to proteins, phosphatases remove them. These antagonistic enzymes work together to coordinate activities controlled by phosphorylation.

Another common intracellular signal transduction pathway involves second messengers derived from the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2), found on the inner layer of the phospholipid bilayer. Different forms of the enzyme phospholipase C are activated either by G proteins or by tyrosine-kinases after the binding of a signal molecule to its cell surface receptor. Phospholipase C cleaves PIP_2 into diacylglycerol (DAG) and inositol triphosphate (IP_3). Because IP_3 is water-soluble, it quickly diffuses through the cytoplasm and binds to IP_3 receptor channels on the endoplasmic reticulum. The cell normally maintains a low cytoplasmic concentration of Ca^{2+} by actively pumping it out of the cell and into the endoplasmic reticulum (ER), but binding of IP_3 to the channels opens them, releasing Ca^{2+} from the ER into the cytoplasm. Ca^{2+} binds to the protein calmodulin, activating it. The Ca^{2+}-calmodulin complex activates several other proteins, resulting in the stimulation of muscle contraction, activation of transcription factors, opening of ion channels, and regulation of metabolic enzymes. Meanwhile, the DAG remains associated with the membrane and activates the enzyme protein kinase C, which in turn phosphorylates serine and threonine residues on target proteins that are often involved in cell growth and differentiation.

Another pathway initiated by tyrosine-kinase receptors activates the small monomeric G protein Ras, an important regulator of cell growth. As with the heterotrimeric G proteins, Ras can bind either GDP or GTP, but it is only active when GTP is bound. A guanine nucleotide-release protein (GNRP) exchanges a bound GDP with a GTP, triggering a cascade of cellular events. One significant event is the activation of mitogen-activated protein kinases (MAP kinases), a family of kinases that are highly conserved throughout eukaryotic kingdoms and play a role in mating and sporulation in yeast and cell growth and differentiation in multicellular organisms. Activated MAP kinases enter the nucleus, where they phosphorylate transcription factors of genes involved in cell growth.

The signal transduction pathways described here have been simplified. In reality, signal transduction pathways are not linear progressions from one starting point to a single end result. Completely separate pathways initiated by different signal molecules can converge to activate a shared protein intermediate. Conversely, a single signal molecule can bind its receptor, initiating one pathway, but then diverge or branch out into several different pathways that result in different cellular actions. Even more complex, cross talk sometimes occurs between two different pathways. Cross talk is the web that is formed when information is transferred back and forth between two pathways.

See also BIOLOGICAL MEMBRANES; BIOMOLECULES; CELL BIOLOGY; ENDOCRINE SYSTEM; ENZYMES; EUKARYOTIC CELLS; NERVOUS SYSTEM; PHYSIOLOGY; PROKARYOTIC CELLS; SENSATION.

FURTHER READING

Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell.* 5th ed. New York: Garland Science, 2007.

Brivanlou, Ali H., and James E. Darnell, Jr. "Signal Transduction and the Control of Gene Expression." *Science* 295 (2002): 813–818.

Campbell, Neil A., Jane B. Reece, Lisa A. Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, and Robert B. Jackson. *Biology.* 8th ed. San Francisco: Pearson Benjamin Cummings, 2008.

Cooper, Geoffrey M., and Robert E. Hausman. *The Cell: A Molecular Approach.* 4th ed. Sunderland, Mass.: Sinauer Associates, 2007.

King, Michael W. "The Medical Biochemistry Page: Signal Transduction." Available online. URL: http://web.indstate.edu/thcme/mwking/signal-transduction.html#intro. Updated March 22, 2006.

cell culture The ability to maintain living cells in the laboratory is an important aspect of life science research. Though the ultimate goal is to understand how biological units function in natural settings, by being able to control the environment, biologists can study different aspects of organisms, tissues, cells, and molecules. In the case of many microorganisms, a single cell is a whole organism; thus in these cases, a researcher grows and observes populations of the organisms. For multicellular organisms, cell culture usually consists of maintaining a piece of tissue or isolated cells from a specific tissue. Depending on the cell type, the cells may actually divide and multiply in number, or

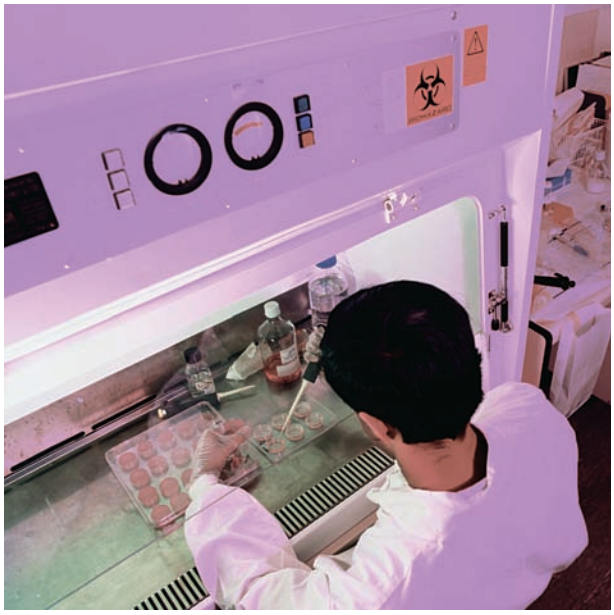
they may simply survive without multiplying when cultured.

In order to grow or maintain cells in vitro, the conditions must generally mimic the in vivo or natural conditions with respect to moisture, salts, pH, and nutrients. The substance that supplies most of these is called the medium, and it may be liquid, solid, or semisolid. The culture is then placed in an incubator that maintains the appropriate temperature and gaseous requirements.

ASEPTIC TECHNIQUE

Controlled experiments on cultured cells demand that only the desired type of cell is present in the culture. Employing aseptic technique ensures that unwanted microorganisms are not accidentally introduced. Aseptic technique is the manipulation of sterile instruments or culture media in a way that maintains sterility. Technicians sterilize the culture medium before it is used, often by autoclaving it. Autoclaves are equipment that use heat and steam to kill any viable microorganisms in culture media or on instruments prior to use. When working with cultures, countertops are often sanitized with bleach, alcohol, or a cleansing solution to reduce the numbers of microorganisms present. Metal instruments such as inoculating loops or forceps can be sterilized by directly flaming them. Other supplies, such as plastic petri dishes, flasks, and pipettes, are often sterilized by treatment with toxic gases or ultraviolet radiation and then wrapped in a manner to preserve the sterility. These types of supplies are often disposable. Briefly flaming the openings of bottles, flasks, and tubes after opening and before closing can reduce contamination as well. During handling, the worker must be careful not to allow nonsterile objects to contact anything that will have contact with the culture itself.

When the preceeding measures are not sufficient, laminar flow hoods provide extra "clean" working conditions. The work area typically consists of a box enclosed on five sides. The sixth side is partly covered, usually with a transparent pane, such as of plexiglass, so the researcher can see inside, but air exhaled during breathing is blocked from entry into the hood. The worker can insert his or her arms through an opening underneath this pane at the front of the hood. The air flow system takes in air through a filter that traps any airborne microorganisms and gently sends it through the hood in a direction leading out the opening in front. The force of the air is just strong enough to prevent any airborne contamination, such as fungal spores or flecks of dust that may be carrying viral particles, from entering through the opening and settling on the work area. Laminar flow hoods also often have an ultraviolet light inside.



Handling and treating cell and tissue cultures within a laminar flow hood reduce contamination. *(James King-Holmes/Photo Researchers, Inc.)*

When nobody is working inside the hood, the light is turned on to kill any microorganisms that might succeed in gaining entry.

BACTERIAL AND FUNGAL CULTURE

Bacteria are cheap and easy to maintain in a laboratory environment. They can be grown either in liquid or on semisolid media containing sugars, amino acids, vitamins, and other necessary nutrients. Broths are liquid media that are used to grow bacteria in test tubes, flasks, or bottles, which are sometimes shaken during growth in order to aerate the media to provide more oxygen. When sterile, most broths are transparent, and as bacterial growth occurs, the medium becomes cloudy. Semisolid media are made by the addition of agar, a gelatinous substance extracted from algae, to broth. When heated, the agar dissolves, and the medium is poured into petri dishes, small circular plates with lids. As it cools, the medium solidifies and bacteria can be spread over the surface to grow. If the bacterial culture is diluted sufficiently before plating, then individual bacterial cells divide and multiply to give rise to single circular-shaped colonies that are usually approximately 0.04 inch (1 mm) in diameter.

To inoculate fresh broth or a fresh agar plate with bacteria, one simply dips a sterilized tool called an inoculating loop (a long metal stick with a wire at one end bent into the shape of a circle with a diameter of about two millimeters) into media containing bacterial growth to pick up a drop of the culture, then dips it into the fresh tube or gently touches

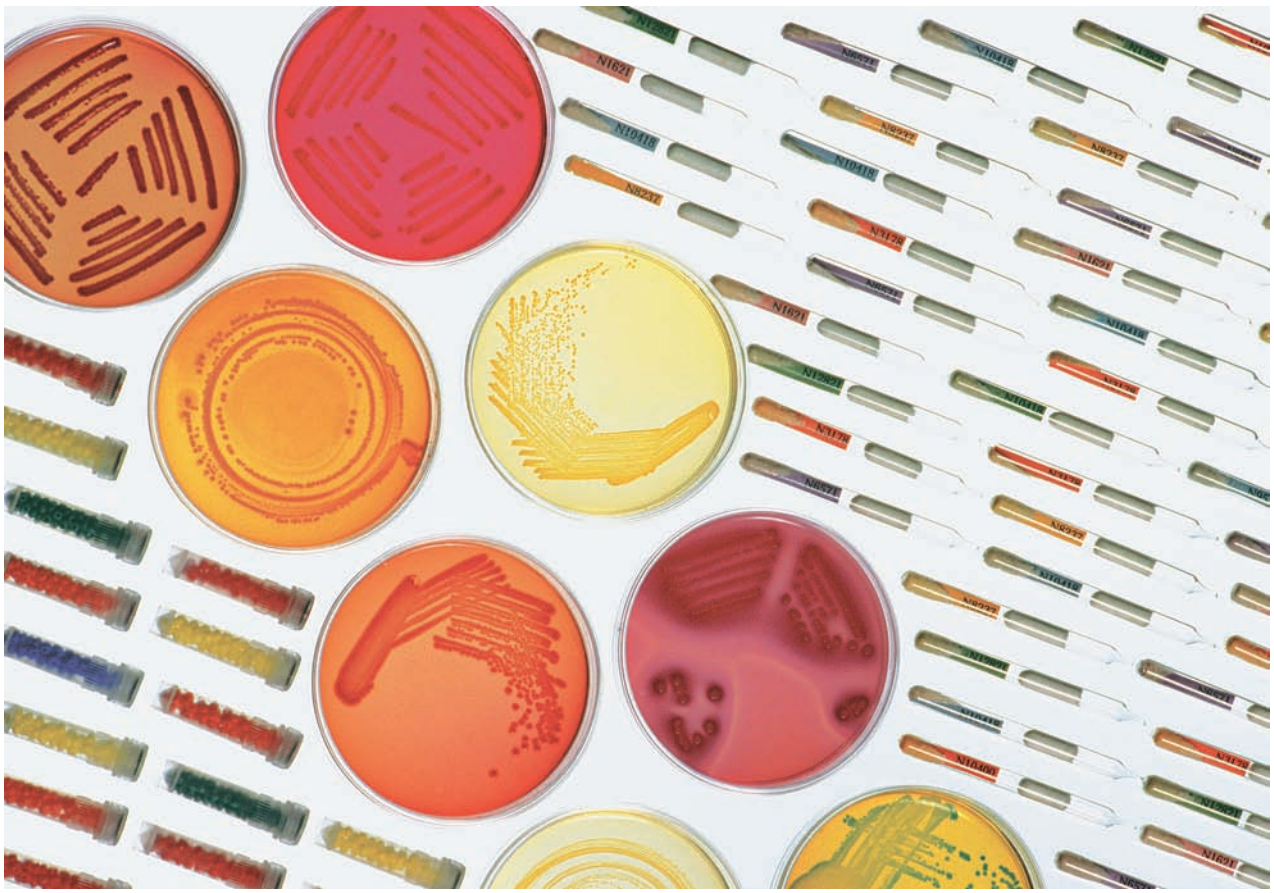
the surface of the agar to transfer the bacteria to the new medium. Alternatively, one could remove a specific volume from a broth culture using a sterile volumetric pipette or a micropipette and dispense it into the fresh medium. Whether bacteria are grown in liquid or semisolid media, they are incubated at the temperature optimal for growth of that species. Bacteria that cause diseases in humans usually grow best at 98.6°F (37°C), normal body temperature. When inoculating media with specific bacteria, it is important to maintain a sterile environment so that no undesired contaminating bacteria are accidentally introduced.

Many types of media are specialized. Selective media inhibit the growth of some types of bacteria while allowing for the growth of others. For example, a high salt concentration may be prohibitive to some species but not others. The addition of antibiotics to a medium also makes it selective. Only bacteria containing genes that confer resistance to that antibiotic will be able to grow in its presence. Differential medium allows one to distinguish between different phenotypes after growth. For example, the medium may contain a dye indicator that changes color at a low pH.

Fungi are also cheap and easy to grow in the laboratory. Molds, filamentous fungi, are usually grown on media containing agar as a semisolid support. The media must contain organic compounds such as simple sugars because fungi are heterotrophs. Because fungi multiply by forming spores that can easily become airborne, containing the mold cultures so they do not contaminate other cultures is difficult. Yeasts are unicellular fungi and are often grown in broth but can be grown on petri plates with agar-containing medium as well.

CELL/TISSUE CULTURE

The cells of most plants and animals can be maintained in culture, allowing a researcher to examine them periodically by microscope, to perform biochemical tests on them, or to examine the effect of adding or removing a component of the growth medium. The typical growth medium for mammalian cells contains amino acids, vitamins, salts, glucose, and growth factors. The addition of antibiotics helps to prevent contamination with microorganisms, and a pH indicator dye reveals whether the pH deviates too far from the optimal pH of 7.4. The cells are grown in an atmosphere of 5 to 10 percent carbon



Many types of media are available for growing and storing bacteria. *(Geoff Tompkinson/Photo Researchers, Inc.)*

dioxide, as the medium usually contains a sodium bicarbonate/carbonic acid buffer system. Often, serum is added to supply any undefined necessary growth factors or hormones. When studies involve assigning a specific macromolecule to a particular cell function or the identification of the factors minimally necessary to support growth, then the medium must be serum-free and chemically defined, meaning the investigator knows every component in the medium and its concentration. The availability of chemically defined media has made possible the study of signaling molecules involved in communication between cells.

Cells are usually grown in flasks laid on their sides or in petri dishes so the cells may attach to the bottom surface and spread out in a monolayer as they grow and so the investigator can view them under a microscope without opening the lids. Many tissue culture flasks are coated with extracellular components such as collagen or laminin to help the cells adhere, since a solid support is required for most tissue cells. Transformed cells often lose their anchorage dependence.

Primary cultures are cultures of cells isolated from tissues that have not proliferated in vitro. As the cells proliferate, the technician may subculture them, that is, transfer small quantities to new flasks. Most vertebrate cells have a limited number of times they can divide in culture; for example, fibroblast cells undergo 25–40 rounds of cell division before they stop. This phenomenon is called cell senescence, and immortalized cell lines have overcome this limitation. Cancer cells are immortal—they have inactivated the checkpoints of the cell cycle and divide continuously, even in culture. When grown in culture, cancer cells can reach high densities and often pile up and float—they do not require a solid support. One can induce cells to become immortal by the introduction of certain chemicals, oncogenes, or oncogenic viruses, but the transformed cells differ from the normal cells, and this must be taken into consideration when drawing conclusions from data obtained using them. Many cell lines from different animals are commercially available—for example, fibroblast cell lines, epithelial cell lines, kidney cells, ovary cells, and embryonic stem cells. Aliquots of cell lines can be stored in vials kept in liquid nitrogen at –320.8°F (–196°C). One of the most widely used human epithelial cell lines, HeLa cells, originated from a cervical cancer from a woman named Henrietta Lacks in 1951. Though she died eight months later, her contribution has advanced research on viruses, genetics, and cancer more than any other cell line.

Cells or tissues from multicellular organisms are often grown in flasks that are incubated on their sides so a small quantity of broth covers a larger surface area of growth. *(James King-Holmes/Photo Researchers, Inc.)*

Plant tissue culture is often performed as a means to propagate plants. Unlike animal cells, many plant cells are totipotent, meaning they have the ability to develop into a complete organism. Cuttings or sections removed from one plant can be cultured on semisolid medium that contains nutrients and hormones to stimulate growth. For example, auxins stimulate root growth, and cytokinins stimulate shoot formation. As the plant grows, new cuttings can be taken and subcultured in new flasks with fresh medium. Each plant that results from the cutting of another is a clone, a genetically identical copy of the parent plant. This is one method horticulturists use to produce large numbers of desirable organisms or to conserve endangered plant species. In the field of plant breeding, researchers use plant tissue culture to screen for beneficial characteristics, such as resistance to a pathogen.

See also CELLULAR REPRODUCTION.

FURTHER READING

Freshney, R. Ian. *Culture of Animal Cells: A Manual of Basic Technique.* 5th ed. New York: John Wiley & Sons, 2005.

cellular metabolism Organisms must be able to obtain energy from their environment in order to carry out the chemical reactions necessary for life.

Metabolism is the sum of all the chemical reactions that serve to break down and build up molecules used in life processes. Anabolism includes all of the processes that act to construct or synthesize materials needed for cellular functions such as growth, repair, or reproduction. The complementary set of reactions, catabolism, serves to break down components into smaller units. The synthesis or breakdown of chemicals inside the cell occurs via multistep pathways catalyzed by enzymes, specialized proteins that increase the rate of biochemical reactions by lowering the activation energy necessary for the reaction to proceed. Catabolic pathways generally release energy stored in chemical bonds whereas anabolic pathways typically require the input of energy to form new chemical bonds. Many intermediates of metabolism can enter either anabolic or catabolic pathways depending on the needs of the cell at the time. This property, amphibolic metabolism, improves cell efficiency.

CELLULAR RESPIRATION AND ATP SYNTHESIS

Almost all energy in living systems ultimately is derived from the Sun, but most energy in living organisms is stored as chemical energy in the form of organic molecules including carbohydrates, proteins, lipids, and nucleic acids. Organisms capable of photosynthesis can harvest the radiant energy in sunlight and convert it to chemical energy stored in biomolecules. The energy stored in organic compounds can be transferred to other organic compounds through metabolism and to other organisms through the consumption of food. Cellular respiration is the process by which organisms extract the energy from organic compounds to synthesize adenosine triphosphate (ATP), the cell's currency of energy. Once the high-energy compound is made, the cell can use it to provide energy and fuel cellular processes.

ATP is an organic molecule that consists of three components: ribose, a five-carbon sugar; adenine, a nitrogenous base; and a chain consisting of three linked phosphate groups. In order to overcome the natural tendency of the negatively charged phosphate groups to repel each other, the covalent linkages between the phosphates must contain large amounts of energy. When these phosphate bonds are broken, the energy within them is released and can be utilized by the cell for processes such as anabolism, movement, or transport of molecules. During aerobic respiration, the cell produces ATP by two mechanisms, substrate level phosphorylation by the direct transfer of a phosphate group from an organic intermediate to adenosine diphosphate (ADP) and oxidative phosphorylation by the addition of an inorganic phosphate to ADP following electron transport.

GLYCOLYSIS

The six-carbon sugar glucose is the cell's major energy supplier. The first stage of aerobic cellular respiration, glycolysis, occurs in the cytoplasm of cells and involves the breakdown, or oxidation, of glucose into two three-carbon molecules of pyruvate. Eight different enzymes catalyze the eight different reactions in the glycolytic pathway. A ninth enzyme catalyzes the interconversion of two different forms of one intermediate. Though glycolysis produces four ATP molecules by substrate level phosphorylation, two are used during the initial steps, giving a net yield of two ATP molecules for each molecule of glucose that enters the pathway. As glucose is oxidized, some of its electrons and accompanying hydrogen atoms are transferred to the electron carrier nicotinamide adenine dinucleotide (NAD^+), forming the reduced form, NADH + H^+, often simply noted as NADH. Each NADH carries a pair of electrons to the electron transport system, where they will play a role in the synthesis of additional ATP by oxidative phosphorylation. During glycolysis, two NADH are formed in addition to a net of two ATP, but most of the energy from the original glucose molecule is now stored in the two molecules of pyruvate. Glycolysis can be summarized as

$$\text{glucose} + 2NAD^+ + 2ADP \rightarrow 2 \text{ pyruvic acid} + 2NADH + 2ATP$$

TRICARBOXYLIC ACID CYCLE

The tricarboxylic acid cycle occurs in the cytoplasm of prokaryotic cells but in the mitochondrial matrix of eukaryotic cells; thus in eukaryotes, pyruvate, the end product of glycolysis, must be transported through the mitochondrial membranes to the matrix. Pyruvate cannot directly feed into the next stage of cellular respiration; it must first be decarboxylated, meaning a carbon is removed, in a reaction requiring coenzyme A (CoA) to form the two-carbon molecule acetyl-CoA. During this preliminary reaction, one molecule of carbon dioxide (CO_2) is released, and one molecule of NAD^+ is reduced to NADH.

The acetyl group of acetyl-CoA enters the tricarboxylic acid cycle, the next main phase of cellular respiration. Also called the Krebs cycle after Sir Hans Krebs, an English biochemist who elucidated its steps, or the citric acid cycle after one of its intermediates, this cyclical pathway has the main purpose of continuing oxidation of organic compounds in order to release more electrons for ATP synthesis via oxidative phosphorylation. Many intermediates of the tricarboxylic acid cycle feed into other anabolic and catabolic pathways. In the initial step, the acetyl group of acetyl-CoA joins a four-carbon molecule called oxaloacetate to form the six-carbon molecule citrate, the ionized form of citric acid. In the seven remaining

steps to complete one turn of the cycle, three NAD^+ are reduced to NADH, one flavin adenine dinucleotide (FAD, an electron carrier similar to NAD^+) is reduced to $FADH_2$, two CO_2 molecules are released, and one ATP is produced directly by substrate-level phosphorylation. The last reaction in the cycle regenerates the four-carbon oxaloacetate, with which an acetyl group that enters the tricarboxylic acid cycle combines. Because a single molecule of glucose yielded two molecules of pyruvate, the preliminary pyruvate decarboxylation step and the tricarboxylic acid cycle yield a total of eight NADH, two $FADH_2$, two ATP, and six CO_2 for each molecule of glucose that is catabolized. This can be represented as

$$2 \text{ pyruvic acid} + 8NAD^+ + 2FAD^+ + 2ADP \rightarrow 6CO_2 + 8NADH + 2FADH_2 + 2ATP$$

ELECTRON TRANSPORT SYSTEM AND CHEMIOSMOSIS

At this point in cellular respiration, the catabolism of one molecule of glucose has generated four total molecules of ATP, but most of the remaining available energy is being carried by NADH and $FADH_2$ in the form of electrons. The reduced NADH and $FADH_2$ carry the electrons extracted from the original glucose that entered cellular respiration to the electron transport system, a chain of molecules that participate in a series of oxidation-reduction reactions coupled to the production of ATP in oxidative phosphorylation. Some common molecules that participate as carriers in electron transport are pyridine nucleotides, flavoproteins, quinines, iron-sulfur proteins, and cytochromes. These molecules are embedded in the inner mitochondrial membrane of eukaryotic cells and the cell membrane of prokaryotic cells. NADH and $FADH_2$ hand off the electrons they have been carrying to a member of the electron transport chain. Each molecule in the chain has its own unique position dependent on its relative tendency to donate or accept electrons. After accepting an electron, a carrier in the chain passes the electron to its neighbor, which has a greater tendency to accept electrons, until the electron reaches a final electron acceptor. Molecular oxygen, being very electronegative and having a great affinity for electrons, acts as the final electron acceptor in aerobic respiration. During the final step of the electron transport system, molecular oxygen is reduced to water by the following reaction:

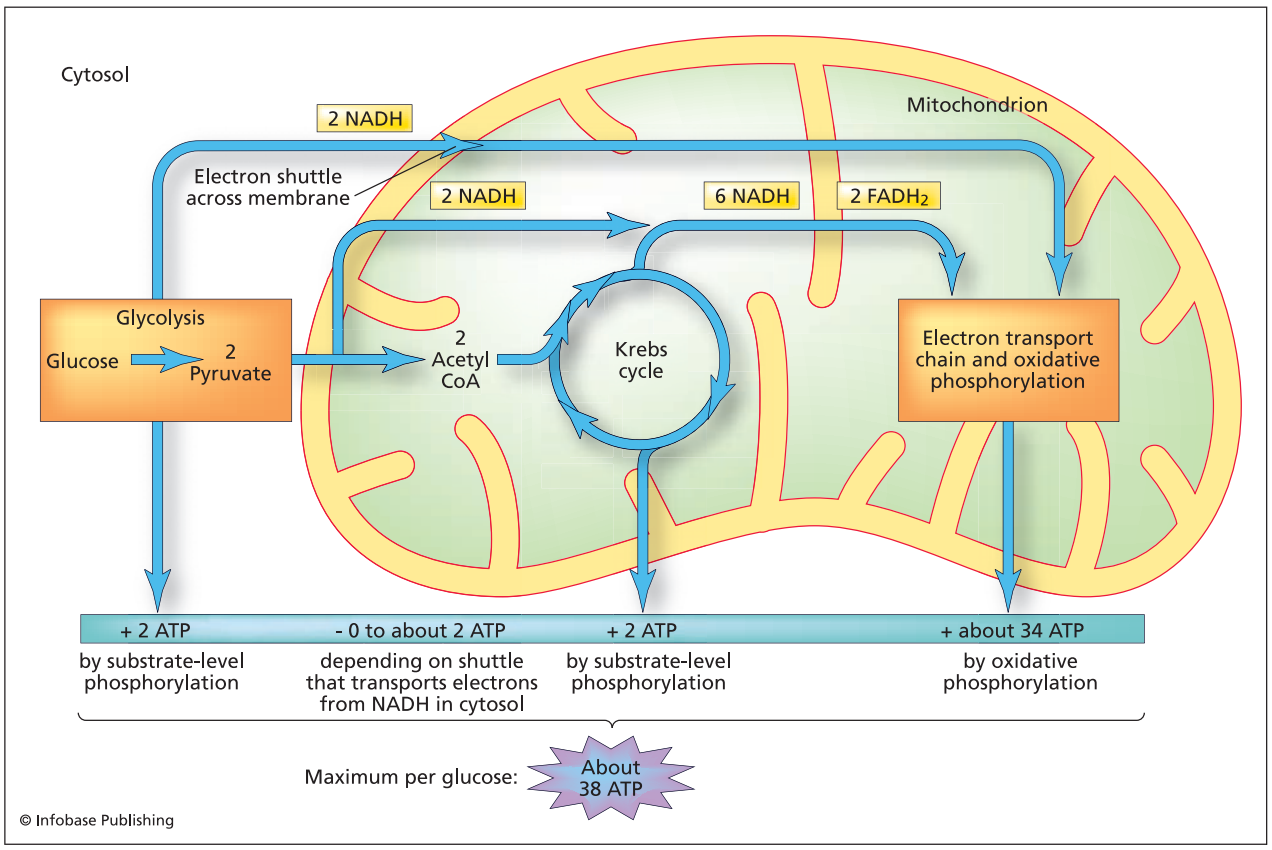
$$2H^+ + \tfrac{1}{2}O_2 \rightarrow H_2O$$

As the electrons pass through this chain to electron transport system members that have progressively higher affinities for electrons, energy released in manageable amounts is used to make ATP by chemiosmosis, the establishment and utilization of a proton gradient across the membrane to generate ATP. NADH + H^+ and $FADH_2$ have been carrying not only electrons liberated by the oxidation of glucose but also the accompanying protons (H^+). As the electron carriers transport the electrons down the chain, they simultaneously pump protons from the mitochondrial matrix into the intermembrane space between the inner and outer mitochondrial membranes in eukaryotic cells or exterior to the cell membrane of prokaryotic cells. This creates a chemical gradient as the concentration of hydrogen ions increases on one side relative to the other side of the membrane and also an electrical gradient as the positive and negative charges are separated across the membrane. The phospholipid bilayer of the membrane prevents the immediate dissipation of the gradient, which would result in wasted energy. At certain locations, unique enzyme complexes called adenosine triphosphatases (ATPases) span the membrane and act as doorways, allowing the hydrogen ions to pass through freely. As the gradient dissipates at these sites, the enzyme complexes use the released energy to form a high-energy bond by linking an inorganic phosphate to a nearby ADP, forming ATP.

Because NADH and $FADH_2$ drop off their electrons at different positions along the transport chains, the amount of energy liberated when those electrons are carried down the gradient varies. As a result, approximately three ATP molecules are synthesized for each pair of electrons carried to the chain by NADH, whereas only two molecules of ATP are synthesized for each pair of electrons carried by $FADH_2$. However, in eukaryotic cells, the NADH molecules created during glycolysis must be actively transported into the mitochondria to take their electrons to the electron transport system. Thus, these two NADH generate a net gain of two ATP rather than three. A maximum of 38 ATP can be generated from the oxidation of one molecule of glucose.

FERMENTATION

In the absence of oxygen, cells can still oxidize nutrients by a process called fermentation. Though the electronegativity of oxygen allows for the most efficient oxidation of organic molecules, anaerobic catabolism by fermentation allows the synthesis of ATP to continue. In the initial stages of glucose fermentation, glycolysis breaks down glucose into two molecules of pyruvate. The oxygen requirement of aerobic respiration occurs at the last stage, when molecular oxygen acts as the final acceptor of the electrons extracted from the food. The glycolytic pathway does not require any oxygen but yields a net of two ATP molecules. Glycolysis will continue to break down glucose into pyruvate as long as



Aerobic respiration yields a maximal output of 38 molecules of ATP, although in eukaryotes the actual yield may be lower due to the "cost" of energy required to transport electrons across the mitochondrial membrane.

the supply of NAD^+ is sufficient. Without NAD^+ available to accept the electrons released during oxidation, glycolysis cannot continue. During fermentation, pyruvate or a derivative of pyruvate acts as the final electron acceptor. Different organisms use different organic molecules, resulting in a variety of end products. Mammalian cells and some other organisms directly transfer the electrons from NADH to pyruvate, forming lactate, the ionized form of lactic acid. Organisms that undergo alcoholic fermentation break down pyruvate to CO_2 and acetaldehyde, which then accepts the electrons from NADH, forming ethanol and regenerating NAD^+, thus allowing glycolysis to continue so that more ATP can be produced. This process, called alcoholic fermentation, is the major step in the process of making beer, wine, and other alcoholic beverages. Fermentation that produces acidic products such as lactic acid or acetic acid is used in the production of certain foods, and fermentation that produces solvents such as acetone or butanol are important in industrial processes.

BIOSYNTHESIS OF MACROMOLECULES

While the function of catabolism is to break down organic nutrients for energy to produce ATP, anabolism serves to create macromolecules needed by cells to survive, grow, and reproduce. Cells obtain the building blocks of proteins, carbohydrates, lipids, and nucleic acids—amino acids, monosaccharides, fatty acids, and nucleotides, respectively—by transporting them in directly from the environment, synthesizing them through metabolic pathways, or releasing them from the breakdown of larger macromolecules. Key intermediates such as pyruvic acid and acetyl-CoA play important roles in catabolism and anabolism. As they are central components of metabolism, small modifications can transform them into compounds that feed pathways that accomplish the cell's most immediate needs.

For example, triglycerides are simple fats composed of a glycerol molecule linked to three fatty acids that contain long hydrocarbon chains. The cell can remove the fatty acids from the glycerol and convert it into pyruvic acid, the end product of glycolysis. The pyruvic acid molecule can then move into the mitochondria and feed into the tricarboxylic acid cycle and electron transport system. Enzymes digest the long hydrocarbon chains of the fatty acids by beta-oxidation. During this process, coenzyme A is attached to the end of the chain, then two carbons

are broken off at a time, creating numerous molecules of acetyl-CoA that can feed directly into the tricarboxylic acid cycle. One triglyceride molecule with three chains containing 18 carbons each yields approximately 450 molecules of ATP.

Carbohydrates and lipids are the major sources of cellular energy, but proteins can be catabolized for energy in starvation conditions. Their building blocks, amino acids, can be deaminated, meaning the amino group is removed. The product can then enter the tricarboxylic acid cycle at one of the intermediate steps.

The ability of a cell to synthesize complicated molecules from simpler ones depends on its genetic makeup. Some organisms can synthesize almost anything from glucose; others do not have the necessary enzymes and require the availability of certain precursors. Essential nutrients are nutrients that must be obtained in preassembled form because of an organism's inability to make them from raw materials. A nutrient that is essential to one organism is not necessarily essential to another. Of the 20 naturally occurring amino acids used to synthesize proteins, adult humans must obtain eight through their diet but can synthesize the other 12 through metabolic pathways.

REGULATION OF METABOLISM

To conserve energy, the cell employs several methods to regulate its metabolism, the most common being feedback mechanisms. When high levels of an end product of a metabolic pathway are present, that end product inhibits an enzyme that catalyzes an early step in the pathway in a process called feedback inhibition. In this manner, the cell diverts key intermediates into pathways whose end products are in short supply. One method of catabolic regulation involves feedback inhibition by ATP of an enzyme that catalyzes the third reaction of glycolysis. If plenty of ATP is available to satisfy the cell's energy requirements, then glycolysis slows down. As ATP levels begin to drop, that inhibition is lifted and cellular respiration speeds up again until the levels increase sufficiently.

See also BIOCHEMICAL REACTIONS; BIOCHEMISTRY; BIOENERGETICS; BIOLOGICAL MEMBRANES; BIOMOLECULES; CHEMICAL BASIS OF LIFE; ENZYMES; EUKARYOTIC CELLS; NUTRITION; PHOTOSYNTHESIS; PROKARYOTIC CELLS.

FURTHER READING

Harris, David E. "Biological Energy Use, Cellular Processes of." In *Macmillan Encyclopedia of Energy*. Vol. 1. Edited by John Zumerchik. New York: Macmillan Reference USA, 2001.

Kusinitz, Marc. "Metabolism." In *Gale Encyclopedia of Science*. 3rd ed, Vol. 4. Edited by K. Lee Lerner and Brenda Wilmoth Lerner. Detroit: Gale, 2004.

Murray, Robert K., Daryl K. Granner, Peter A. Mayes, and Victor W. Rodwell. *Harper's Illustrated Biochemistry*. 26th ed. New York: McGraw Hill, 2003.

Widmaier, Eric P. *The Stuff of Life: Profiles of the Molecules That Make Us Tick*. New York: Times Books, 2002.

cellular reproduction

The continuity of life depends on the ability of cells to reproduce, or to give rise to new cells. A cell grows, duplicates all its contents and information, and divides into two equal cells termed daughters. Cell division is necessary for replacing damaged or unviable cells, forming multicellular organisms from unicellular zygotes, and increasing the population of unicellular organisms. In multicellular organisms, programmed cell death balances cellular reproduction. This genetically directed process of cell destruction, called apoptosis, occurs as a normal part of development and growth. Cells that are old or injured or that have developed an abnormality self-destruct, and mitosis replaces them with healthy, new cells. While a few cell types are constantly either actively dividing or preparing to divide, most cells spend most of their time engaging in other functions, such as transmitting neural impulses in the case of neurons or synthesizing and secreting hormones in the case of endocrine cells. Cells divide in a highly regulated manner at different rates, depending on the type and the environmental conditions. Cancer results when a cell loses the ability to control its divisions.

THE CELL CYCLE

Cells arise by division of their parent cell (also called mother cell) and give rise to their own daughter cells by cell division. The stages between these divisions compose the cell cycle, which can be divided into two major parts: the mitotic (M) phase and interphase. During mitosis, the nucleus duplicates its contents to form two separate nuclei. In coordination with the nuclear division, cytokinesis partitions the cytoplasmic organelles and material, resulting in the formation of two complete cells that are identical to one another and to the parent cell. Though actual separation of the nuclei and cytoplasm occurs during M phase, the cell performs many preparations for mitosis during interphase.

Most cells spend the majority of their time in interphase, which consists of three stages: G_1, S, and G_2. During G_1, the first gap, the cell grows and carries out the particular functions for which the cell is suited. If the cell is an intestinal epithelial cell, it serves as part of a protective lining, acts as a selectively permeable barrier between the lumen of the intestine and the body's internal tissues, and

actively transports materials from the digestive tract into the circulatory system. If the cell forms part of a vegetative fungal hypha, it secretes digestive enzymes into the environment and absorbs nutrients into the fungus. G_1 phase ends in two possible fates: either a cell commits to DNA synthesis and progressing through the cell cycle or the cell exits the cell cycle and enters a quiescent state, called G_0. Most cells in the human body are in G_0. Muscle and nerve cells do not undergo cell division. Other cells, such as liver cells, are typically in G_0 but can reenter the cell cycle under certain conditions. Cells that will ultimately divide continue to experience growth by the production of new proteins and cellular organelles such as mitochondria and endoplasmic reticulum. The cell also accumulates materials necessary for the next phase, the synthesis (S) phase.

As the name implies, synthesis of deoxyribonucleic acid (DNA) occurs during S phase. Chromosomes, located inside the nucleus, contain the DNA. Eukaryotic cells contain linear chromosomes consisting of double-helical DNA wound tightly around proteins called nucleosomes, which package the DNA into manageable units. During G_1 of interphase, the chromosomes are not visible by microscopic examination. The chromosomes exist as extended, dispersed fibers so that the transcription machinery can easily access the genes in order to create templates for the synthesis of new proteins. The uncoiled, extended form also facilitates DNA replication during S phase. A chromatid is a single linear strand of double-helical DNA, and prior to DNA replication, a chromosome consists of a single chromatid. Following replication, chromosomes assume an X shape, due to the physical linkage of the two identical daughter chromatids (called sister chromatids) at a structure called the centromere. Animal cells also duplicate their centrioles during S phase. Centrioles are cellular organelles that play a role in forming the spindle apparatus, which aids in separation of the sister chromatids during mitosis.

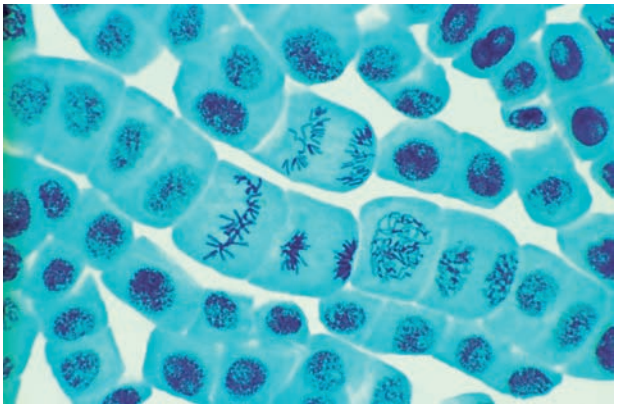
The period between the completion of DNA replication and the beginning of mitotic division is termed G_2. During this last stage of interphase, the cell synthesizes materials needed for mitotic division, such as the proteins necessary to build the spindle apparatus.

MITOSIS

Though sometimes the term *mitosis* is used to mean cell division, technically, it refers to nuclear division. Mitosis occurs in four continuous stages: prophase, metaphase, anaphase, and telophase. Cytokinesis, the division of the cell itself, usually accompanies the last stage of mitosis, resulting in two daughter cells, identical to each other and to the parent cell from which they originated. Mitosis makes up a small segment of the cell cycle, taking only 30 or so minutes, compared with the hours, days, weeks, or years that a cell can remain in interphase.

During the first stage of mitosis, prophase, the chromosomes condense up to several thousand-times, forming dense X-shaped structures that one can easily observe on a stained slide using a microscope. Before compaction, the smallest human chromosome extends up to 0.55 inch (1.4 cm) in length, but after condensation it approaches 7.87×10^{-5} inch (2 µm). Packaging the DNA in this manner makes it much easier to move around the cell during mitosis so it can be equally divided in the daughter cells. Also during prophase, the nuclear envelope begins to disappear, the endoplasmic reticulum and Golgi complex fragment, and the microtubules of the cytoskeleton undergo disassembly. Though nonmitotic cells contain one pair of barrel-shaped centrioles (arranged perpendicular to one another as part of a structure called the centrosome), the cell duplicated the centrioles during S phase, so prophase cells have two pairs. Each pair migrates to one end of the cell and nucleates the assembly of spindle fibers, arrays of microtubules that will assist in the movement of chromosomes later in mitosis.

Metaphase can be divided into two subcategories: prometaphase and metaphase. During prometaphase, microtubules attach to the kinetochores, disklike structures on the outer surface of the centromeres of



This stained preparation of cells from an onion root tip reveals several stages of mitosis. Most of the cells shown here are in interphase, evidenced by round, densely stained nuclei. The cell in the upper middle region of the photo is in anaphase; a metaphase cell with its chromosomes lined up along the equator appears just left of center; telophase is shown in the centermost cell, having two distinct darkly stained patches of chromosomes; and the cell immediately right of center with chromosomes condensed but not yet lined up at the equator is in prophase. *(M. I. Walker/Photo Researchers, Inc.)*

each chromosome. The chromosomes migrate to the metaphase equator, an imaginary plane that divides the cell into two halves. At metaphase, the centromeres of all the chromosomes are lined up at the equatorial plane. Spindle fibers connect each chromosome to both poles, the opposite ends of the cell containing the centrioles.

During anaphase, the centromeres split and the conjoined sister chromatids separate. Motor proteins fueled by adenosine triphosphate (ATP) propel the chromatids to opposite ends of the cell. The poles also move farther apart.

Telophase occurs when the chromatids reach the opposite poles. If everything has proceeded correctly, the poles should contain equivalent sets of chromosomes. In human cells, each pole should have 46 chromatids, and each chromatid at this stage is an independent chromosome. The chromosomes begin to decondense or disperse, and the nuclear envelope reforms around each set of chromosomes, creating two nuclei. The endoplasmic reticulum and the Golgi apparatus also reform, and cytokinesis, or cell division, occurs. In plants a cell plate is synthesized in the center of the cell, and in animal cells partitioning of the cytoplasm takes place by a narrowing or constriction of the cell membrane around the middle of the cell. The result is two distinct new cells.

REGULATION OF THE CELL CYCLE

Tight control mechanisms regulate the cell's progress through the cell cycle. Geneticists and cell biologists have identified numerous genes involved in cell cycle control over the past decade. Mutations in these genes, referred to as cell division cycle (*cdc*) genes, lead to unregulated cell proliferation. Many *cdc* genes encode protein kinases, enzymes that transfer a phosphate group from an ATP molecule to a protein and, in doing so, alter the protein's activity. Cyclins are another key gene product involved in cell cycle control. They are termed cyclins because their concentration increases and decreases as the cell progresses through the cell cycle. Cyclin-dependent kinases, or Cdk proteins, are kinases that are only active when bound to a cyclin protein.

Several feedback mechanisms check to see whether the cell has completed certain events successfully before allowing it to advance to the next stage. Various checkpoints that occur throughout the cell cycle ensure that only cells ready to proceed to the next stage actually do. The most important checkpoint occurs near the end of G_1 and is called the restriction point in mammalian cells. Duplicating the cell's DNA is energetically expensive, and if mutations are present when synthesis begins, they will be perpetuated when the DNA is copied. For these reasons, cells must survey their internal conditions before continuing through the cell cycle. External signals also play a role in controlling cell cycle events through signal transduction pathways. A cell should not enter S phase unless it is committed to completing the cycle and following through until the completion of mitotic division. If the cell has grown sufficiently and the DNA is in good condition, then the concentration of a specific Cdk protein increases, allowing it to combine with its cyclin partner to form a cyclin-Cdk complex that acts as a signal for the cell to proceed into S phase.

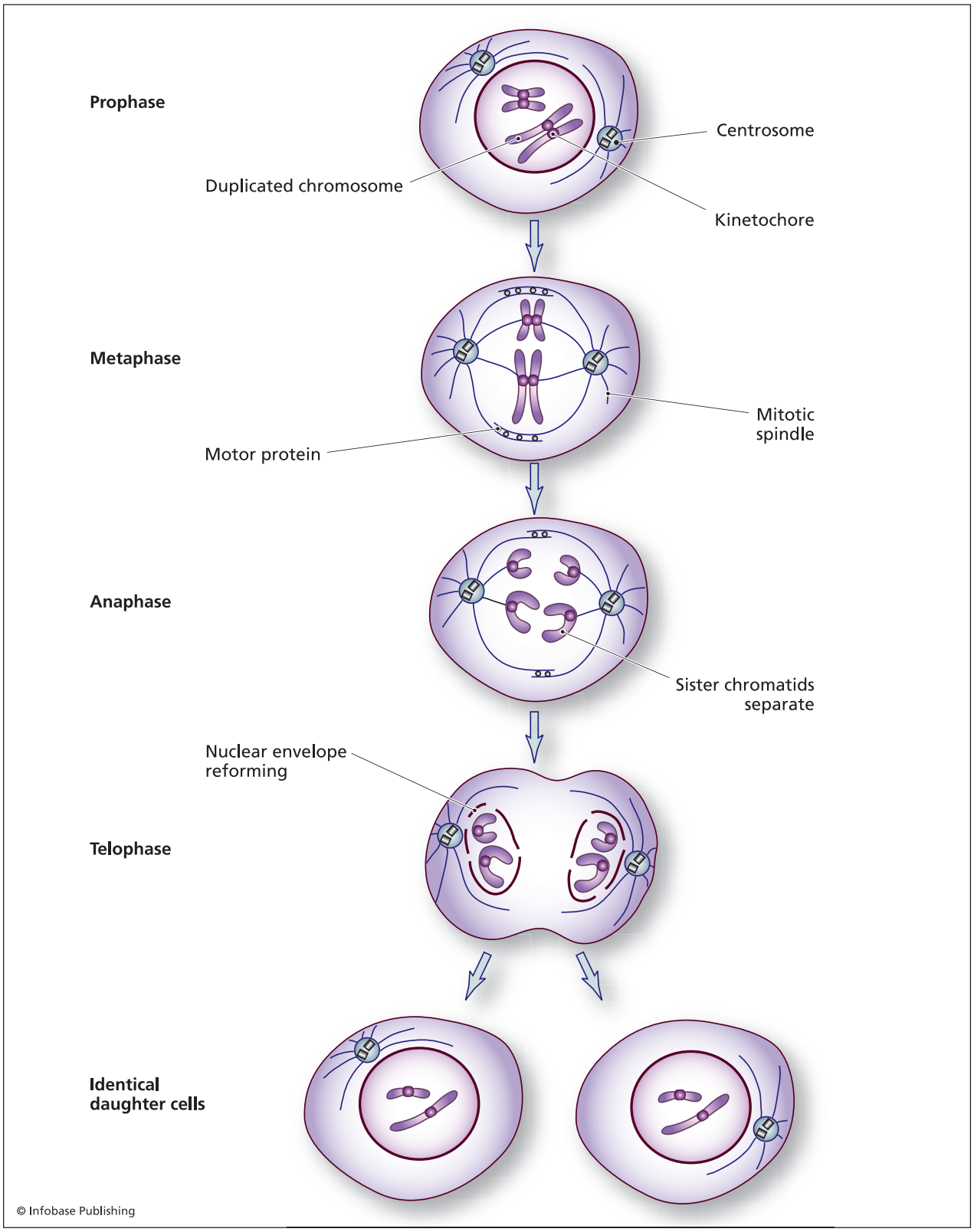
Another important protein in making it past the G_1-S checkpoint is p53, the product of a tumor-suppressor gene. The p53 protein binds to DNA and stimulates the production of proteins that inhibit cell growth. Mutations in tumor-suppressor genes are the most common genetic alterations found in cancerous cells. The protein p53 normally functions in apoptosis, a natural process that prevents the proliferation of mutated cells, such as those that are cancerous. While genetic mutations can lead to loss of function of this protein, outside sources can also interfere with p53 function. For example, viral DNA (specifically, from human adenovirus and papillomavirus) can bind to p53 and inactivate it. In some types of cancer, a particular gene (*mdm2*) is overexpressed, and the gene product binds to and inactivates p53.

A second checkpoint occurs early in G_2, shortly after a cell has completed DNA replication. A Cdk protein binds to a different cyclin to form a complex called maturation-promoting factor (MPF). If a cell has successfully completed DNA replication and repair and is ready to enter mitosis, the concentration of MPF will be high enough to perform its responsibilities such as stimulating the compaction of chromatin, forming the spindle apparatus, and phosphorylating proteins of the nuclear lamina to promote breakdown of the nuclear envelope.

A last checkpoint occurs during metaphase of mitosis. If the spindle apparatus is properly assembled, the chromosomes have all aligned at the equator, and all the chromatids are joined to spindle fibers through their kinetochores, then cell traverses this final checkpoint and proceeds with anaphase. This ensures that chromosomes will not be missing nor be present in duplicate in the daughter cells.

Cancer results when the proliferation of cells goes unchecked. For various reasons, cancer cells do not respond to the mechanisms that regulate progression through the cell cycle. Atypical cells that would normally be eliminated continue to proliferate out of control. Cancer begins when a single cell loses control, or undergoes transformation. If the immune system does not attack and destroy the transformed cell, it will grow and divide numerous times, forming a tumor. If the tumor cells remain at the site of origination, the tumor is called benign and can often be

removed by surgery. Problems arise when the tumor becomes malignant and invades neighboring tissues, impairing their function. When this happens, the individual is said to have cancer. Metastasis occurs when cells break off from the original tumor and spread through lymphatic and blood circulation to



Mitosis involves the separation of duplicated chromatids into two newly formed nuclei and, when accompanied by cytokinesis, results in two genetically identical cells.

other parts of the body, where they can develop into new tumors.

The gene that encodes the p53 protein is linked to slightly more than half of all cancers. When p53 function is lost, damaged cells proliferate rather than die. In some families, a mutated form of this gene passes from generation to generation. Exposure to mutagenic chemicals or radiation increases one's risk of developing cancer because they cause mutations in the DNA, potentially to genes that play a role in regulation of the cell cycle.

See also CANCER, THE BIOLOGY OF; CELL BIOLOGY; CHROMOSOMES; DEOXYRIBONUCLEIC ACID (DNA); EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; EUKARYOTIC CELLS; MOLECULAR BIOLOGY; REPRODUCTION.

FURTHER READING

Becker, Wayne M., Lewis J. Kleinsmith, and Jeff Hardin. *World of the Cell.* 6th ed. San Francisco: Benjamin Cummings, 2006.

Kleinsmith, Lewis J. *Principles of Cancer Biology.* San Francisco: Benjamin Cummings, 2006.

Klug, William S., and Michael R. Cummings. *Essentials of Genetics.* 5th ed. New York: Prentice Hall, 2005.

Panno, Joseph. *Cancer: The Role of Genes, Lifestyle, and Environment.* New York: Facts On File, 2004.

———. *The Cell: Evolution of the First Organism.* New York: Facts On File, 2004.

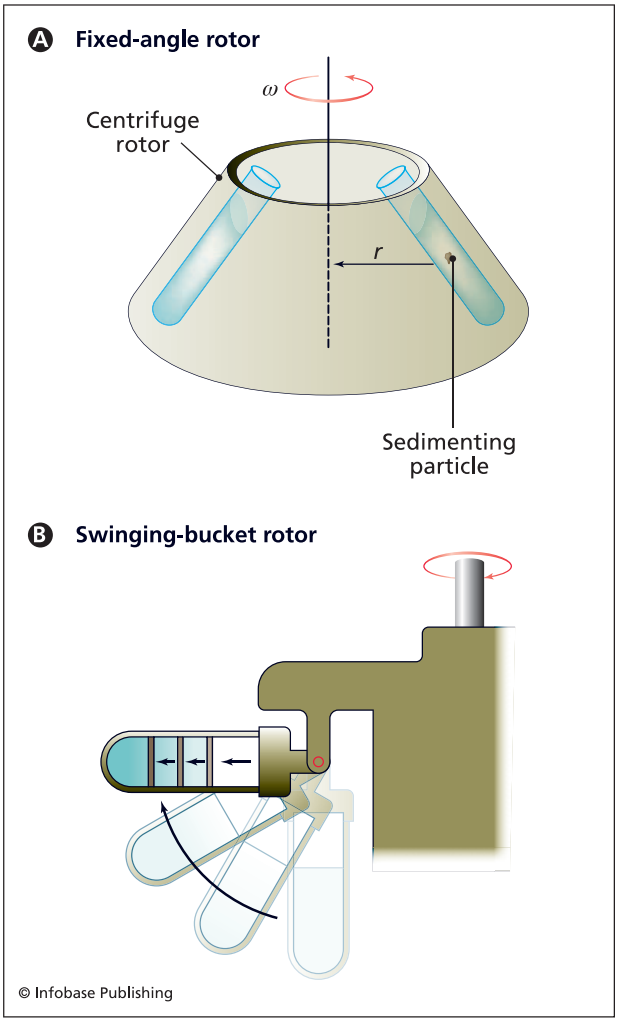
centrifugation Alongside microscopy, centrifugation is one of the most important laboratory techniques in life science research. The process of centrifugation allows for the separation of substances of different densities through the use of centrifugal force, the apparent force felt by an object traveling in a curved path that acts to move the object outward from the center of rotation. Centripetal force, the force that acts to pull the object inward (in opposition to centrifugal force), keeps the object moving in a curved path. A centrifuge, the piece of equipment that performs this task, consists of an electric motor that spins a rotor around a fixed axis. The size of centrifuges used for life science research varies with type; some fit on top of a lab bench, while others are the approximate size of a standard washing machine. The rotor holds the tubes that contain the substances to be separated. Different-sized buckets of various rotors hold different-sized tubes. A rotor can have buckets that swing, allowing the tubes to reach a horizontal position while spinning, so that the contents are forced outward along a path parallel to the sides of the tubes. Other rotors have buckets set at fixed angles, so the tubes are maintained at a constant angle relative to the axis upon which the rotor spins.

Acceleration, or the rate of centrifugation, is often reported in multiples of *g*, the acceleration due to gravity at the surface of Earth, equal to 32.174 $ft \cdot s^{-2}$ (9.80665 $m \cdot s^{-2}$). Acceleration is the product of the radius (from the fixed axis to the end point of the sample tube) and the square of the angular velocity. Reporting centrifugation using *g* allows one to duplicate experimental conditions when using different-sized rotors or centrifuges. Relative centrifugal force (RCF) is a means for reporting the force applied to a sample within a centrifuge.

$$RCF = 0.00001118 \times r \times N^2$$

The variable *r* is the rotational radius measured in centimeters, and *N* is the revolutions per minute.

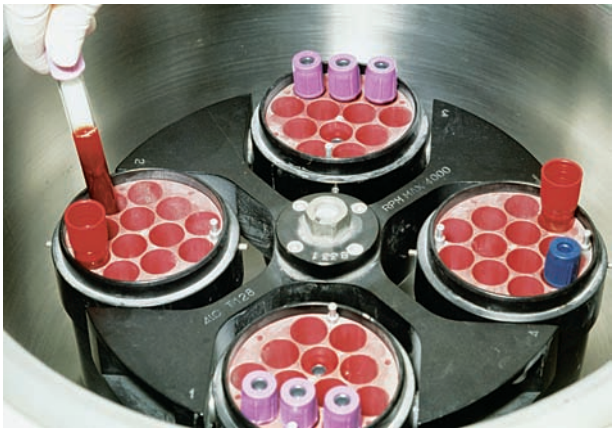
Using centrifugation, a scientist can separate cells, components of cells, and biomolecules; separate solids from liquids in a mixture; or separate



Some centrifuge rotors hold the samples at fixed angles, while others have hinged buckets that swing outward during centrifugation.

liquids of different densities. Microcentrifuges, commonly found in molecular biology or biotechnology laboratories, handle samples with volumes less than 1.5 mL. A much more powerful type of centrifuge, an ultracentrifuge, can achieve very high accelerations and therefore must be operated under a vacuum system to reduce heat due to air friction. Some ultracentrifuges are designed to reach accelerations up to 500,000 × *g* and therefore can be used to separate biomolecules from one another. The importance of ultracentrifugation technology was demonstrated when the Swedish chemist Theodor Svedberg won the Nobel Prize in chemistry in 1925 for inventing and developing this technique. In his honor, the unit of measurement that describes an object's behavior when centrifuged, the sedimentation rate or sedimentation coefficient, bears his name—the *Svedberg unit,* abbreviated as *S,* and equal to 1×10^{-13} second. In all centrifuges, the samples must be loaded in a balanced manner to prevent a force imbalance at high speeds. If unbalanced, the rotor can damage the spindle, upon which it sits, detach, and cause injury in addition to destroying the samples.

Three types of centrifugation important for life science research are differential centrifugation, density gradient (or rate-zonal) centrifugation, and equilibrium density (or buoyant density) centrifugation. Differential centrifugation separates cellular components on the basis of differences in size, shape, and density. In 1974 two Belgian scientists, Albert Claude and Christian de Duve, and the American scientist George E. Palade shared the Nobel Prize in physiology or medicine for their achievement in pioneering subcellular fractionation by differential centrifugation and their subsequent discoveries related to cell structure and function. To fractionate cellular components, the tissue sample or the cells must first be broken open, or lysed. The investigator can accomplish this by osmotically shocking the cells, using ultrasonic vibrations, or physically grinding them up. Employing gentler procedures helps ensure the organelles or other large structures will remain intact. Because cellular organelles vary so much in size and weight, they will sediment, or travel through the sample tube at different speeds when centrifuged. Particles that are large or dense sediment at a faster rate, and they will have a greater sedimentation coefficient. For example, eukaryotic ribosomes are composed of two subunits, a larger 60S subunit and a smaller 40S subunit. Because sedimentation rate depends on shape and density as well as mass, Svedberg units are not additive—an assembled ribosome has a sedimentation coefficient of 80S, not 100S. After the tissue is homogenized in an appropriate ice cold buffer, subcellular fractions are collected by centrifuging the homogenate, then performing repeated

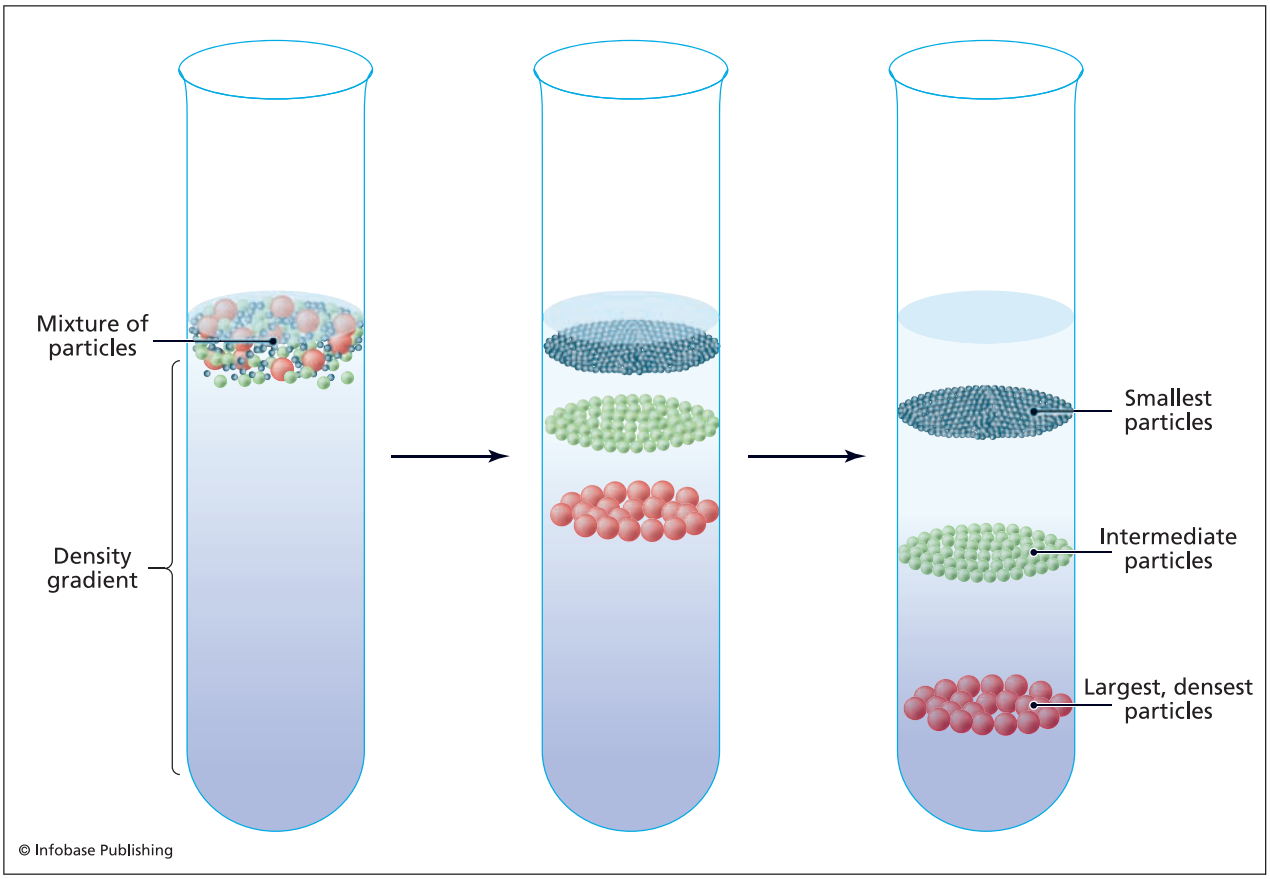


Centrifugation of whole blood separates the blood cells and platelets from the plasma. *(Klaus Guldbrandsen/Photo Researchers, Inc.)*

cycles of centrifugation of the supernatants, spinning out the particles using greater forces with each cycle. The pellets that form at the bottom of the tubes contain successively lighter or less dense cellular components. The supernatant is the fluid that remains in the centrifuge tube after the particulate matter has formed a pellet. When fractionating cellular components, unbroken cells and nuclei will pellet first; then mitochondria, lysosomes, and peroxisomes; followed by fragments of cell membrane and endoplasmic reticulum; and finally, free ribosomes and large macromolecules.

Density gradient centrifugation, also called rate-zonal centrifugation, is a specialized type of centrifugation in which the investigator layers the mixture containing the particles to be separated over a solution that has a higher concentration of solute at the bottom of the tube and a lower concentration at the top. Particles will move through the gradient of solute as discrete bands traveling at different rates depending on their density. As they move through the gradient, the particles encounter higher solute concentrations, and therefore higher densities. Larger particles with greater sedimentation coefficients will move faster through the gradient. The increasing solute concentration keeps particles that are similar in shape and size in a tight band as they travel through the gradient, and at the end of the spin, particles of different sizes will have migrated to different positions on the gradient.

Equilibrium gradient centrifugation, or buoyant density gradient centrifugation, also uses a gradient over which the solution containing the particles to be separated is layered. Though not necessary in rate-zonal centrifugation, in equilibrium gradient centrifugation, the density of the solution at the bottom of the tube must exceed the density of any of the



In density gradient centrifugation, particles travel through a gradient in discrete sedimenting bands.

particles to be separated. In other words, the range of densities in the prepared gradient must span the densities of all the components being separated. Sucrose usually serves as the solute for organelle isolation, whereas cesium chloride works well for biomolecules of different molecular weights or structures. After the mixture is layered on top of the gradient, the samples are centrifuged. The particles travel in discrete bands based on density, just as in rate-zonal centrifugation; however, they stop migrating when they reach a position in the gradient at which the density of the solute equals the density of the particles. The particles stop moving at this point because no force acts on the particles when they are surrounded by a solution of equal density. In rate-zonal centrifugation, the particles will all eventually reach the bottom of the tube if centrifuged for a sufficient period, but in equilibrium gradient centrifugation, after they settle to a characteristic position in the gradient, they cease moving.

After both types of density gradient centrifugation, the fractions are collected by puncturing the bottom of the tube with a sharp object and collecting the material that drips out into a series of tubes. After collection, the fractions can be subjected to further separation techniques, examined using microscopy, or analyzed for various biochemical or biological properties.

See also CELL BIOLOGY; CHROMATOGRAPHY; DUVE, CHRISTIAN DE; EUKARYOTIC CELLS; MICROSCOPY.

FURTHER READING

"Centrifuge." In *Encyclopaedia Britannica.* 2008. From Encyclopaedia Britannica Online. Available online. URL: http://search.eb.com/eb/article-9022102. Accessed January 14, 2008.

Chargaff, Erwin (1905–2002) Austrian-American *Biochemist* Erwin Chargaff is best known for formulating what is known as Chargaff's rules, statements describing the nucleotide composition of deoxyribonucleic acid (DNA). These quantitative relationships were instrumental in the discovery of the double-helical structure of DNA by James D. Watson and Francis Crick.

Erwin Chargaff was born in Czernowiz, Austria, on August 11, 1905. His family was forced to move to Vienna at the outbreak of World War I. He attended the University of Vienna as an undergradu-

ate in chemistry and obtained his doctorate in chemistry in 1928 from Spath's Institute of the University of Vienna, with a thesis on organic silver complexes. During the seven years that followed, Chargaff held several positions including research fellow at Yale University, where he studied lipids of *Mycobacterium tuberculosis;* assistant in the Public Health Department at the University of Berlin; and research associate at the Pasteur Institute in Paris. With 30 published papers to his name, in 1935, he returned to United States to join the faculty at Columbia University, where he remained until his retirement. He started as a research associate in biochemistry (1935), then became an assistant professor (1938), associate professor (1946), and full professor (1952), eventually serving as department chair (1970) and achieving emeritus status in 1974.

When Chargaff settled in at Columbia, chromosomes were known to carry genes and to consist of protein and nucleic acid. Because proteins consist of 20 different amino acids that can be combined in a practically unlimited number of combinations, scientists assumed that proteins were the component of chromosomes that contained the information necessary to direct the expression of an immense amount of variable characteristics. In 1944, after Avery and colleagues published their surprising results showing that DNA encoded for the presence of a polysaccharide capsule in pneumococcal bacteria and that this heritable trait could be transferred from one strain to another via chemically purified DNA, Chargaff's research at Columbia focused on the composition of nucleic acids. Chemists knew that DNA consisted of four different nucleotides. Each nucleotide contained a deoxyribose sugar, a phosphate group, and one of four different nitrogenous bases—adenine (A), guanine (G), cytosine (C), or thymine (T)—but they erroneously believed that the structure of DNA was a nonspecific aggregate of these four subunits, a belief called the tetranucleotide hypothesis. Chargaff isolated DNA from cells of many different types of organisms. He liberated the nitrogenous bases from the nucleotides, then separated them by paper chromatography. Because different nucleotides absorb ultraviolet light of different wavelengths, he was able to determine the quantities of the different nucleotides by measuring how much light each base absorbed. After collecting data from a variety of species including corn, chicken, octopus, rat, and human, he found that, across kingdoms, the amount of A nearly always equaled the amount of T, and the amount of C equaled the amount of G. However, between species, the relative proportions of the two pairs differed. For example, in human DNA, A = 30.9 percent and T = 29.4 percent, whereas G = 19.9 percent and C = 19.8 percent. This information convinced Chargaff that DNA provided sufficient variability to encode genetic information. The regularities in base composition came to be called "Chargaff's rules."

Despite the importance of these results and their implications for DNA structure and self-replication, Chargaff was careful not to overinterpret his data. The results were of key importance in solving the structure of DNA. James D. Watson and Francis Crick interpreted Chargaff's rules to mean that A paired with T by the formation of hydrogen bonds that linked two strands of DNA together, the basis for complementarity between the two strands. Likewise, C specifically formed hydrogen bonds with G. Because A always exists as a member of an A-T base pair in the double helix, the amount of A always equals the amount of T, and because C and G always exist together as a base pair, the amount of C equals the amount of G. When Watson and Crick revealed their stunningly simple double-helical model of DNA to the world, Chargaff was upset that his contributions were not acknowledged. The fact remains, however, that Watson and Crick are the ones who successfully solved the structure, even if they reached their conclusion by incorporating the work of others: the X-ray diffraction data from Rosalind Franklin and Maurice Wilkins, the nucleotide composition observations of Chargaff, the model-building approach of Linus Pauling, the suggestion of their colleague Jerry Donohue that the bases appeared in their keto rather than enol forms, and so on. Chargaff did not recognize the biological significance of his crucial findings concerning the equivalence of the purines and pyrimidines; thus his name is not as well known as those of Watson and Crick.

After the structure of DNA was solved, Chargaff contributed to the understanding of how it encoded for protein construction. Through the years, Chargaff's research also delved into other aspects of biochemistry. He studied blood clotting, lipids and lipoproteins, the metabolism of amino acids and inositol, ribonucleic acid, and phosphotransferases.

Chargaff became an American citizen in 1940. He spoke 15 languages and was respected for his tremendous intellect. He assumed emeritus status at Columbia University in 1974, though he continued to write profusely on a variety of scientific and philosophical topics. Chargaff received many honors and awards during his lifetime including the Pasteur Medal in 1949, the Charles Leopold Mayer Prize from the French Academy of Sciences in 1963, and the Distinguished Service Award from Columbia University in 1982. Erwin Chargaff married Vera Broido in 1928, and they had one son named Thomas. Chargaff died on June 20, 2002.

See also Avery, Oswald; chromatography; Crick, Francis; deoxyribonucleic acid (DNA);

Franklin, Rosalind; Pauling, Linus; Watson, James D.; Wilkins, Maurice H. F.

FURTHER READING

American Philosophical Society. "Erwin Chargaff Papers." Available online. URL: http://www.amphilsoc.org/library/mole/c/chargaff.htm. Accessed January 14, 2008.

Chargaff, E. "Chemical Specificity of Nucleic Acids and Mechanism of Their Enzymatic Degradation." *Experientia* 6 (1950): 201–209.

———. "Structure and Function of Nucleic Acids as Cell Constituents." *Federation Proceedings* 10 (1951): 654–659.

Chase, Martha (1927–2003) American *Geneticist*

Martha Chase is best known for a landmark experiment performed with Alfred Hershey that confirmed deoxyribonucleic acid was the carrier of genetic information in bacteriophage.

Martha Cowles Chase was born in Cleveland Heights, Ohio, on November 30, 1927. She received a bachelor's degree from the College of Wooster in 1950 and took a job as a lab assistant to Alfred D. Hershey, a microbiologist at the Carnegie Institution of Washington in Cold Spring Harbor, on Long Island, in New York.

In 1944 Oswald Avery and his colleagues Colin MacLeod and Maclyn McCarty at the Rockefeller Institute demonstrated that deoxyribonucleic acid (DNA) was the molecule responsible for transforming a nonvirulent strain of pneumococcal bacteria into a virulent strain. This was a surprising result because most geneticists believed proteins carried genetic information. The structure of DNA was still unknown, but chemists assumed that since it was composed of only four different nucleotides, the molecule was too simple to carry all of the necessary information for a cell to perform the complex biological functions of replication and protein synthesis. Proteins, on the other hand, consisted of 20 different amino acid building blocks and thus was the preferred molecular candidate for the carrier of genetic information.

Hershey and Chase performed a series of elegantly designed experiments with bacteriophage that convinced even the skeptics of the scientific community that DNA was in fact the genetic material. Bacteriophages are viruses that specifically infect bacterial cells. The basic structure of a bacteriophage consists of a protein coat surrounding a nucleic acid core. When a phage attaches to a host bacterium, it injects its genetic material through the cell membrane into the cell but the phage particle itself remains external to the cell. Soon afterward, the bacterial cell starts synthesizing viral components, which are then assembled into new viral particles. The bacterial cell eventually bursts open, releasing the newly synthesized viral particles into the environment, where they seek and infect new bacterial cells. The mysterious substance that the bacteriophage injected into the bacterial cell was the genetic material. The experiments performed by Hershey and Chase addressed the nature of that substance.

To identify the injected material, they infected bacterial cultures with T2 bacteriophage that had been labeled with radioactive sulfur (^{35}S) or radioactive phosphorus (^{32}P). Sulfur is a component in proteins, and phosphorus is a component of DNA. The radioactive labeling of these atoms allowed Hershey and Chase to track, or to follow, the location of the viral proteins and nucleic acids during their experiment. After allowing sufficient time for the radioactive bacteriophage to attack the bacteria, they agitated the cultures in a blender and then centrifuged the cultures to separate the bacteria cells and their contents from viral particles. Though most of the viral particles remained in the supernatant, many had already injected their genetic material into the host bacterial cells. In the culture infected with T2 labeled with ^{35}S, the radioactivity remained in the supernatant, indicating that the protein coats did not enter the bacterial cells. When they examined the ^{32}P-labeled culture, they found a significant portion of the radioactivity in the bacterial cell pellet; thus the nucleic acid must have entered into the bacterial cells during the infection process. They concluded from these results that DNA must encode the information necessary to direct the synthesis of new viral particles within a bacterial host cell.

These experiments were published in an article titled "Independent Functions of Viral Protein and Nucleic Acids in Growth of Bacteriophage" in 1952. This landmark paper convinced biologists that nucleic acid, not protein, was the carrier of genetic information. Though not common practice, Hershey included Chase's name as a coauthor of their manuscript. The following year Chase began working at Oak Ridge National Laboratory in Tennessee, then later at the University of Rochester. In 1959 Chase moved to California to begin work toward a doctorate degree in microbial physiology, which she earned from the University of Southern California in 1964. She returned to the Cleveland area and died of pneumonia on August 8, 2003. Martha Chase's name will forever be associated with the famous blender experiment that demonstrated DNA to be the genetic material of phage.

See also Avery, Oswald; biomolecules; centrifugation; deoxyribonucleic acid (DNA); Hershey, Alfred; MacLeod, Colin Munro;

McCarty, Maclyn; viruses and other infectious particles.

FURTHER READING

Hershey, A. D., and Martha Chase. "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage." *Journal of General Physiology* 36 (1952): 39–56.

chemical basis of life Anything that has mass and occupies space is considered matter—water that fills the oceans, the bark of a tree, snowflakes, dust particles floating in the air, or the fossil remains of a long extinct dinosaur. All of the physical material in the universe is composed of chemical elements, substances that cannot be broken down or chemically converted into other substances. Elements consist of a single type of atom, the smallest particle of an element that still retains its chemical properties. When two or more atoms join by covalent linkages, the result is a molecule, the smallest particle of a substance that retains all the properties of the substance. Compounds are substances made of two or more elements combined in definite proportions.

The same chemical elements make up all living and nonliving matter. The element carbon has the same chemical properties whether it exists in the form of diamond, as a component of carbon dioxide gas, or in starch of a potato. Life scientists must study the chemical nature of matter in order to understand life at all levels. Whereas the reason why a biochemist must understand basic chemistry might be obvious, the reasons why a geneticist, ecologist, or any other life scientist must are just as important. The mechanisms by which one generation transmits its genes to the next and the way genes determine one's physical characteristics are based on the molecular processes of deoxyribonucleic acid (DNA) replication, transcription, and translation. Ecologists aim to understand the interactions between organisms and their environment, both the physical surroundings and the other organisms living in the same location. Without understanding the chemical nature of matter, an ecologist could not appreciate the biogeochemical recycling of nutrients such as nitrogen or why some organisms are autotrophic and can make their own food while others are heterotrophic and must ingest food in the form of organic compounds.

When studying life at the molecular or atomic level, as well as at the whole organism or community level, structure and function are intimately related. The structure of atoms and molecules determines their chemical characteristics, such as how they will react with other atoms or molecules and what types of bonds they may form. Though understanding atomic architecture and how different types of chemical bonds hold molecules together is crucial to the development of an appreciation for life science, the question of how life arises from inanimate matter remains unanswered.

ATOMIC STRUCTURE

Atoms consist of protons, neutrons, and electrons. The protons and neutrons have similar masses and reside in the nucleus. Electrons exist in defined orbitals that surround the nucleus, and they do not contribute significantly to the mass of an atom. Protons have a positive electrical charge, and electrons have a negative charge. The atoms of each element have an atomic number that equals the number of protons in the nucleus. Because atoms are electrically neutral, the number of protons in an atom equals the number of electrons. When different numbers are present, the particle carries an electrical charge and is called an ion. Neutrons do not carry a charge and therefore do not contribute to the net electrical charge of an atom, but they do help stabilize the nucleus. The exact number of neutrons in the atoms of a particular element varies. For the elements with lower atomic numbers, the number is usually close to the number of protons, whereas heavier elements require larger ratios of neutrons to protons to achieve stability. The atomic weight of an atom, its mass relative to a hydrogen atom, essentially equals the number of protons plus the number of neutrons. Atoms of the same element (meaning they have the same number of protons) that have different numbers of neutrons are called isotopes and can be distinguished on the basis of their atomic weights. For example, atoms of the element carbon usually have an atomic mass of 12 due to six protons and six neutrons, but carbon 14 isotopes with eight neutrons also exist in nature. Isotopes with too few or too many neutrons have unstable nuclei and may disintegrate spontaneously, a phenomenon known as radioactive decay. The slow but steady rate at which carbon 14 decays to carbon 12 allows researchers such as geologists and paleontologists to determine the age of fossils and other organic matter.

The periodic table of the elements (see appendix) displays more than 100 chemical elements, but more than 96 percent of the matter in living organisms is due to only four: carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). Phosphorus (P) and sulfur (S) are the next most common, and other elements including magnesium (Mg), sodium (Na), chlorine (Cl), potassium (K), and calcium (Ca) each make up less than a fraction of 1 percent of living organisms. These elements combine in different ways to make up biomolecules that assemble into cellular structures. The major classes of biomolecules are carbohydrates,

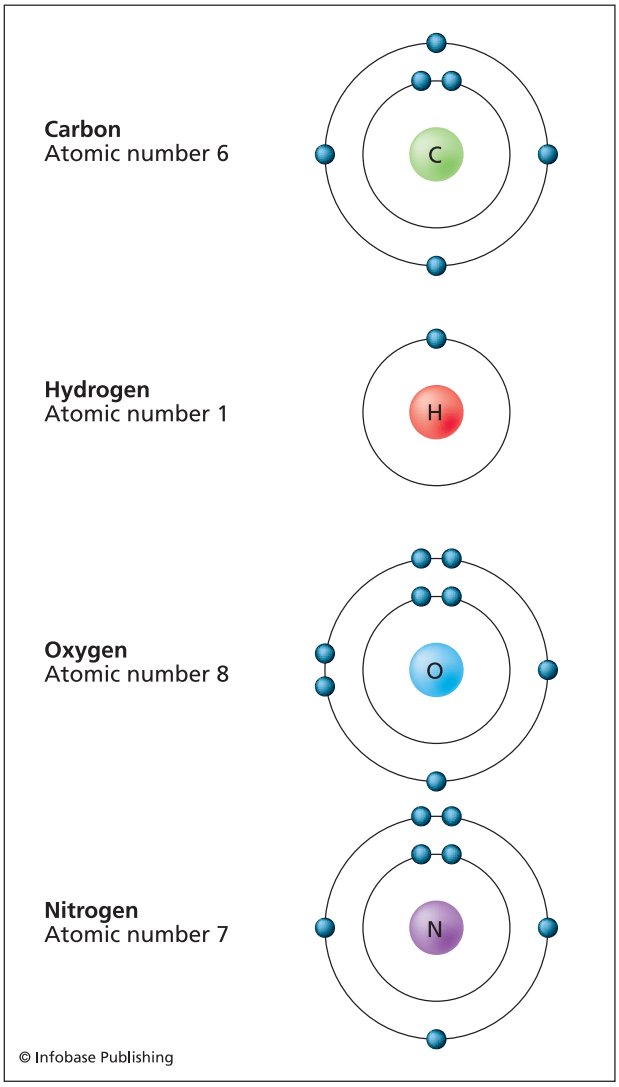
proteins, nucleic acids, and lipids. The manner in which the atoms interact to form molecules depends on the structure of the individual atoms, especially the electrons in the valance shell, the outermost energy level.

The protons and neutrons of an atom cluster together in a region called the nucleus. Electrons continuously move around the nucleus in discrete orbitals, defined as regions where electrons might be found within an atom. Groups of orbitals form main energy levels or electron shells; those nearest to the nucleus have the lowest energies, and those farther away have higher energies. Atoms fill their energy sublevels from the lowest to the highest level, depending on how many electrons they have.

- The lowest-energy level contains a single orbital that can only hold two electrons.
- The second energy level consists of two sublevels that can hold a total of eight electrons.
- The third energy level contains three sublevels and can hold a maximum of 18 electrons.

Though atoms can have more than three energy levels, because of their lower atomic weights, atoms in biological molecules rarely have more. The valence electrons are the electrons located in the outermost shell of an atom. Because of the order in which the sublevels fill, the maximal number of valence electrons in a ground-state (lowest energy state) atom is eight. Atoms are most stable when the valence shell contains eight electrons, a phenomenon called the octet rule. If the outermost shell is the first energy level, two electrons are sufficient to fill it and obey the "octet rule."

To illustrate, consider the atomic structures for carbon, hydrogen, oxygen, and nitrogen. Carbon has an atomic number of 6 and a mass of 12; the nucleus has six protons and six neutrons. Two electrons fill the first energy level, leaving four electrons for the second level. Hydrogen has an atomic number of 1. Because no neutrons are required to stabilize a nucleus that only has one proton, it also has an atomic mass of 1. A single electron orbits the proton. Oxygen has an atomic number of 8 and a mass of 16; its nucleus holds eight protons and eight electrons. After two electrons fill its innermost shell, six remain and exist in the outer shell. Nitrogen has an atomic number of 7 and an atomic mass of 14. Two electrons fill the first energy level, and the valence shell contains the remaining five.



Electron shell diagrams indicate the number of electrons, represented by dots, in each shell of an atom.

CHEMICAL BONDS

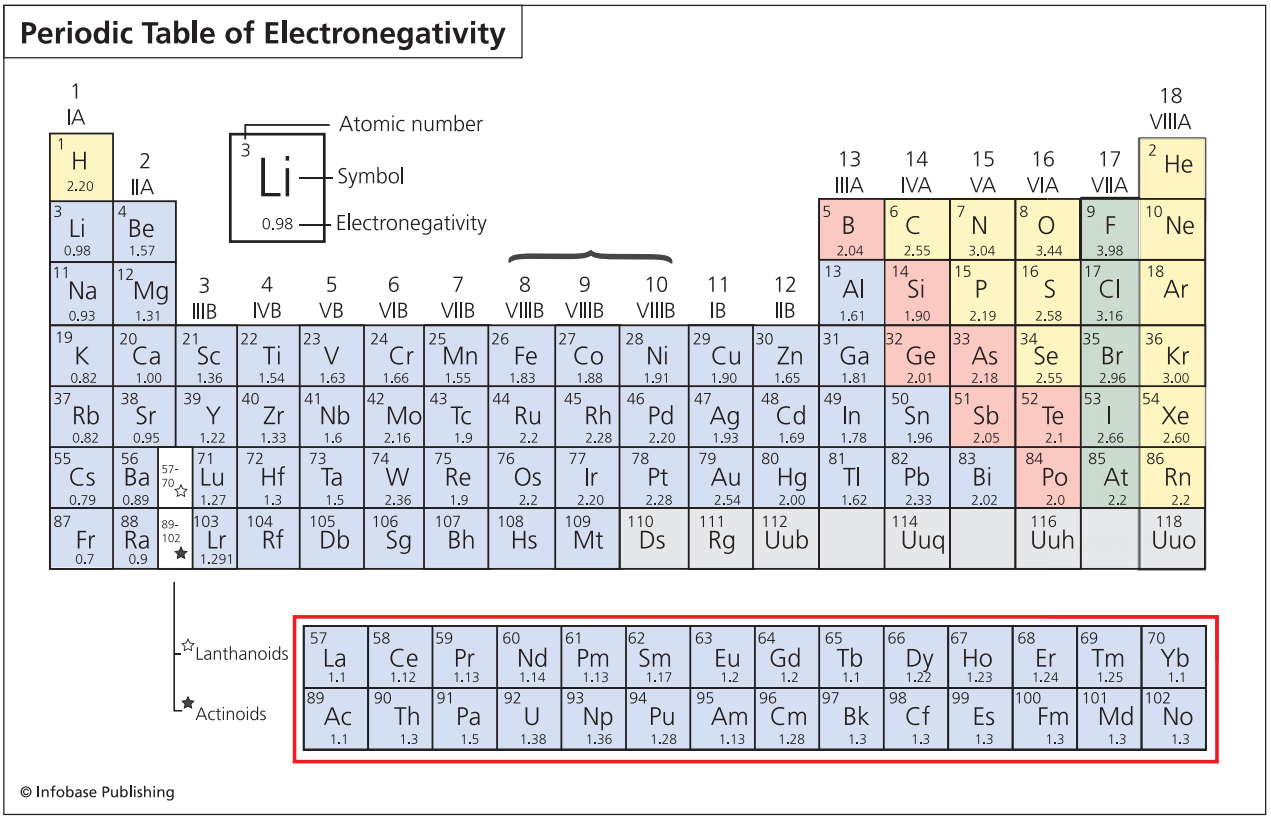
The valence electrons determine how an atom interacts with other atoms. Atoms are most stable when their outermost shell is filled; thus they interact with other atoms in a manner that allows them to achieve this. Interactions are considered chemical bonds when their combined product forms a species that has distinct chemical properties.

Covalent bonds form when two atoms share valence electrons so they both have a filled valence shell, containing either a total of two (if the valence shell is the first energy level) or of eight valence electrons. The number of covalent bonds in which an atom can participate depends on the number of unpaired valence electrons. Each shared pair of electrons constitutes one covalent bond. When two or more atoms are covalently bound, the result is a molecule. For example, in a molecule of methane (CH_4), carbon shares each of its four valence electrons with a hydrogen atom. As a result, carbons shares four pairs of electrons with four hydrogen atoms, each of

which contributes its lone electron to a shared pair. Each hydrogen atom acquires a configuration with two electrons in its valence shell, and the carbon atom achieves a total of eight electrons in its valence shell. Sometimes, two atoms attain octets by sharing more than one pair of electrons. In a double bond, two atoms share two pairs of electrons, and three pairs are shared in a triple bond. In biomolecules, carbon often participates in double and triple bonds. Oxygen can also form double bonds, since it has two unpaired electrons that can participate in covalent bonds, and nitrogen can form double or triple bonds since it has three pairs of unshared electrons. In molecular diagrams, either a pair of dots or a short dash represents a covalent linkage.

Atoms of each element have characteristic electronegativities, or abilities to attract electrons for participation in a chemical bond. In 1932 the American chemist Linus Pauling developed a scale of electronegativities ranging from 0.7 (francium) to 3.98 (fluorine). Two atoms with a difference in electronegativities of less than 0.4 will generally form covalent bonds; in other words, they will share their valence electrons equally. For example, consider a molecule of CH_4. Carbon and hydrogen have electronegativities of 2.55 and 2.20, respectively. The difference is 0.35, which is less than 0.4; thus carbon and hydrogen form covalent bonds. Diatomic molecules, which are composed of two atoms of the same element (H_2, N_2, O_2, F_2, and Cl_2), have pure covalent bonds. As the same element, both atoms have equal electronegativities. Covalent bonds are strong, take a lot of energy to form, and release energy when broken. In living cells, special molecules called enzymes facilitate the formation and breakage of covalent bonds during the synthesis and degradation of biomolecules.

When the difference in electronegativities of two atoms equals or exceeds 1.7, an ionic bond forms. In this case, one atom has a much stronger attraction for the electrons of another atom, often because it has more protons in its nucleus and therefore a stronger positive charge to attract the electrons orbiting another atomic nucleus; or it has a smaller atomic radius, so the attraction of the positively charged nucleus does not have to extend as far to reach and interact with the electrons of another atom. Generally, the most electronegative elements are positioned in the upper right corner of the periodic table. When two atoms with significant differences in their electronegativities interact to form an ionic bond, the atom with the lower electronegativity gives up one or more electrons to the atom with

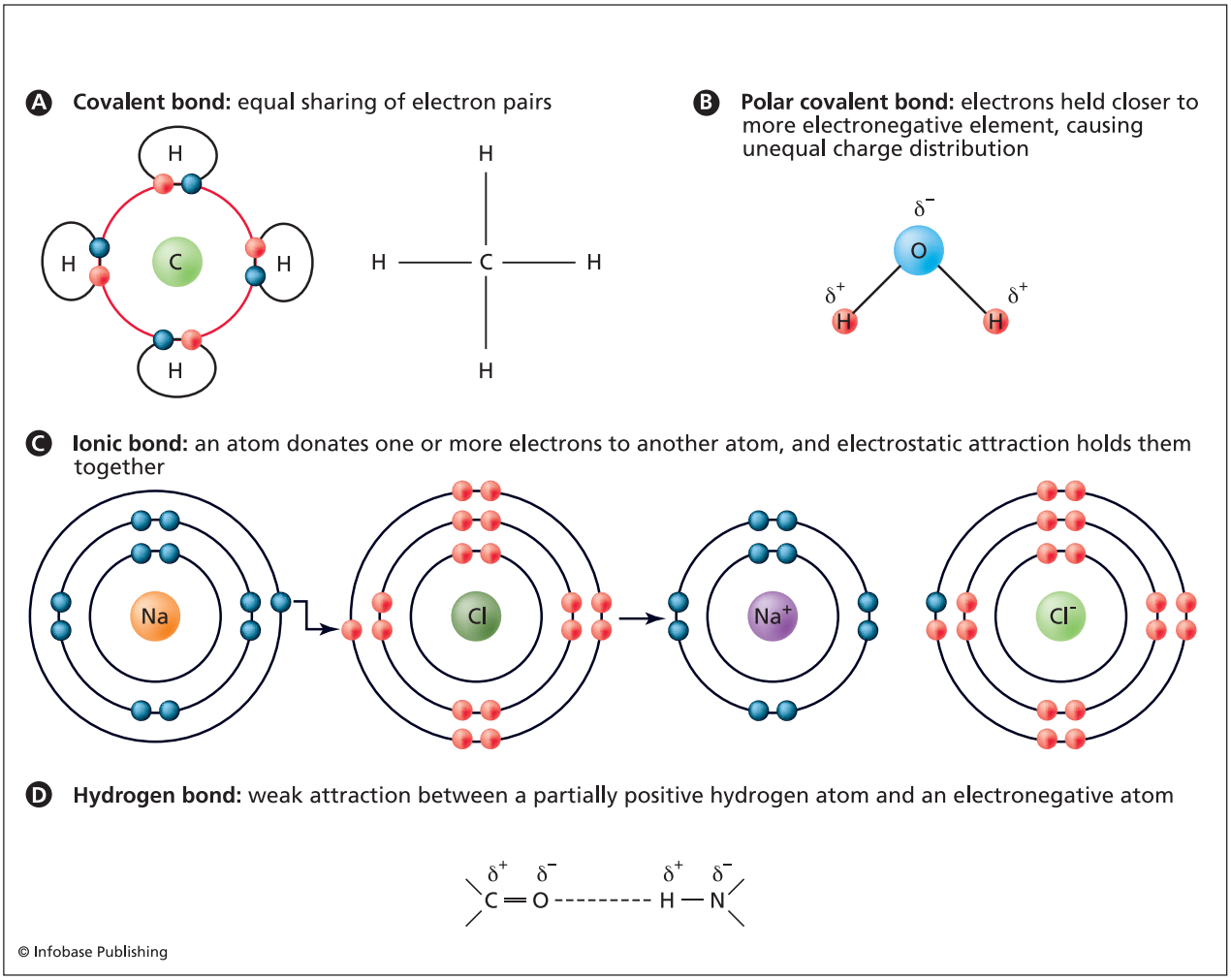


The nature of a chemical bond between two atoms depends on the differences in their electronegativities, shown here as a periodic property.

the greater electronegativity. As in covalent bonding, the driving force is still the tendency of the atom to reach a stable configuration, that is, a configuration with a filled valence shell.

When a neutral atom donates or accepts one or more electrons, it becomes an ion, a charged particle. Ionic bonds are attractions between oppositely charged ions. Sodium chloride (NaCl), also known as table salt, is an example of an ionic compound. Sodium has an electronegativity of 0.9, and chlorine has an electronegativity of 3.0. The difference is 2.1, which is greater than 1.7; thus their interaction results in the formation of an ionic bond. A sodium atom has 11 electrons, so the valence shell has one lone electron. Chlorine has 17 electrons, so its valence shell has seven electrons. By donating its lone electron to chlorine, the sodium atom attains a stable octet in its outermost shell, and because it now has one less electron than proton, it has a positive charge of 1 (Na^{+1}) and is considered an ion. Likewise, chlorine attains a stable octet in its valence shell by accepting the electron donated by the sodium atom. The chloride ion has one more electron than proton, thus has a charge of negative 1 (Cl^{-1}). The sodium ion and the chlorine ion join to form an ionic bond, an attraction between oppositely charged ions. Whereas compounds bonded covalently are called molecules, compounds held together by ionic bonds are usually referred to as salts and assume regular, repeating, three-dimensional structures (crystals) formed by balancing the charges. The environment of living systems is typically aqueous, and ionic compounds rapidly dissociate in the presence of water. When polar water molecules completely surround the ions, the salt is said to be dissolved. Covalent bonds do not break apart in the presence of water.

While water does not cause atoms held together by covalent bonds to dissociate, the nature of a covalent bond does determine the molecule's solubility in water. Covalent bonds, as defined earlier, form



The characteristic electronegativities of different elements determine the types of chemical bonds that they form with other elements.

between atoms that have a difference in electronegativity of less than 0.4, and ionic bonds form between atoms with electronegativity differences greater than 1.7. When the difference falls in the range of 0.4 to 1.7, the bond formed is termed polar covalent. The atoms still share their electrons, but they do not do so equally. The tendency of one of the atoms is great enough to localize the charges by holding the shared electrons closer to its nucleus than they are to the less electronegative atom's nucleus, but the difference is not great enough to pull the electrons off the less electronegative atom completely. In polar covalent bonds, the more electronegative atom will carry a partial negative (δ^-) charge, while the other atom will have a partial positive charge (δ^+). Polar molecules have dipoles, opposite charges at different ends or sides of the molecule. This separation of charges makes polar molecules soluble in water and other aqueous substances; they are said to be hydrophilic because of their affinity for water. Water molecules, consisting of one oxygen atom and two hydrogen atoms, are very polar. Oxygen is very electronegative and holds the electrons shared with the bound hydrogen atoms more tightly than do the hydrogen atoms. This lopsided distribution of electrons gives the oxygen end of the triangle-shaped molecule a partial negative charge and the hydrogen atoms a partial positive charge. In biomolecules, oxygen and hydrogen form polar covalent bonds, as do nitrogen and hydrogen. Carbon, however, has a slightly lower electronegativity than oxygen or nitrogen, and so it shares electrons relatively equally with hydrogen. When two polar covalent molecules are positioned near one another, the opposite partial charges can form a weak ionic interaction with one another. Though the ionic interactions between polar covalent molecules are weak, several can act jointly to hold molecules together. For example, partially charged atoms in the active site of an enzyme can hold a specific substrate in the correct position and orientation to allow a biochemical reaction to take place and then release it after product formation.

A hydrogen bond is a noncovalent interaction that occurs between two atoms that are already participating in polar covalent bonds with other atoms. As the name implies, one of the atoms is a hydrogen atom that is bound to one electronegative atom, and the other is another electronegative atom, usually a nitrogen or oxygen. The hydrogen atom is basically sandwiched between the two electronegative atoms but is only covalently bound to one of them. Hydrogen bonds can form between different molecules or between different parts of one large molecule. They are very weak, and simple thermal motion causes them to break and reform continuously. Hydrogen bonds hold water molecules together and are responsible for the many unique properties of water. The three-dimensional structures and therefore the proper function of many proteins and nucleic acids also depend on hydrogen bonding. The two strands of a double-helical molecule of DNA are held together by hydrogen bonds between complementary nucleotides. The individual hydrogen bonds are weak enough so the two strands can be easily separated in order for replication or transcription to occur, but in a polymer thousands of nucleotides long, the collective strength of the hydrogen bonds holds the two strands of DNA together to form a stable molecule.

Hydrophobic interaction, another biologically important noncovalent interaction, occurs between nonionic, nonpolar substances. Such molecules are not hydrophilic—they are hydrophobic, meaning they do not have an affinity for water and seem to repel it. Composed of atoms that have similar electronegativities, nonpolar molecules (such as the long hydrocarbon chains that are found in lipids) have their electrons equally distributed; thus hydrophobic molecules do not dissolve in water. Hydrophobic interactions are the forces that cause the nonpolar substances to join when placed in an aqueous solution in order to minimize interaction with the polar water molecules.

One last type of noncovalent interaction found between biomolecules are van der Waals forces, weak interactions that result from the attraction of transient dipoles in neutral atoms or molecules. The electrons in an atom or molecule are in constant motion, and the presence of other nearby particles influences their exact location at any instant in time. Thus transient dipoles are constantly forming around the molecules, and the weak intermolecular forces resulting from the attraction of the local charge fluctuations are called van der Waals forces.

See also BIOMOLECULES; WATER, ITS BIOLOGICAL IMPORTANCE.

FURTHER READING

Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell.* 5th ed. New York: Garland, 2007.

Lennarz, William J., and M. Daniel Lane. *Biological Chemistry.* 4 vols. Amsterdam and Boston: Elsevier, 2004.

Myers, Richard. *The Basics of Chemistry.* Westport, Conn.: Greenwood Press, 2003.

chromatography Chromatography is a technique used for separating mixtures. A mobile phase (such as a liquid or gas) containing the mixture in a solvent passes or moves through a stationary phase (such as a paper or a gellike matrix). Different chemical or physical properties of molecules in the mixture

cause them to move through the stationary phase at different rates, and they can be further purified, measured, or analyzed. Different categories of chromatographic methods depend on the type of mobile phase: gas, liquid, or supercritical fluid. Life science research often employs liquid chromatography, in which a solution containing a mixture of biomolecules moves through a medium composed of a variety of possible materials depending on the characteristic used to separate the components. In column chromatography, the stationary phase is typically prepared as a slurry of resin and buffer that is packed into a narrow cylindrical glass or plastic column. In paper chromatography capillary action pulls the liquid up through a strip of paper acting as the stationary phase. An absorbent material spread over a flat glass or plastic plate serves as the stationary phase in thin-layer chromatography.

In liquid column chromatography, gravity pulls the mobile liquid phase through the resin of a column. The resin retains the components of the mixture to different degrees on the basis of different characteristics of the components. The components will thus move through the column at different rates.

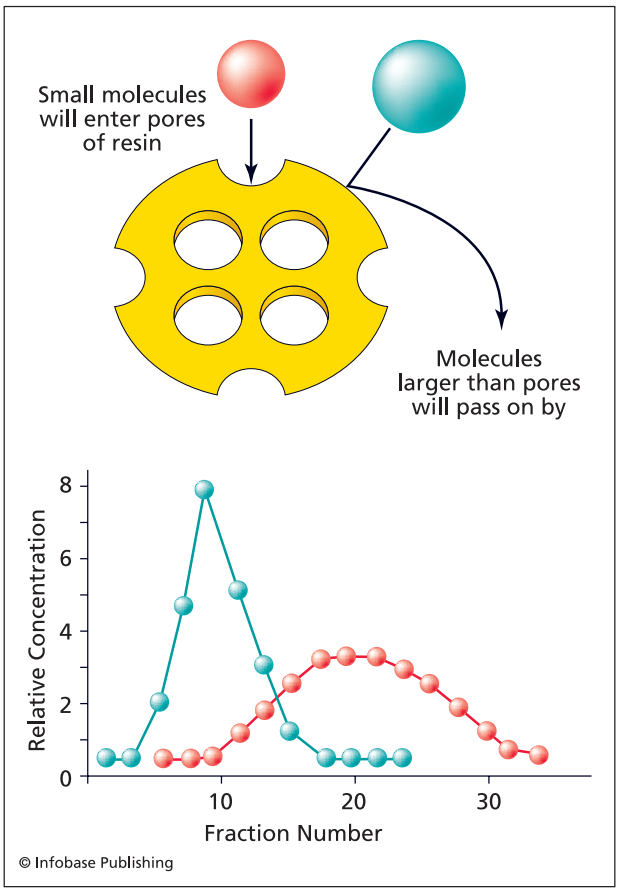
In column chromatography, a liquid mobile phase travels through a column packed with a material that retains mixture components to different degrees. *(Maximilian Stock Ltd./Photo Researchers, Inc.)*

The solution is collected in fractions as it leaves, or elutes from, the column. All of the fractions contain equal amounts of solution, but the concentration of the mixture components in each fraction varies as a result of different retention times of the various molecules. To determine what is present in each fraction, the researcher then assays each one by a variety of methods depending on the purpose. For example, the total protein or nucleic acid concentration can be measured, or the ability of a fraction to carry out a specific biochemical reaction can be assessed.

Different types of column chromatography exploit different properties of the molecules in the mixture to achieve separation. The three most common types in life science research are size exclusion, ion exchange, and affinity chromatography.

Size exclusion chromatography, also called gel filtration chromatography, separates components on the basis of their physical dimensions. The column is packed with porous beads resembling tiny spheres with tunnels running through them. Large molecules will not be able to enter into the pores; they are said to be excluded from the porous beads and flow rapidly through the column. They basically travel in a straight downward path and emerge in the void volume, the volume of the buffer that surrounded the beads of the column. The first few fractions of a size exclusion column will contain all the molecules that were larger than the size of the pores; thus a researcher must carefully choose beads that contain an appropriate size pore on the basis of the biomolecules to be purified. The pore size should be slightly larger than the molecule of interest. Molecules whose size resembles that of the pores in the resin will penetrate some of the pores but not others, so the retention time of these intermediate-sized molecules will be longer than that of the larger molecules. As the size of the molecules decreases, the retention time increases because smaller molecules will spend more time inside the beads, traveling a greater total distance through the column. Common uses of size exclusion chromatography are in fractionating proteins from extracts and purifying oligonucleotides from individual nucleotides after radiolabeling them.

Another type of chromatography commonly used in the purification of biological molecules is ion exchange chromatography, which separates molecules on the basis of their charge. Anion exchange columns use a positively charged stationary phase, such as a resin with diethylaminoethyl (DEAE) groups attached. As negatively charged molecules flow through the column, they interact with the DEAE groups, causing an increased retention time. In cation exchange, a negatively charged resin such as phosphocellulose retains positively charged molecules. Once a charged molecule binds to the column,



The column resin used in size exclusion chromatography contains pores of a size selected to allow some molecules to flow through easily but slow down others. Larger molecules will elute in earlier fractions, whereas the column will retain smaller molecules for longer periods.

it will not elute until the ionic strength or the pH of the buffer solution is changed. Gradually increasing the ionic strength will allow the ions in the buffer to replace the charged side chains of the molecule bound to the column, releasing it into the upcoming fractions. Altering the pH of a solution will either protonate (add a proton) or deprotonate (remove a proton) ionic side chains of a molecule bound to the resin, lowering its affinity and allowing it to elute. Because this method is highly selective and the resins are inexpensive, ion exchange chromatography is often used at early steps in purification protocols.

Affinity chromatography employs resins to which specific compounds have been attached. This is the most selective form of chromatography and can be used to purify specific enzymes on the basis of their binding to a specific substrate, to purify antibodies on the basis of their interaction with a specific antigen, or purify protein receptors on the basis of their recognition of a particular ligand. The compound for which the molecule desired has an affinity is chemically attached to an inert matrix, often agarose, a polysaccharide isolated from algal cell walls. As the solution or extract flows through the column, the molecule of interest binds. In order to liberate the purified molecule, a buffer with a high ionic strength is passed over the column, disrupting the intermolecular ionic interactions holding the molecules together.

See also BIOMOLECULES; CENTRIFUGATION; CHEMICAL BASIS OF LIFE; ELECTROPHORESIS.

FURTHER READING

Heftmann, E., ed. *Chromatography: Fundamentals and Applications of Chromatography and Related Differential Migration Methods*. Boston: Elsevier, 2004.

Poole, Colin F. *The Essence of Chromatography*. Boston: Elsevier, 2003.

chromosomes

Chromosomes are the threadlike structures that carry the genetic information in living organisms. Consisting of chromatin, a complex of deoxyribonucleic acid (DNA) and proteins, eukaryotic chromosomes are found in the membrane-bound nucleus and contain linear DNA molecules. In contrast, typical prokaryotic chromosomes are closed, circular structures that are not enclosed within a membrane-bound compartment but rather are concentrated in a region called the nucleoid. Prokaryotes have a single chromosome, whereas the number of chromosomes in eukaryotic cells is species dependent. Also, most eukaryotic cells contain two each of several chromosome types, a condition called diploidy, with one member of each pair from each parent. Cells that only contain one of each type of chromosome are termed haploid and play an important role in sexual reproduction. The "Chromosome Number by Species" table gives a sampling of the number of chromosomes found in various eukaryotic species. No consistent relationship exists between the number of chromosomes and the complexity of the organism.

CHROMOSOMAL THEORY OF INHERITANCE

The chromosomal theory of inheritance states that chromosomes are the cellular components that carry genes, the functional units of heredity. Though this concept seems simple, the chromosomal theory revolutionized 20th-century biology. The Austrian monk Gregor Mendel laid the foundation in the 1860s when he presented his research on inheritance in pea plants. He collected data from thousands of offspring that resulted from controlled pollinations between plants with specific traits, and he proposed an insightful explanation regarding inheritance. To summarize,

CHROMOSOME NUMBER BY SPECIES

Species	Diploid Number of Chromosomes
Myrmecia pilosula (an ant)	2
mosquito	6
Canis familiaris (dog)	8
Drosophila melanogaster (a fruit fly)	8
petunia	14
Planaria torva (a flatworm)	16
Aspergillus nidulans (a mold)	16
corn	20
Saccharomyces cerevisiae (a yeast)	32
Homo sapiens (human)	46
Pan troglodytes (chimpanzee)	48
chicken	78
king crab	208
Ophioglossum reticulatum (Indian fern)	1,260

he said that every diploid organism possesses two determinants (today called genes) for each character (trait). The two copies of a gene (now called alleles) may or may not be identical. By definition, a dominant allele will mask the presence of a recessive allele if an individual has one copy of each, meaning the individual will exhibit the phenotype (the observable characteristic) associated with the dominant allele. If an individual has two identical copies of a gene, then the phenotype associated with the alleles the individual possesses will manifest. Mendel also proposed the law of segregation, stating that though every individual has two copies, only one passes from parent to offspring through the gametes; in other words, the two copies of a gene separate during the formation of gametes. At fertilization, offspring receive one allele from each parent and thus have two copies. Random chance determines which allele passes to the offspring through an egg or the sperm cell that fertilizes it. Mendel also put forth the law of independent assortment, which states that the alleles for one gene separate independently of the alleles for other genes. (Scientists later discovered this only holds true for alleles located on different chromosomes or sufficiently distant on the same chromosome.) Mendel presented his research in 1865, and it was published the following year, but the work was unnoticed until the turn of the century.

Meanwhile, unaware of Mendel's findings, cell biologists sought the hereditary material. Since egg and sperm were thought to contribute equally to the offspring, and the sperm contained very little cytoplasm, the biologists focused on the nuclear contents. In 1882 Walther Flemming at the University of Kiel in Germany described his observations of chromosomes and their movement during cell division, a process he named mitosis. In 1883 the German physiologist August Weismann proposed that chromosomes were the bearers of the genetic information.

In 1900, while performing literature searches related to their own studies, three scientists independently rediscovered Mendel's work: Hugo de Vries, Erich Von Tschermak, and Carl Correns. An American graduate student named Walter Sutton and a German biologist named Theodor Boveri separately but simultaneously had been studying chromosomes and their behavior during mitosis (the process resulting in duplication and separation of the nuclear contents prior to cell division) and meiosis (the process that during the formation of gametes results in nuclei that only contain half of the total number of chromosomes). The attention drawn to Mendel's experimental data, Correns's suggestion that chromosomes might be the carriers of inherited traits, and Sutton's own research on chromosome structure and function in grasshoppers led to Sutton's 1902 *Biological Bulletin* paper, "On the Morphology of the Chromosome Group in *Brachystola magna*." In that paper and one he published the following year titled "The Chromosomes in Heredity," Sutton concluded that genes are located on chromosomes, described how chromosomal behavior during meiosis explained Mendel's laws of heredity, and provided supportive data from his own research. Also in 1903, Boveri drew the same conclusion from his studies in roundworms. Science credits both Sutton and Boveri with formulating the chromosomal theory of inheritance. With a powerful theory to explain the transmission of inherited characteristics, genetics advanced quickly in the early 1900s. The work performed by Thomas Hunt Morgan, in particular, provided a preponderance of confirmatory evidence. Biologists did not determine that DNA, rather than protein, was the component of chromosomes that acted as the molecular carrier of genetic information until the 1940s.

CHROMOSOME STRUCTURE

The molecule of heredity is DNA, which consists of a long polymer of four alternating nucleotides, referred to by the nitrogenous base the nucleotide contains: *A* for adenine, *C* for cytosine, *G* for guanine, or *T* for

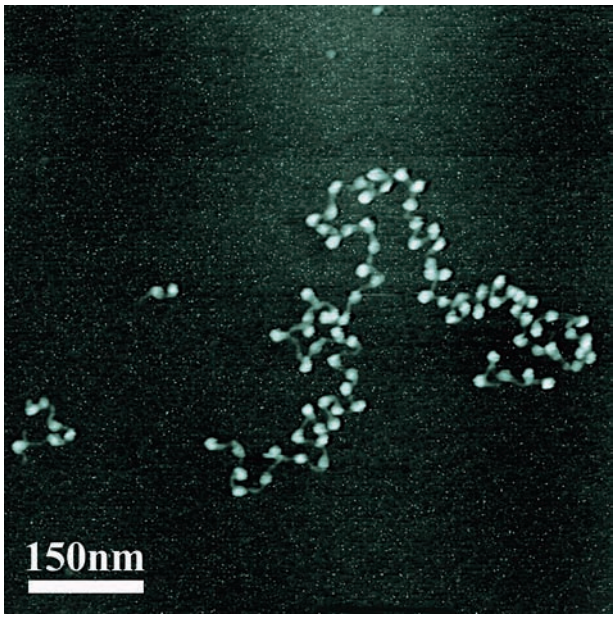
thymine. Molecules of DNA are double-stranded, and the nucleotides between the two strands pair up in a specific manner. Nucleotides containing the nitrogenous base A always pair with nucleotides containing the nitrogenous base T, and C always pairs with G. The sequence of the four nucleotides carries the information within genes, which exist as distinct segments along the length of a chromosome. All types of living organisms have DNA as their genetic material, but the manner in which the DNA is organized and stored within a cell varies. The genetic material of viruses can be either DNA or ribonucleic acid (RNA), which is very similar in structure to DNA and can be single-stranded or double-stranded, linear or circular, but viruses are not considered living organisms.

Prokaryotic cells include members of the domains Archaea and Bacteria. They usually have only one chromosome, consisting of a single, circular molecule of double-stranded DNA. The length is much shorter than that of the typical eukaryotic chromosome. The chromosome of *Escherichia coli,* the most extensively researched bacteria, is just under 5 million base pairs long, which if straightened and measured, would extend about 0.05 inch (1.2 mm), the approximate width of a letter on this page. Sometimes bacteria also have plasmids, much smaller circular pieces of DNA found in the cytoplasm. Plasmids are considered extrachromosomal pieces of DNA, though they can be considered part of the organism's genome, the entire complement of genes within an organism. An average rod-shaped *E. coli* cell measures 0.5 × 2.0 micrometers (μm, 1 μm = 1×10^{-6} m); thus compaction of the chromosome is necessary for it to fit within the cell. Topoisomerase enzymes that twist the DNA, causing it to coil into a tight bundle, package the chromosome, which is attached to the inner cell membrane. Positively charged proteins called HU and H bind the negatively charged phosphate groups of the bacterial DNA, helping it fit within the relatively small volume of a bacterial cell while still allowing it to function as a template for DNA replication and for transcription during protein synthesis.

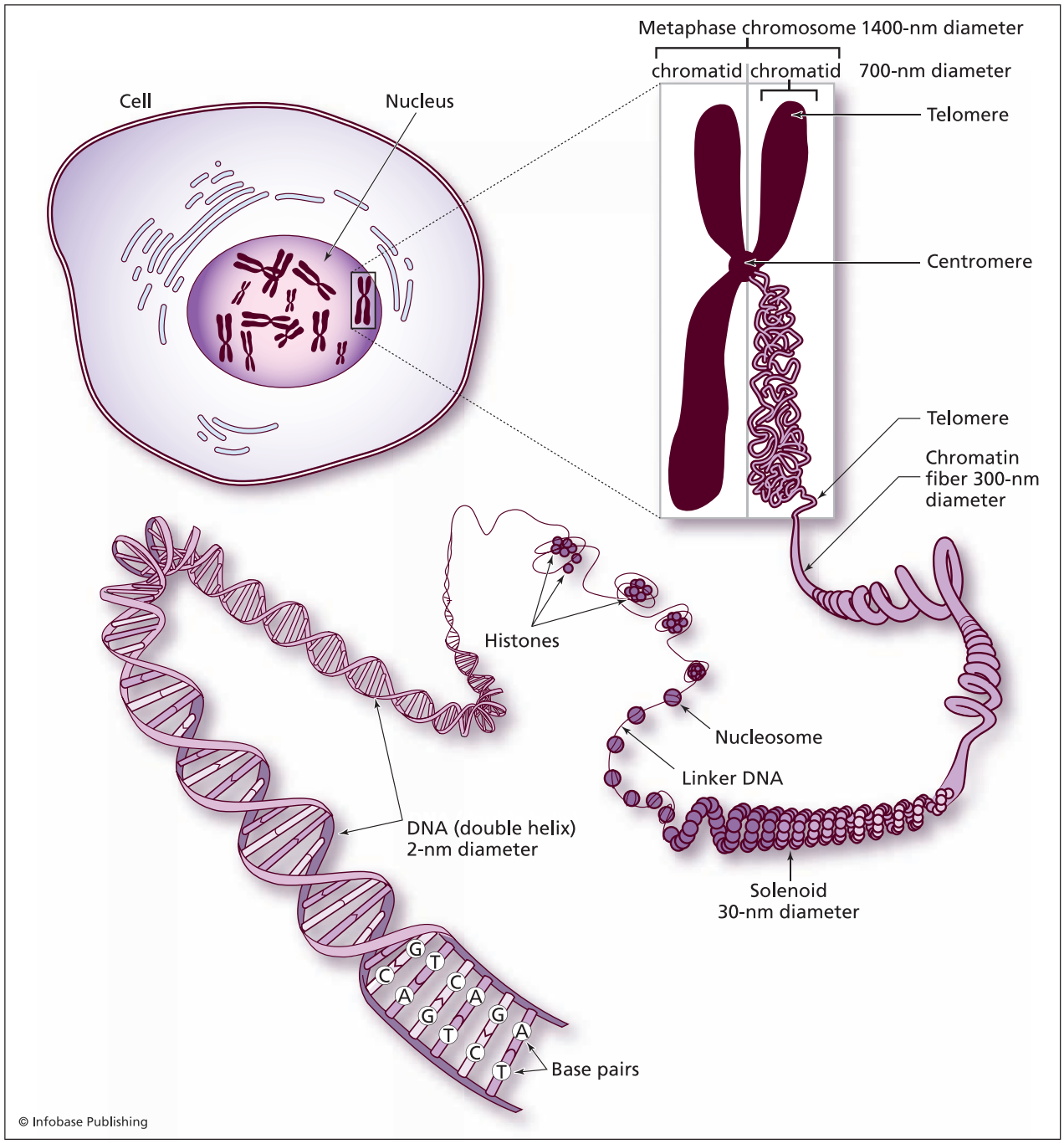
Eukaryotic chromosome structure is much more complicated than prokaryotic chromosome structure. Except during mitosis, the chromosomes of a eukaryotic cell exist as chromatin dispersed within the nucleus. Eukaryotic chromosomes vary in length; even different chromosomes of one species can differ. For example, human chromosomes range from 51 million to 245 million base pairs long, which translates into 19–73 millimeters (mm, 1 mm = 10^{-3} m) if fully extended. Since the diameter of a typical nucleus is only 5–10 μm, extensive packaging of the chromatin is necessary so it will fit within the confines of the nuclear envelope.

Eukaryotic chromosomes contain a much higher proportion of protein than prokaryotic chromosomes. Histone proteins are positively charged proteins that interact with the DNA of eukaryotic chromosomes. The five major types are H1, H2A, H2, H3, and H4. Two each of the latter four histone proteins combine to form an octamer that serves as the core of a structure called a nucleosome, around which a segment of about 147 base pairs of DNA wraps 1.7 times. The amino terminus of each histone protein extends outward and plays a role in the regulation of gene expression. Linker DNA, stretches of eight to 114 base pairs, exists between the nucleosome core particles. When viewed with an electron microscope, DNA packaged at this level resembles beads on a string, with the beads representing the nucleosomes and string representing the linker DNA. The extended chromatin fibers measure about 10 nanometers (nm, 1 nm = 10^{-9} m) in width at this stage, and the DNA has been compacted about threefold.

The next level of packaging requires the binding of a fifth type of histone protein, H1, which binds to the DNA near the nucleosome and further compacts the DNA. H1 binding causes the chromatin to coil into a thicker and shorter 30-nm solenoid structure. Each turn of the solenoid consists of about six nucleosomes, resulting in compaction of about five-times. At the next level of packaging, the 30-nm fiber forms



At the lowest level of packaging, DNA wraps around histone octamers, forming nucleosomes, a structure that resembles beads on a string. This transmission electron micrograph was obtained using purified chromatin fragments from chicken erythroid cells. *(Dr. Zhifeng Shao, University of Virginia)*

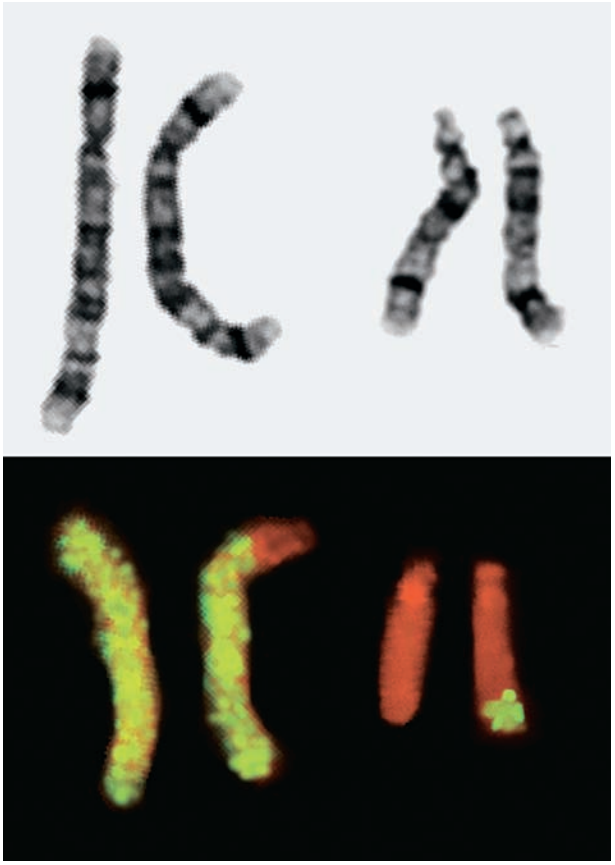


DNA in eukaryotic cells undergoes a series of successive levels of packaging.

looped domains that attach to a protein scaffolding, forming a 300-nm chromatin fiber. When further compaction is required, as when a cell prepares for mitosis, these looped domains and the scaffolding coil and fold, forming the arms of the chromosome and resulting in a fiber of approximately 700 nm in width (this measurement varies). Because the chromosomes of cells undergoing mitosis are duplicated at this stage, the width of the two attached sister chromatids is twice the value. The final degree of compaction varies from approximately 1,000-fold to 10,000-fold.

Prior to mitosis, the chromosomes in a nucleus duplicate, and during mitosis, the chromosomes separate and migrate toward opposite poles of the cell. Cytokinesis ensues, and the two resulting daughter cells each contain a complete set of the chromosomes. Most of the time, the chromosomes are not fully condensed but are long and threadlike and thus are not visible using light microscopy. They do, how-

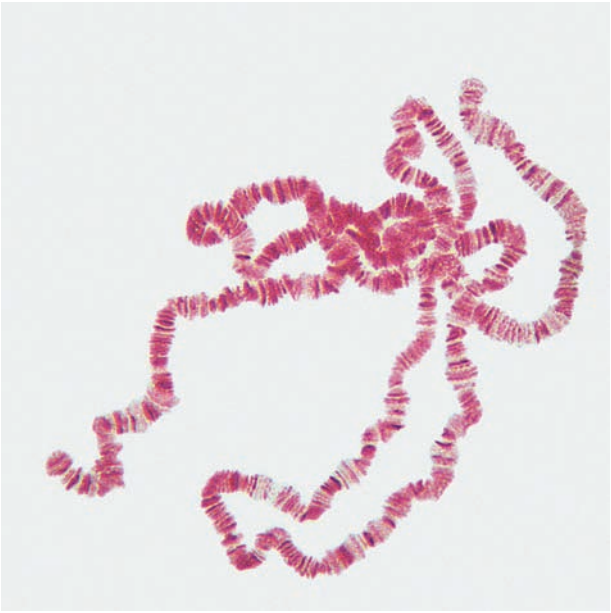
ever, retain an organized higher level of structure. In the absence of scaffolding, looped domains of chromosomes attach to the inside of the nuclear envelope and to fibers of the nuclear matrix, maintaining organization and preventing entanglement with other looped domains and other chromosomes. During mitosis, the chromosomes are fully condensed and, if stained, can be easily observed with a light microscope. Because the chromosomes are still duplicated, each chromosome consists of two sister chromatids attached at a centromere. The position of the centromere and the relative length of the mitotic chromosomes allow cytogeneticists to distinguish among chromosomes types. When performing a karyotype analysis, the cytogeneticist identifies all the chromosomes and arranges them in pairs according to their number. This enables the diagnosis of genetic disorders due to abnormalities in chromosome number or structure. The use of specialized staining techniques results in unique banding patterns on the chromosomes. These bands facilitate the identification of each type. Fluorescent dyes are useful in detecting abnormalities in chromosomal structure. For example, in translocations, a piece of one chromosome swaps locations with another.



Specialized staining techniques create unique chromosomal banding patterns that aid in identification of each chromosome. The use of fluorescent dyes helps in the detection of chromosomal structural abnormalities, as shown in this translocation between human chromosome numbers 5 and 14. *(Addenbrookes Hospital/Photo Researchers, Inc.)*

The major function of DNA is to encode for the synthesis of proteins. This occurs in several steps, the first of which is transcription, whereby the two stands of DNA in a region that contains a gene separate, and RNA polymerase reads the template strand of DNA to build a complementary strand of messenger RNA. In its most compact state, the DNA of a chromosome is inaccessible to the enzymes and other proteins that perform this task. In some cells, segments of chromosomes that either do not encode for proteins or encode for proteins those cells do not need to express are turned off. These sections, called heterochromatin, remain tightly compacted and stain darker than sections that are packed more loosely, called euchromatin. Protective structures called telomeres, repetitive sequences of DNA found at the ends of linear chromosomes, and centromeres also remain tightly packaged.

Two unique types of chromosomes are polytene chromosomes and lampbrush chromosomes. The French embryologist Edouard-Gérard Balbiani first observed polytene chromosomes in 1881, but their hereditary nature was not explored until the 1930s. Found in certain tissues of developing fly larvae, polytene chromosomes result from multiple successive rounds of replication without separation. The long DNA molecules remain attached and align with the many other sister chromatids. The condensation of the genetic material at different positions along the length of the chromosome forms distinct bands, sometimes called chromomeres. The interbands, regions between the bands, are uncoiled and genetically active, as evidenced by large puffs called Balbiani rings. Polytene chromosomes are interesting because they are highly visible even during interphase, whereas most chromosomes are not visible unless a cell is undergoing mitosis. Biologists believe that polytene chromosomes serve to increase the nuclear and cellular volume in developing organisms and allow higher levels of gene expression than if the cells were diploid, a factor that may be important for developing larvae. Lampbrush chromosomes are so named for their resemblance to the brushes once used to clean kerosene-lamp chimneys. Found mostly in oocytes but also in some spermatocytes, lampbrush chromosomes are meiotic, meaning they only exist in cells that undergo meiosis, the specialized type of cell division that results in a halving of the genetic information to create haploid sex cells such as eggs and sperm. Lampbrush chromosomes are unique in that they exist in an extended form rather



The banding patterns of polytene chromosomes, which are large enough to observe using light microscopy, are distinctive for each species. *(Andrew Syred/Photo Researchers, Inc.)*

than condensed, as most chromosomes are during nuclear divisions. Their structure is characterized by lateral loops that emanate from each chromomere.

CHROMATIN REMODELING

Histones, the proteins responsible for the first level of packaging, are nonspecific and can bind to any DNA sequence in vitro. The positioning of nucleosomes along a DNA molecule within the nucleus of a cell, however, is influenced by several factors. Certain regions must remain exposed to function properly and the chromosomes in similar cell types are all packaged similarly. The chromatin must also be capable of remodeling, during which the chromatin relaxes its packing temporarily to provide access to replication and transcription enzymes and machinery and then returns to its packaged state when no longer active. In 1993 Bryan M. Turner suggested that epigenetic information resides in histone tail modifications. Epigenetic information is genetic information that is not encoded within the DNA sequence. In 2001 Thomas Jenuwein and C. David Allis proposed the "histone code," a system of postranslational modifications to histone amino termini that acts as an epigenetic regulatory mechanism for processes that act on chromatin. In 2006 Eran Segal of the Weizmann Institute of Science in Israel, Jonathan Widom of Northwestern University, and their colleagues published a study showing that combinations of DNA sequences direct nucleosome positioning by rendering certain regions of the DNA more flexible for wrapping around histone octamers. The pattern appears to have very loose requirements; thus the sequences that direct positioning do not conflict with protein coding sequences of genes. The existence of such a nucleosome code explains features of transcriptional control previously not understood.

Three chemical modifications to the amino acids in histone tails, the amino termini that extend outward from the nucleosome core, influence the activity of a chromosome's genes and its chromatin structure: acetylation, methylation, and phosphorylation. An enzyme called histone acetyltransferase adds acetyl groups ($-COCH_3$) to the positively charged amino acid lysine in the histones. This neutralizes the charge and reduces the electrostatic interaction with the negatively charged DNA. Loosening up the histones binding to the DNA makes the DNA more accessible; thus acetylation is a means of activating chromatin. Females have two X chromosomes, but the genes of one are sufficient; males only have one. The extra X chromosome in females becomes inactivated by lack of acetylation, forming a structure called a Barr body. Methyltransferases add methyl groups ($-CH_3$) to the positively charged amino acids lysine and arginine of the histone and, as a result, inactivate genes. The DNA itself also can be methylated, particularly cytosines adjacent to a guanine. Kinases transfer phosphate groups ($-PO_4^-$) to the amino acids serine and histidine, thus making the protein more negative. Though the exact effect is not understood, phosphorylation is related to relaxing and compacting the chromatin before and after DNA replication and in gene activation in nonmitotic cells.

Because histones do not completely dissociate during DNA replication, modifications of chromatin at the level of the histones can be passed on to future generations. Until recently, geneticists believed all heritable traits were due to changes in the nucleotide sequence. A relatively new concept, epigenetic inheritance, describes the transmission of genetic information in the form of reversible changes in DNA rather than alterations in the DNA sequence. Heritable chemical modifications to the histones or to the DNA itself affect the regulation of gene expression without altering the sequence. Epigenetic effects can occur during embryogenesis or during normal cellular reproduction, and recently scientists have shown that environmental factors such as diet can induce changes to one's epigenome that can be transmitted to the next generation.

See also CELLULAR REPRODUCTION; DEOXYRIBONUCLEIC ACID (DNA); EUKARYOTIC CELLS; GENE EXPRESSION; GENETIC DISORDERS; GENOMES; PROKARYOTIC CELLS.

FURTHER READING

Calladine, Chris R., Horace R. Drew, Ben F. Luisi, and Andrew Travers. *Understanding DNA: The Molecule and How It Works*. 3rd ed. San Diego: Elsevier Academic Press, 2004.

Gibbs, W. Wyat. "The Unseen Genome: Beyond DNA." *Scientific American* 289, no. 6 (December 2003): 106–113.

Gregory, T. Ryan, ed. *The Evolution of the Genome*. Burlington, Mass.: Elsevier Academic, 2005.

Olins, Donald E., and Ada L. Olins. "Chromatin History: Our View from the Bridge." *Nature Reviews* 4 (October 2003): 809–814.

Richmond, Timothy J., and Curt A. Davey. "The Structure of DNA in the Nucelosome Core." *Nature* 423 (2003): 145–150.

Wagner, Robert P., Marjorie P. Maguire, and Raymond Stallings. *Chromosomes: A Synthesis*. New York: Wiley-Liss, 1993.

Watters, Ethan. "DNA Is Not Destiny." *Discover* 27, no. 11 (November 2006): 33–37, 75.

Wolffe, Alan. *Chromatin: Structure and Function*. 3rd ed. San Diego: Academic Press, 1998.

circulatory system Living cells must exchange nutrients and waste products across their membranes; thus the surface area to volume ratio restricts the maximal size a cell can reach. As the diameter of a cell increases arithmetically, its volume increases exponentially. The surface area of the cell membrane must be large enough to support the diffusion of substances to meet the metabolic needs of contents within the entire volume of the cell. In single-celled organisms, substances diffuse directly between the cytoplasm and the external environment. The cells of lower invertebrates (animals without a backbone) such as sponges and cnidarians have close enough contact with the environment for nutrients to reach all of the organisms' cells and waste products to leave by simple diffusion and osmosis.

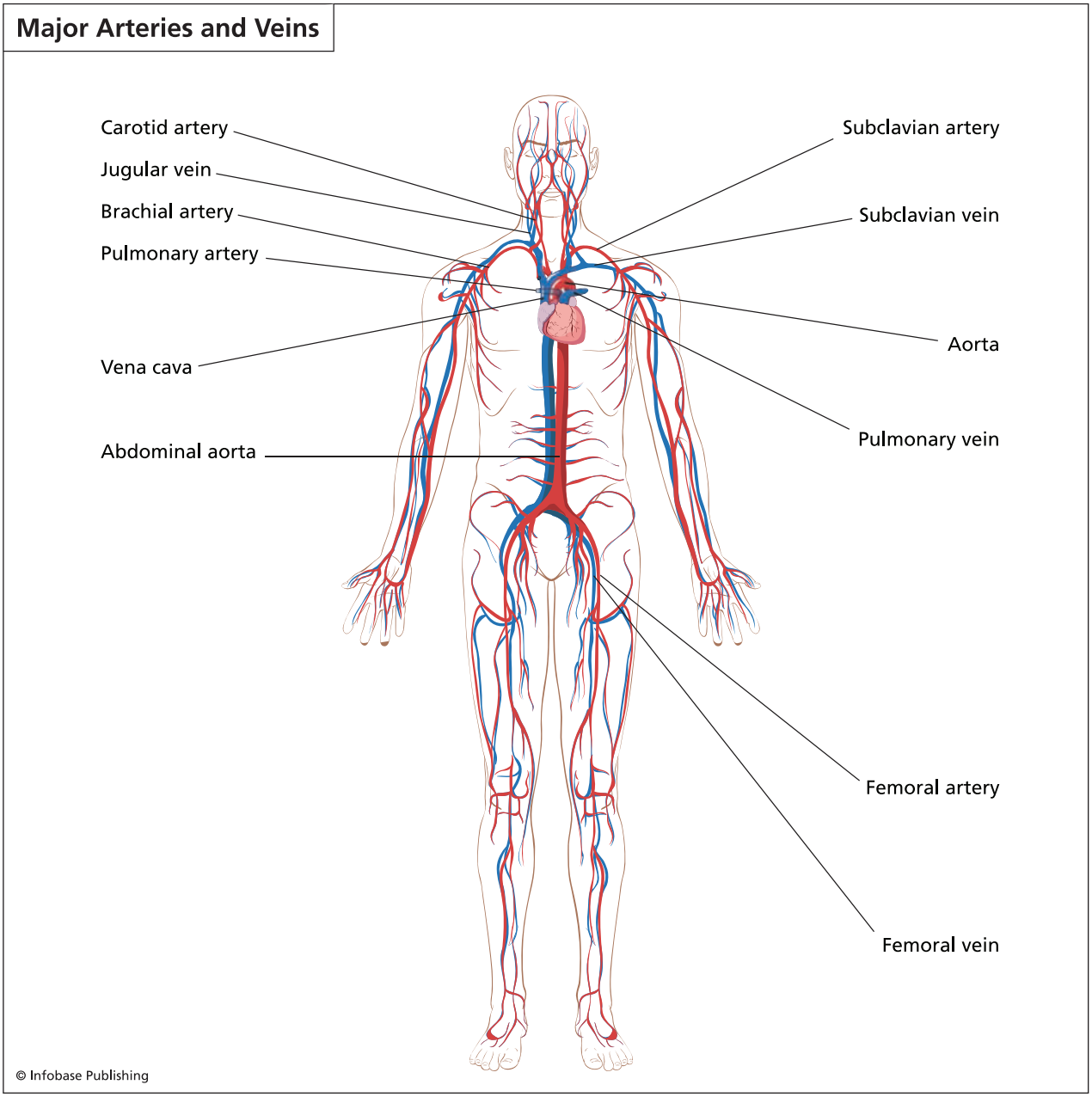
Invertebrates with multiple layers of body cells such as clams, insects, and spiders require special adaptations for nutrient and waste exchange because some of the cells are too far away from the external environment. Circulatory systems connect the organs or tissues of gas and nutrient exchange with all the other body cells when the distance is too great for simple diffusion alone to supply the body cells with the oxygen and nutrients necessary for metabolism. Open circulatory systems consist of a heart that pumps hemolymph, fluid containing essential substances, throughout a network of vessels and open spaces, or sinuses. The hemolymph bathes the body tissues, exchange occurs, and the fluid returns to the heart. Other invertebrates such as starfish, earthworms, and octopuses and all vertebrate animals have more efficient closed circulatory systems that transport substances throughout the body. In closed circulatory systems, the circulating fluid, called blood, travels via blood vessels throughout the body but never leaves the body tissues. Instead, substances diffuse through the walls of smaller branched vessels into the extracellular fluids of tissues, where exchange occurs.

ORGANIZATION OF THE HUMAN CIRCULATORY SYSTEM

In mammals gas exchange occurs in the lungs, nutrients are absorbed through the digestive tract, and nitrogenous waste products exit the body via the excretory system. All the cells in the body need to exchange gases, nutrients, and waste products, but many are too far from the specialized organs for transport by diffusion to achieve this efficiently. The human cardiovascular system comprises a muscular heart, blood, and numerous branched vessels that functionally connect all the body tissues and organs.

Blood circulates through an extensive branched network of vessels, including arteries, capillaries, and veins. Arteries transport blood from the heart to the body's tissues. As arteries approach the tissue they supply, they branch into arterioles, smaller vessels leading to capillaries that infiltrate the tissues. Capillaries converge into venules that converge into veins, the vessels that carry blood back to the heart.

Arteries are thicker and more muscular than other blood vessels because they receive blood directly from the heart; thus the blood flows through them with greater force than through the other vessels. The walls of arteries must be flexible so they can expand and contract to accommodate the force of the blood expelled from the heart and help regulate blood pressure. Arterial walls consist of three layers of tissue. A single layer of cells called the endothelium lines the innermost region of the artery, giving it a smooth interior to minimize resistance during blood flow. The thick middle layer consists of bundles of smooth muscle and elastic fibers that surround the endothelial layer. The outer layer of connective tissue also contains elastic fibers that wrap around and protect the vessels. Capillaries have only a thin epithelial layer and its basement membrane. These very thin vessel walls facilitate osmosis and diffusion of gases, nutrients, hormones, and other molecules. Their diameter is very narrow, barely wider than that of a single blood cell. The construction of veins is similar to that of arteries except the walls are thinner and valves are present. Whereas the force of the heart's contracting propels blood through the arteries at high speeds and great pressure, both the velocity and the pressure decrease as the blood travels through capillaries and then into venules and veins. Flaps of



The major arteries of the human circulatory system carry blood from the heart to all the body's tissues. The veins return blood to the heart, completing the closed systemic circuit. Note: Not all of the major arteries and veins are shown here.

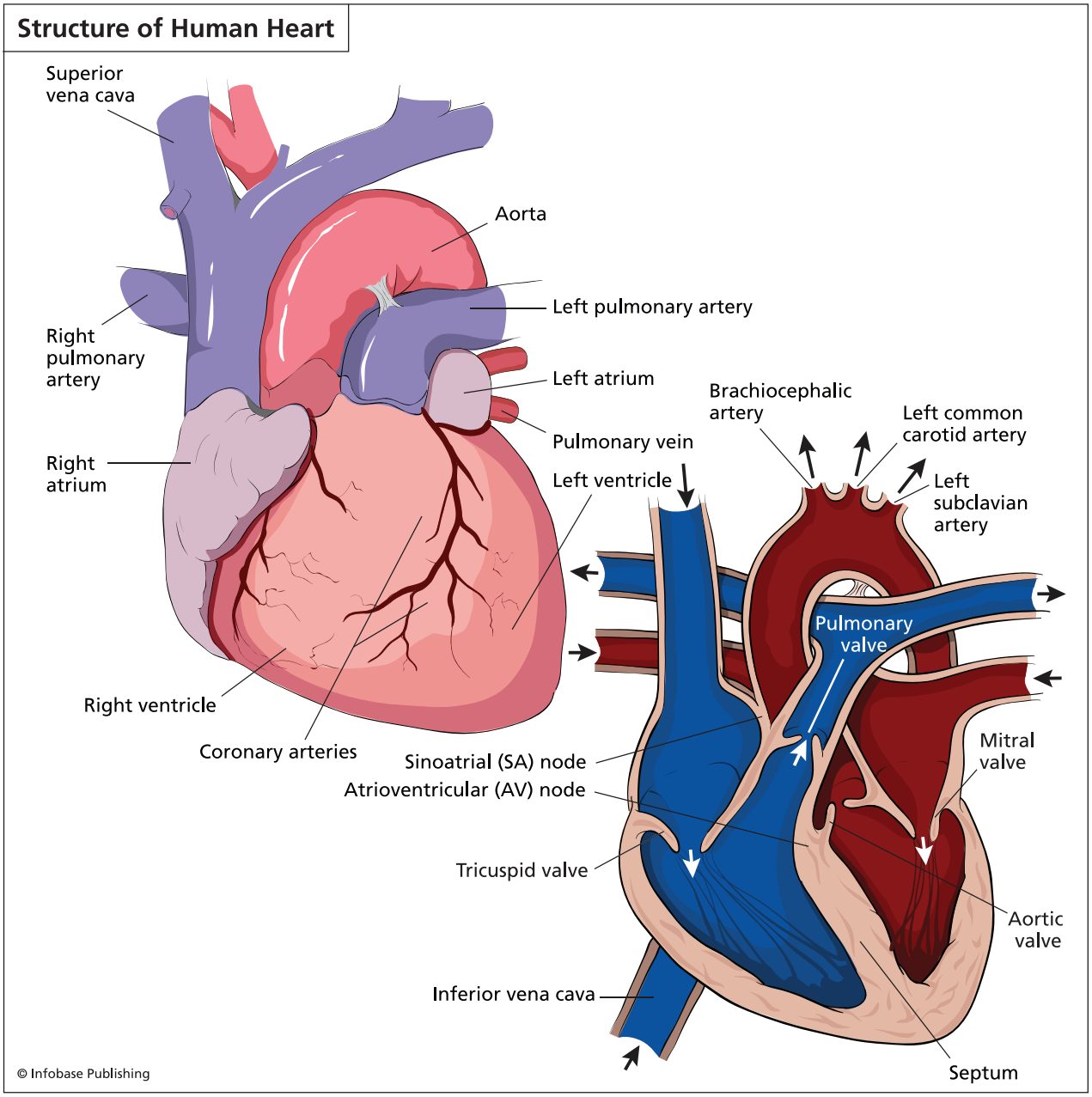
tissue called valves prevent the backflow of blood in the veins, keeping it moving in the direction toward the heart. Contraction of skeletal muscles squeezes blood inside the veins, forcing open the one-way valves and allowing blood to pass. When the muscles relax, the valves close. This explains how movement assists in circulation.

A separate second system of branched vessels functions to return excess fluid to blood circulation. The fluid that leaks from capillaries into the body tissues is called interstitial fluid. The lymphatic system collects the excess fluids from body tissues and dumps the fluid, now called lymph, back into the bloodstream near the junction of the jugular and subclavian veins on each side of the body. Lymph flows through lymphatic vessels mostly by skeletal muscular contraction and nearby arterial pulsing, squeezing the lymph vessels. Valves similar to the valves found in veins prevent backflow of the lymph. Swelling, or edema, occurs when interstitial fluid persists in the tissues, especially during times of inactivity when lack of skeletal muscular contraction reduces move-

ment of lymph through the lymphatic vessels. This is why finger rings that fit fine before going to bed feel tight after waking up in the morning. The lymphatic system also plays an important role in defense against infection.

HEART STRUCTURE AND FUNCTION

The human heart contains four chambers and is similar in size to a closed fist. Made mostly of cardiac muscle, the heart functions to pump blood through the circulatory system in order to transport oxygen and nutrients to the body's tissues and carry carbon dioxide and other waste products away from the body's tissues to organs specialized in their elimination from the body. Three layers make up the walls of the heart: the thin endocardium lines the interior of the heart, the myocardium is the bulky muscular portion, and the epicardium is the thin outer layer that holds the coronary arteries responsible for supplying blood to the heart tissue. A thin sac called the pericardium encases the entire heart, protecting it and separating it from the rest of the internal organs. The septum, a muscular wall, separates the heart into two sides, each consisting of two chambers that fill



The human heart contains four chambers, two atria and two ventricles, through which blood flows in a unidirectional manner.

with blood, atria and ventricles. The smaller atria receive blood from different parts of the body via veins, and the ventricles pump the blood through arteries out to the body. Four valves inside the heart prevent the backflow of blood.

The heart pumps blood through a unidirectional circuit. The two largest veins of the body, the superior and inferior vena cava, feed oxygen-poor blood into the right atrium. The blood travels through the tricuspid valve into the right ventricle, which pumps the blood into the pulmonary arteries through the pulmonary semilunar valve. After obtaining a fresh supply of oxygen and releasing carbon dioxide in the lungs, the blood returns to the left atrium of the heart via the pulmonary veins. The blood travels through the mitral valve into the left ventricle, the most muscular chamber, which pumps blood through the aortic semilunar valve into the largest artery, the aorta, which branches into the body's major arteries. After passing through the capillary beds of the tissues, blood moves through the veins to the vena cava, completing the circuit.

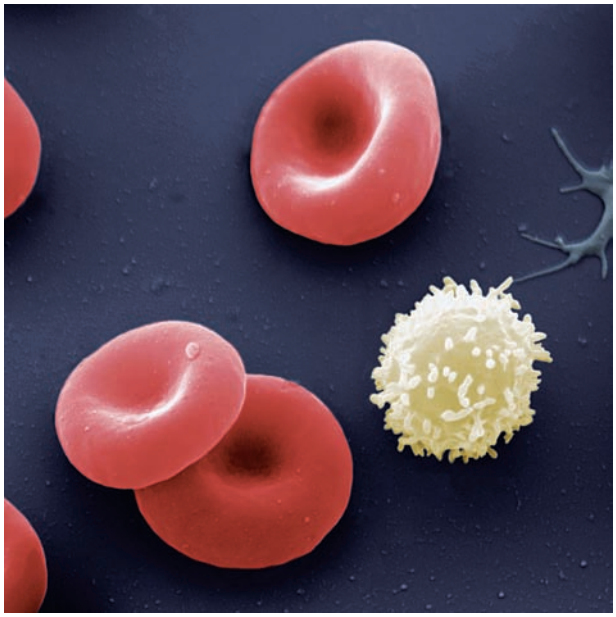
Contraction of the heart muscle decreases the cavity size of the chambers, forcing blood along its path. The rhythmic beating of the heart causes the arteries to pulse. The force of blood rushing through the arteries causes them to expand to accommodate the incoming blood with each contraction of the heart and then relax as the fluid moves along. A cardiac cycle is one complete cycle of the heart chambers' filling and draining. The contraction stage is called systole, and the relaxation stage is called diastole. A stethoscope is an instrument that facilitates the detection of a heartbeat. The first sound one hears, the "lub" in the "lub-dub" of each beat, is the recoil of the blood against the closed valves between the atria and ventricles. The second sound, the "dub," is the recoil against the valves that prevent backflow of blood leaving the ventricles. A healthy heart beats an average of 72 times each minute. The heart rate increases during exercise and decreases during rest.

The sinoatrial node, a small cluster of cardiac muscle cells located in the right atrium, acts as a natural pacemaker that controls the regular contractions of the heart. Certain vertebate cardiac muscle cells are unique in that they can contract without receiving a neural impulse. Gap junctions that allow almost instantaneous transmission of electrical impulses separate cardiac muscle cells. When the sinoatrial node initiates an electrical impulse, the signal immediately travels throughout the atria, resulting in simultaneous contraction of all atrial cells. The atrioventricular node, located between the right atrium and right ventricle, delays transmission of the electrical signal for about one-tenth of a second, then relays it to the muscle cells in the ventricles. This delay allows the atria to empty into the ventricles completely before the ventricles contract. Though cardiac muscle cells can contract without external input, other factors such as hormonal influences or body temperature can affect the heart rate.

COMPOSITION OF THE BLOOD

Blood is a type of connective tissue consisting of several types of cellular components (erythrocytes, leukocytes, and platelets) suspended in a liquid matrix (plasma). The average 150-pound (68-kg) adult human circulatory system holds approximately 5.5 quarts (5.2 L) of blood. About 60 percent of the blood volume is plasma, the liquid portion of the blood. Plasma is 90 percent water but contains numerous solutes. Sodium, chloride, bicarbonate, and other ions dissolved in the plasma maintain the osmotic balance and act as buffers that keep the pH of the blood at its optimum of 7.4. Proteins present in plasma also function to maintain osmotic balance as well as transport lipids, contribute to blood viscosity, and aid in clotting. Immunoglobulins, or antibodies, are proteins that fight infection, and protein hormones act as molecular signals that communicate information between cells of distant body parts. Steroid hormones also travel around the body via blood circulation, as do vitamins, amino acids, simple sugars, and dissolved gases such as oxygen and carbon dioxide.

Cellular components make up the remaining 40 percent of the total blood volume. Erythrocytes, also known as red blood cells, are the most abundant cell type present in the blood. Their main function is to



Human red blood cells (erythrocytes) are shaped like biconcave disks and function in oxygen transport. White blood cells (lymphocytes) help fight against infection. *(Eye of Science/Photo Researchers, Inc.)*

CELLULAR ELEMENTS OF BLOOD

Cellular Element	Major Function	Number (per mm^3 of blood)
ERYTHROCYTES (RED BLOOD CELLS)	transport oxygen and also carbon dioxide	5–6 million
LEUKOCYTES (WHITE BLOOD CELLS)	various immune functions	5,000–10,000
neutrophils	phagocytosis	3,000–7,000
eosinophils	defend against parasitic worms, counteract allergic responses	100–400
basophils	secrete histamine and heparin	20–50
lymphocytes	specific immune response, produce antibodies	1,500–3,000
monocytes	phagocytosis	100–700
PLATELETS (CELL FRAGMENTS)	blood clotting	250,000–400,000

carry oxygen from the lungs to all the body tissues. Shaped like a biconcave disk that is thinner in its center than around the edges, a red blood cell contains about 250 million molecules of hemoglobin, an iron-containing protein that binds oxygen. Mature human erythrocytes have no nuclei and therefore cannot repair themselves or undergo mitosis. They also have no mitochondria and cannot aerobically respire; that makes sense, considering that their function is to carry oxygen (that aerobic metabolism consumes) to other body tissues. Because erythrocytes have a life span of only a few months, stem cells in the bone marrow must constantly replenish them.

Approximately 1 percent of all blood cells are leukocytes, or white blood cells, that function in the immune response. Different types of white blood cells perform different tasks in preventing and fighting infection. Some white blood cells such as neutrophils and monocytes are phagocytic and engulf bacteria or other foreign particles. Basophils produce and secrete chemicals such as histamine that play a role in inflammation, and eosinophils help fight infections caused by parasites. Two types of lymphocytes, B cells and T cells, play central roles in the specific immune response.

Platelets are fragments of cells made when pieces of cytoplasm are pinched off large cells in the bone marrow. They circulate in the blood and stick to damaged epithelium of an injured vessel. At the injured site, the platelets secrete a sticky substance and attach to the proteins on the wall, plugging up any holes at the site of injury. A clotting enzyme released by platelets initiates a cascade of reaction that result in the formation of fibrin, a protein that forms a network of fibers that aid in blood clot formation, a process called coagulation.

Red blood cells express unique surface antigens composed of complex branched chains of sugars, or oligosaccharides. These antigens form the basis for one blood typing system used to determine compatibility before performing a blood transfusion. The ABO blood typing system involves two different oligosaccharides called A and B. People who express only the A oligosaccharide on the cell membrane of their red blood cells have type A blood. Individuals with the B oligosaccharide on their cells have type B. People who express both types A and B on their cells have type AB blood, and people with neither form are said to be type O. The Rh factor is another type of antigen found on the surface of some red blood cells. People whose blood cells have the antigen are Rh^+ and those who do not are Rh^-.

CARDIOVASCULAR HEALTH

Cardiovascular disease (CVD), afflictions related to the heart and blood vessels, is the leading cause of death of people of all racial and ethnic groups living in the United States. A heart attack occurs when part of the organ stops working and dies. If the coronary arteries that supply blood to the heart become blocked and blood cannot pass through, the tissue supported by those vessels will suffer from oxygen deprivation. The seriousness of the heart attack depends on the size of the area affected. A victim might die immediately or live for decades after a heart attack. During a heart attack some victims experience intense crushing pain in their chest, mild pain in an arm, or nausea or break into a sweat.

A stroke results when part of the brain dies as a result of interruption of its blood supply either by blockage or by rupture of a blood vessel. Body movement, function, and sensation can be impaired after a stroke, and death results in approximately one of three cases. Signs that someone might be experiencing a stroke include sudden numbness or weakness (especially if on one side of the body), confusion, dizziness, loss of sight, loss of balance, or severe headache with no known cause.

Two factors that increase the risk of heart attack and stroke are high blood pressure and atherosclerosis. Blood pressure is related to the amount of blood the heart pumps and the resistance it encounters as it flows through the arteries. High blood pressure often produces no symptoms until it is advanced, but it can be easily measured in a doctor's office. A blood pressure reading consists of two numbers, and normal is 120/80 or below. The first number, called the systolic pressure, indicates the pressure generated by the heart when it contracts and pushes blood through the arteries. The second number, the diastolic pressure, indicates how much pressure is on the arteries when the heart is at rest. Anything that narrows the diameter of the arteries will cause an increase in blood pressure. The body can do this naturally to compensate for a low blood volume or during periods of increased activity by contracting the smooth muscles surrounding the arteries, causing them to constrict and resulting in an increased blood pressure. High blood pressure increases the risk of heart attack and stroke.

Atherosclerosis is a disease in which fatty deposits such as cholesterol build up on the interior surface of arteries forming growths called plaques. The buildup decreases the diameter through which the blood flows, increasing the pressure. When calcium is deposited on the fatty acid buildup, the result is a hardening of the arteries, a condition termed arteriosclerosis. Hardened arteries do not have the elasticity of healthy arteries and cannot expand during the contraction of the heart, so the heart must work harder to push the blood through the vessels.

A thrombus, or clot, is often the cause of blockage in an artery that leads to a heart attack or stroke. The thrombus can develop in a coronary artery or an artery leading to the brain, or it can form elsewhere and travel through circulation in the blood until it becomes trapped in a narrow vessel. A traveling clot, called an embolus, often develops at sites where plaques have formed inside the arteries. Circulating platelets recognize plaques as injured tissue and begin the clotting process.

Many risk factors for CVD are easy to control. Cholesterol travels through the blood bound to proteins. High levels of low-density lipoprotein (LDL) cholesterol in the blood are associated with cholesterol deposition and plaque formation. Smoking, overweight, poor diet, and lack of exercise increase LDL levels. High levels of high-density lipoprotein (HDL) seem to protect against heart attack. Researchers believe HDL either carries cholesterol from the arteries back to the liver or removes excess cholesterol deposits from plaques, slowing their buildup. Exercise increases HDL levels. Not smoking, exercising regularly, maintaining a healthy diet and weight, and having medical checkups all decrease the risk of developing CVD. Hereditary factors cannot be controlled, but awareness of a family history of CVD can help someone develop a plan to decrease other CVD risk factors.

See also ANATOMY; ANIMAL FORM; HARVEY, WILLIAM; HOMEOSTASIS; HOST DEFENSES; INVERTEBRATES; PHYSIOLOGY; VERTEBRATES.

FURTHER READING

American Heart Association homepage. Available online. URL: http://www.americanheart.org/. Accessed January 14, 2008.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 1. Detroit: U*X*L, 2000.

Romaine, Deborah H., and Otelio S. Randall. *The Encyclopedia of the Heart and Heart Disease*. New York: Facts On File, 2004.

Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*, 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

Clark, Eugenie (1922–) American *Ichthyologist*

Eugenie Clark is a world-renowned ichthyologist who has discovered numerous new species as well as being an expert on sharks. Her pioneering studies on shark behavior dispelled several falsehoods and demonstrated that sharks are intelligent creatures rather than ferocious manhunters.

CHILDHOOD, EDUCATION, AND TRAINING

Eugenie Clark was born on May 4, 1922, in New York, New York, to Charles and Yumiko Clark. Her mother was a swim instructor, and her father was the manager of a private pool. He died when Genie was only two years old, and by then, she already knew how to swim. They lived with Genie's Japanese grandmother in Queens but often went to the beach on Long Island, where they used chewing gum to plug their ears before plunging into the ocean. Her mother then worked to support the family at a newspaper and cigar stand in the lobby of the Down-

town Athletic Club. Genie often accompanied her on Saturdays and waited for lunchtime at the nearby New York Aquarium, where she amused herself by watching the fish swim in their long tanks. She soon began collecting fish at home in a 15-gallon (68-L) tank, became the youngest member accepted into the Queens County Aquarium Society, and learned to keep methodical records of all her pets. By the time she entered high school, she also kept pet snakes, toads, salamanders, and alligators, and biology was her favorite subject.

After graduation Genie enrolled at Hunter College in New York City. In addition to taking every zoology course offered, she took field courses in zoology and botany at the University of Michigan Biological Station during the summers. After obtaining a bachelor's degree in zoology in 1942, she worked as a chemist for the Celanese Corporation of America at their plastics research labs in New Jersey.

Clark entered graduate school at New York University, where she specialized in ichthyology, the scientific study of fishes. She researched the puffing mechanism of blowfish (the order *Tetraodontiformes* or *Plectognathi* includes the triggerfishes, puffers, filefishes, boxfishes, globefishes, and ocean sunfishes) under the guidance of Dr. Charles Breder, who was the curator of the Department of Fishes and taught an ichthyology class at the American Museum of Natural History. He was so impressed with her work that he published her research results in the museum's scientific magazine, *Bulletin of the American Museum of Natural History,* "A Contribution to the Visceral Anatomy Development, and Relationships of the Plectognathi" (1947).

Clark met Dr. Carl Hubbs, of the Scripps Institution of Oceanography of the University of California, at the 1945 meeting of the American Society of Ichthyologists and Herpetologists in Pittsburgh, Pennsylvania. When she completed her master's degree in zoology at NYU in 1946, she joined him as a part-time research assistant and began research toward a doctorate degree. Hubbs taught Clark to dive with a face mask and to walk on the ocean floor using a metal helmet connected by a long hose to a compressed air supply aboard a ship. Scuba gear was not yet widely available.

In 1947 the United States Fish and Wildlife Service (FWS) hired Clark to study fish in the waters surrounding the Philippines, but en route, she was delayed in Hawaii only to learn that they had changed their mind about hiring her because of her gender. Though disappointed, Clark used her time in Hawaii to explore the waters around the islands and study tiny tropical puffers. She returned to NYU and continued her dissertation studies on the mating habits of platies and swordtails under the supervision of

Eugenie Clark is a renowned American ichthyologist. *(Al Danegger/University of Maryland)*

Professor Myron Gordon. In aquariums, platies and swordfishes mated to create hybrid fish, but hybrids were never found in the wild. Clark described the act of true copulation in platies and determined that in a competition between sperm of the two different species, the same-species sperm had an advantage over sperm from a different species. This meant that even if both species deposited sperm inside a female, the sperm from the same species as the female would successfully fertilize the eggs. Clark also performed the first successful artificial insemination of a fish in the United States.

While working toward her Ph.D. in zoology, in 1949 Clark accepted a job studying the fish of the South Seas for the Scientific Investigation in Micronesia program of the United States Office of Naval Research (ONR). Since the United States had acquired many of the South Pacific islands after World War II, the navy wanted to know whether commercial fishing in the area would be profitable and to determine which fish were safe to eat and which were poisonous. One method Clark used for collecting fish was to add rotenone to tide pools. Rotenone is a chemical that can be extracted from plant roots that stuns the fish, causing them to float

to the surface. The fish are still safe for consumption, however, and the vegetation is not harmed. Clark caught many interesting specimens, some of which were normally hidden or too small to be captured by other methods. She shipped the preserved specimens to the American Museum of Natural History. From the islands of Micronesia, Guam, Kwajalein, and Palau, Clark collected hundreds of specimens from the tide pools and also several puffers that she sent back to California for poison analysis. On the Palauan island of Koror, Clark learned how to hold her breath for a long time underwater and to spear-fish near the coral reefs. She found many new types of plectognaths and other fish she had never seen before and encountered sharks and razor-toothed barracudas.

THE RED SEA AND CAPE HAZE

Clark earned her doctorate in zoology in 1950 and received a Fulbright Scholarship to study fish of the Red Sea in the Middle East. The Red Sea is very warm and salty, and its name is derived from the reddish appearance caused by tiny red algae that live on its surface. Clark had read an article describing the symbiotic relationship between a sea anemone and a clown fish and found many similarities between the Red Sea and the tropical Pacific Ocean. Many of the tropical plectognaths she studied years earlier had been described originally by ichthyologists from the Red Sea, but no one had scientifically analyzed the Red Sea fish in 70 years. Using the Marine Biological Station in Ghardaqa, Egypt, as a base, she traveled around, collected specimens of more than 300 species, wrote detailed descriptions, discovered three new species, and performed dissections of the many forms of marine life. The only poisonous fish she found were puffers. Several scientific papers and a bestselling autobiography, *Lady with a Spear* (1953), resulted from her year spent exploring the Red Sea.

Back in New York, Clark taught biology at Hunter College and conducted ichthyological research at the American Museum of Natural History. She also lectured at educational institutions around the country.

One reader of Clark's autobiography happened to be a wealthy Floridian with a son who was interested in marine biology. Anne Vanderbilt and her husband William invited Clark to their home on the Cape Haze peninsula. The meeting began a long association between Clark and the Vanderbilts, who funded the Cape Haze Marine Laboratory for her to direct in Placida, Florida, on the Gulf of Mexico. The lab opened in 1955.

The first marine mystery Clark solved at her new lab involved a fish called the belted sand bass from the genus *Serranus*. She observed several egg-carrying fish and assumed they were females, but she could not locate any of the males. Dissection and microscopic examination revealed not only eggs but sperm. The fish were hermaphroditic, meaning they had the reproductive organs of both males and females. The fish could switch from male to female in less than 10 seconds, and their coloring changed at the same time. They could even fertilize their own eggs.

"SHARK LADY"

Prompted by a request for fresh shark livers by Dr. John Heller, a medical researcher at the New England Institute of Medicine, Clark broadened her focus from fish reproduction to sharks. She identified 18 species living off the coast, including hammerheads, blackfins, dog fish, nurse, tiger, bull, lemon, and sandbar sharks. She performed hundreds of dissections and examined their stomach contents to learn about their diet, finding mostly small fish, crabs, eels, octopuses, and other sharks. She built pens at the end of the dock so she could keep live sharks for observation. A shark expert, Dr. Perry Gilbert from Cornell University, taught Clark to spray a chemical at the mouth and over the gills of sharks to render them unconscious for about 10 minutes, during which the workers could haul the sharks safely into the pens. Over the 12 years that Clark directed the Cape Haze Marine Laboratory (which moved to Sarasota in the early 1960s), she became an expert on sharks and earned the nickname "Shark Lady." The National Science Foundation and the ONR provided financial assistance in the form of grants that Clark used to expand the lab, build more shark pens, and execute research.

Though many people believed sharks were dangerous and stupid creatures, no one ever had inves-

Clark helped dispel several myths about sharks, such as this white-tip reef shark from the Northwestern Hawaiian Islands reserve shown here. *(National Oceanic & Atmospheric Administration/Department of Commerce)*

tigated their behavior. Clark observed that lemon sharks swam toward people approaching their tanks, as if they expected to be fed, and believed they were capable of learning. Her research demonstrated that sharks were actually intelligent creatures with the ability to learn and remember. To teach a shark to associate a ringing bell with food, she painted a square wooden target white, hung fish from it, and lowered it by rope into the shark pen. When the shark went for the bait, he bumped into the target and a bell sounded. After three days, she lowered the fish only after the shark bumped the target rather than attaching it, then progressively lowered the fish farther and farther away from the target until the shark had to swim to the other side of the pen to retrieve his food. Clark also performed experiments that showed that sharks can distinguish colors and shapes.

When Prince Akihito of Japan, who was interested in ichthyology and had studied many types of fish, invited Clark to be his guest in 1965, she gave him a nurse shark that she had trained to ask for food by bumping a target. She was surprised to learn that he had never been diving, so a few years later, in 1967, when he stopped by Sarasota on the way from South America back to Japan, Clark met him at 5:30 A.M., before any reporters were around, and taught him to skin-dive.

Though sharks were the main focus of her research, Clark also engaged in other scientific activities. Exploration of nearby freshwater springs led to the discovery of many ancient human bones and other traces of Native American life from over 7,000 years ago. In 1959 Clark broke the record for the deepest dive with compressed air for a woman at 210 feet (64 m). During a trip with her family to the Middle East sponsored by the National Geographic Society in 1964, she investigated a colony of garden eels near Elat, Israel, and identified and named a new species of sandfish found in the Red Sea *Trichonotous nikii* (after her youngest son, Niki).

Clark left the lab in the late 1960s to move back to New York and recommended Perry Gilbert at Cornell to succeed her as director. With financial assistance from a businessman named William Mote, the lab was expanded and renamed the Mote Marine Laboratory. Today scientists continue to research a variety of marine disciplines at the laboratory, which consists of research centers for sharks, marine mammals and sea turtles, fisheries enhancement, ecotoxicology, coastal ecology, aquaculture research and development, and tropical research (located in Summerland Key). Mote also offers a series of educational programs, houses aquarium exhibits, and runs a dolphin and whale hospital.

For two years Clark taught zoology at the City College of New York and was a visiting professor at the New England Institute for Medical Research. She accepted a position in the Zoology Department at the University of Maryland in 1968 and was promoted to full professor in 1973. The following year she published a second autobiography, titled *The Lady and the Sharks*. Many of her discoveries were published in articles that she wrote for *National Geographic* magazine.

In 1972 Clark examined fluid secreted by the Moses sole, a sand-dwelling flat fish that she had first observed a dozen years before. The whitish substance that oozed from pores by the fins made her fingers tingly and numb and killed sea urchins and reef fishes in small doses. When she placed a Moses sole in a shark tank as bait, the mouths of sharks seemed to freeze open as they approached the fish, and the sharks wildly shook their heads back and forth. When she put one on an 80-foot (24-m) line with nine other types of fish and lowered it into the sea, sharks consumed all the fish except the Moses sole. She pulled it up and rubbed the scales with alcohol then lowered it again, and it was immediately eaten. Putting a small shark in a tank with the fluid killed the shark in six hours. She wondered whether the Moses sole produced a substance that could act as a shark repellent. Though initial studies seemed promising, the substance was unstable at room temperature and could not be sold for general use. As Clark learned more about sharks, she did not believe shark repellents were necessary anyhow; people were more dangerous to sharks than sharks were to people. She felt that understanding the creatures' behavior and acting accordingly were a better measures against shark attacks. The Moses sole also produced an antidote for its own poison that was later found also to protect against bee, scorpion, and snake venom, but the antidote had to be injected at the same time as the poison itself to be effective.

In 1972 a friend in Mexico sent Clark photographs of sharks in underwater caves off the Yucatan Peninsula and described some unusual behavior. The sharks seemed to be sleeping or dazed. Clark was interested and in 1975 traveled to Mexico to investigate the phenomenon. Biologists thought that sharks needed to swim constantly in order to survive, but these sharks remained motionless inside the caves for extended periods. To obtain oxygen, they pumped water over their gills while they remained stationary. Clark noticed that freshwater was leaking into the caves, lowering the salt concentration. She suggested the change in salinity caused a trancelike state in the sharks. Her team also noticed that remoras could easily remove all the parasites from the sharks' skin while in the less salty water. The question of whether or not sharks or other fish actually sleep, as defined by a distinctive change in brain waves, has never been resolved.

As the 1970s drew to a close, pollution threatened many of the world's waters. Clark was particularly concerned with the fate of the Red Sea since one of her favorite places to dive was Ras Muhammad, located at the southern tip of the Sinai Peninsula. She initiated efforts to have the site declared a national park, to protect it from the traffic that caused damage to its coral reefs and pollution. Her vision became a reality in 1983, and the park now is often referred to as an underwater Garden of Eden.

In 1981, on the coast of Baja California, Clark took her first ride on a whale shark. These gentle creatures grow up to 40 feet (12 m) long and mostly eat plankton. Later she would discourage others from doing the same, to leave the sea creatures in peace.

From 1987 to 1990, Clark was the chief scientist for the Beebe Project, funded by *National Geographic*. She was in charge of 71 deep ocean dives in deep ocean submersibles, underwater vessels that can travel distances of up to 20,000 feet (6 km). Her longest dive was 17.5 hours and her deepest was to 12,000 feet (3,658 m). She also served as a consultant for several television specials about marine life including "The Sharks" in 1982, a program that was sponsored by National Geographic and received the highest Nielsen rating for a television documentary. She wrote a children's book, *The Desert beneath the Sea,* in 1991, with the author Ann McGovern. That same year, she visited Ningaloo Reef Marine Park in Australia to study whale sharks. She saw 200 in a single month and observed their eating habits. Though she retired as a professor emerita from the University of Maryland in 1992, she continues to travel to exotic locations around the world. Since 1996 Clark has been studying the behavior of a small reef fish off the coast of Papua New Guinea and off the island of Mabul off North Borneo. *Pholidichthys leucotaenia,* called convict fish because of the striped pattern, burrow into a labyrinth of tunnels as adults and stay there. The juveniles, however, leave the tunnels to eat plankton and then return to the tunnels, where the parents take the juveniles into their mouths. The young regurgitate their food into the parent, and the parent releases them from their mouth.

PERSONAL LIFE AND ACCOMPLISHMENTS

In 1942 Clark married a pilot named Hideo "Roy" Umaki, who served in the army and was stationed overseas. They divorced in 1949. The following year she married a Greek orthopedic surgeon named Ilias Papakonstantinou (later shortened to *Konstantinou*). They had two daughters and two sons during the next seven years. Clark divorced Konstantinou and married a writer named Chandler Brossard in 1967. The marriage between Clark and Brossard dissolved, and in 1970 Clark married a scientist from the National Institutes of Health named Igor Klatzo. That marriage also ended in divorce. In 1997 she married a longtime friend, Henry Yoshinobu Kon, who died in 2000.

In a career that has spanned seven decades, Clark has contributed significantly to knowledge in the fields of fish and shark behavior, taxonomy, and ecology. She has discovered 11 new species, authored more than 165 scientific and popular articles about marine science, and consulted for or participated in more than 200 radio and television programs dealing with conservation, marine biology, fish, diving, and career women. She has received numerous medals and awards for her research. The National Geographic Society, the Society of Women Geographers, the Maryland Women's Hall of Fame, the American Society of Oceanographers, and other organizations have acknowledged her. The University of Massachusetts awarded her an honorary doctorate in 1992, and the University of Guelph and Long Island University awarded her honorary doctorates in 1995. Four new fish species bear names in her honor: *Callogobius clarki, Sticharium clarkae, Enneapterygius clarkae,* and *Atrobucca geniae*. As Mote director emerita, Dr. Eugenie Clark remains committed to teaching others to protect and appreciate marine life.

See also MARINE BIOLOGY.

FURTHER READING

Clark, Eugenie. *The Lady and the Sharks*. New York: Harper & Row, 1969.

Dr. Eugenie Clark home page. Available online. URL: http://www.sharklady.com/. Accessed January 18, 2008.

Mote Marine Laboratory welcome page. Available online. URL: http://www.mote.org/. Accessed January 18, 2008.

Reis, Ronald A. *Eugenie Clark: Marine Biologist*. New York: Ferguson, 2005.

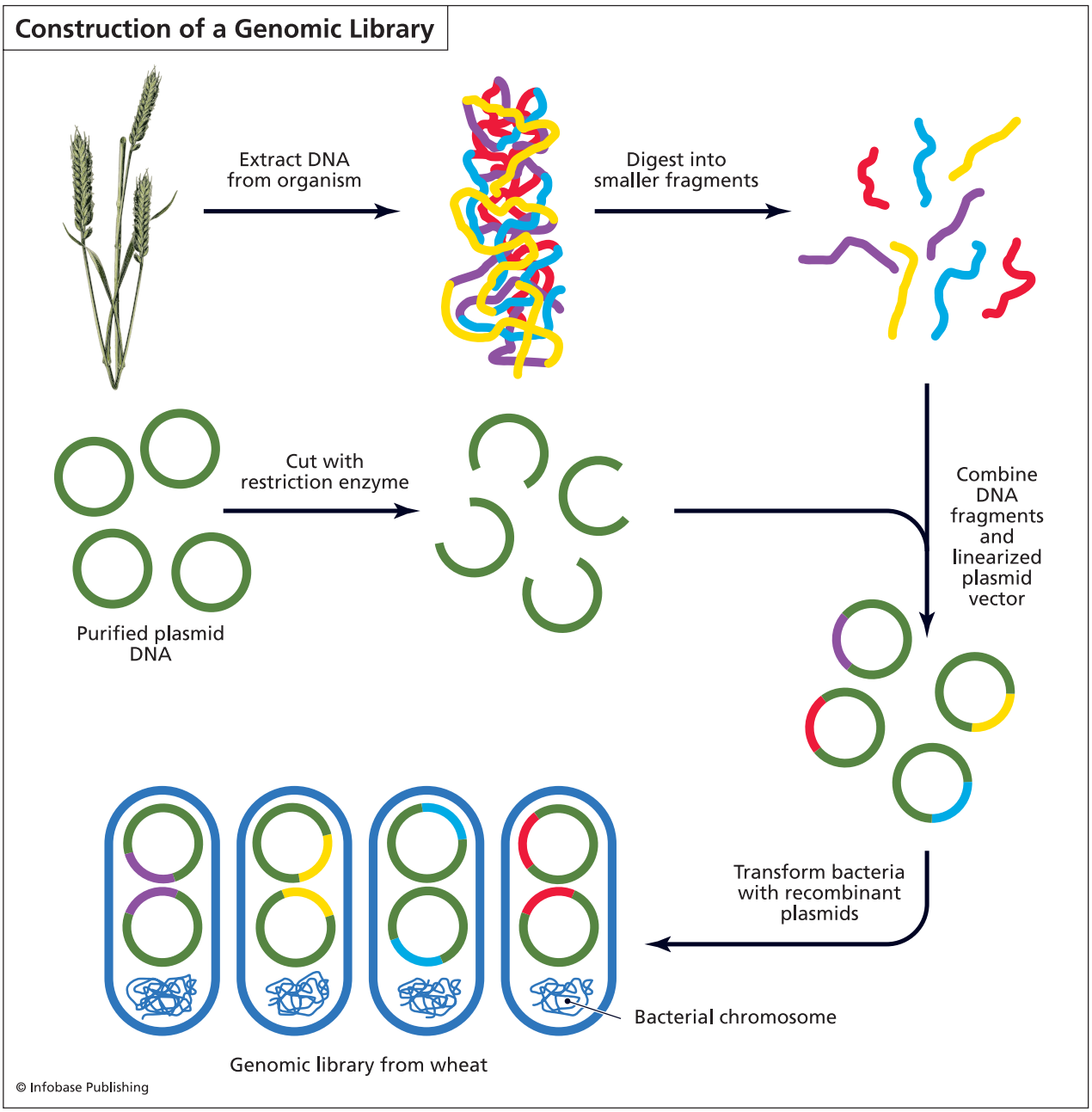
cloning of DNA To clone something means to create an identical copy. Cloning often refers to whole organisms, in other words, making genetically identical individuals. Cloning deoxyribonucleic acid (DNA) means making molecules that are identical copies of an original DNA molecule or portion of one. If the cloned DNA encodes a gene product, then the procedure is referred to as cloning a gene. The process of cloning a gene involves recombinant DNA technology to isolate a foreign gene, insert it into another DNA molecule called a vector, and put it into bacterial cells or the cells of another simple organism such as yeast. When the microorganisms grow and divide to produce new cells (clones), the cloned DNA will also be replicated; thus cloning

DNA allows for the production of large numbers of copies of a gene for further genetic analysis, medical research, or other studies.

In 1972 Paul Berg synthesized the first recombinant DNA, made by using restriction enzymes to cleave DNA from two different organisms and joining the molecules. His hybrid genome contained viral DNA and genes from *Escherichia coli* (bacteria), an achievement that won him the 1980 Nobel Prize in chemistry. The American biochemists Stanley Cohen at Stanford University and Herbert Boyer at the University of California at San Francisco built upon Berg's accomplishment to clone the first gene in 1973. Cohen worked with bacterial plasmids, and Boyer worked on restriction enzymes. Boyer's research group had discovered the commonly used restriction enzyme *Eco*RI in 1968. In a collaborative effort, they used an enzyme to cut a plasmid that already encoded for resistance to the antibiotic tetracycline and, in the cut site, specifically inserted a gene encoding resistance to the antibiotic kanamycin obtained from another bacterium. After sealing the nicks in the plasmid, they introduced the recombinant plasmid into *E. coli*. The bacteria exhibited resistance to both antibiotics. Next, they successfully inserted genes from the toad *Xenopus laevis* into bacteria, the first transplantation of genes from an animal to a bacterial species. In 1980 the Lasker Foundation awarded the Albert Lasker Award for Basic Medical Research to Berg, Boyer, Cohen, and the virologist Dale Kaiser, who also advanced recombinant DNA methodology through his studies of bacteriophage lambda DNA.

Today, cloning a gene often simply means putting a particular gene into a plasmid. Scientists can obtain genes or DNA segments for cloning experiments from gene libraries. Breaking up an organism's DNA into smaller pieces makes it easier to manipulate in the laboratory. Restriction enzymes recognize and cut double-stranded DNA at specific sequences. Most restriction enzymes leave behind single-stranded overhangs that facilitate subsequent attachment of the DNA to other DNA molecules that have been cleaved with the same restriction enzyme. Once the DNA segments are of manageable size, scientists incorporate the fragments into plasmid vectors—small circular DNA molecules that carry DNA between cells of different organisms. A genomic plasmid library is a collection of bacterial colonies that each possess specific plasmid that contains a different DNA segment from a given species, such as human or fruit fly. Collectively, a copy of the organism's entire genome is represented. A complementary DNA (cDNA) library is more specific in that it only contains genes that encode for proteins—noncoding regions of DNA are excluded. The *c* stands for "complementary," as cDNA molecules are molecules of DNA made from messenger RNA (mRNA) molecules using the enzyme reverse transcriptase. Cells synthesize mRNA molecules by reading the DNA and adding ribonucleotides that are complementary to the template strand of DNA to a growing chain of RNA. This process is called transcription, and in eukaryotic cells, the product is a molecule of RNA that contains alternating exons and introns. Exons are the regions of the newly made RNA that encode for protein, and introns are intervening regions that do not encode for protein. Before protein synthesis, splicing occurs; the introns are removed and the exons are joined. The result is a mature mRNA molecule that ribosomes translate into a chain of amino acids during protein synthesis. To make a cDNA library, a researcher extracts and purifies mRNA from cells, then uses that mRNA as a template for reverse transcription, in which the enzyme reverse transcriptase reads the sequence of ribonucleotides on the mRNA and incorporates complementary deoxyribonucleotides into one strand of DNA, which it then uses to make a complementary second strand. The result is a double-stranded segment of DNA that contains only the coding region of a gene that encodes a protein that the original cell type expresses. Because of this, libraries can be specific for a specific cell or tissue type; for example, a pituitary library would only contain the genes expressed by the pituitary gland, which would differ from the set of genes expressed by the testes, for example. The DNA segments are maintained in plasmid vectors for transforming bacteria to generate clones. The library comprises the collection of bacteria containing the DNA from the cells or organisms of interest.

Having access to a premade gene library expedites the process of cloning. The next step is to find the bacterial clone that contains the gene to be cloned. The bacteria are spread out onto semisolid medium called agar to grow. This method of culturing the bacteria allows for individual colonies to be isolated, and each colony contains hundreds of thousands of clones of the original bacterial clone. The researcher must screen the colonies to locate one that contains the gene of interest using one of several different possible methods depending on the goal and on the biological reagents that are available. If the researcher is looking for a gene in one species that has already been isolated and characterized from another species, the researcher can use that DNA in hybridization methods for identifying colonies from the library that contain similar DNA. Hybridization occurs by the formation of hydrogen bonds between nitrogenous bases of complementary nucleotides. Molecules of single-stranded nucleic acid hybridize to other molecules that have



Genomic libraries contain a copy of an organism's entire genome as fragments of DNA inserted into plasmid vectors maintained in bacterial clones.

complementary sequences. A probe is a molecule that is labeled with either a radioactive isotope or a fluorescent dye and is used to search for complementary molecules on a substrate such as a nitrocellulose or nylon membrane to which DNA has been bound. One way to accomplish this is to blot the surface of an agar plate with a membrane so that the bacterial colonies contact the membrane, forming a replica of the pattern of the colonies on the agar plate. The researcher then treats the membrane in a manner that immobilizes the DNA in specific positions on the membrane and denatures the DNA to ensure it is single-stranded and therefore available for hybridization. Subsequent incubation of the probe diluted in a solution with the membrane allows complementary molecules to recognize and bind to, or hybridize with, one another. The radiolabel or fluorescent dye enables detection of the locations on the membrane where the probe hybridized on a piece of X-ray film. Aligning the pattern of dark spots on the film with the colonies on the plate reveals which colonies contain the DNA of interest.

A variation of this method involves the use of antibodies to assay for specific protein products of the genes in the library. Antibodies are proteins made by the immune system that specifically recognize and bind to other molecules including proteins. The antibodies can be tagged with fluorescent labels so the researcher can detect their position on a membrane to which proteins have been immobilized. If the gene product is an enzyme, the researcher can simply assay for the product of the biochemical reaction catalyzed by the enzyme.

Once the investigator has identified the bacterial clones that contain the DNA of interest, he or she can culture the bacteria to grow large quantities. The bacterial cells can be harvested and the plasmid DNA extracted and purified. The researcher may need to move the cloned gene to a different plasmid vector that is more suitable for future investigations. Some vectors are specially designed to express the gene product. Called expression vectors, these plasmids contain a strong bacterial promoter to initiate transcription and an adenine-thymine-guanine (ATG), the triplet codon that initiates translation by ribosomes. The gene might need to be cloned into a different organism in order for the gene product to be properly processed after translation of the mRNA into a polypeptide chain, or the gene might need to be cloned into a different cell type or organism in order to carry out functional studies.

For investigations into the control of gene expression, DNA segments from the regulatory region of a gene may be of more interest than segments from the coding region or the gene product itself. For these studies, the region regulatory elements of a gene may be cloned and inserted into a plasmid in front of a reporter gene, something that is easy to measure. One commonly used reporter gene is β galatosidase, an enzyme that hydrolyzes the disaccharide lactose into glucose and galactose subunits. The activity of this enzyme is often associated with a chemical reaction that causes colonies expressing this protein to turn blue if a certain substrate is included in the medium, making them easy to identify. Green fluorescent protein (GFP), originally isolated from a type of jellyfish, is another popular reporter gene that fluoresces a bright green color when exposed to blue light. One advantage of GFP is that its activity can be measured in living cells, allowing a researcher to monitor the gene's expression under different conditions. When the GFP coding region is attached in frame to the gene of a protein of interest, one can use a microscope to look at cells under blue light and observe the intracellular localization of the protein.

After the cloning of a gene, the potential applications are practically limitless and benefit many people and improve daily life. The advent of cloning has enabled pharmaceutical companies to produce hormones, such as insulin and growth hormone, and blood clotting factors for treating patients whose glands or tissues do not synthesize sufficient quantities. The human genes that encode these proteins have been inserted into bacteria that can be grown in large quantities easily and cheaply. Medical researchers also clone genes from viruses, bacteria, and toxins that bacteria synthesize to make recombinant vaccines to protect people from infectious diseases. Cloning technology has benefited basic research tremendously by allowing biochemists, geneticists, and cell and molecular biologists to study single genes or proteins in controlled conditions. Having the gene inserted into a vector also allows researchers to induce specific mutations and to observe their effects. Commercial industries use cloned genes to manufacture enzymes for use in products such as laundry detergents and biomedical research. Agricultural applications include using cloned genes for genetically engineering crops or livestock that possess traits that improve yields or make them more marketable. The introduction of cloned genes into certain microorganisms increases their efficiency in degrading chemical pollutants for bioremediation purposes.

The polymerase chain reaction (PCR) is a technique used to amplify segments of DNA. Whereas recombinant DNA technology requires several days to generate large quantities of a gene or DNA segment, PCR takes only a few hours. Depending on the future research plans for a particular cloned gene, PCR may eliminate the need to clone the DNA in some cases.

See also BIOTECHNOLOGY; CLONING OF ORGANISMS; DEOXYRIBONUCLEIC ACID (DNA); GENETIC ENGINEERING; POLYMERASE CHAIN REACTION; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

Birren, Bruce, Eric D. Green, Sue Klapholz, Richard M. Myers, and Jane Roskams. *Genome Analysis: A Laboratory Manual Series*. 4 Vols. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1997.

Sambrook, Joseph, and David Russell. *Molecular Cloning: A Laboratory Manual*. 3rd ed. 3 Vols. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2001.

cloning of organisms Biologically defined, a *clone* is a genetically identical copy of an organism. Fragments of genetic material, or deoxyribonucleic acid (DNA), can also be cloned, meaning identical copies have been made and usually inserted into

bacteria for maintenance. Molecular biologists, biochemists, geneticists, and other researchers regularly clone genes or pieces of DNA as part of standard laboratory procedures. Cloning organisms, however, is much different and falls under the realm of developmental biology rather than genetics. In this case, cloning means creating a whole organism that is genetically identical to another; the individuals are referred to as clones.

For organisms that reproduce asexually, cloning is no big deal. Asexual reproduction is the creation of new individuals from one parent and is the normal means of reproduction for many bacteria, fungi, protists, and numerous plants. Most unicellular organisms replicate by synthesizing an identical copy of their DNA, then splitting into two cells, with each cell receiving the same genetic information. Some multicellular organisms can also reproduce asexually. For example, fungi can reproduce both asexually and sexually, depending on whether two different organisms contribute genetic material to the spores that develop into new complete organisms. Plants can also naturally create genetically identical but separate organisms by several different methods. For example, strawberries asexually reproduce via stolons, extensions that grow outward from the base of a plant along a horizontal surface. Every so often, the stolon makes contact with the ground and forms an organized structure from which roots grow downward to penetrate the soil and shoots grow upward, forming a new plant that can grow independently if separated from the parent clone. The eyes of potatoes are really buds that can also sprout to form new plants. Gardeners and horticulturists often take cuttings to propagate a favorite plant asexually.

Though in all the preceding examples the new organisms that are formed are indeed clones of their parent, in most discussions, *cloning* refers to cloning animals, a complex process that requires technological intervention. While nature can accomplish this by the spontaneous division of a fertilized egg into two separate embryos that become identical twins, purposeful animal cloning is a feat of cell biology and embryology. Success is due to the hard work of numerous experimental embryologists who each contributed their insight and skills to put into practice today what a century ago was considered the domain of God, the creation of designed life.

BRIEF HISTORY OF ANIMAL CLONING

The German embryologist Hans Spemann performed one of the first vertebrate animal cloning experiments more than 100 years ago, in 1902, when he split apart a two-celled salamander embryo and demonstrated that each embryonic cell contained all of the information necessary to create a new organism. He went on to show that as development progressed and cells became more differentiated, they lost the ability to form entire new organisms. Spemann won the Nobel Prize in physiology or medicine in 1935 for discovering the organizer effect, an important aspect of early embryogenesis by which one group of cells influences the developmental pathway taken by another group of nearby cells, a phenomenon today called embryonic induction. In 1938 Spemann also first conceived of nuclear transfer, the transfer of a nucleus from one cell into another from which the nucleus has been removed. Nuclear transfer later became the basis for successful animal cloning procedures. In 1952 the American biologists Robert Briggs and Thomas J. King cloned tadpoles. Their protocol involved removing a nucleus from a blastula-stage cell (an embryonic stage during which the cells form a fluid-filled sphere but have not yet begun to differentiate into specialized tissues) and inserting it into a fertilized egg from which they had already removed the nucleus. After numerous attempts failed, they eventually produced 27 tadpoles from 104 nuclear transfers.

In 1958 the American biologist F. C. Steward grew whole carrot plants from differentiated root cells. This was a significant event, because in vivo, after a cell becomes completely differentiated, there is no turning back. A liver cell remains a liver cell until it dies; a fat cell cannot convert into a neuron to replace damaged brain tissue. Steward's root cells formed all of the new tissue types necessary to create a whole plant. An animal is different from a plant, however, given that many plants naturally reproduce by creating clones of themselves. Only a few years later, in 1962, John Gurdon cloned frogs from differentiated intestinal cells. This would have been a major milestone, but critics doubted whether what Gurdon thought had happened really did happen. Scientists still do not know whether in fact Gurdon's reported frogs did result from successful cloning using differentiated cells, but the event put cloning in the limelight.

Over the next two decades, recombinant DNA technology took center stage, leading to the creation of genetically engineered bacteria in 1973. Meanwhile, the Danish scientist Steen Willadsen perfected a method for freezing and thawing livestock embryos that resulted in live births. The next real advance in cloning was Willadsen's 1984 success in producing a live lamb by fusing cells from an eight-cell lamb embryo with unfertilized eggs. He coated the embryos in agar for protection, grew them in a sheep oviduct for one week, and then transferred the developing embryos to a surrogate mother sheep, one of which resulted in a live birth, the first cloned mammal. The following year Willadsen birthed a new

industry cloning cattle for Grenada Genetics, and in 1986, he had success using nuclei from differentiated cells of a one-week-old cow embryo. A team in Wisconsin, Neal First, Randal Prather, and Willard Eyestone, used a slightly different method to clone cattle. They fused single-cell embryos to fertilized eggs using an electric current, allowed them to develop to the eight-cell stage within oviducts that were removed and kept alive in culture, and then transplanted them into a surrogate.

In 1995 Sir Ian Wilmut and Keith Campbell at the Roslin Institute in Scotland produced two cloned sheep from differentiated embryo cells by nuclear transfer. They discovered that a key step was to synchronize the cell cycles of the nuclear donor and recipient cells. The following year Wilmut and Campbell cloned the first sheep, named Dolly, from differentiated adult udder cells. Though experiments had been under way for years with the goal of cloning animals from differentiated cells, the announcement of Dolly's birth stunned the world. To the general public, this event made the possibility of cloning humans closer to home. One year after Dolly, the Roslin group reported success in creating the first transgenic lamb, Polly, who expressed a human gene for factor IX, a blood-clotting protein used to treat hemophilia.

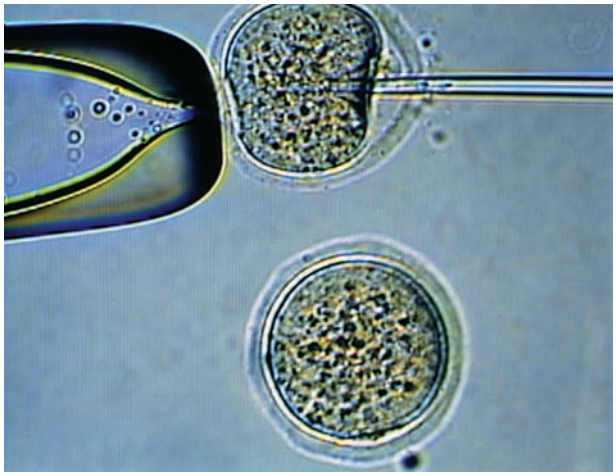
Dolly the sheep was the first mammal cloned from adult differentiated cells. *(AP Images)*

Since the successes at the Roslin Institute, numerous other mammals have been cloned. In 1998 Ryuzo Yanagimachi and Teruhiko Wakayama, at the University of Hawaii, reported that they cloned 50 mice, including some clones of clones. The technique, now called the Honolulu technique, was more efficient and involved the injection of nuclei from cumulus cells into an enucleated egg. Cumulus cells are cells that surround eggs during development inside the ovaries. After letting the cells recover for six hours, they added chemicals to the medium to stimulate cell division, and they transplanted the embryos into a surrogate mother after reaching the blastocyst stage. A year later, they cloned the first male, using nuclei from cells removed from the tip of a male mouse's tail. To date, the following mammals have been cloned: mice, sheep, cows, pigs, cats, a rhesus monkey, rabbits, mules, a deer, a dog, a horse, rats, and a guar.

HOW IT IS DONE

Somatic cell nuclear transfer, the method used to create Dolly and many other cloned animals, requires a great amount of skill and time. The goal is to create an egg that contains a nucleus from a donor cell of the individual to be cloned. The term *somatic* means the cell is not from a reproductive cell such as an egg or sperm, but is from another part of the body. A skilled technician uses a micromanipulator to insert a needle into an egg cell and gently sucks out the nucleus, which contains all of the DNA. Enucleation, the process of removing the nucleus, essentially turns an egg cell into a cloning factory. The genetic material is gone, but the rest of the egg contents provide the necessary building blocks for biomolecules: amino acids, sugars, nucleotides, and fatty acids. The egg cell also contains the machinery and enzymes necessary to synthesize proteins and factors that carry out cell division.

The technician then uses another needle to insert a different nucleus from a cell of the animal to be cloned into the enucleated egg. Alternatively, after removing the nucleus from an egg, the egg can be fused with a whole cell from the animal to be cloned. Because the nucleus is from a differentiated cell, reprogramming of its genetic material is necessary in order to create a whole new individual. During normal development, the DNA of differentiated cells becomes chemically modified by the addition of methyl groups ($-CH_3$) to the nitrogenous bases of DNA, an event that leads to the inactivation of genes that a specialized cell will not need to express. Early embryonic cells are totipotent, meaning they each individually have the capacity to give rise to



In the process of nuclear transfer, a micropipettor is used to remove the nucleus from one cell and replace it with the nucleus removed from another cell. *(James King-Holmes/Photo Reseachers, Inc.)*

a complete individual. As cells become specialized, they lose this ability (in part because of methylation of the chromatin); that is why successful cloning from differentiated cells was such a milestone. The egg cell "reprograms" the donor nucleus by removing the previously added methyl groups and then reestablishing new methylation patterns.

Administration of an electrical shock stimulates the manipulated cell to divide, beginning the process of embryogenesis. Each of the embryo's cells, called blastomeres, contains nuclear DNA identical to that of the parent clone, the organism from which the nucleus was taken: in other words, the embryo is a clone.

For some applications, such as research purposes or therapeutic cloning, the cloned embryonic cells are the final goal. When the goal is to create a complete cloned organism, transplantation of the embryo clone into a surrogate mother follows. If transplantation is successful and the surrogate mother's uterus implants the embryo and accepts the pregnancy, gestation follows the normal course. Despite all the progress by cell and developmental biologists, the success rates are still very low, less than 1 percent on average, with variation depending on the species and the techniques used.

In January 2008 a group of scientists published a report of their success producing cloned human embryos from adult skin cells using somatic cell nuclear transfer (French et al., 2008). While this is an important step in the development of stem cells from somatic cells, the scientific community eagerly awaits the creation of cloned human embryos that mature and produce patient-specific embryonic stem cells from which a stem cell line could be created.

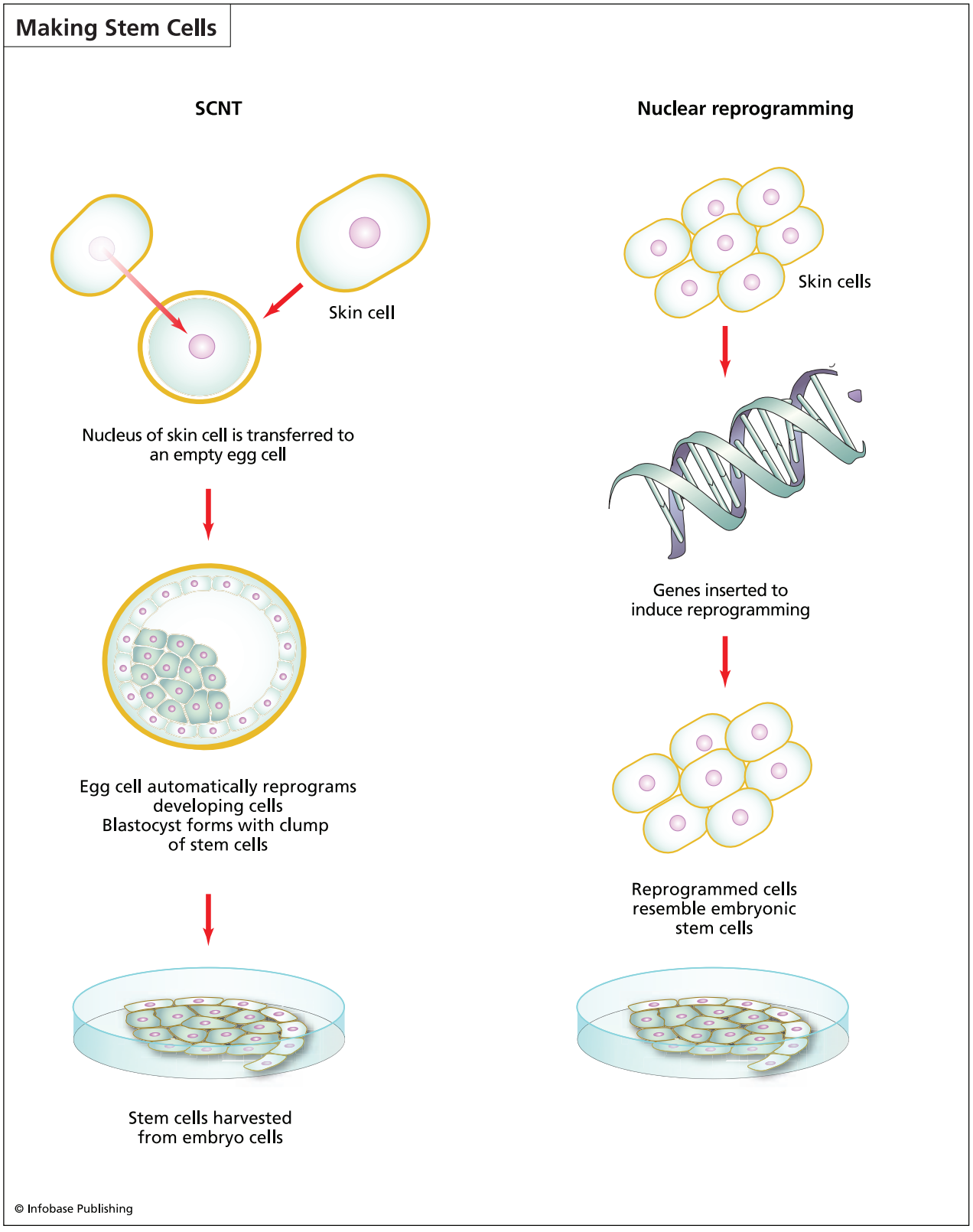
In November 2007 two groups reported success at reprogramming human skin cells into cells that resembled embryonic stem cells. In a study led by James Thomson of the University of Wisconsin-Madison, researchers showed that four factors were sufficient for reprogramming somatic cells to become pluripotent stem cells. Called "induced pluripotent cells," the cells exhibited the essential characteristics of embryonic stem cells and had the developmental potential to differentiate into cells of all three primary germ layers. In the second study, led by Shinya Yamanaka at Kyoto University in Japan, researchers inserted four genes (two of them different from those of the first study) into adult human fibroblasts. Their induced pluripotent cells also exhibited stem cell–like characteristics. Because reprogramming does not utilize eggs or embryos, this newer technique diminishes some of the ethical objections that cloning raises, but reprogrammed cells carry an increased theoretical risk for cancer. John Gearhart, the scientist who discovered human fetal embryonic stem cells, claims this alternative approach "is the future of stem cell research."

APPLICATIONS OF ANIMAL CLONING

While polls show that the majority of people overwhelmingly disapprove of the application of cloning technology for the purpose of reproducing humans, there are many other beneficial applications for cloning. Animal cloning methodology can be used for the commercial production of useful gene products, for cloning farm animals with desirable characteristics, and for therapeutic medical uses and has advanced and will continue to advance research in many different fields, especially developmental biology.

One beneficial application of cloning technology is the bioengineering of animals to produce useful proteins. A transgenic animal is one whose genome has been deliberately modified, such as by the insertion of a gene from another species. The first transgenic sheep, Polly, expressed a human gene that encoded a clotting factor used to treat hemophilia patients. Transgenic cattle have also been or may soon be created to produce and secrete in their milk proteins such as insulin and growth hormone and proteins for treating diseases such as emphysema, cystic fibrosis, or phenylketonuria. Transgenic animals created by cloning can also be designed to exhibit specific disease symptoms, so medical researchers can use them as living disease models to understand the disease better and to explore treatments.

For thousands of years farmers have used selective breeding to improve the yields and quality of agricultural products of animals, such as milk, meat, and wool. Farmers would profit from cloning their



In somatic cell nuclear transfer (SCNT), after transferring the nucleus from the parent clone to an enucleated egg, the resulting embryo cells can be used for research purposes, as in therapeutic cloning. In nuclear reprogramming, genetic factors are inserted into adult cells, causing them to "regress" back into a state that resembles stem cells capable of differentiating into any cell type.

fattest cattle and pigs, and the U.S. Food and Drug Administration preliminarily ruled in late 2006 that milk and meat from cloned cows, goats, and pigs is perfectly safe for human consumption. The general public, however, hesitates to embrace the idea of eating cloned animals, mostly because of lack of understanding of the process of cloning and the perception that cloning makes something unnaturally grotesque or monsterlike. Another desirable characteristic that could be potentially engineered into farm animals is disease resistance.

The potential benefits of therapeutic cloning may force people to overcome their negative feelings about cloning. Therapeutic cloning is the use of somatic cell nuclear transfer and other cloning methodology for the purpose of carrying out stem cell research and studying and curing diseases. One possible application for this methodology is to treat diabetes. To accomplish this, a cell from a diabetes patient is cloned using an unfertilized egg cell. After stimulating the cell to start dividing in vitro, the stem cells can be harvested and transplanted back into the patient. Because the transplanted cells would contain the same genetic information, the patient's immune system would not recognize them as foreign and thus would not reject them by mounting an immune attack. Inside the patient, the cells could differentiate into cells that produce and secrete insulin and respond to the appropriate regulatory control mechanisms, minimizing the need for constant monitoring and adjustments in hormonal administration. Patients with neurodegenerative diseases such as Parkinson's and Alzheimer's diseases as well as spinal cord injuries or brain damage from a stroke may also benefit from therapeutic cloning. Nerve cells do not proliferate after differentiation; thus once they become damaged, the body cannot replace them. By cloning cells from patients with these conditions, scientists could create undifferentiated stem cells with a patient's own genetic material that upon transplantation into the patient could potentially replace the damaged cells and restore nervous system function.

Therapeutic cloning would also allow researchers to study the development of certain diseases, to learn how cells become diseased, and to follow the molecular and cellular events associated with the progression from normal to malfunctioning cells. A better understanding of these processes could lead to novel therapies and treatments, which could also be studied using cloned cells, tissues, and organisms that have been designed to model the disease.

Thousands of patients who await organ transplantation each year could also benefit from the applications of therapeutic cloning research. The number of organs that become available by the death of others falls far below the number needed. Another problem is that when organs are available, they must be a perfect match for molecular markers expressed by the recipient's tissues; otherwise the recipient's immune system will attack and attempt to destroy the transplanted organ. Even when the tissues match fairly well, the recipient must take immunosuppressant drugs for the rest of his or her life, which make him or her susceptible to infections and other maladies. One means to overcome the availability problem is to use organs or tissues from other species, such as pigs. This procedure, called xenotransplantation, still carries the risk of rejection. Using therapeutic cloning to create an organism that expresses the same molecular markers as the transplant recipient could prevent the patient's immune system from recognizing the transplanted tissue or organ as foreign and thus prevent rejection. In the future, perhaps scientists could grow whole new organs from embryonic cells created by somatic cell nuclear transplantation, both eliminating the risk of rejection and reducing the wait time for donor organs.

One can imagine that reproductive cloning could help restore populations of endangered species. In 2001 a biotech company cloned a guar, a type of endangered ox, using a cow as the surrogate mother for gestation. The guar died at two days old of dysentery. In 2001 Italian scientists reported using adult cells to clone a mouflon, an endangered species of wild sheep, which now resides at a wildlife center in Sardinia. One problem with cloning endangered species is that the egg and the surrogate used in the process are different species from the animal being cloned. Also, cloned populations would not have the genetic variability that is necessary for species to survive environmental challenges.

HUMAN CLONING

The cloning of humans raises many complex issues. Many concerns are ethical, but numerous unanswered scientific questions also remain, making it impossible for any well-educated panel to make intelligent, informed decisions regarding human cloning. Scientists do not know how human cloning will differ from animal cloning, which itself continues to be plagued by unresolved questions and difficulties. The success rate of cloning from nuclear transfer is very low because of tremendous stress placed on the nucleus introduced into an egg. Dolly was the single successful live birth resulting from 277 nuclear transfers. How many sacrificed embryos would be necessary to achieve success in cloning a human? Scientists also cannot predict the long-term effects of cloning. Cloned animals have demonstrated symptoms associated with an age much greater than the time since birth should permit. If an animal clone results from the transfer of a nucleus taken from

a cell of a five-year-old, is the cellular age at birth already five years? Cell biologists believe that cells may have predetermined life spans, so are clones doomed to shorter life expectancies? Will clones have any acquired characteristics of their clone parent? Would any changes that occurred to the parental clone's DNA, such as rearranged genes, chemical modifications to subunits of the DNA, and the manner of packaging the DNA into chromosomes, be transmitted to cloned offspring? Nuclei from cells at earlier stages of development should have fewer permanent changes.

A few of the ethical questions include, When does life begin? Considering the low success rate, what number of sacrificed embryos is acceptable in order to achieve a live birth? Who should be cloned? Must certain criteria regarding degree of intelligence, health, fertility, lack of known genetic defects, or family history be met? Who makes these decisions? Who pays for the process? What rights do the clones have?

A few international organizations have policies regarding cloning. The United Nations (UN) approved a nonbinding ban on all human cloning in 2005, but the wording does not specifically address somatic cell nuclear transfer. The Royal Society, one of the world's oldest and most respected scientific organizations, denounced the UN for adopting the "ambiguous and badly-worded" declaration. The European Court of Human Rights of the Council of Europe added a protocol to their Convention on Human Rights and Biomedicine that was entered into force in 2001 banning any intervention that seeks to create genetically identical human beings, and though the treaty specifically mentions nuclear transfer, it is unclear whether this procedure is permitted for therapeutic cloning. The United Kingdom passed legislation in 2001 that allows somatic cell nuclear transfer for research purposes, as long as permission is first obtained from the Human Fertilization and Embryology Authority. That same year, U.S. president George W. Bush announced that federal money could not be used in research that involved harvesting new cells from embryonic stem cells (including for therapeutic cloning) but allowed for continued research on cell lines from embryonic cells previously harvested. No federal laws address reproductive or therapeutic cloning using private money, but some states have taken action on this issue. To date 13 states have enacted legislation that prohibits reproductive cloning, and two others prohibit the use of public money for such research. Seven of the 15 states that have laws addressing cloning specifically allow therapeutic cloning. Some fear that the U.S. ban on stem cell development and research is leading to a decline in the country's competitive edge in scientific research, but some very reputable U.S. research institutions including Harvard University and the University of California at San Francisco are getting around the ban by establishing privately funded programs. Other countries have recently lifted bans on therapeutic cloning. Australia lifted its ban on therapeutic cloning for research in late 2006 but requires that embryos be destroyed within 14 days. In 2004 France approved a law allowing stem cell research using human embryos and banning reproductive cloning. A Japanese government panel also approved the use of human clones for research purposes that same year.

See also CELLULAR REPRODUCTION; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; REPRODUCTION; WILMUT, SIR IAN.

FURTHER READING

Edwards, J. L., F. N. Schrick, M. D. McCracken, S. R. van Amstel, F. M. Hopkins, M. G. Welborn, and C. J. Davies. "Cloning Adult Farm Animals: A Review of the Possibilities and Problems Associated with Somatic Cell Nuclear Transfer." *American Journal of Reproductive Immunology* 50 (2003): 113–123.

French, Andrew J., Catharine A. Adams, Linda S. Anderson, John R. Kitchen, Marcus R. Hughes, and Samuel H. Woods. "Development of Human Cloned Blastocysts following Somatic Cell Nuclear Transfer (SCNT) with Adult Fibroblasts." *Stem Cells* published online (January 17, 2008). Available online. URL: http://stemcells.alphamedpress.org/cgi/reprint/2007-0252v1.pdf.

Panno, Joseph. *Animal Cloning: The Science of Nuclear Transfer*. New York: Facts On File, 2004.

Takahashi, Kazutoshi, Koji Tanabe, Mari Ohnuki, Megumi Narita, Tomoko Ichisaka, Kiichiro Tomoda, and Shinya Yamanaka. "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors." *Cell* 131, no. 5 (November 2007): 861–872.

U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program. "Human Genome Project Information: Cloning Fact Sheet." Available online. URL: http://www.ornl.gov/sci/techresources/Human_Genome/elsi/cloning.shtml. Updated August 29, 2006.

Yu, Junying, Maxim A. Vodyanik, Kim Smuga-Otto, Jessica Antosiewicz-Bourget, Jennifer L. Frane, Shutan Han, Jeff Nie, Gudrun A. Jonsdottir, Victor Ruotti, Ron Stewart, Igor I. Stukvin, and James A. Thomson. "Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells." *Science* 318, no. 5858 (December 21, 2007): 1,917–1,920.

community ecology Ecology is the study of the interactions between organisms and their environment, including both living and nonliving

components. Populations of different species that live near and interact with one another form biological communities. The boundaries that define a biological community may be small, such as a community of phytoplankton, aquatic plants, zooplankton, invertebrates, and fish that live in a pond. Alternatively, the boundaries may be vast, such as those demarcating the African savannah. The size depends on the research focus of the individual scientist studying the community.

Community ecology is the study of how interactions of living components within an ecosystem affect that community's structure and organization. Within communities, the organisms depend on one another for survival and reproduction. For example, insects, birds, and bats pollinate plants, helping them to reproduce with other individuals that may be located at too great a distance for wind alone to accomplish this. The plants provide food in the form of nectar or pollen for the pollinators. Their green tissues serve as food for plant eaters in a community, and their seeds as food for other organisms. The roots of plants help stabilize the soil from erosion, and they also provide shade and shelter for other animals. The numerous, complex interactions are not always obvious; unraveling them is the major goal of community ecologists.

INTERSPECIFIC INTERACTIONS

The individuals and populations of species within a biological community interact in a variety of ways. In a mature community, each species plays an important role in maintaining the community's health, or stability. This does not mean that all the individuals benefit from interactions with individuals of other species; rather, the community as a whole benefits, as does the ecosystem to which it belongs. In some cases, the link between two or more species is so close that the species coevolve: that is, their dependence on each other limits the extent to which modifications may occur. Relationships between two species (the simplest situation) include competition, predation, herbivory, symbiosis, and disease.

Competition is the demand of two or more organisms for an environmental resource that is in short supply. The 19th-century naturalists Charles Darwin and Alfred Russel Wallace evoked competition as a major factor driving natural selection. They suggested that because environmental resources such as food and space are limited, and because individuals produce more offspring than can survive, species must compete for such resources. In doing so, over evolutionary time species develop adaptations that confer an advantage to them in that particular environment. Intraspecific competition occurs between members of the same species. For example, damselfish compete for nooks and crannies in coral reefs that they use for shelter and spawning and from which they obtain food. Interspecific competition is the condition when populations of different species compete.

Each species has its own niche within a biological community. A niche is the sum of the biotic and abiotic environmental factors that affect the survival, growth, and reproduction of a species and includes the relationships between that species and others in its community. In 1934 G. F. Gause formulated the competitive exclusion principle, which states that two species cannot occupy the same niche in a community. Many species have similar needs or have overlapping roles in a community, and this feature of a community helps maintain the function of that ecosystem. The more biodiverse a community is, the more overlapping roles there will be, and the better chance that if the population of one species decreases, the function it performed will still be carried out by another species. Though some overlap in roles is good, the roles they play must differ in some respect, or one of the species (the one more favorably suited for that environment) will eventually overtake the other in the competition for resources. The two species cannot occupy identical niches. For example, the Galápagos have numerous species of finches that differ in their food source. Even though many feed on seeds, the beaks of certain species are adapted to feed on different-sizes of seeds and seeds of varying hardness, thus reducing the competition between the finch species, allowing them to coexist. The degree of niche overlap correlates with the degree of competition that a species faces, and over time competition for a niche can lead to a phenomenon called character displacement, in which species evolve to occupy different niches.

Factors that influence interspecific competition in plants include exposure to sunlight, water availability, and nutrient availability in the soil. Other factors such as composition or acidity of the soil can affect which species will "win" the competition and dominate a region when planted together. This condition can lead to a gradient of species at boundaries where environmental conditions change, such as from a low to a higher altitude or from a coastal region moving inland.

Another type of interspecific interaction is predation. Predators hunt and kill other organisms to obtain their food. In response to hearing the word *predator,* one often thinks of large animals such as mountain lions, sharks, or snakes. Indeed these are examples of successful predators, but so are sea stars that prey on mussels, and so are stoneflies that feed on mayflies, caddis flies, and true flies. As do all interspecific interactions in a community, predation plays

Many leguminous plants, such as the broad bean shown here, participate in symbiotic associations with nitrogen-fixing bacteria that form nodules on the roots. *(Adam Hart-Davis/Photo Researchers, Inc.)*

an important role in maintaining the structure of a community by controlling population size. Because predators are consumers, and often secondary or tertiary consumers, they have a significant impact on all the trophic levels of an ecosystem, particularly considering the amount of biomass and energy necessary to support organisms at higher trophic levels.

Organisms that feed off plants or algae, even though they sometimes kill them by doing so, are usually not referred to as predators but simply herbivores. (Most herbivores feed off living plants.) Like predation, herbivory is a type of interspecific interaction in which one organism exploits another to increase its own fitness at the expense of the fitness of another species. Herbivores in both terrestrial and aquatic environments have unique adaptations that allow them to ingest and grind up plant material, process and digest it (this often involves symbioses, discussed later), and distinguish between poisonous and nonpoisonous food sources.

Symbiotic associations are intimate interspecific interactions. Two or more species live in direct contact to the benefit of at least one of them. Mutualistic symbioses benefit both species, as exemplified by the bacterium *Rhizobium* and broad bean plants. Although nitrogen is abundant in the atmosphere in the form of nitrogen gas (N_2), most organisms cannot use this form because of the strong triple covalent bond that holds the two nitrogen atoms together. Certain species of bacteria, such as *Rhizobium,* are capable of fixing this nitrogen by converting it into ammonia (NH_3), which plants can readily incorporate into organic molecules such as amino acids and nucleic acids. Some nitrogen-fixing bacteria live freely in the soil, but others live in symbiotic associations within the roots of plants, mostly legumes. The plant benefits because it has a built-in source of available nitrogen, which is often a limiting nutrient for plants. Though the bacteria expend much energy fixing more nitrogen than they will use themselves, in return they receive a steady supply of carbon, which the plants fix through photosynthesis.

Another common example of symbiosis involves herbivores and microorganisms that live in their guts. The microbes produce an enzyme that allows them to digest cellulose, the main component of plant cell walls. Herbivores that do not produce this enzyme but harbor microorganisms that do can extract more energy from their food. The microorganisms benefit from the warm, moist environment in the animals and the constant supply of food.

In commensalistic symbiotic associations, one species benefits, but the other is neither harmed nor helped. These sorts of relationships are difficult to document in nature, as close linkages between species typically have some effect, either positive or negative, on both species. The western clown anemone fish and the sea anemone demonstrate one possible commensalistic symbiosis. The fish hides within the tentacles of the sea anemone to escape predators. The anemone protects the fish because its tentacles cause

The emperor shrimp hangs on to the skin of an inedible animal, such as the sea cucumber shown here, in order to hide from predators. Because the shrimp benefits, but the sea cucumber neither benefits nor is harmed, the relationship is called commensalistic. *(Georgette Douwma/Photo Researchers, Inc.)*

Many wasps, such as *Aleiodes indiscretus* shown here, parasitize caterpillars, such as this gypsy moth caterpillar, by drinking their blood, or hemolymph. *(USDA/Agricultural Research Service/Scott Bauer)*

poisonous stings to most species, but the anemone receives no obvious advantage from the fish.

In parasitism, another type of symbiosis, one partner benefits from the association, but the other one suffers from it. The parasite feeds off the host's bodily fluids or tissues. Endoparasites live inside a host; for example, pinworms live inside the intestines of dogs. Ectoparasites feed from an external surface on the host. For example, mosquitoes drink blood from a host, but they do not enter the host's body. Another type of parasitism involves an insect that lays eggs on or in a host, so when the eggs hatch, the larvae have a ready food source.

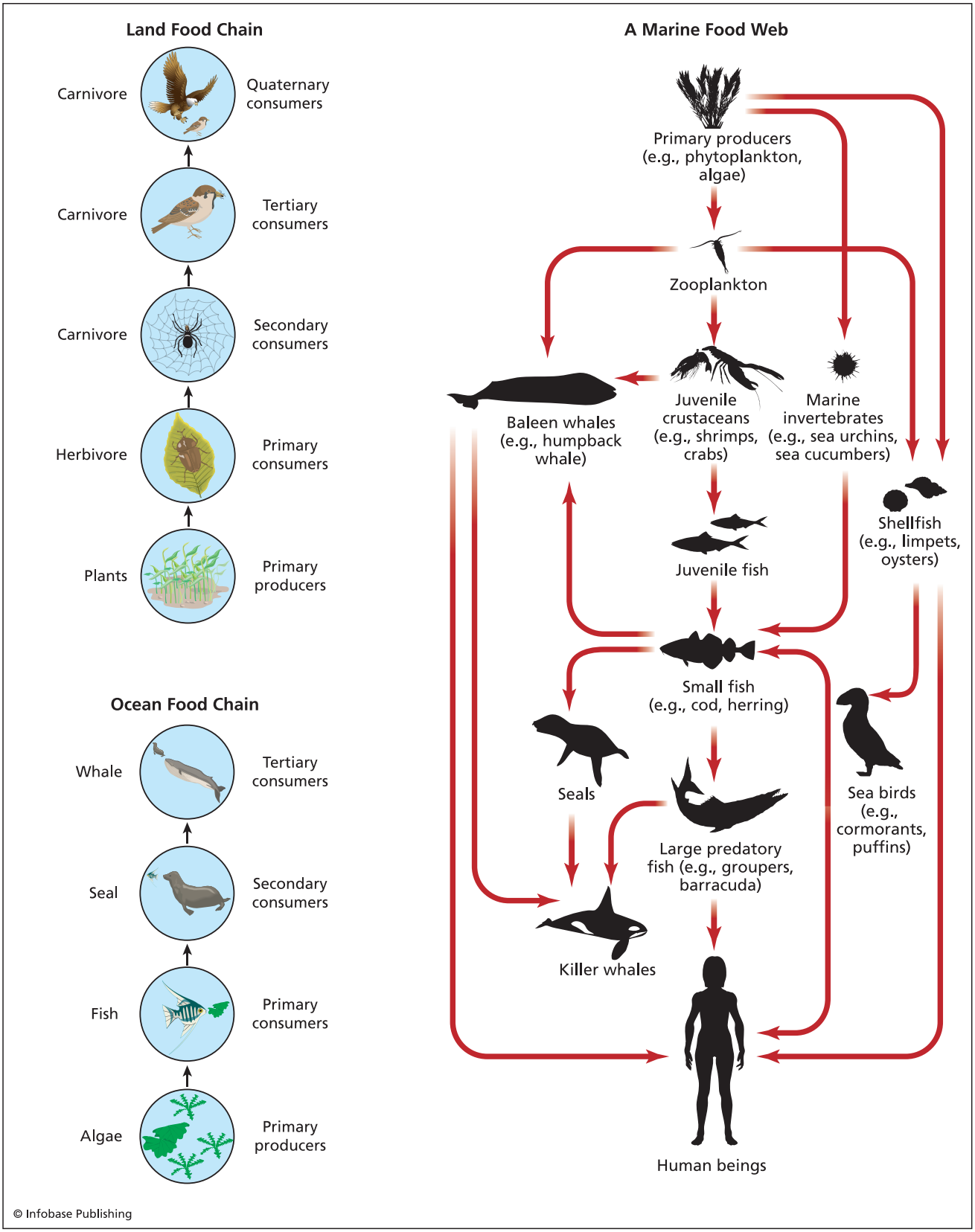
Pathogens are similar to parasites in that they benefit while causing harm to their host organism. Because parasites depend on a living host for continued nourishment, however, they typically do not kill their host, whereas pathogens cause diseases and often kill their host as a result. Another difference between pathogens and parasites is that pathogens are usually microorganisms such as bacteria, fungi, or viruses, whereas parasites are usually unicellular eukaryotic organisms or multicellular animals such as worms. Examples of pathogens and the diseases they cause are the bacterium *Treponema pallidum,* which causes syphilis, and the influenza virus that causes flu.

COMMUNITY STRUCTURE

Community structure refers to the sum and the result of all the relationships among populations of different species in a community. Structural characteristics of a community include features such as the composition (which species are present), the abundance (how many of each species are present), and the diversity (how many types of species are present). The interspecific interactions affect these aspects of a community, particularly the trophic structure, or feeding relationships. Food chains depict the feeding relationships in a community. Primary producers, organisms that can fix carbon from inorganic sources to form organic molecules, are found at the bottom of a food chain. These are most commonly photosynthetic organisms but can also be chemosynthetic organisms, as is the case in hydrothermal vent communities. Organisms that feed off primary producers are called primary consumers. Secondary consumers feed off primary consumers, and tertiary consumers feed off secondary consumers, and so on. Because a tremendous amount of energy is lost at each level, the maximum for most communities is four or five links in a food chain. Food webs are more accurate representations of the real feeding relationships in a community. They recognize and depict the fact that consumers feed off several types of food and also can be eaten by more than one type of predator.

Some species have a greater impact on the community structure and function than others. In most communities, one or a few species are more abundant than the others. Called the dominant species, these species usually garner a larger share of the community resources, but in return they contribute most to the ecosystem's productivity. Ecosystems are often named for their characteristic dominant species; for example, a few common C_4 grasses (grasses that undergo a specialized form of photosynthesis as an adaptation of living in hot, dry climates) dominate tallgrass prairies, and the large brown seaweed called kelp dominates the kelp forests of the ocean. Recent research has shown that dominant species of a community play as much if not more of a role than species diversity in maintaining an ecosystem's stability and function.

Keystone species are species that have a great impact on community structure even though they represent a low proportion of the community's biomass or contribute a small percentage to the community's productivity. For example, though the removal of any species within a community will cause a shift or disturbance in the community's structure, the



Food chains depict the movement of energy from lower to higher trophic levels, whereas food webs depict a more complete representation of the numerous complex interactions occurring among the different populations of a community.

removal of a keystone species alters it tremendously. Because of this, keystone species are an important focus of conservation biologists. One example of a keystone species is the African elephant that roams the grasslands of Africa. If the elephants were removed, such as by hunting, woody plants would overgrow because elephants normally pull out young trees by their roots. If allowed to grow, the woody plants would block the sunlight from the grass, and the grasslands would transform into forests or shrub-dominated ecosystems.

Foundation species are those that control the physical structure of a habitat in which a community lives. For example, beavers can convert forests into flooded wetlands by chopping down trees and building dams. When a foundation species is removed or becomes greatly reduced, such as by global climate changes, the introduction of exotic species, or pests, the physical structure changes in ways that disturb the stable local conditions and ecosystem processes. For example, the human introduction of the hemlock woolly adelgid, an insect native to Asia, has reduced the number of eastern hemlock trees in riparian forest communities (those near riverbanks) in the eastern United States. Hemlocks can grow up to 150 feet tall (45 m) and measure up to six feet (1.8 m) in diameter. These insects destroy hemlocks by feeding on the tree sap, disrupting the nutrient flow, and the needles fall off. Without the needles, the tree dies within about five years of infestation. The massive size of the hemlocks provides much-needed shade at the riverbanks or along creeks. This helps moderate the local temperature, crucial to the survival of trout and cold-water species of the water body, which play a key role in the trophic structure of the aquatic ecosystems. Because of this, hemlocks are considered foundation species, or ecosystem engineers.

Ecologists use different models to describe community organization. In bottom-up control, the species at lower trophic levels exert unidirectional influence on the structure of the community. For example, the availability of nutrients in the soil affects plant growth, which in turn affects the numbers of herbivores and other species moving up the trophic levels. Top-down models purport that predators have the largest effect on community organization. For example, reduction in the number of a species at higher trophic levels will allow species at a lower trophic level to overgrow. The species at the next lower level will decline as a result because it serves as food for the now overpopulated species. More realistic models are intermediate, involving controls that exert their influence in both directions, but the simplified models facilitate analysis. Communities probably fluctuate temporally in the degree of bottom-up or top-down control, something that continued research will help ecologists better understand.

DISTURBANCE

Biologists once thought that mature communities reached a point of equilibrium, at which the relative species composition, abundance, and diversity were established. Ecologists are realizing that many communities are as dynamic as the organisms that inhabit them. Communities evolve when challenged, as do species. Any event that alters a community is called a disturbance. Natural phenomena such as a hurricane, a forest fire, an epidemic of a disease, or destruction of a habitat as a result of human influence can all disturb a community. Disturbance is not necessarily bad; it can open up opportunities for new species to join a community or fill a niche previously occupied by a different species. Human-induced disturbances, such as urban development and clearing of vegetation for agricultural purposes, do often cause negative effects, such as loss of biodiversity or reduction in ecosystem services.

Natural disturbances such as a volcanic eruption or glacial formation and action remove all or most of the vegetation in an area. The processes by which the habitat reforms after the volcanic activity ceases or after a glacier retreats are somewhat predictable. Succession is the stepwise process in which communities reform at a habitat that has been dis-

The combined characteristics of the photosynthetic organism and the fungus that make up a lichen allow lichens to inhabit areas that do not contain fertile soil; thus lichens are usually among the first organisms to inhabit relatively lifeless areas during primary succession. *(Robert F. Balazik, 2007, used under license from Shutterstock, Inc.)*

Retreating glaciers, such as this one in Alaska, leave behind relatively lifeless habitats that become recolonized through ecological succession. *(Charles D. Winters/Photo Researchers, Inc.)*

turbed. The species that initially recolonize the area are eventually displaced by a new group of species, and then another group, as a result of the physical effects the living organisms have on the environment. Primary succession is the initial recolonization of an area that has been left lifeless and where no soil is present. When a disturbance clears a community but leaves habitable soil behind, such as occurs when a fire decimates a forest, the process by which a community becomes reestablished is called secondary succession.

The first type of organism to colonize the area are autotrophic prokaryotes, followed by mosses and lichens, which arrive at the new location by windblown spores. Lichens are symbiotic associations between a photosynthetic organism and a fungus. The photosynthetic organism, either an alga or a cyanobacterium, fixes carbon and provides organic nutrients for the fungus. The fungus absorbs water and minerals for the phototroph and provides a structure to which the phototroph can adhere. In other environmental conditions, the relationship between the two symbionts may be described as commensal or even parasitic, but under the conditions in which primary succession occurs, the relationship is mutualistic.

Lichens and mosses do not require soil to thrive, as they can grow attached to substrates such as a rock or tree bark, but as they grow, reproduce, and decompose, they contribute to the organic matter of soil. Erosion of rocks also contributes to the formation of soil. Eventually grasses and then shrubs can take root. As they grow, they too enrich the soil, and over time the species that will characterize the community become established. Wind and animals carry in new spores and seeds. The process may take hundreds or thousands of years.

In succession, the organisms that colonize the habitat early alter the environment in a way that facilitates the arrival or success of later-arriving species. As mentioned, one way they do is by forming soil that can support the growth of plants. After soil is formed, its composition continues to change as other plants and soil bacteria establish themselves. For example, the amount of available nitrogen may increase and the acidity may change. Another way that early-arriving species prepare the habitat for later-arriving species is by providing the vegetation sufficient to support primary consumers, or herbivores. Also, as trees grow, they provide shade and microhabitats for smaller animals. The types of

organisms that arrive during an early stage of ecological succession may also inhibit or prevent the establishment of other specific types of organisms.

See also BIODIVERSITY; DARWIN, CHARLES; ECOLOGY; ECOSYSTEMS; HYDROTHERMAL VENTS; POPULATION ECOLOGY; WALLACE, ALFRED RUSSEL.

FURTHER READING

Molles, Manuel C., Jr. *Ecology: Concepts and Applications*. 3rd ed. New York: McGraw Hill, 2005.

conservation biology Conservation biology is the scientific study of processes that affect the maintenance, loss, and restoration of biological diversity, or biodiversity. Though conservation biology just emerged as a scientific discipline in the 1980s, the interdisciplinary field that draws on life sciences such as ecology, evolution, and genetics as well as other disciplines, including economics, sociology, political science, and philosophy, has quickly gained impetus as scientists have recognized that rates of extinction are higher than ever before on the planet. Biologists realized that because of human influence, species were disappearing before scientists had a chance to describe and scientifically study them. *Biodiversity* refers to all the variation present in living systems, including genetic variation within and among populations of a species, the total number of different species, and the varied composition and functions of all ecosystems in the biosphere. Conservation biologists believe that natural diversity is valuable in itself, but preserving biodiversity is also crucial to supporting life and maintaining the ecological health of the biosphere. The biodiversity intrinsic to functioning ecosystems is responsible for cycling nutrients, regenerating water supplies, purifying air, decomposing wastes, dispersing seeds, protecting shorelines and preventing erosion, controlling atmospheric chemistry, regulating climate and weather, pollinating crops, controlling agricultural pests by natural enemies, and making the soil fertile. The aim of biological conservation efforts is to preserve genetic variation, populations, species, biological communities, and entire ecosystems by educating the public about the negative impact of the reduction in biodiversity, identifying existing and potential threats to biological diversity, preventing further reduction of biodiversity, and restoring that already lost.

CONSERVATION GOALS AND EFFORTS

The major goals of research in conservation biology are to attain a better understanding of all life-forms, how they affect one another, and the mechanisms by which biodiversity contributes to the function of ecosystems, including Earth's largest, all-encompassing ecosystem, the biosphere. With this knowledge, conservation biologists can educate the public and the governments about conservation efforts, apply ecological principles to design strategies to preserve biodiversity, and assist in the development of conservation programs and the formulation of legislation and policies related to conservation.

Conservation biologists have identified numerous threats to Earth's biological diversity. The worst threat is habitat destruction, which can result from urban development, agriculture, forestry, mining, and pollution. The global warming trend has led to the physical alteration of many habitats and forced the displacement of many species and endangering of others that remain homeless. The introduction of foreign or nonnative species to new geographic areas threatens the native populations of a biological community. Whether the introduction is purposeful or inadvertent, without the natural population controls such as predators and pathogens that exist in their original habitat, the introduced species may grow uncontrollably and outcompete the native species for resources or even prey on the native species in the new habitat. Overexploitation occurs when humans harvest or kill a species at a rate faster than the species reproduces. The threatened species usually contains or provides a product that has great commercial or medicinal value. Species that play a significant or highly specialized role in an ecosystem are called keystone species. Their removal from a habitat or their extinction can cause a domino effect, impacting many other species of the community and thus the function of the entire ecosystem. For example, ecologists have shown that the removal of large carnivorous animals, the top predators, from ecosystems lifts a natural control on the population size of the prey. This, in turn, decreases the supply of the prey's food. Because food resources are often shared, the resultant increased competition for the food may have a domino effect, reaching other branches of a food web. These major threats to biological diversity are an integral consideration of the strategies aimed to preserve it.

Data collection must precede the development of a conservation management plan. The first step is to identify a species, a population, or an area of conservation interest. Reasons a species becomes of conservation interest extend beyond the scientific; efforts also aim to protect species of symbolic, economic, or cultural importance. After determining which species are in need of conservation, means to monitor success must be defined and specific target goals must be set. Researchers must identify the most important factors in preventing extinction of the species and figuring out how to affect those factors to achieve the desired result. Conservation actions may

include taking steps toward restoring lost habitats, establishing protected areas and habitats to prevent further pollution or destruction, stimulating changes in society's attitudes and actions that threaten biodiversity, assisting migration, breeding animals in captivity for eventual release back into the wild, and taking steps to increase the natural success of breeding based on observations concerning mating and breeding behaviors.

Careful landscape planning complements conservation management programs. The boundaries between ecosystems and the structures within them affect the biological richness of the ecosystems. Boundaries can be natural, such as where a grassland meets a forest, or artificial, such as where a city meets the edges of what once was a forest. The species that reside in or near boundaries may require characteristics of both ecosystems, and thus increasing man-made boundaries may increase their populations and thus might negatively affect the balance of the ecosystems. Human actions also act to fragment ecosystems, such as by creating an artificial waterway, building a road, or chopping down parts of forests and leaving patches behind. This affects both the populations that reside on the boundaries and those that live in the interior. Research suggests that smaller areas support fewer species in the interior, disrupting the balance of an ecosystem and potentially contributing to the reduction in biodiversity. Developers and others who alter the natural landscape of an area should consult conservation biologists in the planning stages as a preventative measure. The creation of corridors through which animals can move between fragmented sections of a formerly connected ecosystem is one way to address the issue of fragmentation. These strips or patches of habitat that join fragments allow animals to interbreed with larger populations and thus help maintain genetic variation.

The establishment of protected areas and laws preventing development within national parks and on nature reserves helps preserve biodiversity. For this strategy to be most effective, conservation biologists must identify biodiversity hot spots, areas where numerous species, especially those that are at risk or are endangered, inhabit a small area. Since only a percentage of the Earth's surface is set aside for the purpose of conservation, preserving the hot spots will have the greatest influence. One concern is that selection of targeted areas focuses too much on vertebrates and plants compared to invertebrates and microorganisms that carry out many crucial ecosystem services such as nutrient recycling. The size of the protected area is also important, as the biological boundary necessary to sustain a species may exceed the available area defined by legal or political boundaries; thus disturbances in the surrounding areas can still considerably affect a defined nature reserve.

Efforts to conserve biodiversity and prevent further loss are useful and necessary, but a comprehensive approach to conservation efforts includes the restoration of habitats that human activities have already destroyed and continue to destroy—for example, cleaning up oil spills. One method of restoration favored by environmentalists because it provides a natural means to correct an unnatural problem is bioremediation, which utilizes the natural ability of some microorganisms to metabolize chemicals that are considered pollutants or that are toxic to other species. After inoculation of an oil spill or contaminated water source, the microorganisms break down the undesirable substance and release innocuous substances or at least less harmful ones back into the environment. For example, a microbe might ingest oil, break it down, and release carbon dioxide and water. One avenue of research in environmental microbiology aims to expand the repertoire of substances that microorganisms can safely remove from the environment through recombinant DNA technology and finding and characterizing new species with unique metabolic abilities; thus not only is habitat restoration important to biodiversity, but biodiversity is important to finding new ways to restore habitats.

The purpose of biological augmentation is also to restore habitats, but in contrast to bioremediation, which removes pollutants, the means it uses is adding nutrients in order to assist and accelerate recovery. If a nutrient has been depleted from an ecosystem, any life that depends on that nutrient will be threatened. Adding back the limiting factor can encourage growth of the organisms dependent on that factor and possibly lead to the restoration of the ecosystem's structure and therefore function.

CONSERVATION LEGISLATION AND ORGANIZATIONS

The growing concern over the loss of biological diversity in the 20th century led to the enactment of legislation that helps conservation biologists achieve their goals. Many countries have laws that make it a crime to kill, capture, or harm threatened or endangered species or to damage their habitats or range. The U.S. Environmental Protection Agency (EPA), founded in 1970, is a governmental agency charged with the mission of protecting human health and the environment, a goal consistent with biological conservation efforts. In addition to developing and enforcing environmental laws, the EPA supports education and performs research related to environmental concerns such as acid rain, beaches, pesticides,

(continues on page 224)

PARTNERS IN PROTECTION: COOPERATIVE APPROACHES TO PROTECTING WIDE-RANGING SPECIES

by Rachel Mazur, Ph.D.
Wildlife Biologist
Sequoia and Kings Canyon National Parks

Sequoia and Kings Canyon National Parks are contiguous national parks, found on the western slope of the Sierra Nevada Mountains in California. They are famed for containing both the highest mountain in the continental United States and the largest trees in the world. The parks are also home to more than 450 species of birds and mammals. Not surprisingly, these two parks, along with all of the other parks in the system, are legislatively mandated to "conserve the scenery and the natural and history objects and the wildlife therein." With a total area of 860,000 acres (348,030 ha) between Sequoia and Kings Canyon National Parks, one might think that this would be a simple task. After all, the boundaries of the park are established to allow for conservation, are they not?

The answer is yes *and* no. Yes, in that efforts were made to establish boundaries containing the famous sequoia groves, the high alpine scenery, and the wildlife species. No, in that politics and money also played a role in boundary establishment, thus cutting critical areas of scenery and habitat out of the final designation. Boundaries are human constructs that nature has no obligation to recognize. Wind continues to blow air pollution into the parks. Climate change causes vegetation to shift its distribution into, or out of, the parks.

Then there is the wildlife. Even the largest parks in the world are too small to protect the diverse habitats used by wide-ranging mammals or migratory birds fully. This article presents two case studies demonstrating how biologists at Sequoia and Kings Canyon National Parks reached outside their political boundaries to solve these issues.

STUDY 1: Creation of the Sierra Interagency Black Bear Group

The mountains of the Sierra Nevada are home to a growing population of black bears (*Ursus americanus*). Black bears are found at a range of elevations but spend the majority of their time in the coniferous zone. Here, they find grasses in the meadows during spring, berries and insects during summer, and abundant pine seeds in the fall. When there is a good acorn crop, black bears will move to lower elevations to feast on this high-calorie treat and then return to the coniferous zone to den. Historically, there was little reason for black bears ever to venture into the high country, where scenery is abundant but food is scarce. Their entire range was literally defined by sources of high-calorie foods, which they are adept at finding and accessing with their natural curiosity, intelligence, and strength.

In the mid-1900s, however, backpackers began to visit the high country for its abundant scenery and with them carried high-calorie foods. Bears began to exploit this new food source by stealing it from unsuspecting hikers, who in turn began to sleep with their food to protect it. At night the bears would sneak up quietly and pull it away. When hikers hung their food from trees with ropes, bears shook the trees until the food bags fell. Bears figured out how to foil each new method of food storage almost as soon as it was invented. The situation escalated until bears became so bold that they would simply snatch food from hikers. By the late 1980s, hikers were continually losing food to bears, sustaining injuries from encounters with bears, and leaving a mess (e.g., ropes hanging from trees) in the high country. At the same time, some bears became so bold, destructive, and potentially dangerous that they had to be destroyed.

There did appear to be one solution. A machinist named Richard Garcia in Visalia, California, had worked with national park personnel to develop a portable, bear-resistant canister. After many failures he came up with the Garcia canister, which could fit in a backpack, hold a one-week supply of food, and withstand battering by a hungry bear. Its greatest disadvantage was its weight—small price for protecting one's food and keeping bears wild. Because of its great success, one national forest and one national park quickly mandated areas of the high country where canisters were required, but there were three persistent problems. First, regulations were created by each agency without consulting the other, so while the agency boundaries were adjacent, the restricted areas were not, a situation that made little ecological sense. Second, other manufacturers saw the Garcia canister's potential and began producing their own canisters for sale. But some of these newer canisters were easily opened by bears, so users became disillusioned and lost interest in using any canister. Finally, there was no central area where the public could find information about multiple agencies' regulations. Instead, each agency had to be consulted separately—quite a task for a hiker planning a long backcountry trek that would cross the boundaries of different agencies.

In 2000, a particularly bad year for human-bear conflict in the front country, exhausted biologists from Sequoia, Kings Canyon, and Yosemite National Parks and Inyo National Forest decided to get together to talk. The resulting meeting in fall 2000 produced the Sierra Interagency Black Bear Group. The group drafted a "Memorandum of Understanding" with the stated goal of working "to preserve a healthy black bear population free of human influence on a regional-scale by sharing information, techniques, and ideas; coordinating policies and information; and eliminating political barriers to progress."

At first, work by the group was a labor of love. None of the members had funding for meetings. They met during their free time and funded their own gatherings, hoping that together they would make a difference. They created a Web page where visitors and staff could conveniently access information about bear biology and food storage regulations for each agency. More difficult was the creation of a joint protocol for testing and approval of portable food-storage containers. The group agreed to coordinate on any new policies so they would be collaborative, and based on ecological rather than administrative boundaries.

Since its creation, the group has expanded to six agencies, with a seventh expected to join in 2009. As a result of this effort, the public is better informed about food storage restrictions and bear biology, knowing where and how to access information. Bear management specialists have more technical training and are better informed. The increased efficiency of all agencies working together has reduced workloads to the point where they meet as a part of their work schedules. The group is considered an authority on human-bear conflict and is now called upon to advise other national parks and forests across the country. Coordinated food-storage restrictions now cover ecological areas, even if that means crossing agency boundaries. Most importantly, human-bear incidents are down by more than 50 percent.

STUDY 2: Partners in Protection: A Cooperative Approach to Protecting Migratory Birds

Migrant songbirds are another group of well-known residents of Sequoia and Kings Canyon National Parks, although they spend only a portion of their lives within the confines of the parks. They arrive in the spring, set up and defend territories, breed, raise their young, and are gone by late summer. Where do they go? Most fly thousands of miles south to Central America, although some simply migrate farther south in California and others go all the way to South America. There, the birds spend the winter where there are milder weather and abundant food. The following spring, they migrate back north to breed. To conserve these species, habitats at the birds' breeding and wintering grounds must remain intact, as well as those stopover points along the migration route where birds rest and eat.

The challenges of conserving a species that migrates internationally are significant, involving various governmental agencies with different cultures and languages. Biologists in each nation's national parks recognized these difficulties but until recently were restricted to working within their own political boundaries. Then, in 2001, the U.S. National Park Service initiated Park Flight, an innovative program with the goal of working with Central American parks to protect habitats shared by their migratory bird species. The program was funded by generous grants from American Airlines, the National Park Foundation, and the National Fish and Wildlife Foundation.

The program awarded grants to U.S. and Central American parks that were interested in participating. Sequoia and Kings Canyon National Parks were among the first to receive one of these grants and have been involved ever since. The program, "Partners in Protection: A Cooperative Approach to Protecting Migratory Birds," revolves around three main components: education, biological monitoring, and international exchange.

International exchange is the heart of the program. Each year Sequoia and Kings Canyon National Parks host an intern from Central America or Mexico who is training to work in the field of conservation and has great interest in bird biology. They have hosted interns from Guatemala, Nicaragua, Honduras, and Mexico. Some arrive speaking no English; others are bilingual. All have a great love of birds, but their monitoring experience varies widely. Most interns have been working as biologists in their home countries and want to expand their bird expertise and language skills. All have a wealth of knowledge about the birds' wintering grounds, conservation challenges, and monitoring techniques. Park employees have learned an immense amount from them.

The intern is paired with a U.S. biological technician. Both the intern and the technician attend a week of training on bird identification, data collection, and bird safety. Together they run two bird-banding stations. Birds are caught when they fly into large nets that they cannot see. Biologists gently remove the birds from the nets; measure them; determine their species, age, and breeding status; find out whether the bird is already banded and, if not, affix a small metal band around one leg; and then release them. These data are used to monitor trends in species numbers and to assess any declines (or increases) in their breeding or wintering grounds.

Another critical component of the program involves monitoring peregrine falcons, which were once on the brink of extinction. Cliffs that are known to attract both peregrines and human climbers are watched for falcon activity. When a peregrine aerie (nest) is located, nearby climbing routes are temporarily closed until the young successfully fledge from the nest. That way, the birds are not disturbed by climbers, and the climbers are still allowed access to most of the cliff face.

Another type of monitoring involves conducting yearly point-count transects. To do this, one stops at preset intervals along a pathway and records all the birds that are seen or heard. By comparing data over several years, one can determine whether various bird species are increasing or decreasing. All of these monitoring programs may be, and many have been, implemented by the interns when they return to their home parks.

The education component includes the offering of bilingual bird walks to the public, which allows us to reach our many Spanish-speaking visitors. The

(continues)

(continued)

parks celebrate International Migratory Bird Day by teaching people how they can prevent birds from hitting windows, that they should take their birdfeeders inside during the winter and keep cats indoors.

As of this writing, Sequoia and Kings Canyon National Parks have hosted nine international interns through Partners in Protection. Many of these interns have learned new monitoring techniques, improved their English, and, most importantly, become conservation leaders in their countries. At the same time, our employees have learned about conservation issues in the birds' wintering grounds, improved their Spanish, and even gone to Central America to serve as interns for people who were once our interns. Park employees look forward to hosting a 10th intern during summer 2009.

FUTURE DIRECTIONS

Both the Sierra Interagency Black Bear Group and the Partners in Protection program are now integral to the wildlife management program at Sequoia and Kings Canyon National Parks. Both programs required large initial investments of time and commitment but have reaped enormous rewards. Clearly, the different nations' parks are stronger working together. Around the world, thousands of collaborative projects such as these are under way, all with the ultimate goal of protecting a species, group of species, ecosystem, or natural process. To succeed, these projects first require conservation biologists to conduct sound scientific research into the problem. They then share their results with a range of specialists, from economists to politicians to social scientists, who work collaboratively to find a solution. This type of cooperation moves the global community closer to realizing the goal of conservation biology—the protection of the biological diversity of the Earth.

FURTHER READING

National Park Service. U.S. Department of the Interior. "Global Conservation: Park Flight." Available online. URL: http://www.nature.nps.gov/globalconservation/parkflight.cfm. Accessed February 2, 2008.

Sierra Interagency Black Bear Group Web site. Available online. URL: www.sierrawildbear.gov. Accessed February 2, 2008.

(continued from page 221)

wetlands, oil spills, the ozone layer, climate change, and clean air—all relevant to habitat preservation and the effects of human impacts on biodiversity. First passed in 1973, the Endangered Species Act (ESA) is one of the major environmental laws forming the legal foundation for EPA programs and actions. The ESA prohibits actions that result in the taking, harming, or killing of threatened or endangered species; actions that adversely affect their habitats; or trade of listed species. Two governmental agencies jointly administer the ESA: the Fish and Wildlife Service, whose mission is to conserve, protect, and enhance fish, wildlife, and plants and their habitats, and the National Oceanic and Atmospheric Administration (NOAA) Fisheries Service, who work to conserve, protect, and manage living marine resources through science-based conservation programs and promotion of healthy ecosystems. Much of the text of the ESA is taken from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), an international agreement that restricts the trade of more than 28,000 species of plants and 5,000 species of animals, living or dead, as well as their parts. In August 2008 CITES had 173 participating countries.

Recognizing the unique need for international cooperation to address environmental issues effectively, the United Nations (UN) has implemented several programs. The objectives of the United Nations Environment Programme (UNEP) are to advocate, educate, and promote conservation and sustainable use of all the Earth's natural resources. The UN Convention of Biological Diversity specifically supports the conservation of biodiversity and promotes the sustainable use of biological resources. The UN Convention on the Conservation of Migratory Species of Wild Animals (CMS) protects terrestrial, marine, and avian species whose natural migration patterns cross political boundaries.

The world's largest conservation network is IUCN—the World Conservation Union, whose self-reported mission is "to influence, encourage, and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable." IUCN—the World Conservation Union publishes a comprehensive "red list" that tracks and evaluates the extinction risks for thousands of plants and animals. On the basis of criteria such as population size, rate of decline, geographic distribution, and degree of population fragmentation, organisms are placed into one of nine categories: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient, and not evaluated. Conservation biologists worldwide use this list to develop conservation management plans and recovery programs. IUCN—the World Conservation Union also set up a World Commission of Protected Areas (WCPA),

with the goal of selecting, establishing, and managing national parks and protected areas.

Several private organizations have made significant contributions toward conservation efforts. The World Wildlife Fund, the largest privately financed, multinational conservation organization with a membership of more than 5 million, oversees 2,000 projects in 100 countries, all with the aim of nature conservation. Wildlife conservation is also a major goal of the Association of Zoos and Aquariums (AZA), which supports zoos and aquariums that spearhead efforts to protect wild animals and restore threatened populations. Another governmental service that plays a key role in conservation efforts in the United States is the National Park Service, which established the Conservation Study Institute in 1998 in order to stay current with contemporary issues in conservation research and efforts and to develop partnerships, tools, and strategies for future conservation efforts.

Often, the efforts to conserve populations or protect areas where threatened or endangered species live conflict with private property rights or economic ventures. Educating landowners and local, state, and federal officials about conservation biology is imperative for cooperation in conservation efforts. The fact that so many organizations and agencies are working toward a shared goal of biological conservation holds promise for the future of conservation biology. As scientists gain an improved understanding of the ecological principles that govern ecosystems, communities, and populations, perhaps the world will develop better methods for managing and conserving the Earth's biological diversity.

See also BIODIVERSITY; ENDANGERED SPECIES; ENVIRONMENTAL PROTECTION AGENCY.

FURTHER READING

Anderson, Anthony B., and Clinton N. Jenkins. *Applying Nature's Design: Corridors as a Strategy for Biodiversity Conservation*. New York: Columbia University Press, 2006.

Center for Applied Biodiversity Science home page. Available online. URL: http://science.conservation.org. Accessed January 19, 2008.

Groom, Martha J., Gary K. Meffe, and C. Ronald Carroll. *Principles of Conservation Biology*. 3rd ed. Sunderland, Mass.: Sinauer, 2006.

IUCN—The World Conservation Union home page. Available online. URL: http://www.iucn.org. Accessed on January 19, 2008.

Primack, R. N. *Essentials of Conservation Biology*. 4th ed. Sunderland, Mass.: Sinauer, 2006.

Society for Conservation Biology (SCB) home page. Available online. URL: http://www.conbio.org/. Accessed January 19, 2008.

Soulé, Michael E. "What Is Conservation Biology?" *Bioscience* 37, no. 11 (1985): 727–734.

Crick, Francis (1916–2004) British *Molecular Biologist* Francis Crick codiscovered the structure of deoxyribonucleic acid with James D. Watson. He shared the 1962 Nobel Prize in physiology or medicine with Watson and Maurice Wilkins "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material."

TRAINED IN PHYSICS

Francis Harry Compton Crick was born on June 8, 1916, in Northampton, England. He received a bachelor of science in physics from University College in London in 1937 and for a time researched the viscosity of water under pressure. His graduate studies were interrupted by World War II, when he went to work for the British Admiralty designing magnetic and acoustic mines. After the war ended, in part stimulated by reading Erwin Shrödinger's *What Is Life?,* a book that emphasized the importance of learning about genes, Crick completely changed his focus to molecular biology. He received a small scholarship to attend Cambridge in fall 1947.

While working at the Strangeways Laboratory at Cambridge, he studied the physical properties of the cytoplasm of chick fibroblast cells and began learning about biology, organic chemistry, and crystallography. He joined the new Medical Research Council Unit at the Cavendish Laboratory of Cambridge University as a doctoral candidate in 1949. His supervisor, Max Perutz, was a well-known protein chemist and soon had Crick examining the structure of hemoglobin using X-ray crystallographic techniques. An enthusiastic graduate student, he was easily distracted from his project and often irritated his coworkers with his incessant chatter.

SOLVES STRUCTURE OF DNA WITH JAMES WATSON

When James D. Watson entered the lab as a postdoctoral fellow in the laboratory of John Kendrew in 1951, the two quickly became friends, partly because of their common enjoyment of scientific discussions, but also their youthful arrogance. Crick needed frequent breaks from his research to talk about science, and Watson enjoyed the chance to offer his opinions of Crick's ideas. Excitement over deoxyribonucleic acid (DNA), which had only recently been identified as the carrier of genetic information, became the focus of their conversations. Both believed genes were composed of DNA and soon became obsessed

with the way genes copied themselves, a problem they hoped to address by solving its structure.

The fact that neither Crick nor Watson had the background training one would expect for someone tackling such a problem did not deter either of them. The problem was that all DNA-related research belonged, by unspoken scientific etiquette, to Maurice Wilkins of King's College in London. Wilkins was not only the first scientist in Great Britain to show an interest and make progress in the subject, but he was also a personal friend of Crick. Sir Lawrence Bragg, head of the Cavendish Laboratory, even directed them to stick to their own research subjects—proteins—but the two eager scientists could not dismiss thoughts of DNA.

Scientists knew two types of nucleic acids existed, DNA and ribonucleic acid (RNA). The structures of each of the five nucleotide building blocks (the purines adenine and guanine and the pyrimidines cytosine, thymine, and uracil) were also known. The Austrian biochemist Erwin Chargaff had determined that, in DNA, the percentage composition of adenine equaled that of thymine and the percentage composition of cytosine equaled that of guanine. X-ray diffraction evidence from photographs taken by Rosalind Franklin in Wilkins's laboratory suggested that DNA was helical.

Using molecular models that resembled toy building blocks, Watson and Crick attempted to recreate the biological structure. Watson attended a lecture given by Wilkins in spring 1951. During the talk, Wilkins showed an X-ray diffraction photograph obtained from crystallized DNA. Though the picture was not remarkable, the fact that DNA could be crystallized and yield X-ray diffraction patterns at all meant that DNA had a regular structure and therefore was solvable. This knowledge excited him greatly, because knowing the structure of DNA would surely lead to an understanding of the way genes worked. Watson attended a colloquium that fall, in which Franklin presented some of her X-ray diffraction data. Though Watson had attempted to learn as much crystallography as possible before hearing her presentation, he failed to remember some important details regarding water content. Unaware, Crick and Watson constructed a model that they believed was the true structure, a triple helix with a sugar-phosphate backbone running down the center. After they eagerly invited Wilkins and Franklin to view their model, Franklin at once saw their structure contained at least 10-fold less water than the molecule had and chastised them. This embarrassing fiasco led to a chewing out by Bragg, who demanded Crick get back to his thesis task of examining the effect of different salt solutions on hemoglobin crystals.

They laid low for a while and limited their DNA discussions to lunchtime. In 1952 Crick and Watson had the opportunity to meet with Chargaff personally. Though Chargaff was unimpressed with their knowledge about nucleic acid chemistry, he discussed his data regarding the composition of DNA, now referred to as Chargaff's rules, with them. Crick recognized the data were of key importance. In December of that year, Linus Pauling wrote to his son, Peter, who had joined Kendrew as a Ph.D. student six months prior. Pauling said that he solved the structure for DNA and would soon send a copy of the manuscript. When it arrived the first week of February 1953, Peter showed the crestfallen Watson and Crick the manuscript in which Pauling described DNA as a triple helix with a sugar phosphate backbone in the center, very similar to their design that Franklin scoffed at 15 months before. To their extreme delight and disbelief, they saw that Pauling had ignored the fact that DNA was an acid and had attached hydrogen atoms to the phosphate groups, neutralizing their negative charge. Furthermore, Pauling had made these hydrogen atoms participate in bonds that held the structure together. The error was unmistakable, and they knew that Pauling would soon recognize it. After trying unsuccessfully to convince Wilkins to work with them to beat Pauling in determining the correct structure, they frantically resumed their model building. Wilkins did assist them by sharing some of Franklin's X-ray diffraction data that suggested the structure was indeed a helix and giving the unit repeat length. They immediately ordered new purines and pyrimidines from the Cavendish machine shop, meanwhile working with cardboard cutouts.

Though Crick knew more about physical science and Watson about biology and genetics, Crick credits himself with figuring out that Chargaff's rules meant that adenine paired with thymine and that cytosine paired with guanine and credits Watson with determining how the nucleotide base pairs fit together chemically. On February 28, 1953, all the puzzle pieces seemed to fall into place, and by March 7, 1953, they constructed a double-stranded model that resembled a twisted ladder, with the rungs being the paired nucleotides. The nitrogenous bases of the nucleotides were linked by specific hydrogen bonds between adenine and thymine and between cytosine and guanine. Strands that pair in this manner are termed complementary. The simplicity of the model was surprising and made apparent a mechanism for replication. Each of the two strands could serve as a template for the synthesis of a complementary strand. The model was biologically as well as chemically attractive.

Coincidentally, Perutz, Crick's supervisor, was serving on a committee whose job was to oversee the biophysics research at King's College. He had access to a comprehensive summary of Franklin's research as part of a report and shared it with Crick and Watson, so they could check their model against the X-ray diffraction data. This event later led to some controversy in the distribution of credit for the eventual discovery of the structure of DNA.

Once again, Wilkins went to Cambridge to examine their proposed model. Surprisingly, he did not act bitter, and within a few days he called to confirm that both his and Franklin's data strongly supported the double helix. Though many were working to solve the structure, Crick and Watson were successful because of their persistence and determination. They chose a biologically important problem, immersed themselves in the necessary background information, and were successful. Their short article "A Structure for Deoxyribose Nucleic Acid" was published in *Nature* in April 1953.

After determining the structure of DNA, in 1953, Crick earned his doctorate from Caius College of Cambridge for a thesis on X-ray diffraction of polypeptides and proteins and went on to serve science in many capacities. He worked in Brooklyn for one year, then returned to Cambridge. After spending a sabbatical at the Salk Institute for Biological Studies in San Diego, Crick joined the faculty in 1977, obtaining an endowed professorship, and became an emeritus president of the institute's Kieckhefer Center for Theoretical Biology. His studies included work on the structures of other biological molecules such as collagen, polypeptides, and polynucleotide chains. He also contributed to the elucidation of how DNA and RNA direct and carry out the synthesis of proteins and how variations in the sequence of nucleotides lead to genetic mutations. In later years he switched to neurophysiology and explored the mammalian visual system.

The Royal Society of London elected Crick to membership in 1959. His contributions to the discovery of the structure for DNA led to his sharing the Nobel Prize in physiology or medicine and the Lasker Foundation Award in 1962 with Watson and Wilkins in addition to many other honors. In 1992 Queen Elizabeth II awarded Crick the Order of Merit.

Crick was married to Ruth Doreen Dodd from 1940 to 1947. They had one son together, Michael. In 1949 he married Odile Speed, with whom he had two daughters, Gabrielle and Jacqueline. Crick died on July 28, 2004.

See also BIOMOLECULES; CHARGAFF, ERWIN; DEOXYRIBONUCLEIC ACID (DNA); FRANKLIN, ROSALIND; PAULING, LINUS; WATSON, JAMES D.; WILKINS, MAURICE H. F.; X-RAY CRYSTALLOGRAPHY.

FURTHER READING

Crick, Francis. *What Mad Pursuit: A Personal View of Scientific Discovery*. New York: Basic Books, 1988.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1962." Available online. URL: http://nobelprize.org/medicine/laureates/1962. Accessed January 14, 2008.

Watson, J. D., and F. H. C. Crick. "A Structure for Deoxyribose Nucleic Acid." *Nature* 171 (April 25, 1953): 737–738.

———. "Genetical Implications of the Structure of DNA." *Nature* 171 (May 30, 1953): 964–967.

Cuvier, Georges, Baron (1769–1832) French *Comparative Anatomist*

Georges Cuvier established the reality of the existence and extinction of past life-forms at a time when people hesitated to believe that animals never seen by human eyes had once crawled, walked, hopped, swum, and flown over Earth's surface. As an expert comparative anatomist, he advanced the field of paleontology through his studies of fossil mammals, which he suggested disappeared from the Earth as a result of catastrophic geological events.

BECOMES A NATURLIST

Georges Cuvier was born on August 23, 1769, in Montbéliard, Württemberg. At the time, Montbéliard was under German jurisdiction, but it was a French-speaking community. In 1793 the territory was annexed, and Cuvier became a French citizen. He was baptized as Jean-Leopold-Nicholas-Frédéric Cuvier. Later his mother added Dagobert to his name. When his elder brother Georges died as a child, he adopted the name Georges and used it for the rest of his life. As a child Cuvier spent time sketching various animals he read about in a 44-volume encyclopedia about the natural world, *Natural History,* written by Georges-Louis Leclerc, comte de Buffon. Cuvier was a skilled artist and later in life provided many of the drawings for his own published works.

Though his parents wanted him to enter the ministry, Cuvier's teachers did not recommend him for a scholarship to theology school. Georges's father was a soldier, and the family did not have enough money to send him to a university. Fortunately, he gained admission to a school founded by the duke of Württemberg—Caroline University in Stuttgart, Germany. From 1784 to 1788, he studied a variety of subjects ranging from administration and economics to the scientific discipline of zoology and the art of dissection. Georges also mastered the German language.

After Georges's graduation his father helped him obtain a position as a private tutor for an aristocratic family in Normandy, France. This protected him

from the immediate effects of the French Revolution and allowed him to indulge in independent studies of natural history during his free time. He collected fish, mollusk, and shorebird specimens from the nearby port of Fécamp. He kept detailed notes of his dissections, observations, and sketches in scientific diaries. During this time, Cuvier regularly corresponded with a friend from Caroline University, Christian Heinrich Pfaff. These letters mentioned many of the scientific ideas for which Cuvier became famous in the early 1800s. In them, he described his scientific endeavors and wrote about topics such as the geology of Normandy and his opinion of the proposed notion that all living beings formed a continuous chain of increasingly complexity. Cuvier and his notebooks attracted the attention of French naturalists, who encouraged him to go to Paris.

In 1795 he found himself teaching animal anatomy at the recently reformed Muséum d'Histoire Naturelle, the largest institution dedicated to scientific research at the time. Having a proper forum, he promptly presented the results of his Normandy researches. Among these was his proposal of a new means to classify invertebrates. Whereas previously zoologists had divided all invertebrates into two groups, worms or insects, Cuvier distinguished among mollusks, crustaceans, insects, worms, echinoderms, and zoophytes.

CAREER AND PERSONAL ACHIEVEMENTS

Cuvier was a gifted teacher and soon obtained the position of professor of zoology at the Écoles Centrales. His abilities and reputation led to several other responsibilities and appointments. In 1796 he became the youngest member of the Class of Physical Sciences at the Institute of France (hereafter referred to as the *Institute*), which partly replaced the Royal Academy of Sciences. In 1800 he was appointed professor at the Collège de France, and he was appointed professor of comparative anatomy at the muséum in 1802. He became permanent secretary of the physical sciences for the Institute in 1803. Napoleon appointed him university counselor in 1808 and sent him to reorganize higher education in Italy, the Netherlands and southern Germany. As compensation, in 1811 Cuvier received the title of chevalier, awarding him the privileges of a low-ranking nobleman. In 1814 Cuvier became councillor of state and head of the Interior Department of the Council of State from 1819 until his death. Cuvier was elected a member of the Académie Française in 1818. He was made a baron in 1819 and given the title of *grand officier* of the Legion of Honor in 1824. In 1831 Cuvier was given the status of peer of France, an honor of high-ranking noblemen.

Cuvier married a widow of a victim of the Revolution, Mme. Davaucelle, in 1804. She already had four children from her previous marriage. Together they had four more children. Tragically, Cuvier was preceded in death by all of his children.

IDENTIFICATION OF ANIMALS FROM FOSSIL REMAINS

For much of his career, Cuvier gathered information on the structure of living beings from fossil remains. The term *fossil* refers to the remains or traces of a formerly living organism. Ancient bones and animal tracks found in sediment are both considered fossils. Fossils from aquatic life are more common than fossils from terrestrial life, as deposition of loose sediment washed away by erosion occurs in bodies of water. Normally microorganisms decompose the remains of dead organisms, but sometimes the remains are preserved. Hard materials such as bones and teeth may be found essentially unaltered. Even softer parts may be found intact if the organism was quickly frozen in a block of ice. Other fossil remains become petrified, meaning that minerals have replaced the organic matter and hardened. This process usually preserves the basic shape. In other instances, holes or cavities may become filled in with hardened mineral deposits. Another common type of fossil occurs when an organism is compressed within the Earth's crust. Over time the organism decomposes, and a thin layer of carbon is left behind. The last type of fossil results when an organism becomes trapped in a layer of sediment and the sediment hardens around it. If acidic liquid gains access to the organism, the organism may dissolve, leaving behind an imprint in the hardened sedimentary rock. Depending on the type and quality of the fossil, an anatomist can extract much information about the former organism's anatomy from the fossil.

The scientific study of fossils is called paleontology. Paleontologists are interested in a variety of topics. For example, some paleontologists use fossil evidence to investigate geologic time. The history of the Earth is divided into a set of geologic periods. Each division of time is characterized by a unique group of fossil remains; thus fossils may be used to determine when the rock layer in which they are embedded was formed. Paleontologists also use fossils to learn about tectonics, the movement of landmasses, throughout the history of the Earth. Cuvier used his knowledge of anatomy to investigate the relationships among organisms, past and present.

In 1796 Cuvier presented to the Institute his treatise *Mémoire sur les éspèces d'elephans tant vivantes que fossiles* (Memoir on the species of elephants, both living and fossils). He gave a detailed description of the osteological (related to the study of bones) features of two known elephant species, African and Indian. He discussed their teeth, skulls, and jaws,

among other structures. Then he confidently claimed that fossil elephant remains belonged to a distinct third species, identified as *Elephas primigenius,* an extinct hairy maned mammoth. Cuvier was the first to suggest that comparative anatomy could be used to learn about geological history. For example, one popular geological theory was that the Earth had been gradually cooling since its formation. Scientists assumed that locations where elephant remains were found but where elephants no longer live must have previously been warmer. But Cuvier responded that since the remains belonged to an entirely new species, the extinct animal might have been better adapted to cooler climates than the living species, so the Earth might not necessarily be cooling. Cuvier claimed there must have been a primitive prehuman world that was destroyed by some major catastrophe, but he left the determination of the specific nature of the cataclysmic event to experts in geology.

Later that year Cuvier was sent plates of fossil bones from a large animal found in South America. Again, using careful anatomical comparison, he concluded that this elephant-sized beast that he named *megatherium* was also extinct. He concluded that it was another animal from the ancient world. These studies increased Cuvier's interest in fossil anatomy and set him on a mission to study all fossil animals.

The nearby gypsum quarries of Montmartre and Mesnilmontant contained an abundance of well-preserved fossil remains. Cuvier appealed to a quarrier (someone who excavates stone from a quarry or pit) to give him fossils uncovered during excavation. Cuvier had to draw on his expert skills to examine these fossils since they were embedded in hard plaster stone rather than loose sediment. Many of the fossils were from unknown species. He surmised that the Earth must have been previously crawling with vertebrate animals that no longer existed. In other words, he asserted that animals became extinct.

Cuvier awed other scientists with his ability to identify organisms from only fragments of their skeletal remains. He claimed this was possible because organisms were well-integrated wholes: their parts were not independent of one another. For example, if he found a tooth, he deduced the animal's diet from its construction. If it were a meat eater, the animal's form would have to allow it to move in a manner that would permit it to capture its prey and its jaws strong enough to crush it. Carnivory would require a digestive system that could efficiently extract necessary nutrients from this type of food source. Thus each body part told enough of the full story that an anatomist familiar with the fundamental laws of comparative anatomy could reconstruct the entire organism with astounding accuracy. Cuvier went so far as to claim even the musculature could be reconstructed from imprints left on the bone. Since organisms are functionally integrated, he depended on their structures to infer their habitat and even the physical history of the Earth at the time that they roamed it.

EXTINCTION AND CATASTROPHISM

Cuvier began to wonder why animals became extinct. By now he realized that the fossils of extinct organisms were not all the same age, and that difference in age pointed to a series of revolutions throughout Earth's history. What happened in the geological past that these animals could no longer survive? Why were their forms no longer sufficient for survival in the habitats that had previously supported their needs? Consequently, Cuvier's interests shifted toward geology in order to learn about the physical environment during the time that the extinct lifeforms lived. He studied the material in which the fossils were found and tried to figure out what was happening at the time the strata were laid down. He was looking for keys to the geological history of Paris, clues to what might have happened that wiped out entire species. He appealed to other natural historians for collaboration. Meanwhile, he continued to publish prolifically on the bones of fossil animals. He identified several new extinct species including mammals similar to present-day otters, gazelles, hares, tapirs, opossums, and others.

Regarding the Earth's history, Cuvier accepted the divisions of periods of time, called epochs. He believed there was a primary, universal, lifeless ocean prior to the formation of continents. Marine life appeared, and then terrestrial life. The lack of human-type fossils and of intermediate fossils convinced him of the reality of extinction and creation of life in its original form. His lectures on geology contained few original ideas except when linked to fossil evidence. Even the idea of several cataclysmic revolutions of the Earth was not new.

Cuvier's training was in anatomy. A new interest in a field does not necessarily qualify one for productive study in that field. Cuvier was simply a biologist with an interest and natural talent in geology. In Alexandre Brongniart he found his complement, a geologist interested in biology. Brongniart had a background in mining engineering. Beginning in 1804, these two men undertook a study of the Seine basin in northern France. They traveled around France examining the succession of strata, paying particular attention to the distinct groups of fossils embedded in each. Each fossil bed demonstrated that the surface of the Earth was not as it always had been.

In 1808 they presented a joint preliminary report to the Institute, *Essai sur la geographie mineralogique*

des environs de Paris (Essay on the mineral geography of the Paris region). A fuller version was published in 1811. Cuvier graciously gave the majority of credit to his associate for the efforts that resulted in this essay. This work outlined the principles of paleontological stratigraphy and included a color-coded mineralogical map of the strata and detailed descriptions of nine different successive formations and their corresponding fossils. One goal was to identify the chronology of the Montmartre fossil beds. A significant finding was that there were major differences between the groups of fossils found in different beds. Beds contained significantly different fossils from the beds above and below them. Cuvier and Brongniart also reported finding both saltwater and freshwater organism remains in the same location. They suggested that fossils could be used to determine geological chronologies. For example, they could determine that a particular region first was submerged in salt water, then became dry land, then later was covered by freshwater. The strata may have looked similar, but if the fossils differed, the chronologies did as well. Cuvier also described some of the quadrupeds, or four-legged animals, that he found.

As one ascends a stratigraphical column, sediments near the bottom contain fossils from the oldest periods in geologic time. As Cuvier and Brongniart traveled upward toward more recently laid strata, they noticed that mammal remains suddenly appeared, though they were not remains of extant creatures, that is, creatures still in existence. As they continued moving up the column, remains of current recognizable species were finally observed. The succession was not gradual, but erratic. Cuvier concluded that the breaks represented actual geological breaks and were indicative of major revolutions in Earth's history; he did not believe that gradual geological cycling sufficiently explained his observations. These revolutions wiped out entire species, such that the species alive today do not represent the complete assortment of this planet's animals. Cuvier did not focus on whether new creations occurred after each catastrophe, though others thought this was a logical possibility.

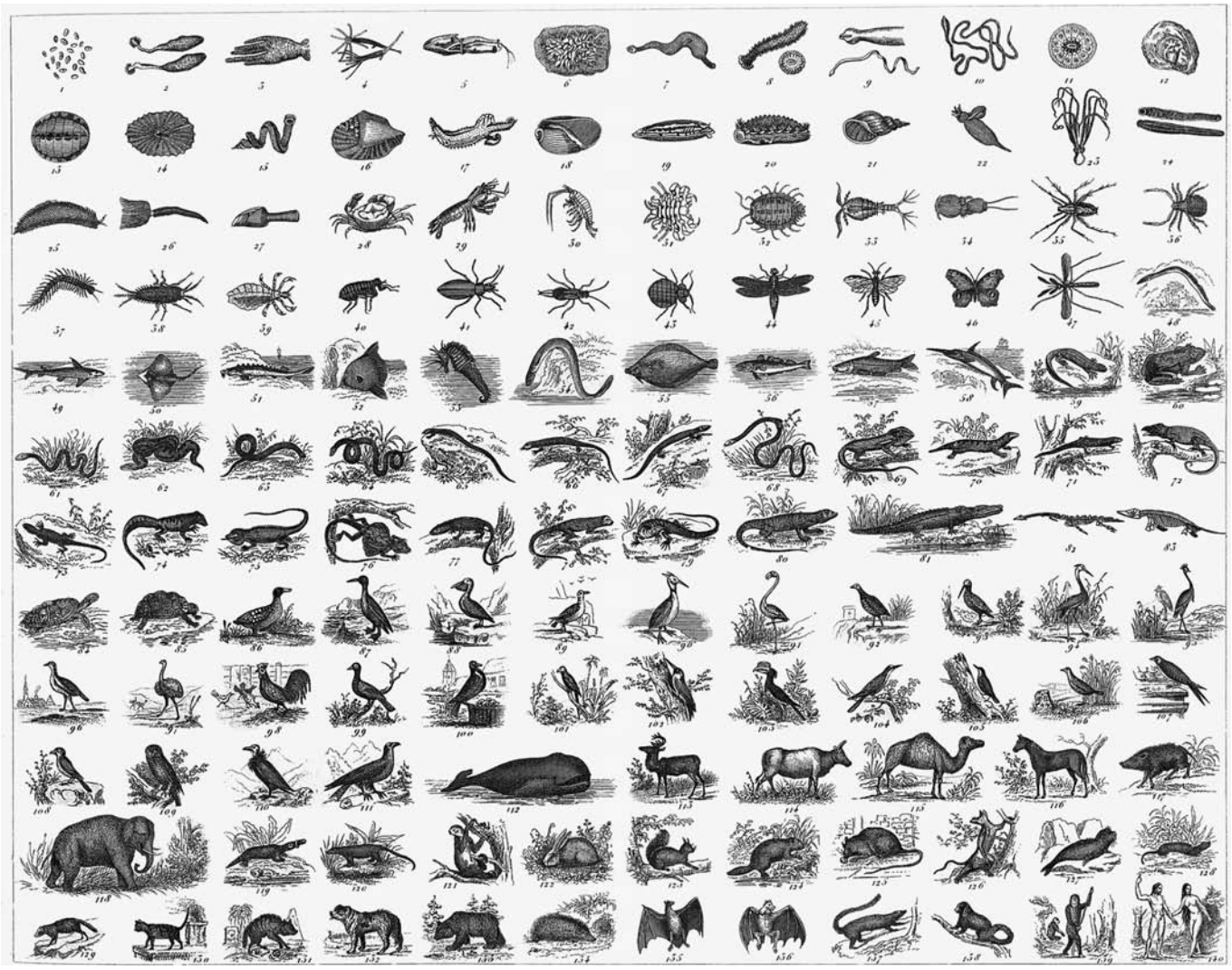
Cuvier was a catastrophist. Catastrophism suggests that certain geological features of the Earth's crust are the result of past cataclysmic events such as volcanic activity or flooding. Cuvier thought mass extinctions might result from such geologic catastrophes. The term *catastrophism* had not yet been used. Instead, Cuvier referred to "global revolutions." He thought periodic revolutions reasonably explained why remains of saltwater and freshwater organisms were found at the same location and why there were apparent breaks in geological time according to rock strata. Moreover, though Cuvier did not specifically identify any revolutions with biblical events, catastrophism did not require that he abandon his Protestant upbringing. The biblical account of creation and the great flood was compatible with a catastrophic viewpoint. The great flood was simply the most recent great catastrophe. However, Cuvier's paleontological findings did convince him that creation must have occurred in many stages.

As far as Cuvier's claim that former life-forms were no longer in existence, doubters questioned why God would create something and then allow it to disappear. Some thought that the fossils were the remains of living organisms, just incorrectly identified. Others asserted that the species to which the fossil remains belonged had simply not yet been observed or identified by humans. Perhaps the species resided in an unexplored part of the world, they speculated.

By 1812 Cuvier's fossil research had almost ended. He collected, reordered, and reissued many of his previous related papers into the four-volume work *Recherches sur les ossemens fossiles de quadrupèdes (Researches on Fossil Bones of Quadrupeds)*. To this work, he added a *Discours préliminaire* (Preliminary discourse) immediately after the preface. This introductory text, which was technically accessible to the public, summarized the evidence of global revolutions, geological structures and formations, research on fossil bones, their utility in uncovering Earth's history, and the extinction of life-forms. In 1826 this piece was published separately with a new title, *Discours sur les révolutions de la surface du globe, et sur les changemens qu'elles ont produit dans le règne animal* (*A Discourse on the Revolutions of the Surface of the Globe, and the Changes Thereby Produced in the Animal Kingdom,* 1829). It was ultimately reprinted many times in several languages and was considered a masterpiece on its own. By the time Cuvier died it was in its sixth edition.

COMPARATIVE VERTEBRATE ANATOMY

Next Cuvier refocused on his original field of comparative anatomy. In 1817 he published a zoological masterpiece, *Règne animal distribute d'après son organization* (*The Animal Kingdom, Distributed According to Its Organization,* 1834–37). This work included descriptions of the entire animal kingdom. In it, he modified the classification system proposed by the Swedish naturalist Carl Linnaeus. Cuvier recommended four major divisions of animal life: vertebrata, mollusca (including shellfish), articulata (including insects), and radiata (including echinoderms). This system may seem crude today, but at the time it emphasized the diversity of animal life, particularly the invertebrates, and marked a change in the approach to the classification of animals.



Georges Cuvier's classification system considered single-celled organisms (number 1, top left corner) to be the simplest of animals and humans (number 140, bottom right corner) to be the most advanced. *(Sheila Terry/Photo Researchers, Inc.)*

In his 1809 *Zoological Philosophy,* the French naturalist Jean-Baptiste Lamarck suggested that living things transmutated, that is, evolved. Their forms gradually changed to become better adapted to their environment, and these changes were passed on to the next generation. According to Lamarck, animals were becoming more and more complex. At the time it was popular to believe in the stability of life-forms, that each creature existed as God originally had created it. The form of each creature was not subject to mutation. Change would not only violate moral law, but decrease the ability of an animal to survive in the particular environment for which it was divinely suited. Besides, if animals were mutable, then the entire science of taxonomy would have no basis. Étienne Geoffroy Saint-Hilaire, who had initially written to Cuvier inducing him to move to Paris, and who himself believed that animals were subject to change, gave Cuvier some mummified ibises (a type of bird) from Egypt in 1802. After studying them, Cuvier found that though the mummified birds were more than 3,000 years old, their morphology was identical to that of current ibises. At the time 3,000 years was considered a very long time. Most people believed the Earth itself was only about 6,000 years old. Cuvier thought that if they had not changed in 3,000 years, they were never going to change.

In 1829 two of Geoffroy's followers tried to demonstrate to the academy that a link existed between cephalopods (invertebrates) and fish (vertebrates); Cuvier interfered. This eventually led to a huge public debate between the former collaborators and friends, Geoffroy and Cuvier, at the Royal Academy of Sciences in Paris in 1830. The issue was whether form determined mechanical function or function dictated form. Geoffroy believed that all vertebrates

had a common form of basic organization, with slight modifications. He claimed that vestigial organs such as the appendix demonstrated that all vertebrates had originally shared a common ancestral form. He thought that if structures were connected to other structures in the same manner, then differences in size or shape were not so important. Cuvier responded that similarities in form were simply the result of similar functions. He believed in the integration of parts into functional wholes. Today scientists accept both concepts, depending on the structures and species that are being compared. For example, the wings of a bird and the wings of an insect both allow the organisms' function of flight. However, these two types of animals do not share a common structural archetypical ancestor. These structures are considered analogous. On the other hand, the wings of birds and the wings of a bat do share a common vertebrate structural ancestor; they are more closely linked evolutionarily. Being derived from a common ancestral structure, the structures are referred to as homologous.

Another enormous zoological work by Cuvier was *Histoire naturelle des poisons* (Natural history of fish). Written in collaboration with Achille Valenciennes, it summarized all of the content knowledge of the field of ichthyology, the study of fish. The first volume was published in 1828, with eight more being completed prior to Cuvier's death. The 22nd and final volume was published in 1849. The classification system contained in this work remains the basis of modern ichthyologic classification.

CUVIER'S LEGACY

In May 1832, Cuvier suffered an attack of paralysis and died within a few days. His legacies include his library, boasting more than 19,000 volumes and thousands of pamphlets, and an increase in the collections at the muséum from a few hundred to more than 13,000 specimens, all arranged according to his own classification system. Cuvier is most remembered for supporting catastrophism, establishing the reality of extinction of past life-forms, and expanding Linnaeus's classification system of animals. However, his contributions in the form of progress reports on science and scientific biographies submitted in the capacity of permanent secretary to the institute are also noteworthy.

Constantly striving to overcome his humble beginnings, Cuvier was reported to be somewhat arrogant, hurried, and eager to receive flattery. He did achieve prominence during his lifetime and was appropriately honored with many appointments and titles. He was admired for his intelligence, but he was too stubborn to open his mind concerning the variability of species. Because of this, once the theory of evolution by means of natural selection became widely accepted, Cuvier's reputation diminished. However, he will always hold a place in scientific history for bridging the gap between the life sciences and the Earth sciences by founding the science of vertebrate paleontology.

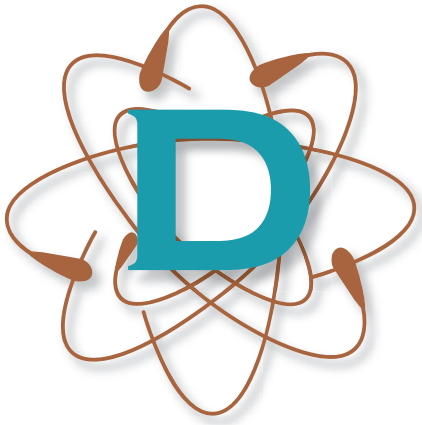
See also ANATOMY; BUFFON, GEORGES-LOUIS LECLERC, COMTE DE; GEOFFROY SAINT-HILAIRE, ÉTIENNE; LAMARCK, JEAN-BAPTISTE; LINNAEUS, CARL.

FURTHER READING

Oldroyd, David. *Thinking about the Earth: A History of Ideas in Geology*. Cambridge, Mass.: Harvard University Press, 1996.

Rudwick, Martin J. S. *Georges Cuvier, Fossil Bones, and Geological Catastrophes: New Translations and Interpretations of the Primary Texts*. Chicago: University of Chicago Press, 1997.

Waggoner, Ben. "Georges Cuvier (1769–1832)." University of California Museum of Paleontology. Available online. URL: http://www.ucmp.berkeley.edu/history/cuvier.html. Accessed January 23, 2008.



Darwin, Charles (1809–1882) English *Naturalist* Charles Darwin formulated the theory of evolution by means of natural selection following a five-year voyage around the world aboard the H.M.S. *Beagle*. When he published his theory in *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* in 1859, a controversy began; it would last until the latter part of the 20th century. While Darwin was not the first to propose that life-forms change or evolve, he was the first to propose a scientific mechanism for the process of evolution and to provide an overwhelming amount of organized evidence in support of it.

CHILDHOOD AND EDUCATION

Charles Robert Darwin was born on February 12, 1809, in Shrewsbury, England. His father, Robert, was the son of Erasmus Darwin, a respected physician and nature writer. His mother, Susannah, was the daughter of Josiah Wedgwood, a renowned potter and philanthropist. Both families instilled in their children a high regard for education. This was passed on to Robert and Susannah's own six children, but young Charles was not a promising student.

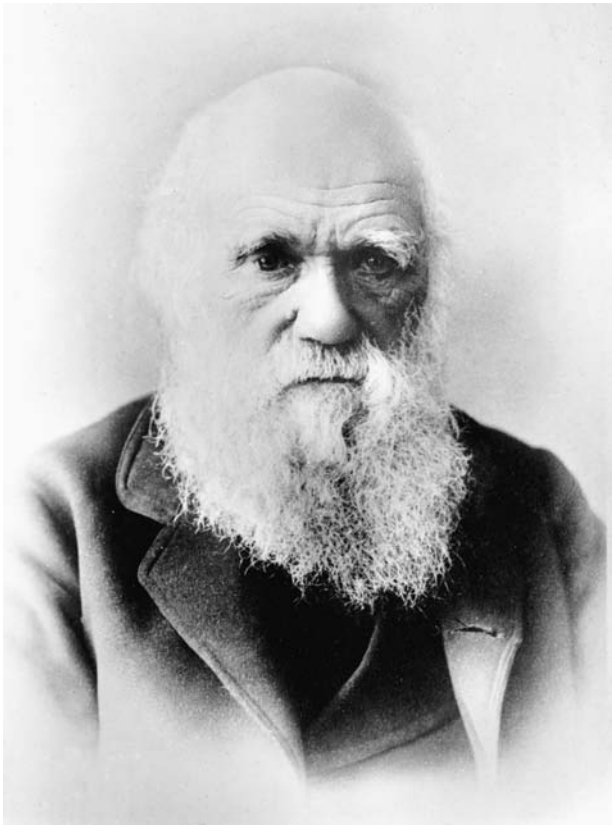
One year after his mother died, when Charles was nine, his father enrolled him at Shrewsbury School, where his teachers struggled to teach him Latin, the classics, and history. Charles, who preferred to spend his time in a chemistry lab his older brother had fashioned from a tool shed, was terribly bored. Even after the headmaster publicly admonished Charles for wasting his time on scientific pursuits when he should have been studying, Charles did not take a more serious interest in his studies.

When Charles was 16 years old, his father enrolled him at Edinburgh University to study medicine. Though his grandfather and father were both successful physicians, medicine was not to be Charles's destiny. He was disgusted by the animal dissections, and during a mandatory observation of human surgery, he was so repulsed that he ran out of the room.

Two years later Charles Darwin enrolled as a divinity student at Christ's College at Cambridge University, but he continued his personal scientific studies. He also joined the Glutton Club, whose members ate, drank, and played cards frequently. In his spare time he hunted birds and foxes and collected beetles. He successfully completed the requirements for a divinity degree in 1831, but he still needed additional elective credits, so Darwin enrolled in Professor Adam Sedgwick's geology class. For this class he read *A Personal Narrative of Travels to the Equinoctial Regions of the New Continent during the Years 1799–1804* by Alexander von Humboldt. He was fascinated by the voyage of discovery. A botany professor, the Reverend John Stevens Henslow, and Sedgwick both recognized Darwin's scientific mind and encouraged Darwin to pursue natural history as a career. Hesitant to challenge his father's wishes, Darwin earned his bachelor's degree in theology in 1831.

VOYAGE ON THE H.M.S. *BEAGLE*

At the same time, Commander Robert Fitzroy (1805–65) was preparing to depart for South America. Fitzroy was the commander of the H.M.S. *Beagle,* and he was looking for an intellectual companion during an upcoming voyage to explore the coasts of South



The British naturalist Charles Darwin was the first to provide overwhelming evidence in support of biological evolution and to propose a mechanism for evolution. *(Library of Congress)*

America and the Pacific Islands. His friends recommended he take Darwin along as the voyage's naturalist. Darwin's father initially balked at this idea but eventually gave in and provided financial support for his son on what would become one of the most influential scientific expeditions of all time.

The H.M.S. *Beagle* set sail from Plymouth, England, on December 27, 1831. Darwin suffered from severe seasickness and spent the first several weeks in the hammock of his cramped cabin. To pass time on board, Darwin read a recently published textbook by the Scottish geologist Charles Lyell (1797–1875), *The Principles of Geology*. Lyell opposed the popular ideas of the day regarding the history of the Earth. Most scientists believed in a strict biblical account of the creation of the world and the origin of life—God had created the world approximately 6,000 years ago and created all organisms in their present-day form. Fossils told a different story, one that included the prior existence of life-forms no longer present on Earth. Catastrophists believed that huge earthquakes and floods, such as the flood described in Genesis, accounted for the extinction of some species. Lyell argued that the Earth's current physical form was the result of gradual forces such as erosion and volcanic activity acting over a period of millions of years. Darwin agreed with Lyell. When he voiced his opinions to Fitzroy, Fitzroy was outraged by what he considered blasphemy.

On January 16, 1832, the *Beagle* stopped at the Cape Verde Islands off the northwest coast of Africa. Darwin performed the tasks he was taken along to accomplish—collecting specimens and making detailed notes of his observations. Darwin also observed evidence in the strata that supported Lyell's views on the gradual nature of Earth's change.

After a stop at Tenerife of the Canary Islands, the ship arrived in Brazil on February 28, 1832. Darwin was amazed by the wealth of life in the tropical rain forests and struck by the different types of life-forms. The crew reached Rio de Janeiro in April, and Darwin noticed the nearby rain forests had been destroyed to accommodate the city's growing population. He was also appalled at the treatment of slaves. He mentioned this to Fitzroy, who vehemently disagreed, causing conflict between the two men. Though Fitzroy later apologized, it was apparent the two did not have as much in common as they initially thought.

As they traveled down the east coast of South America, Darwin took time to explore the Punta Alta beach, where on September 23, 1832, he discovered the head of a large animal. The remains were from an extinct toxodon, a rodent the size of an elephant, similar to the present-day capybara, which is about two feet in length. A few days later he found the bones of a 20-foot- (6-m-) tall ground sloth. Darwin wondered why God bothered to create such similar animals. Why did God destroy the larger ones only to replace them with smaller versions?

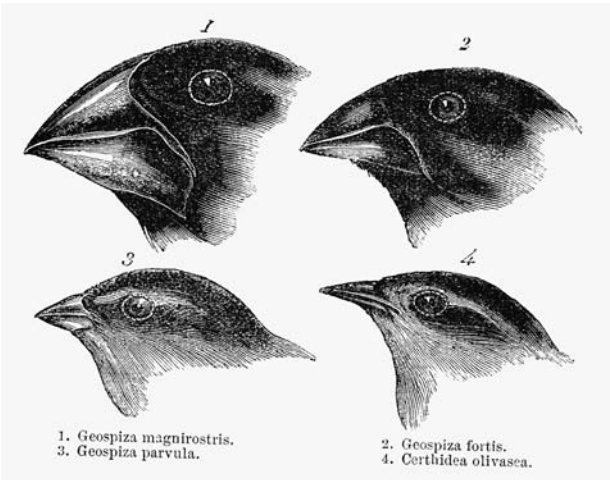
Darwin also noticed snakes with tail rattles that were not as efficient as those displayed by the North American rattlesnakes. In Patagonia he observed two different forms of unusual ostriches. He also took note of the mountains, valleys, and other geological features of the region. In February 1835, while in Chile, he experienced an earthquake that destroyed villages and killed inhabitants. The earthquake visibly raised the land level and altered other geological formations. Darwin had witnessed firsthand how an ordinary natural disaster affected the Earth's surface and the life it supported.

On September 15, 1835, the H.M.S. *Beagle* landed at the Galápagos Islands. This isolated equatorial chain of over one dozen volcanic islands lay 500 miles (800 km) off the west coast of Ecuador. The islands were home to numerous strange animals, animals that were never observed on the mainland of South America and certainly not in Europe. One of the most legendary species inhabiting the Galápagos

The giant tortoises Darwin observed on the Galápagos Islands seemed prehistoric to him. *(Paul Guther/ U.S. Fish and Wildlife Service)*

Islands is the giant tortoise. These tortoises weigh approximately 500 pounds (227 kg) and have shells with a circumference of eight feet (2.4 m). Darwin and his colleagues were easily able to ride on their backs. The tortoises seemed somehow prehistoric to them.

Another famous native of this archipelago is the finch. Darwin observed and sketched 13 different small birds, some of which resembled finches, along with others that had atypical beaks. He noticed that not only did these birds differ from the mainland birds, but each island seemed to have its own unique species. Darwin found it odd that geologically similar islands would have different species. They all resembled each other, but all varieties had different beak shapes. Some were clearly designed for cracking nuts and seed shells. Others were ideally suited for eating insects or fruit. One beak type resembled that of a woodpecker; its shape allowed the bird to extract larvae from tree bark. Darwin again wondered why God would create so many creatures that were very similar, yet had discernible differences. He suspected that some natural principle was at work, and that God was not the cause.



Darwin concluded that the variations in beak shape exhibited by the Galápagos finches were adaptations to the available local food supplies on different islands. *(HIP/Art Resource, NY)*

Late in the year of 1835, the *Beagle* left the Galápagos. They crossed the Pacific Ocean and stopped at Australia. Darwin wondered why kangaroos, wombats, and wallabies only lived in Australia. Darwin had a lifetime's worth of data to examine and lots of unanswered questions.

EVOLUTION BY MEANS OF NATURAL SELECTION

The ship returned to England on October 2, 1836. Knowing he was not interested in working as a clergyman, Darwin was worried about facing his father. His spirit was in science. Upon his return, he was pleasantly surprised to learn that his father was proud of his work as a naturalist. Professor Henslow had been circulating Darwin's correspondence, and he was respected among intellectuals. Darwin was relieved not to have to become a minister and set to work immediately writing up the narrative of his travels. *The Journal of Researches into the Geology and Natural History of the Various Countries Visited by the H.M.S.* Beagle *under the Command of Captain Fitzroy, R. N., from 1832–1836* was published in 1839.

Darwin settled back into life in England with many observations and specimens that he still needed to process and evaluate. He returned to the question of why so many subtly different forms of animals such as tortoises, birds, ostriches, and snakes existed. He concluded that the different forms descended from a single common ancestor. Physical variations slowly accumulated, they way geological changes gradually shaped Earth. The idea of evolution was not novel. In fact, during the 1770s, Darwin's own grandfather, Erasmus Darwin, had published a book discussing the concept, but the world was not ready to accept evolution. No probable method had been proposed, and no one had gathered or compiled enough factual evidence to support the theory. An even greater hindrance was that acceptance of evolution required abandonment of strict biblical teaching. Most people believed that God created the world and all of its species in their present form. Darwin knew that in order to convince the world of his theory, he would have to address a persuasive argument posed by those who opposed the idea of evolution. The argument stated that organisms were each perfectly suited for the environment in which they lived, and if they did accumulate modifications, these variations would lead them to become less well suited for their

habitat. Darwin had to figure out a way that organisms could change so that it appeared that they were designed that way.

Darwin had his work cut out for him. Fortuitously, he had the benefit of several intellectual colleagues with whom he could discuss his ideas. One such colleague was Lyell, whose geology text had influenced Darwin's thinking during the *Beagle* expedition. The two became good friends. Another friend was John Gould, a respected ornithologist. Gould confirmed for Darwin that the Galápagos finches he took back were all distinct species, not simply slightly different varieties of the same species.

By 1838 Darwin had already spent much time contemplating how offspring differ from their parents. Offspring had subtle yet discernible variations. Darwin thought that the accumulation of enough variations might lead to the formation of a new species over thousands of generations. He thought about artificial selection, the process by which farmers select domesticated animals or cultivated plants for breeding on the basis of their possession of a desirable characteristic. Over several generations, the incidence or degree of the favored characteristic increases. For example, a farmer may choose sheep that yield a superior amount of wool for breeding purposes. In the next few generations, the sheep's offspring also would yield more wool.

Darwin also considered extinction. Organisms were thought to become extinct because a change in climate or environmental conditions meant they were no longer perfectly adapted to their environment. Darwin concluded that if some of the offspring had accumulated enough variations, they might have an advantage over those that had not. Then the variant organisms might be better suited to survive in the new environmental conditions. Nature selected against the offspring that were not able to adapt.

In September 1838 Darwin started reading a popular book to give his mind a rest from thinking about evolution. That book was *An Essay on the Principles of Population* by Thomas R. Malthus, an English economist and clergyman. Malthus described how plants and animals produce more offspring than can survive. He discussed human populations and how poverty, famine, and disease acted to keep population size under control. Darwin recognized the significance of the natural struggle for existence, which may be summarized as follows: Animals produce many more offspring than can possibly survive. Those that do survive face a constant struggle for food and territory. Even if they successfully reach adulthood, they then must compete for mates. Offspring with variations that give them some advantage in their particular environment have a better chance to survive to reproductive age and to breed. Thus, those individuals who are best suited for survival in their environment are the members of that species that pass on their characteristics to the next generation. Since offspring are very similar to their parents, the likelihood is high that they possess the same characteristics that gave their parent an advantage. In other words, nature selects variations that are advantageous for survival and reproduction in a particular environment, just as farmers artificially select for economically desirable characteristics. Darwin called this process natural selection and believed it was the method by which evolution occurred over thousands of generations. Though this insight would eventually cause a revolution in science, Darwin hesitated to make it public.

Darwin had married his cousin, an intelligent woman named Emma Wedgwood, in January 1839. They moved to London and enjoyed the upper-class lifestyle courtesy of wedding gifts from their parents. They eventually had 10 children, only seven of whom survived infancy. Shortly after his wedding, Darwin became mysteriously ill. He was plagued with headaches, fatigue, and sleeplessness. Modern physicians have suggested that he suffered from Chagas disease, a tropical parasitic infection, but none of Darwin's doctors could diagnose his illness. The family moved to Down House, Kent, in September 1842, and Darwin retreated from the public.

The year he moved to the Kent countryside, Darwin sketched out his theory of evolution by natural selection in a 35-page outline. In 1844 he expanded it to 230 pages, but he delayed publishing it. Instead, he delved into the study of barnacles for the next eight years. Emma suspected this was a way of avoiding the expected controversy. Darwin knew that though he had collected adequate evidence for evolution and had formulated a plausible method, the majority of people would reject his theory on religious grounds. Darwin was a shy and now frail man. He did divulge his ideas to a few friends including Lyell and an English botanist named Joseph Hooker. They encouraged him to continue collecting evidence and developing his theory.

By 1856 Darwin still had not shared his ideas concerning evolution with the rest of the scientific world. Lyell and Hooker exhorted him to publish something before anyone else did. They told him it would be a shame for him to be preempted after two decades of tireless work, so Darwin began slowly writing. Lyell and Hooker urged him to work more rapidly, but Darwin wanted to be thorough.

Then, on June 18, 1858, Darwin received a letter from a young naturalist named Alfred Russel Wallace, who was in the Malay Archipelago at the time. Wallace had developed an idea for how spe-

cies might change with time, influenced by environmental changes acting to select for advantageous variations in offspring. An essay titled "On the Tendency of Varieties to Depart Indefinitely from the Original Type" was enclosed. Wallace wanted to know whether Darwin thought it worthy of publication. Darwin was stunned to read in Wallace's essay many of the same ideas contained in his own book, which was still in progress. He appealed to Lyell and Hooker for advice. They acted quickly by presenting both Wallace's essay and Darwin's outline to the Linnean Society on July 17, 1858. Hooker helped to establish priority for his friend by asserting that they had discussed the same ideas more than a dozen years prior. Surprisingly, Wallace was very chivalrous about this.

Now Darwin wrote furiously, producing a 200,000-page manuscript by March 1859. That November, *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* was published. All 1,250 printed copies sold the first day. The book was very detailed and was composed of three main sections. The first section described the process of natural selection of favorable variations. The second section dealt with objections to common arguments against evolution such as the lack of transitional forms and the development of complex specialized organs such as eyes. The third section elucidated how the theory of evolution by natural selection explained many previously unexplained phenomena such as extinction and the slight resemblance of modern and ancient species. *On the Origin of Species* presented immense substantiation for Darwin's theory of evolution by natural selection.

Despite the well-defined supportive arguments and the massive evidence Darwin provided for evolution, his theory engendered a storm of brutal criticism. Famous scientists including his former professor Sedgwick, the English zoologist Richard Owen, and the Swiss-American naturalist Louis Agassiz were all outraged and viciously reviled Darwin and his theory. Darwin had dreaded this outcome and was predictably upset. Luckily, he found equally aggressive and competent defense from his loyal friends. One such ally was Thomas Henry Huxley, a well-known biologist and educator. Huxley was an excellent public speaker and welcomed the challenge of a public debate against Bishop Samuel Wilberforce.

The famous debate took place on June 30, 1860, at Oxford University during the annual meeting of the British Association for the Advancement of Science in front of a crowd sporting more than 700 anxious people. Wilberforce spoke first, denouncing evolution and criticizing Darwin. His speech consisted mostly of personal opinions. The audience applauded loudly and cheered when Wilberforce ended his dialogue by asking Huxley whether it was through his grandmother or grandfather that he was descended from a monkey.

Huxley spoke next. He pointed out that Wilberforce did not state anything new and did not even appear to understand Darwin's theory or arguments. After carefully reviewing Darwin's theory and clearly presenting the arguments in favor of evolution by natural selection, he ended his speech with the statement that he would rather be descended from an ape than be related to a man bestowed with great intellectual gifts who used them to obscure the truth and mock serious scientific debate. The audience was uncontrollable. Some women fainted. Fitzroy was present. He wildly waved his Bible in the air and yelled abominations against Darwin.

Hooker then calmly made his way to the podium. He was disgusted by the behavior of the audience. He proceeded systematically to destroy all of Wilberforce's arguments over a two-hour period. In the end, the force of truth prevailed.

The controversy continued, however. Darwin left the defense of his ideas to his qualified contemporaries and spent his time in the gardens of Down House. Though he had deliberately left out any mention of the human species in *On the Origin of Species,* it had become the focus of the debate between creationism and evolution. In 1867 Darwin tackled this directly by composing *The Descent of Man,* published in 1871. Darwin declared that humans and apes had evolved from a common ancestor, but this idea often is represented incorrectly as that humans descended from apes. Darwin braced himself for more attacks, but this book did not generate the controversy that *On the Origin of Species* did. Most of the scientific world had already dealt with the notion of human evolution and accepted it as part of evolutionary theory.

The remainder of Darwin's life was peaceful. He published other works including *The Expression of the Emotions of Man and Animals* (1872), *Insectivorous Plants* (1875), and *The Movements and Habits of Climbing Plants* (1875). He also wrote an autobiography for his children in 1876 and enjoyed time with his family. His unexplained illness disappeared. In December 1881, he suffered his first heart seizure. Charles Darwin suffered a second heart seizure and died on April 19, 1882. Though he was elected a member of the Royal Society of London and even awarded their Copley Medal in 1864, he never received formal recognition from the British government while he was alive because his work offended the leaders of the Church of England. After his death, Parliament requested that he be buried in Westminster Abbey near Sir Isaac Newton.

Today most people have heard of Charles Darwin and consider Darwinism synonymous with evolution. Fundamentalists still decry evolution and fight to suppress its teachings. Amazingly, the negative feelings the general populace harbors concerning evolution are strong enough to force strict guidelines addressing the manner in which it is taught in public schools. In the scientific community, however, evolution by natural selection is a fundamental unifying theory of all the life sciences.

See also Dobzhansky, Theodosius; evolutionary biology; evolution, theory of; Gould, Stephen Jay; Humboldt, Alexander von; Wallace, Alfred Russel.

FURTHER READING

The Charles Darwin Foundation for the Galápagos Islands home page. Available online. URL: http://www.darwinfoundation.org. Accessed January 19, 2008.

Darwin, Charles. *On the Origin of Species*. Cambridge, Mass.: Harvard University Press, 1964.

Dobzhansky, Theodosius. "Nothing in Biology Makes Sense Except in the Light of Evolution." *The American Biology Teacher* 35 (1973): 125–129.

Jastrow, Robert, ed. *The Essential Darwin*. Boston, Mass.: Little, Brown, 1984.

Wilson, Edward O., ed. *From So Simple a Beginning: The Four Great Books of Charles Darwin*. New York: Norton, 2006.

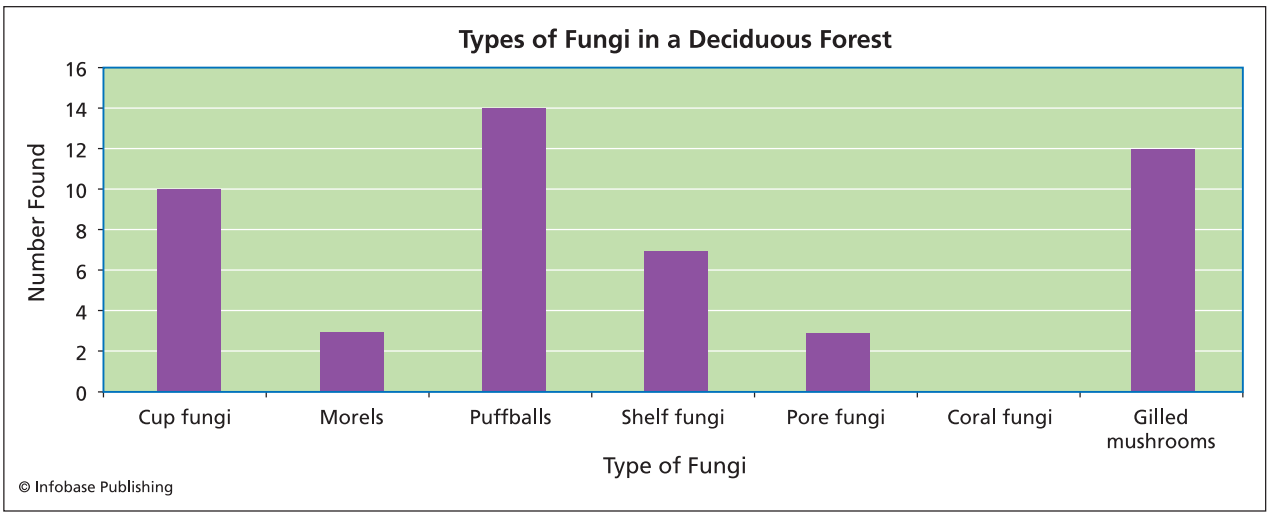
data presentation and analysis Data are the objective information collected during the course of an experiment and may take the form of measurements or descriptive observations. Data analysis is the conversion of the raw data into useful information from which a researcher can draw conclusions.

GRAPHS

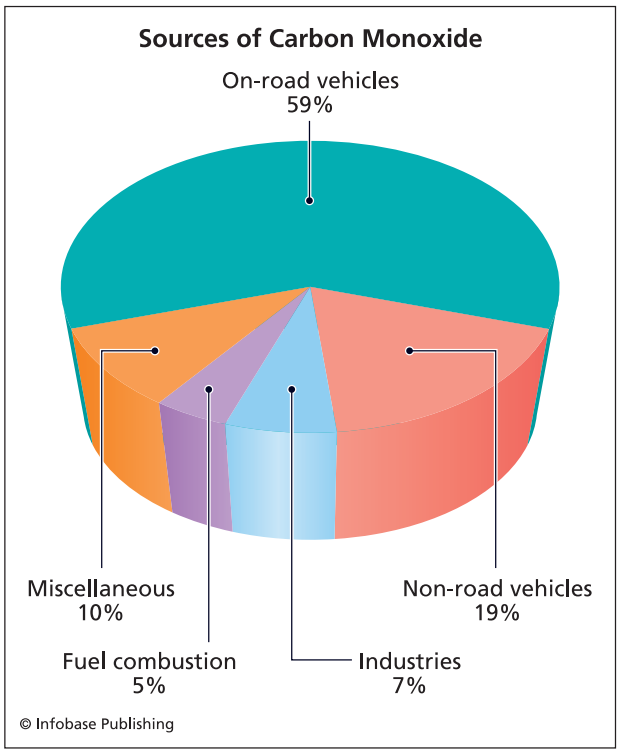
Charts, tables, and graphs are useful ways to present data. Selecting the most appropriate method for presenting data facilitates analysis by accentuating patterns. Charts are often used for raw data collection; the researcher records the information directly as lists, notes on a diagram or on a map, or on other defined areas of a chart. After recording all the data on a chart, the researcher may compile the information in table format or in a spreadsheet. Tables consist of rows and columns that contain combined data or information. Generating a table facilitates graphing, especially when the researcher uses computer software to generate the graphs or pictorial representations. The use of the many different forms of graphs to present data reveals trends or patterns that might not be as apparent when using a chart or a table to present the information.

Bar graphs depict quantitative data from discontinuous categories. The horizontal axis contains the distinct categories, and the vertical axis shows the number of occurrences. Vertical bars shaped like rectangles extend upward to the demarcation for the number of units associated with that data set. For example, a researcher surveying a defined area of a deciduous forest for different types of fungal species might depict data in the form of a bar graph. One can look at the graph below and immediately see that puffballs were the most common type of fungus in the observed area of forest, and that coral fungi were not observed at all.

Pie charts or pie graphs show the relative frequencies of subdivisions of a set of data. The area of each



A typical bar graph



A typical pie chart

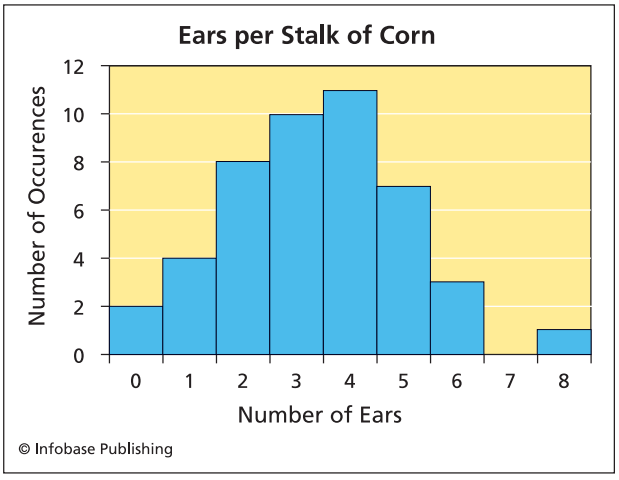
"slice" of the circular pie represents the percentage of the whole that a particular subdivision makes up. For example, an environmental scientist may report the sources of carbon monoxide emissions in a particular city in the form of a pie chart like the one above. This allows one to see at a glance that the major source of emissions is on-road vehicles, with nonroad vehicles (such as boats or construction equipment and machinery) contributing the second highest amount.

Histograms also depict frequencies, but they reveal how the frequency of the data relates to another particular variable. One can study a histogram to see the distribution of the frequencies over the entire range of the variable. For example, a botanist might use a histogram to show the frequency of number of ears per stalk for a new strain of corn. The number of ears on a stalk would be indicated on the *x* axis, and the rectangular bars would extend up to the number of occurrences for each number. From the histogram depicted at right, one can see that four ears per stalk occurs most frequently and that the numbers ranged from zero to eight ears per stalk.

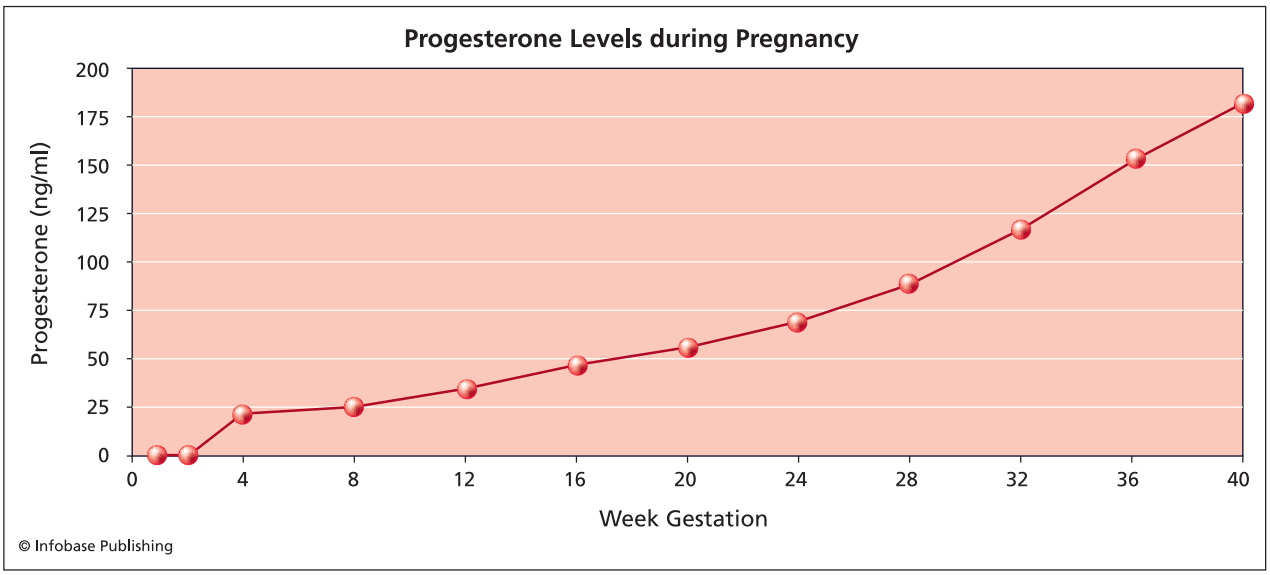
Line graphs reveal relationships between two sets of continuous data and are especially useful for displaying data over time. The data points are plotted and connected by lines that may reveal informative trends. For example, the line graph on page 240 shows that circulating levels of the hormone progesterone progressively increase during gestation.

One can infer values for unobserved data by extending the line of a line graph beyond the last data point. Scientists often use this process, called extrapolation, to make predictions about the future effect of a trend if it continues along the same pathway. For example, if data show that the number of fish species in a particular lake is declining at a rate of one per year, and the lake currently supports 17 different species of fish, then one could use extrapolation to predict that if the current trend continues, within 17 years the lake will be devoid of fish. Interpolation is the process of determining a value at an interval between two observed data points. For example, if the ichthyologist responsible for counting the number of fish species in the lake recorded data for every year between 1993 and 2007 with the exception of the year 2002, then one could use interpolation to infer the number of fish that were present in the lake in 2002 from the data obtained before and after that time point.

In a scientific experiment, the independent variable is the factor that the experimenter intentionally manipulates, and the dependent variable is what the experimenter evaluates for change. If the experiment is carefully controlled, meaning the only difference between experimental systems is the independent variable, then the general direction and the steepness of the line drawn between data points demonstrate how changing the independent variable affects the dependent variable, if at all. The independent variable is graphed on the horizontal axis, which is referred to as the *x* axis. The dependent variable is graphed on the vertical axis, which is referred to as the *y* axis. In the line graph, the dependent variable is the number of mutant bacterial colonies that grow on a plate of medium and is a function of the concentration of the chemical mutagen added to the medium, at least for the range of concentrations tested.



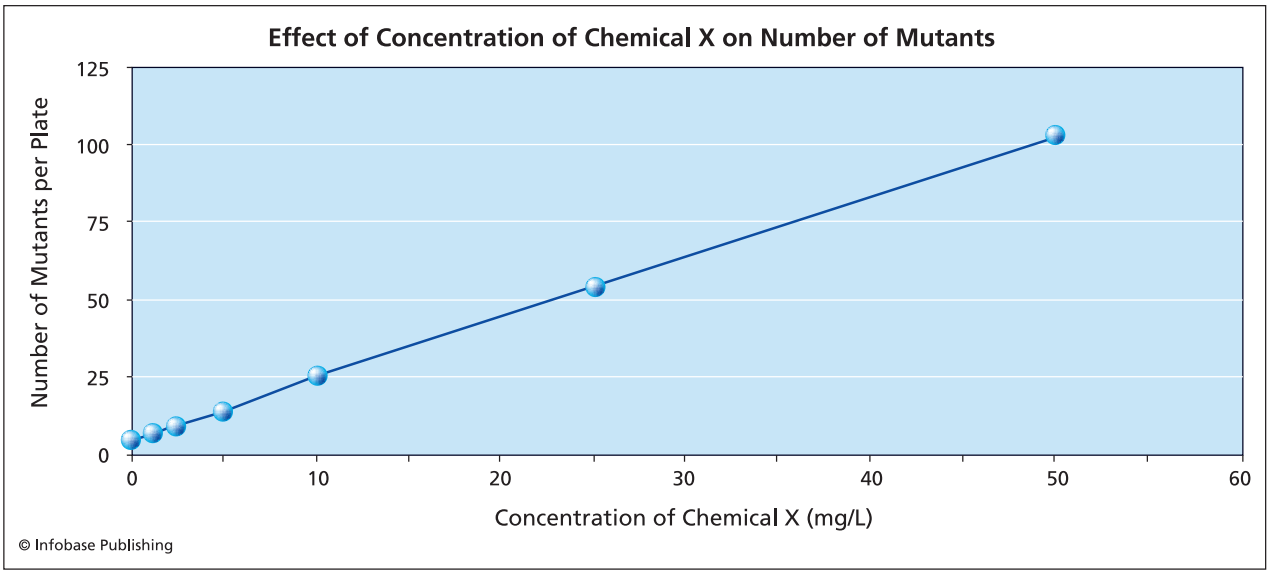
A typical histogram



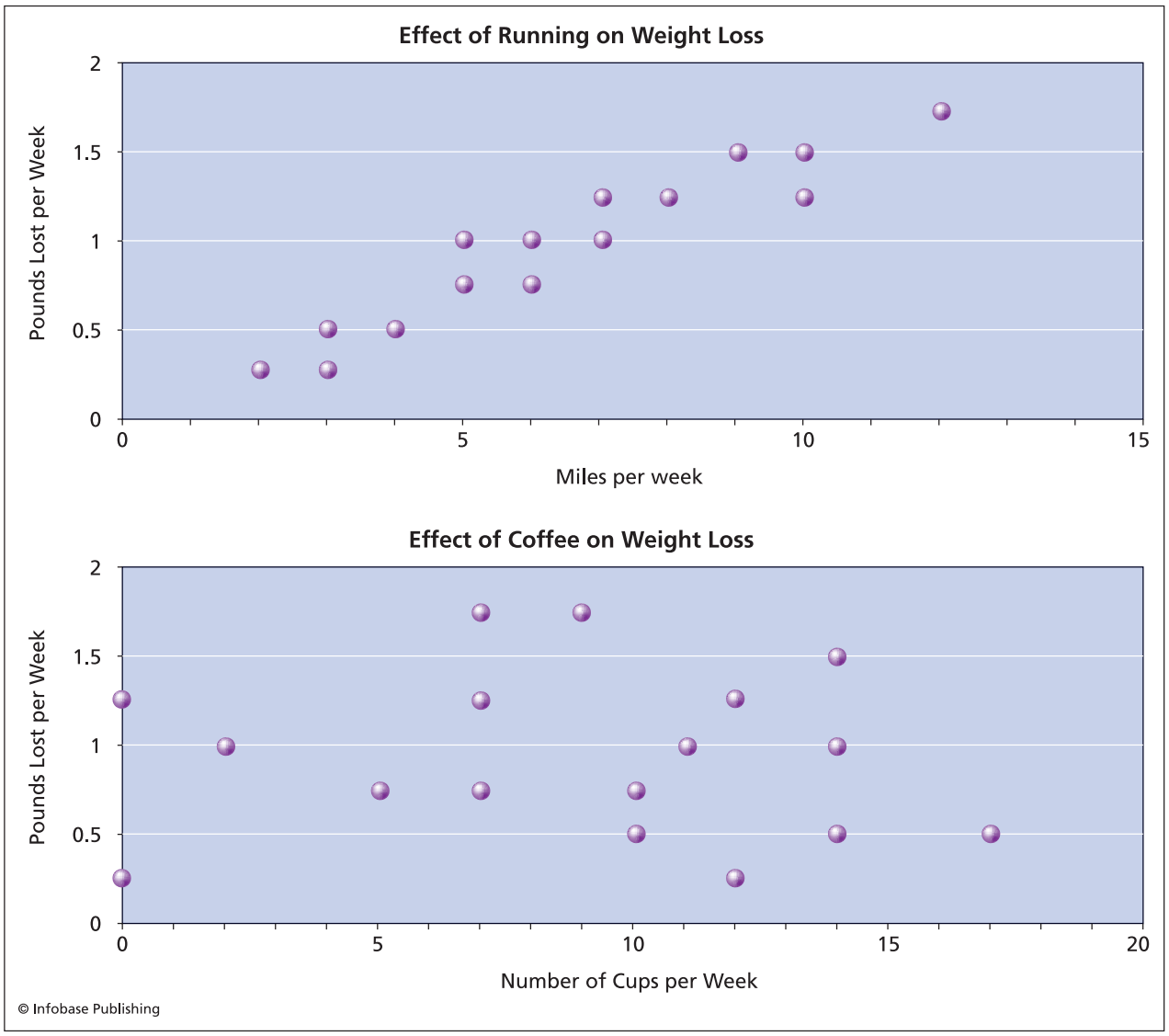
This line graph reveals a trend—progesterone levels increase with time of gestation.

One must be careful, however, in concluding from a line graph that one variable is causing the measured variable to change. Some plots may reveal a trend or relationship without one of the variables directly influencing the other. The variables change together in a way not expected on the basis of chance alone, but one does not necessarily cause the other to change. In some cases the researcher cannot ensure that only a single factor is altered during an experiment. Data collection might involve observing a phenomenon that occurs in nature and cannot be manipulated. For example, the average temperature of the ocean may vary at different times of the day, but the researcher cannot experimentally control the many factors that cause this effect (e.g., the intensity of the radiation from the Sun as it reaches the water, oceanic circulation patterns). In this case, the time of day does not cause the change in temperature—other factors that also change over time cause the resulting temperature variations—but plotting the data on a line graph will still reveal the temporal patterns in temperature fluctuations.

Scatter plots are similar to line graphs but are used to determine whether and how one variable relates to another. After the data are entered onto the scatter plot, a computer can generate a line of best fit, also



Line graphs illustrate the relationship between independent and dependent variables.



Scatter plots reveal possible correlations between variables.

called a regression line, through the data points. If the data points lie on a reasonably straight line, they are said to be correlated. A widely scattered pattern indicates that no correlation exists between the two factors. If the *y* values increase as the *x* values increase, then the variables have a positive correlation, and if the *y* values decrease as the *x* values increase, the variables are said to be negatively correlated. To illustrate this, from the scatter plots (generated from hypothetical data), one can conclude that running larger distances correlates with greater weight loss, whereas the number of cups of coffee someone drinks each week does not appear to be correlated to weight loss.

STATISTICAL ANALYSIS

Scientists use statistical analysis to interpret numerical information obtained during the course of an experiment. Statistics is a branch of mathematics devoted to collecting data and extracting meaningful information from those data. Because the data obtained during an experiment must be analyzed afterward, the researcher must carefully design an experiment that will allow one to perform an efficient and meaningful statistical analysis. Statistics helps the experimenter do this, for example, by indicating how many data must be collected in order to draw reasonable conclusions from the results. Another purpose of statistical analysis is to determine the reliability of a data set. Ideally, the information obtained from the observed or tested sample reflects information that is true for a larger population. In order to determine whether a sample accurately depicts the behavior or a result characteristic for the larger population, one must first be able to summarize the

experimental data. The mean and standard deviation are two important statistical descriptions of a sample data set.

The mean (χ) is the average of a sample of numerical values and can be calculated by the following formula:

$$\chi = \frac{\sum_{i=1}^{i=n} x_i}{n}$$

Simply, the mean equals the sum of all the numerical values in the data set divided by the number of values (n). As the sample size increases, that is, as the number of values in the data set increases, the mean of the sample approaches the mean of the population (μ) that the sample represents. (Statisticians use Roman letters to indicate sample values, referred to as statistics, and use Greek letters to represent population variables, referred to as parameters.)

Standard deviation (s) and variance (s^2) indicate the spread of the population, in other words, the degree to which the individual data points differ from the mean. A small standard deviation means that all the measured variables are very close in value. The standard deviation of a sample (s) of n data is an estimate of the standard deviation of the entire population (σ). The variance is its square.

$$s = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})^2}{n-1}}$$

$$s^2 = \frac{\sum_{i=1}^{i=n} (x_i - \bar{x})^2}{n-1}$$

Because the difference between each datum and the mean is squared in the formulae, the value will increase the standard deviation whether the datum is less than or greater than the mean. The standard deviation will have the same units as the mean, but the variance will have the units of the mean squared.

To illustrate the concepts of mean, standard deviation, and variance, consider the following hypothetical data for number of trunk segments in *Geophilus* species (centipedes), presented in the table at right Number of Centipedes with Different Numbers of Segments. The mean equals 54.06 segments (though the actual number of trunk segments in centipedes is always odd). The standard deviation equals 4.77 segments, and the variance is 22.72 segments^2.

The standard deviation is basically the average of the squared differences of the individual data points and the mean, but the sum of the squares is divided by the degrees of freedom rather than n. Degrees of freedom (df) is a mathematical restriction that indicates the number of independent variables upon which a calculation is based and equals n - 1, since the mean must be determined before computing standard deviation, and calculating the mean uses up one degree. In other words, the degrees of freedom are the number of values in a calculation that are free to vary, a measure of the amount of precision an estimate of variation has. The more parameters that must be estimated, the fewer the degrees of freedom.

Standard deviation relates to another statistical descriptor called the confidence interval, a range of values about a sample mean that is likely to contain the population mean with a stated probability, such as 95 or 99 percent. Both indicate a degree of reliability for a set of data. Confidence limits are the values at the extreme ends of the defined confidence interval. Remember that when collecting data, a sample is meant to represent the entire population. The mean of the representative sample will probably differ slightly from the mean of the population, but one can define a range of values and declare that the population mean will fall within that range defined by the sample data 95 percent of the time. One can extend the limits slightly and predict with 99 percent certainty that the

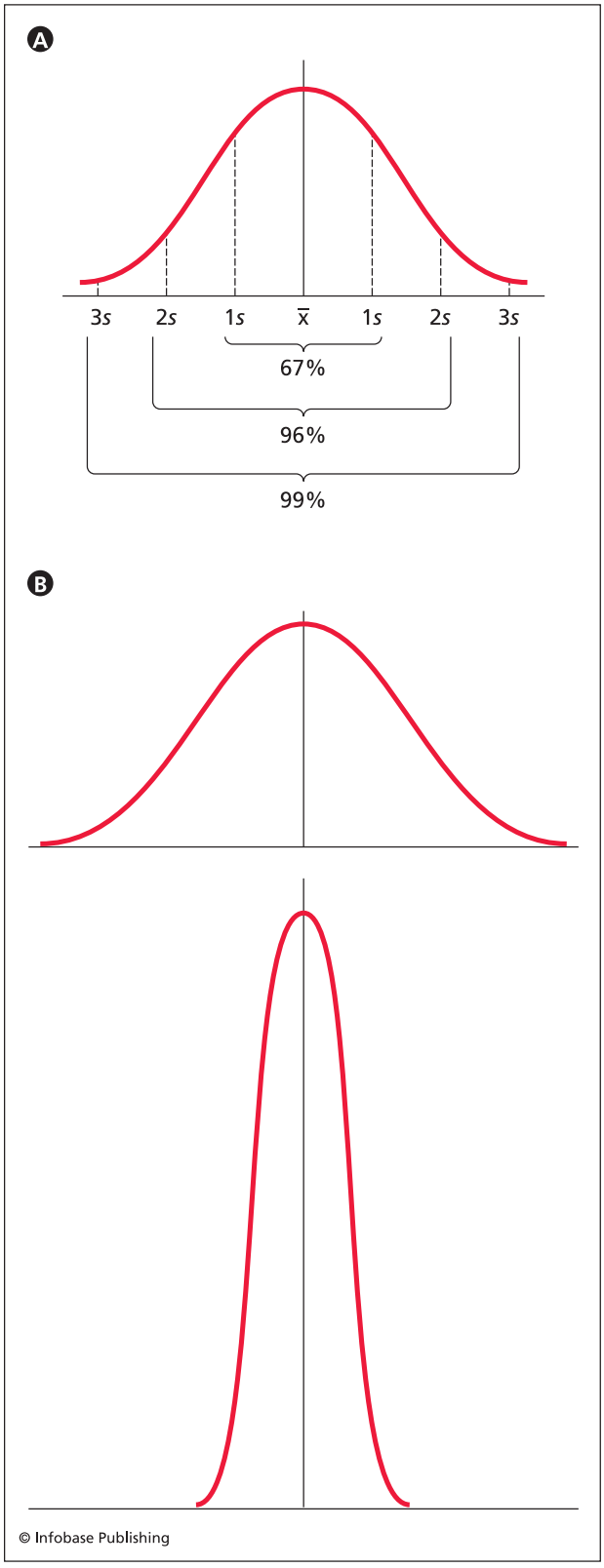
NUMBER OF CENTIPEDES WITH DIFFERENT NUMBERS OF SEGMENTS

Number of Segments	Occurrences
43	2
45	5
47	6
49	9
51	28
53	36
55	39
57	12
59	10
61	4
63	3
65	2
67	4
69	2

population mean will fall within the slightly larger range. As the sample size increases, the sample mean will better estimate the population mean and the standard deviation will decrease, allowing for the prediction of a tighter range for the population mean while maintaining the same degree of confidence.

In order to determine how much variation to expect, an investigator calculates the frequencies with which various results could occur for a given experiment. For example, if a geneticist crosses two plants heterozygous for a trait, the expected ratio of offspring that exhibit the dominant characteristic to offspring that exhibit the recessive characteristic is 3:1. Using simple rules of probability and a mathematical expression called a binomial expansion, one can determine the probability that in a sample of four offspring, three would express the dominant characteristic and one would express the recessive characteristic. If one calculates the probabilities for all the possible outcomes (all four offspring dominant, all four recessive, two of each, three dominant and one recessive, or three recessive and one dominant) and plots them as expected frequencies for each outcome on a graph, the result is called a normal frequency distribution. The peak of a normal distribution curve represents the mean, and the spread or dispersion increases or decreases as the standard deviation increases or decreases.

Because the *y* axis depicts frequencies rather than actual numbers, the distribution curve reflects possible outcomes for all sample sizes. If the investigator repeats the experiment numerous times, most often the results will be close to 3:1, but on occasion, they may deviate significantly. A normal distribution curve shows how often one would expect such deviations to occur on the basis of chance. To illustrate the concept of a normal distribution, consider another example, in which someone flips a coin 10 times. Assuming the probability of landing on heads is the same as that of landing on tails, of all the possible outcomes, the probability that of 10 coin flips, five would be heads and five would be tails is the highest. However, few people would be surprised if the result were six heads and four tails, or even seven heads and three tails. Most people would be surprised, however, if all 10 flips resulted in heads. Though this is a possible outcome, randomly flipping a coin and obtaining the same result 10 times would be an unlikely outcome. The normal distribution curve created by calculating and plotting the probabilities for



A) The curve of a normal distribution resembles a bell, with 67 percent of the area within 1*s* of either side of the mean, 96 percent within 2*s* of the mean, and 99 percent of the area within 3*s* of the mean. B) Both of these plots are normal distributions about the same mean, but the sample depicted in the lower diagram has a smaller standard deviation than the data depicted in the upper diagram, as indicated by the difference in the spread of the curves.

all the possible outcomes would peak at the position reflecting the outcome of 50 percent heads and 50 percent tails. The area underneath the curve represents all of the possible outcomes. One can draw vertical lines at each end of the curve that mark off 5 percent of the total area (2.5 percent at each end). The remaining area represents 95 percent of the possible outcomes. Thus, one can predict that 95 percent of the time, a data set will give results that fall under this portion of the curve.

One common application of statistics is to test the validity of a hypothesis. Frequency distributions allow a researcher to determine whether to accept or reject a hypothesis. A null hypothesis is the hypothesis against which the data are tested; it assumes the observed data do not deviate significantly from the expected data; that the differences are due to chance alone. After data collection, the researcher calculates the probability of obtaining those data assuming the null hypothesis to be true. If the probability is high, the data support the hypothesis. If the probability is too low, the researcher rejects the hypothesis. If something is statistically significant, it is unlikely to occur by chance. A researcher must decide the level of significance to use when drawing conclusions from their data. The significance level is a fixed probability of wrongly rejecting the null hypothesis when it is true. Scientists often base their determinations on a 5 percent level of significance. This means that the data will deviate from the expected results enough to reject the hypothesis 5 percent of the time on the basis of random chance alone, and the scientists risk rejecting a true hypothesis under those circumstances.

The chi-square test is one method life science researchers use to see whether their data are consistent with the null hypothesis or whether the data reject the null hypothesis. The formula for calculating a chi-square value (χ^2) is

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

where O is the observed number for a category and E is the expected number on the basis of the hypothesis being tested. Comparison of the calculated value with a value from an available table of chi-square values for specific degrees of freedom and probabilities tells the researcher whether the data reject or fail to reject the hypothesis. The values from the table are the probabilities of obtaining a chi-square value calculated from experimental data given certain numbers of variable parameters or categories (degrees of freedom) for a normal frequency distribution. One must decide the level of significance to use. Note that as the degrees of freedom increase, the critical values for chi-square increase, as more parameters increase the expected standard deviation, even if the sample data fit well with the expected outcome. Because one must add the calculated chi-square values from each category in order to obtain the total chi-square value, simply increasing the number of categories will increase the critical chi-square value. A calculated total value that is less than the critical chi-square value indicated by the table means that the data support the hypothesis; a calculated value that is higher than the value given by the table means the data differ significantly from the outcome predicted by the hypothesis, and thus, reject the hypothesis. If the data fail to reject the hypothesis, one can conclude that the data support the hypothesis, but not that they prove it.

Imagine a population biologist counting the number of male and female babies born in a particular city. Since fertilization is a completely random event, one may hypothesize that the data will reveal equal numbers of boys and girls. In other words, the null hypothesis would be a 1:1 ratio of boys to girls. Because this example contains two categories (boys and girls), there is one degree of freedom. Only one of the categories can freely vary—if the result in one category is known, so is the other, since there are only two possibilities. For example, if the outcome of one event is not a boy, it must be a girl. Suppose the

CHI-SQUARE (χ^2) VALUES

Degrees of Freedom	*Probabilities*						
	0.99	0.95	0.80	0.50	0.20	0.05	0.01
1	0.000	0.004	0.064	0.455	1.642	3.841	6.635
2	0.020	0.103	0.446	1.386	3.219	5.991	9.210
3	0.115	0.352	1.005	2.366	4.642	7.815	11.345
4	0.297	0.711	1.649	3.357	5.989	9.488	13.277

researcher collects data on 1,000 total children, 528 of whom are boys and 472 of whom are girls. Given the null hypothesis of 1:1, the expected number of boys is 500, and the expected number of girls is 500. Chi-square for this example is calculated as follows:

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$
$$= \frac{(528-500)^2}{500} + \frac{(472-500)^2}{500}$$
$$= 3.136$$

The critical chi-square value for one degree of freedom with a 5 percent level of significance is 3.841. This means that given the null hypothesis of a 1:1 ratio of boys to girls, the probability of getting a chi-square value greater than or equal to 3.184 by chance alone is 5 percent. Because 3.136 is less than the critical value of 3.841, the researcher should not reject the hypothesis of 1:1 on the basis of the sample data.

When the sample size is small, one can use a test called the *t*-test to determine whether the sample mean differs significantly from the population mean. This type of test assumes the dependent variable is normally distributed. The *t* value can be calculated and compared to a table of values to determine the probability that the *t* value would be exceeded in a larger number of replicates. The two-sample *t*-test is another common test of significance used to determine whether two independently observed groups of sample data are from populations that have the same mean or whether they are statistically different. After calculating the *t* value, a table of significance will reveal whether the value is significant.

FURTHER READING

Hibbert, D. Brynn, and J. Justin Gooding. *Data Analysis for Chemistry: An Introductory Guide for Students and Laboratory Scientists*. New York: Oxford University Press, 2006.

McCleery, Robin H., Trudy A. Watt, and Tom Hart. *Introduction to Statistics for Biology*. 3d ed. Boca Raton, Fla.: Chapman and Hall/CRC, 2007.

Newman, Isadore, Carole Newman, Russell Brown, and Sharon McNeely. *Conceptual Statistics for Beginners*. 3rd ed. Lanham, Md.: University Press of American, 2006.

deoxyribonucleic acid (DNA) Deoxyribonucleic acid (DNA), the molecular basis for heredity, contains within its sequence of nucleotides all of the information necessary for an organism's growth, maintenance, and reproduction. In eukaryotic cells, the DNA is compartmentalized in the nucleus, and in prokaryotic cells, the DNA exists in the cytoplasm. The unique structure of DNA allows for duplication of the genetic material as well as encoding of all the genetic information for the synthesis of proteins and other macromolecules that result in the outward expression of species-specific and individual characteristic traits.

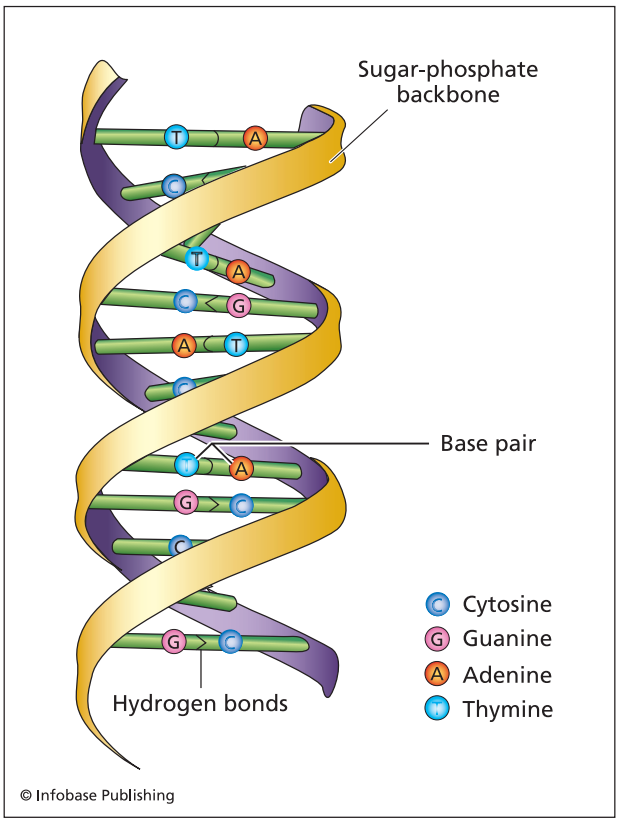
STRUCTURE

The double-helical molecule of DNA is composed of two individual strands, each a chain constructed from four different nucleotides. The two strands wind around each other and are held together by hydrogen bonds between complementary nitrogenous bases.

The individual nucleotide subunits that compose DNA consist of a deoxyribose sugar, a phosphate group, and a nitrogenous base. The nitrogenous bases of adenine (A) and guanine (G) make up the purines, nucleotides that contain two linked rings—one with five members and one with six members. Cytosine (C) and thymine (T), the pyrimidine nucleotides, each have a nitrogenous base that consists of a single six-membered ring. A phosphate group linked to the 5'-C of the deoxyribose component of one nucleotide forms a phosphodiester linkage with the hydroxyl group extending from the 3'-C of the deoxyribose sugar of the adjacent nucleotide. The nitrogenous base extends from the 1'-C of the deoxyribose sugar.

The two strands that make up a double-helical molecule of DNA are complementary, meaning that the sequence of one strand can be predicted from the sequence of the other. Because A always forms hydrogen bonds with T and G always forms hydrogen bonds with C, the sequence of one strand determines that of the complementary strand. Two hydrogen bonds link A and T, whereas three hydrogen bonds connect C and G. In order for the correct hydrogen bonds to form, the two strands must run antiparallel to one another, meaning the orientations of the two strands are opposite. Following along the backbone of a single strand, the 5'-phosphate group of one nucleotide leads to the pentose sugar, then to the 3'-hydroxyl group of that same nucleotide, which is attached to the 5'-phosphate of the adjacent nucleotide, and so on. Looking at the complementary strand, however, the directionality is reversed. If the one strand runs 5' to 3', then the opposite strand runs 3' to 5'. Alternate arrangements of hydrogen bonding can occur between nucleotides, but the regular pairing of a purine with a pyrimidine maintains the constant width of approximately 20 angstroms in this form of DNA.

The helix formed by wrapping the two strands of DNA around each other is right-handed. In other



DNA consists of two strands, wrapped around one another and connected by hydrogen bonds between specific pairings of nitrogenous bases of the nucleotides.

words, when viewed from the top of its axis down the center, the helix turns in a clockwise direction. If unwound, the double-stranded DNA would resemble a ladder, with the base pairs represented by the horizontal rungs of the ladder and positioned perpendicular to the sugar-phosphate backbone, represented by the vertical side rails of the ladder. The helix completes one turn every 10 base pairs, or 34 angstroms. The form of DNA described thus far is called the B form, but alternate forms of DNA do exist. In the Z form, the helix adopts a left-handed character and a zigzagged backbone. This form could potentially occur naturally in GC-rich areas by the addition of methyl groups to the cytosine moieties, and it might be involved in regulating gene expression. Another form, A DNA, is characterized by slightly tilted base pairs and has more base pairs per turn than B DNA.

DNA REPLICATION

The characteristic of specific pairing between nucleotides supports a semiconservative mechanism for DNA replication, in which each linear chain of nucleotides serves as a template for the creation of a new, complementary strand. Double-helical daughter molecules contain one parental strand and one newly synthesized strand.

Prior to division, the two strands of DNA separate at specific sequences termed origins of replication. First, an enzyme called helicase unwinds the DNA, the hydrogen bonds are broken, and the two strands open up in that region, forming two Y-shaped replication forks from which replication will proceed in both directions. Proteins called single-stranded binding proteins bind to each strand of DNA to prevent the hydrogen bonds from reforming. Replication begins when an enzyme called primase creates primers of approximately 10 ribonucleotides in length at the replication fork. This step is necessary because DNA polymerase, the enzyme that forms covalent linkages between deoxynucleotides, cannot initiate synthesis of nucleic acid without a preexisting free 3'-hydroxyl group. After the synthesis of primers, DNA polymerase reads the parental template strand of DNA, then adds the appropriate complementary deoxynucleotide to the nascent strand, forming an ester linkage between the existing 3'-hydroxyl group and the 5'-phosphate of the incoming nucleotide.

DNA polymerase can only synthesize a new strand of DNA in the 5' → 3' direction. The enzyme scans or reads the template strand 3' → 5' and elongates 5' → 3'. This limitation in conjunction with the antiparallel nature of a double-stranded DNA molecule complicate the progress of replication. The two parental strands of DNA at each replication fork run antiparallel to one another. Thus, only one of them can be scanned continuously in the 3' → 5' direction by DNA polymerase toward the junction of the replication fork. The newly synthesized daughter strand that is complementary to this one is termed the leading strand. The other daughter strand is called the lagging strand because it is synthesized in short, interrupted fragments termed Okazaki fragments. The enzyme primase initiates the synthesis of numerous primers to which DNA polymerase adds nucleotides, progressing away from the replication fork until it reaches the end of another RNA primer. A different DNA polymerase then removes the ribonucleotides from the primer and replaces them with deoxyribonucleotides. The short fragments of DNA are eventually joined together by yet another enzyme, DNA ligase, in order to form one continuous new strand of DNA.

The 5' ends of a DNA molecule present a unique problem for DNA polymerase. Because the enzyme requires a 3'-OH to add nucleotides, as the DNA polymerase approaches the 5' end of a linear DNA molecule, such as a eukaryotic chromosome, the lagging strand cannot be synthesized to the very end by usual mechanisms. The small gap that would be left at the 5' end of the lagging strand would

grow larger with each successive round of replication. To prevent this gradual erosion at the telomeres (the physical ends of the chromosomes), eukaryotic organisms have between 100 and 1,000 repeated DNA sequences at the ends of their chromosomes. An enzyme called telomerase adds these terminal repeats to maintain chromosome length over time.

The crucial function of DNA as the carrier of a cell's genetic information demands a high fidelity. The error rate for DNA replication approaches only one in 1 billion nucleotides. Immediately after the initial pairing of an incoming nucleotide with its complementary partner on the original DNA template, DNA polymerase proofreads, or double-checks, to ensure that proper base pairing rules have been obeyed. If an incorrect nucleotide was incorporated, the polymerase excises it and replaces it with the correct complementary nucleotide. Other mechanisms repair mutations on existing DNA.

ESTABLISHMENT OF DNA AS THE GENETIC MATERIAL

In the early 1900s, scientists believed protein was the most likely candidate for the molecular carrier of genes. Composed of chains of 20 different amino acids, proteins seemed the most varied of biological macromolecules in structure and function, making them the probable means of encoding all of a cell's genetic information. Not many suspected DNA, thinking it was a tetranucleotide, that is, a short chain of the four different deoxyribonucleotides linked to one another. It did not seem as if that simple structure could encode the tens of thousands of different proteins made by a cell and allow all the genetic diversity observed among organisms. Thus, the discovery that DNA was the carrier of genetic information was quite a surprise to the scientific community.

The establishment of DNA as the genetic material was the result of the work performed by many individuals. The process started with a British researcher named Frederick Griffith, who studied the different types of *Streptococcus pneumoniae,* a type of bacterium that causes pneumonia. In the late 1920s he was particularly interested in two strains, one that was deadly to humans and one that was harmless. The virulent bacterial strain was encapsulated with a polysaccharide covering that gave its colonies a smooth appearance; thus it was named *S* for "smooth." Because the nonvirulent strain produced rough-looking colonies in comparison, it was referred to as *R,* for "rough." When the S strain was injected into mice, the mice developed pneumonia and quickly died, whereas the R strain had no deleterious effect. When Griffith first killed the S type by heating it, injecting the bacterial cells into mice did not kill them. However, when he injected mice with both living R cells and heat-killed S cells, the mice died. Griffith demonstrated that the R bacteria had acquired polysaccharide capsules as the S bacteria had and that the new characteristic was passed on to progeny of the transformed bacteria. He figured that the gene that encoded the information to make the capsule was lost in the R strain, and the molecule that carried this genetic information was transferred from the S strain to the R strain, transforming it. He published his results in 1928 without having any idea of what molecule was responsible.

This question was painstakingly addressed by Oswald Avery, Colin MacLeod, and Maclyn McCarty at the Hospital of the Rockefeller Institute for Medical Research. Avery's assistants first duplicated Griffith's results in vitro. They made extracts of the S strain by repeated freezing and thawing and used these extracts to transform R strain bacteria. To identify the substance in the extract that was responsible for transformation, they purified different substances from the extracts by treating them with various enzymes known to break down specific types of molecules. Expecting a protein to be the transforming factor, they attempted to perform transformations with the purified substances, but the only substance that successfully transformed R into S bacteria was DNA. The carrier of genetic information was DNA. This result astonished them, but further tests confirmed their results, and they published their data in 1944.

The worldwide scientific community did not immediately embrace the idea that DNA was the genetic material. Four main factors contributed to the lack of enthusiastic reception of the proclaimed biological activity of pneumococcal DNA.

- The timing, during the height of the United States' involvement in the war, was unfortunate.
- The readership of the journal in which Avery published his paper was limited.
- The members of the Rockefeller team were bacteriologists, not geneticists.
- Finally, the erroneous assumption that protein was the genetic material was just too hard for many to surrender.

Though some scientists gradually accepted the enormity of the conclusions of Avery and his colleagues, the next major event that convinced the remaining nonbelievers to accept DNA as the genetic material occurred in 1952. Alfred Hershey, an investigator at Cold Spring Harbor Laboratory, and his assistant, Martha Chase, published their results from some ingenious experiments performed

with T2 bacteriophage, a virus that infects bacterial cells. They demonstrated that DNA physically entered bacterial cells and directed the synthesis of new viral particles with their well-known blender experiment. Briefly, they radioactively labeled the bacteriophage by inoculating bacterial cultures growing in the presence of either radioactive phosphorus (^{32}P) or radioactive sulfur (^{35}S) with T2. Phosphorus is a component of nucleic acid, and sulfur is found in proteins. They used the radiolabeled T2 to infect fresh cultures of bacteria and found the ^{32}P label inside bacterial cells and within new viral progeny, but the ^{35}S remained outside the bacterial cells, showing that nucleic acid but not protein entered the bacteria during the infection process.

The common theme that structure determines function pervades the biological sciences, and the molecular level provides no exception. By the time that Hershey and Chase published their results, the race was on to determine the structure of this biological molecule now known to be of utmost importance. The top contenders were the British biophysicists Maurice Wilkins and Rosalind Franklin at King's College in London, the American biochemist Linus Pauling at the California Institute of Technology, and James D. Watson and Francis Crick at Cambridge University. In 1950 the Austrian biochemist Erwin Chargaff found that the amount of the nitrogenous base adenine always equaled the amount of thymine and that the amount of cytosine always equaled the amount of guanine. Another clue to the puzzle was X-ray diffraction evidence obtained by Franklin and Wilkins suggesting the molecule was helical. Watson and Crick employed a model-building strategy, repeatedly constructing and disassembling structures to fit better with all the biochemical information, chemical bonding requirements, and structural data. In early 1953 they proposed their famous double-helical model. Knowing the structure of DNA, biologists were soon able to figure out the mechanisms for its replication and for the coding of genetic information, leaving no room for any doubt as to its profound significance and role as the molecule of life.

See also Avery, Oswald; biomolecules; cellular reproduction; Chargaff, Erwin; Chase, Martha; chemical basis of life; Crick, Francis; Franklin, Rosalind; gene expression; genetics; genomes; Griffith, Frederick; Hershey, Alfred; MacLeod, Colin Munro; McCarty, Maclyn; molecular biology; Pauling, Linus; Watson, James D.; Wilkins, Maurice H. F.

FURTHER READING

Calladine, Chris. R., Horace R. Drew, Ben F. Luisi, and Andrew A. Travers. *Understanding DNA: The Molecule and How It Works,* 3rd ed. San Diego: Elsevier Academic Press, 2004.

Lewin, Benjamin. *Genes IX*. Sudbury, Mass.: Jones and Bartlett, 2008.

Watson, James D., Tania A. Baker, Stephen P. Bell, Alexander Gann, Michael Levine, and Richard Losick. *Molecular Biology of the Gene*. 6th ed. San Francisco: Benjamin Cummings, 2007.

diabetes The common endocrine system disorder diabetes is characterized by inadequate secretion or utilization of the hormone insulin. This results in excessive urine production, thirst, weight loss, and high glucose (sugar) levels in the urine and blood. Insulin, made by the pancreas, helps the body utilize glucose, the major energy source for the body's cells. Diabetes results when the pancreas does not produce sufficient insulin, or when the body's cells do not respond to it as they should. There are several types of diabetes: Type 1, formerly known as insulin-dependent diabetes mellitus; Type 2, previously called non-insulin-dependent diabetes mellitus; and gestational diabetes, which occurs during pregnancy. Other types of diabetes can result from genetic abnormalities, surgery, drugs, or other causes. According to the International Diabetes Federation, diabetes afflicts 246 million people worldwide and kills 3.8 million people each year.

ROLE OF INSULIN

Living organisms must maintain relatively constant internal conditions despite fluctuations in the external environment. For example, most organisms can only survive within a certain range of temperature. A decrease or increase in temperature may stimulate a behavioral or physiological response that functions to counteract the change in order to maintain the internal body temperature within a safe range. Blood sugar level is another factor that the body must maintain within a limited range. After the digestive system breaks down carbohydrates, glucose diffuses into the bloodstream, which then transports the glucose throughout the body. In the tissues, the cells uptake the glucose and utilize it as an energy source to carry out cellular functions.

Insulin helps keep the concentration of glucose in the blood within a healthy range whether someone has just eaten a meal or has not eaten in several hours. It is a protein hormone that facilitates the cellular uptake of glucose molecules. Because glucose is polar, it cannot simply diffuse through the phospholipid bilayer of a cellular membrane; specific carrier proteins allow the glucose to move through the membrane without contacting the interior hydrophobic layer of the membrane. In the presence of

insulin, the transporters that carry glucose across the cell membrane are present at the cell surface. In the absence of insulin, the transporters are stored in vesicles inside the cell. In this manner, insulin facilitates the movement of glucose from the blood into the body's cells.

A portion of the pancreas called the islets of Langerhans consists of groups of endocrine cells that produce and release the hormones that regulate blood glucose levels. Beta (β) cells make and secrete insulin, and alpha (α) cells make and secrete glucagon. After a meal, the increased blood glucose levels directly stimulate the release of insulin. This results in glucose uptake into the cells, which decreases the circulating levels once again. When the blood glucose levels are no longer higher than normal, the release of insulin stops. Within a few hours after eating, the blood glucose levels return to normal. This negative feedback system maintains relatively constant levels of blood glucose in healthy individuals.

TYPES AND SYMPTOMS OF DIABETES

Type 1 diabetes results from the destruction of more than 90 percent of the insulin-producing cells of the pancreas. Because the pancreas cannot produce enough insulin, this form of diabetes used to be called insulin-dependent diabetes. Approximately 5–10 percent of all people who have diabetes have Type 1, and most of them develop symptoms before they reach 30 years old. Because of this, Type 1 is also sometimes referred to as juvenile-onset diabetes.

Type 2 diabetes, also called non-insulin-dependent diabetes or adult-onset diabetes, results when a person's cells develop a resistance to insulin. The pancreas still produces and secretes insulin, but as the disease progresses, much more insulin is needed to move the transporters to the cell membrane to uptake glucose from the blood. Type 2 diabetes typically affects adults, and 80–90 percent of people who have this form are obese. People who take corticosteroids or who have high levels of natural corticosteroids may also develop Type 2 diabetes, or if they already have diabetes, steroid injections for other conditions (such as asthma, acute poison ivy, or arthritis) may worsen the diabetes.

Pregnant women who have never had diabetes may develop it late in their pregnancy. This form of diabetes is called gestational diabetes, and approximately 4 percent of pregnant women in the United States develop it. The cause is not known, but hormones naturally synthesized by the placenta during pregnancy are known to interfere with insulin action. The woman may require up to three times the normal amount of insulin to overcome this resistance. Gestational diabetes can result in too much weight gain in the fetus because of the excessive amounts of sugar available in the blood. This may cause difficulties during childbirth, and these children have a higher risk for becoming obese and developing Type 2 diabetes later in their lives. The mother's diabetes usually ends after the pregnancy, but she is at higher risk for developing Type 2 diabetes in her future.

Whatever the type of diabetes or the cause, without sufficient insulin, the body cells starve. Though the person may eat plenty, the glucose cannot enter the cells. Hyperglycemia, or high blood sugar levels, leads to increased urine production. The excess sugar spills into the urine, and the presence of excess solute in the urine filtrate prevents water from being reabsorbed. The volume of urine increases, and the person feels very thirsty from the fluid loss. This dehydration can cause circulatory failure due to decreased blood volume and may even result in death if cerebral blood flow is reduced. Fatigue, hunger, nausea, and blurred vision may also occur.

In the case of Type 1 diabetes, the "starved" cells switch on an alternate metabolic pathway that allows them to obtain energy from fat cells. Compounds called ketones are made as fat stores break down, and acidic by-products accumulate, leading to a condition called ketoacidosis. In addition to extreme thirst and increased urine production, the person may experience nausea, vomiting, fatigue, and abdominal pain. Respirations will be deep and rapid in order to rid the body of extra carbon dioxide in an attempt to maintain a more neutral blood pH. Ketoacidosis causes the breath to have a distinct odor. In the absence of treatment, the patient may die within hours.

Whereas the onset of Type 1 diabetes can occur rapidly, Type 2 usually develops slowly, gradually becoming worse over a period of several years. The person will exhibit similar symptoms to those exhibited by Type 1 diabetics, but symptoms worsen slowly to the point at which the person feels the need to seek medical attention.

DIAGNOSIS AND TREATMENT

When a patient seeks medical attention for symptoms including constant thirst and frequent urination, the physician will look for sugar in the urine by dipping a paper strip that has been treated with special chemicals into a sample obtained from the patient. If the result of this test is positive, then blood will be drawn after a period of fasting and the levels of sugar present will be measured. If the levels are higher than the normal range, the person may have diabetes mellitus. Other symptoms and factors will help the physician diagnose Type 1 or Type 2 diabetes and will determine the course of treatment.

For all types of diabetes, controlling blood sugar levels requires a healthy diet, meaning one high in

fiber, low in saturated fats, and low in simple sugars. Treatment of Type 1 diabetes usually involves the administration of insulin, either by injections or by a pump. Eating a healthy diet and a consistent one with respect to amount of total calories consumed and having regular mealtimes makes it easier to determine the proper doses of insulin. Exercise is also beneficial. Because diabetes compounds the negative effects of alcohol and smoking, people who have diabetes should abstain from or limit these activities.

Successful treatment of Type 2 diabetes may not require medication; the disease may respond sufficiently to a controlled diet, weight loss, and exercise. In addition to insulin, medications to treat Type 2 diabetes include drugs that stimulate the pancreas to produce more insulin or to do so more quickly, increase sensitivity to insulin, decrease the amount of glucose produced by the liver, and slow the absorption of ingested starches.

If blood sugar levels fluctuate too much or remain too high, as when the diabetes is untreated, complications may result. High blood sugar level can cause the interior diameter of the blood vessels to shrink by leading to increased deposition of fatty substances on their walls, a condition called atherosclerosis. This prevents good blood flow and inhibits circulation. Because of this diabetics are at higher risk for heart attacks and stroke, and heart attacks are more likely to be severe and lead to death in diabetics.

Diabetes can also lead to neuropathies, disorders in which nerve damage leads to numbness or pain in the extremities. Vascular and nerve damage can reduce one's sensitivity to pain in the feet, cause poor circulation, and slow the healing of foot ulcers. When these problems become serious, amputation may be necessary. Eye problems and kidney disease can also result from high blood glucose level and high blood pressure from diabetes. Improper dosages of medications used to treat diabetes can lead to blood sugar levels that are too low, called hypoglycemia.

DIABETES INSIPIDUS

Diabetes insipidus produces similar symptoms to diabetes mellitus, extreme thirst and excessive urine production, but has a different underlying cause. In this type of diabetes, either the body fails to synthesize or secrete sufficient quantities of antidiuretic hormone (ADH) or the kidneys fail to recognize or respond to it. The hypothalamus normally synthesizes ADH, and the posterior pituitary gland stores it until the hypothalamus chemically stimulates the pituitary to release it during a water deficit. ADH then travels through blood circulation until it reaches the kidney, which has special receptors that recognize and bind the hormone. In response, the kidneys reabsorb more water during urine production. Diabetes insipidus can result from damage to the hypothalamus or pituitary gland, interfering with ADH synthesis or release. Failure of the kidneys to respond to ADH also can cause diabetes insipidus.

See also Banting, Sir Frederick G.; endocrine system; homeostasis.

FURTHER READING

American Diabetes Association home page. Available online. URL http://www.diabetes.org/home.jsp. Accessed August 12, 2007.

International Diabetes Foundation home page. Available online. URL http://www.idf.org. Accessed January 23, 2008.

U.S. Department of Health and Human Services. Centers for Disease Control and Prevention. National Center for Chronic Disease Prevention and Health Promotion. "Diabetes Public Health Resource." Available online. URL: http://www.cdc.gov/diabetes/. Updated December 4, 2008.

"World Health Organization Diabetes Programme." Available online. URL: http://www.who.int/diabetes. Accessed January 23, 2008.

digestive system

The purpose of the digestive system is to take food into the body and convert it to a usable form that can be transported throughout the body. Animals are heterotrophs, meaning they must take in food from their external environment as a source of energy and nutrients. One way to classify animals is their usual source of food. Herbivores eat autotrophs, organisms with the ability to synthesize organic molecules from inorganic carbon sources, such as plants or algae. Carnivores consume the flesh of other animals. Omnivores obtain their nutrition from plants or algae and other animals. Each type of feeder has unique adaptations that allow it to obtain, ingest, and digest food from its usual source. Animals also can be classified according to the mechanism used for obtaining food. Suspension feeders filter food particles such as plankton or small invertebrates from the water. Substrate feeders live on or inside the substance they eat. Fluid feeders obtain their nutrition from organic fluids such as blood or nectar obtained from a living host. Bulk feeders consume large pieces of food, a task that often requires adaptations for chasing and killing prey.

STAGES OF DIGESTION

The four processes of digestion are ingestion, digestion, absorption, and elimination. Ingestion is the process by which an animal takes food substances into the body, for example, by tearing pieces of leaves from a plant with mouthparts, sucking nectar from a flower, filtering surrounding water for food

particles, or grasping and placing large pieces of food into the mouth. Usually, the body cannot utilize the form of food introduced. Mechanical processes such as chewing or grinding help break food into smaller, manageable pieces that digestive juices can efficiently dissolve. Macromolecules such as proteins, lipids, nucleic acids, starches, or other polysaccharides are too large to be absorbed by body cells. The process of digestion breaks down the food into usable fragments. Enzymes secreted by cells of digestive tissues or accessory glands reduce the macromolecules into individual subunits such as amino acids, monosaccharides, fatty acids, or nucleotides. Absorption is the uptake of these smaller nutrient substances from the digestive compartment. In primitive invertebrates, individual body cells absorb the nutrients directly from a gastrovascular cavity, but in most animals, a separate circulatory system often transports the nutrients throughout the body. The indigestible material remaining in the digestive tract after absorption exits the body as feces through a process called elimination.

DIVERSITY OF DIGESTIVE SYSTEMS

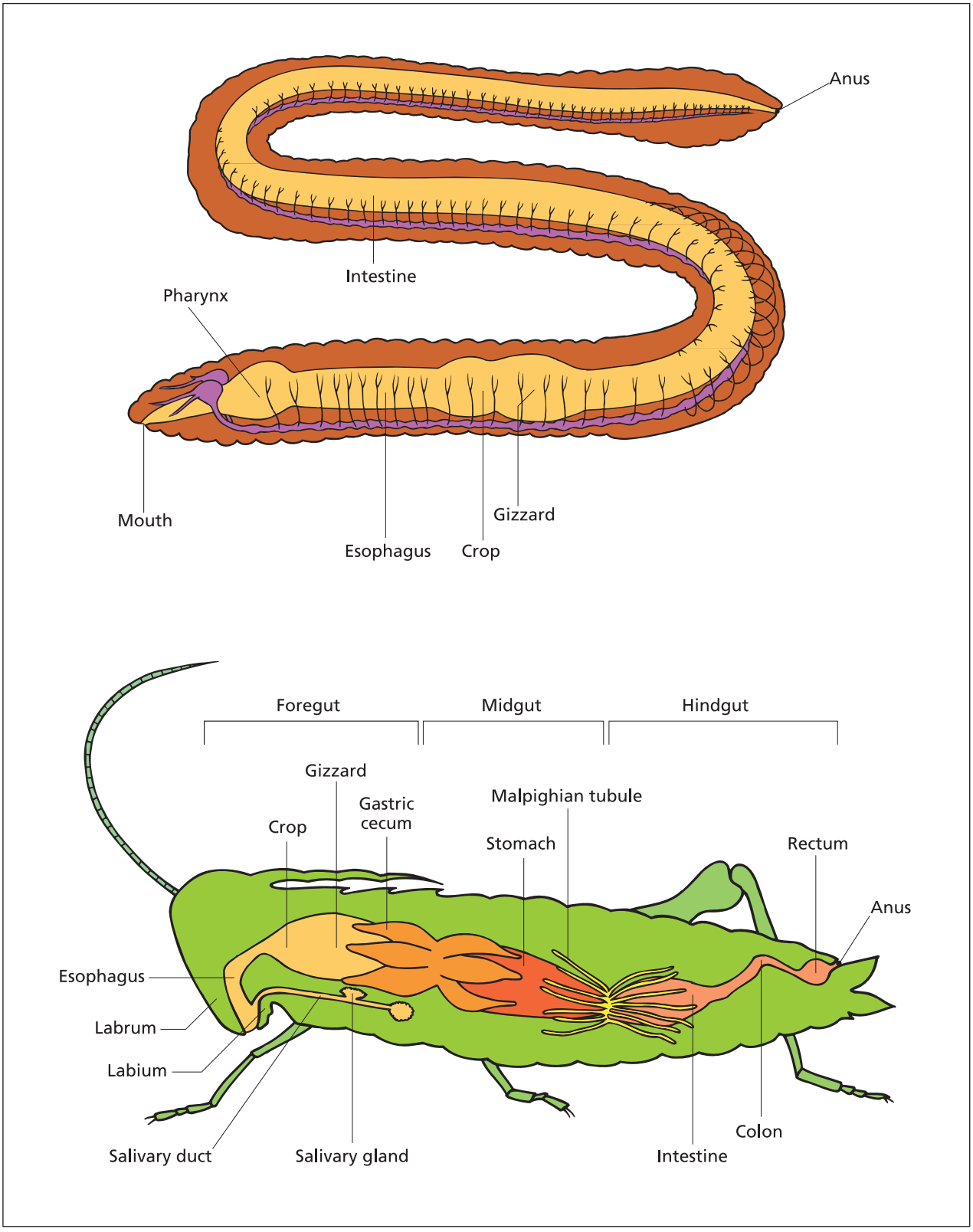
Some animals perform intracellular digestion while others digest food extracellularly. In intracellular digestion, each cell obtains in its own food and digests it. More primitive animals such as sponges and cnidarians digest food in this manner. Extracellular digestion is more complex and often requires a body system with several parts to accomplish each process of digestion. The animal breaks down the food outside cells, inside a body cavity that is continuous with the external environment, and then individual cells absorb the nutrients.

Many animals, such as flatworms and cnidarians, have a single opening, a mouth, through which food enters and travels to a gastrovascular cavity. Because most of the body cells have direct contact with the gastrovascular cavity, no special system or mechanism is necessary to transport the nutrients. Other animals have a digestive tract, a tube with openings at both ends. Food enters at the mouth, then travels through a long tube that has parts specialized in different digestive functions. For example, earthworms have a muscular pharynx that sucks in food through the mouth. The food moves down the esophagus into the crop for storage, before moving into the gizzard, where bits of gravel grind up the food into smaller particles. Chemical digestion and absorption occur mostly in the intestine, and undigested material exits the body through the anus. Birds have digestive tracts similar to those of earthworms but also have a stomach located between the crop and gizzard.

Insect digestive systems consist of three regions: a foregut, a midgut, and a hindgut. In grasshoppers, the foregut includes the mouth, pharynx, esophagus, crop, and gizzard. Mouthparts vary among insect species depending on whether the animal feeds on plant foliage and needs parts adapted for tearing and chewing or obtains nutrition from fluids, requiring mouthparts adapted for piercing and sucking. The labrum and labium are liplike structures surrounding the mouth, and the maxillae and mandibles are jawlike parts for chewing. Some insects have a long tube called a proboscis for sucking nectar from flowers. The salivary glands produce and secrete saliva containing some digestive enzymes. The tubelike esophagus leads from the pharynx to the crop, which stores food until the muscular gizzard pulverizes it. The midgut, where most digestion and absorption occur, includes the gastric cecum and the stomach. Bacteria and protozoa that aid in digestion reside in the gastric cecum, which also produces digestive enzymes. The stomach mixes the digestive enzymes with the ground food particles. Absorbed materials enter the hemolymph, the fluid that bathes the tissues of animals with open circulatory systems. The hindgut contains the openings of the Malpighian tubes, the intestine, colon, rectum, and anus. Hemolymph diffuses into the Malphigian tubes, which serve primarily as excretory organs to remove nitrogenous wastes and in osmoregulation. Water reabsorption occurs in the hindgut, and undigested food and nitrogenous wastes exit through the anus.

HUMAN DIGESTIVE SYSTEM

The digestive tract (gastrointestinal tract or GI tract) of humans consists of a long tube through which food passes as it is broken down, digested, and absorbed or eliminated and several accessory organs and glands. The digestive tract includes the mouth, pharynx, esophagus, stomach, small intestine, large intestine, rectum, and anus. The accessory glands are the salivary glands, the pancreas, the liver, and the gallbladder. The layers of the digestive tract share similar qualities all the way from the esophagus to the anus. Epithelial cells that line the tract transport substances into and out of the lumen and produce and secrete enzymes, mucus, and hormones. Epithelial cells lining the GI tract only live for a few days before sloughing off and being replaced. Layers of smooth muscle function to move substances within the lumen, either to churn it (as in the stomach), increase the flow of substances across the surface area of the epithelial cells for increased contact, or push it through the tract. Contractions force the food forward in successive waves by peristalsis. Connective tissue supplies blood vessels, lymph vessels, and nerves to the wall of the GI tract. The serosa, a thin layer of connective tissue that surrounds the entire tract, holds the digestive organs in place and



Earthworms and grasshoppers have one-way digestive tracts with regions specialized to perform different digestive functions.

is continuous with the peritoneum, which lines the entire abdominal cavity.

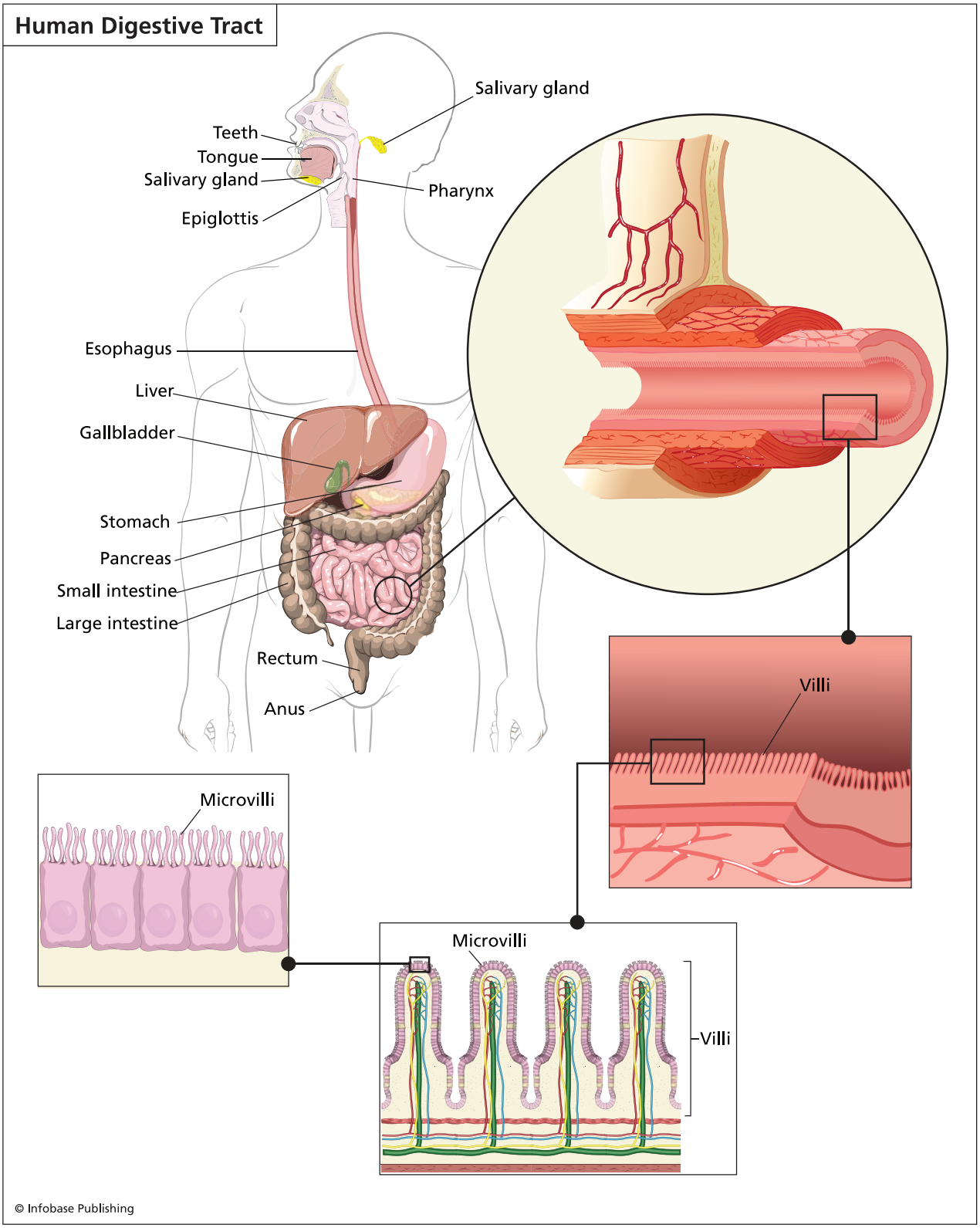
Digestion begins when food enters the mouth, where chewing begins the process of mechanical breakdown. Three pairs of salivary glands secrete saliva into the mouth, lubricating the food and initiating the process of chemical digestion. Digestive enzymes secreted by the accessory organs and glands catalyze the breakdown of macromolecular polymers. The enzyme amylase, present in the saliva, breaks down large starch molecules into maltose, a disaccharide made from two glucose subunits. When a bolus of chewed food or liquid reaches the pharynx, the swallowing reflex is triggered, and a little flap of cartilaginous tissue called the epiglottis moves to its down position and blocks the entrance to the respiratory tract. A muscular ring called a sphincter relaxes, and food moves into the esophagus. Peristalsis moves the bolus down the esophagus into the stomach within five to 10 seconds.

While the stomach churns the food, cells of its gastric glands secrete a variety of chemicals and enzymes that work chemically to digest it. Hydrochloric acid (HCl) denatures proteins, kills most microorganisms that have been ingested, and disrupts the matrix that holds many cells together in meat and plant tissue. The gastric glands secrete pepsinogen, an inactive digestive enzyme. The acidic environment created by the HCl in the stomach causes the cleavage of pepsinogen, converting it to pepsin, an active enzyme that cleaves peptide bonds between amino acids of protein chains. The gastric glands also secrete mucus and bicarbonate, substances that protect the digestive juices from digesting the stomach lining. Contractions of the smooth muscles surrounding the stomach move the contents, now called chyme, toward the pyloric sphincter, the opening to the small intestine.

The pyloric sphincter limits the amount of chyme that enters the duodenum, the first portion of the small intestine. In humans, the duodenum is about 10 inches (25 cm), and the entire length of the small intestine is approximately 20 feet (6 m). The pancreas, gallbladder, liver, and intestinal gland cells all discharge digestive enzymes and other substances into the duodenum. By a combination of hormonal and neural mechanisms, distension of the intestine stimulates the pancreas to secrete pancreatic amylase, lipases that digest lipids, and several types of proteases that digest proteins through a duct into the duodenum. Some enzymes are secreted as zymogens, meaning they are secreted in an inactive form and must undergo a chemical change to become active. The pancreas also secretes bicarbonate, a substance that buffers the acidic pH of chyme entering from the stomach. The liver produces a solution called bile from bile salts, bile pigments, and cholesterol. The bile travels via hepatic ducts to the gallbladder for storage until food enters the digestive tract, then flows through the common bile duct into the duodenum. Bile contains no enzymes, but the bile salts help emulsify lipids to dissolve them in the fluids flowing through the GI tract. The brush border, the epithelial lining of the duodenum, also secretes many digestive enzymes into the lumen, but other enzymes including peptidases that hydrolyze peptides, disaccharidases that split disaccharides into monosaccharides, and a protease called enteropeptidase remain attached to the surface of the brush border while the chyme and the enzymes it contains move forward by peristalsis and other contractions. By the time the chyme reaches the end of the duodenum, enzymes have digested most of the carbohydrates, proteins, lipids, and nucleic acids into monosaccharides, amino acids, fatty acids and glycerol, and nucleotides, respectively.

The remaining two regions of the small intestine, the jejunum and the ileum, function mainly in absorption of nutrients. To increase the surface area across which absorption occurs, the lining forms large circular folds that are covered with fingerlike projections called villi. Microscopic extensions called microvilli extend from the villi, effectively increasing the surface area of the internal lining and, consequently, the rate of absorption. Though the small intestine of an average adult human is about 20 feet (6 m) long, the surface area approaches 360 square yards (about 300 m^2). Transport from the lumen of the small intestine across the epithelial cells occurs by a combination of passive diffusion and active transport. Inside the epithelial cells, fatty acids and glycerol molecules are reassembled into triglycerdes and combined with cholesterol molecules to form chylomicrons. Exocytosis moves the chylomicrons from the epithelial cells into lacteals, small ducts that drain into lymphatic vessels. The lymphatic system eventually drains into blood circulation. The sugars, amino acids, nucleotides, and vitamins diffuse into tiny capillaries that extend into each villus. The capillaries carry the nutrients to veins that converge into the hepatic portal vein, which transports the absorbed materials to the liver.

Approximately 5.8 quarts (5.5 L) of food and beverages and 3.7 quarts (3.5 L) of secretions pass through the small intestine of a human each day. The small intestine reabsorbs most of this in addition to various ions, but about 1.6 quarts (1.5 L) of chyme moves on to the large intestine. After passing through the ileocecal valve, chyme enters a pouch called the cecum, from which the appendix projects. Chyme then passes through the ascending colon, traverse colon, descending colon, sigmoid colon, and rectum, where undigested material is stored until it is

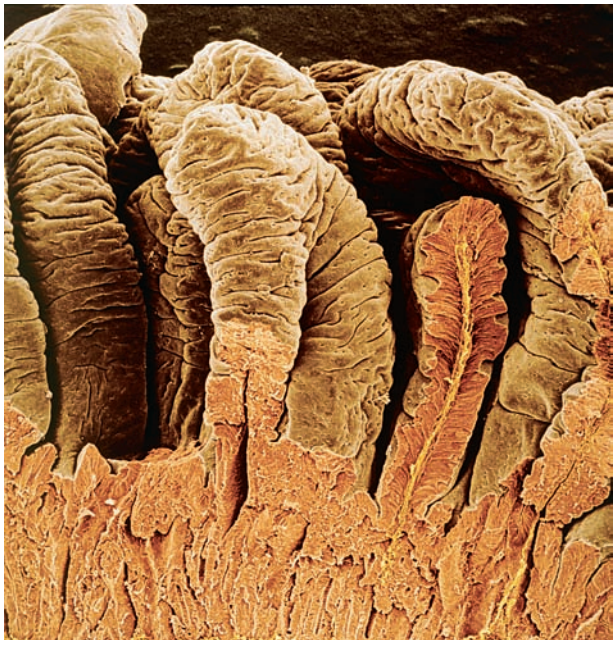


In humans, food ingested through the mouth passes through the esophagus, the stomach, the small intestine, the large intestine, and the rectum during the process of digestion. Several additional accessory glands including salivary glands, the liver, the gallbladder, and the pancreas secrete substances that aid in the chemical breakdown of the ingested material.

eliminated. The major function of the large intestine, which measures about five feet (1.5 m), is to recover water as the chyme travels its roundabout route. The material solidifies as it moves through the tract, sodium is absorbed, and potassium is added to the waste material. Billions of bacteria inhabit the large intestine and aid in digestion of complex carbohydrates and proteins. Some also produce vitamins, such as vitamin K, that the human host absorbs, and intestinal gas as a by-product of their metabolism. One voluntary and one involuntary sphincter control the movement of waste from the rectum through the anus, the posterior opening of the digestive tract. Peristaltic contractions push the feces toward the anus with the assistance of voluntary abdominal contractions. Constipation, the infrequent discharge of feces, results in dry, hard bowel movements as a result of increased water reabsorption. Diarrhea is characterized by watery stools and results when substances move through the large intestine too quickly, preventing the efficient reabsorption of water. Persistent diarrhea can cause dehydration, a dangerous medical condition.

Hormones control the release of digestive enzymes, ensuring they are only secreted when food is present. The presence of amino acids in the stomach or distension of the stomach stimulates cells in the gastric glands to release the hormone gastrin, which travels through the bloodstream before acting back on the stomach. A pH below 1.5, indicating no food is present, inhibits the release of gastrin. The protein hormone gastrin promotes acid release, and acid triggers somatostatin release, creating a negative feedback loop that inhibits further gastric acid release, gastrin secretion, and pepsinogen secretion. When fatty acids and amino acids are present, the duodenum secretes cholecystokinin (CCK), a hormone that causes the pancreas to secrete digestive enzymes and the gallbladder to release bile. Enterogastrone, secreted by the duodenum, slows peristalsis when a diet rich in fats is ingested, giving the intestine more time to digest the lipids. The duodenum also secretes secretin, a hormone that signals the pancreas to release sodium bicarbonate to neutralize the acidic chyme.

Mammals and other vertebrates with specialized diets have adaptations that meet different needs. Carnivores require sharp teeth that can rip and tear into flesh, while herbivores have teeth with broad surfaces for grinding plant tissue. Omnivores have teeth that possess both characteristics. Herbivores and omnivores also have longer digestive tracts because plant tissue takes longer to digest than animal tissue. Many herbivores also have additional structures or chambers for housing symbiotic microorganisms such as bacteria and protozoa that aid in the digestion of cellulose, a carbohydrate that animals cannot digest because of the lack of the enzyme cellulase. Because snakes swallow prey whole, they have an adaptation that allows them to unhinge the lower jaw in order to ingest large animals.



Small fingerlike projections called villi cover the interior surface of the intestinal wall and function to increase the surface area across which nutrient transport occurs. *(David Scharf/Photo Researchers, Inc.)*

See also ANATOMY; ANIMAL FORM; BIOMOLECULES; CIRCULATORY SYSTEM; DIABETES; NUTRITION; PHYSIOLOGY.

FURTHER READING

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton.* Vol. 1. Detroit: U*X*L, 2000.

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U.S. Dept. of Health and Human Services. *Your Digestive System and How It Works.* National Digestive Diseases Information Clearinghouse. Bethesda, Md.: April 2008. Available online. URL: http://digestive.niddk.nih.gov/ddiseases/pubs/yrdd/. Accessed August 2, 2008.

Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology.* 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach.* 4th ed. San Francisco: Benjamin Cummings, 2006.

dissection Dissection is an important tool for studying plant and animal anatomy. The person performing the dissection carefully separates the specimen into pieces or takes it apart in a manner that exposes the internal structures for detailed analysis. Students often perform dissections on preserved specimens in order to learn about the structure, organization, and relationships of tissues and organs in a body. Grasshoppers, earthworms, starfish, frogs, and fetal pigs are common dissection specimens in high school teaching laboratories. More advanced anatomy classes often use cats, and dental and medical students use human cadavers. Physiologists, pathologists, or medical researchers may dissect animals in order to observe the effects of a drug treatment on certain organs or tissues or to look for internal damage caused by a particular disease. An autopsy is a specialized type of dissection performed by a coroner whose purpose is to investigate the cause of death when there is reason to suspect that it was not due to natural causes. Vivisection is a dissection performed on a living animal for physiological or pathological study.

Groups have expressed differences in opinion as to the ethics of animal dissection. Though labeled illustrations and computer simulations depict the same structures, many educators believe that no alternative adequately provides a similar enough experience to an actual dissection. Schools, universities, and research institutions have guidelines for the proper use and care of animals in teaching and research, and the U.S. Department of Agriculture establishes and enforces procedures used in the procurement of animals. Many educators believe that when these are followed, the advantages to the student—the memorable experience of a hands-on exploration that demonstrates the complexity of the interrelationships and internal workings of the body's organs and structures—far outweigh the disadvantages. Artificial models and diagrams cannot convey or teach characteristics such as texture, the true colors, and individual variations. Other groups believe that dissection is not necessary and that it is unethical to use animals for teaching or research purposes.

Ancient Greek philosophers such as Aristotle and early physicians such as Herophilus and Erasistratus were the first to perform dissections to study animal anatomy and physiology, believing direct observation was the best way to learn about a subject. Because many people believed an intact body was necessary to enter the afterlife, public pressure ended the practice of dissecting human bodies from about 250 B.C.E. until the 16th century, when the Flemish physician Andreas Vesalius drew attention to the fact that human anatomy differed from that of other animals, and the practice resumed. Vesalius published the results of his extensive dissections in what is recognized as the first anatomy textbook, *On the Structure of the Human Body,* in 1543. His detailed instructions on the methods for dissection helped revitalize the field of anatomy.

The most common tools used for dissection are a teasing or dissection needle for pulling apart muscle tissue or gently working connective tissue away from the organs it envelopes, dissecting scissors for cutting through tissues and exposing organs, and a very sharp scalpel, for cleanly slicing thin layers of tissue. Pins are often used to hold structures in place during viewing or further dissection.

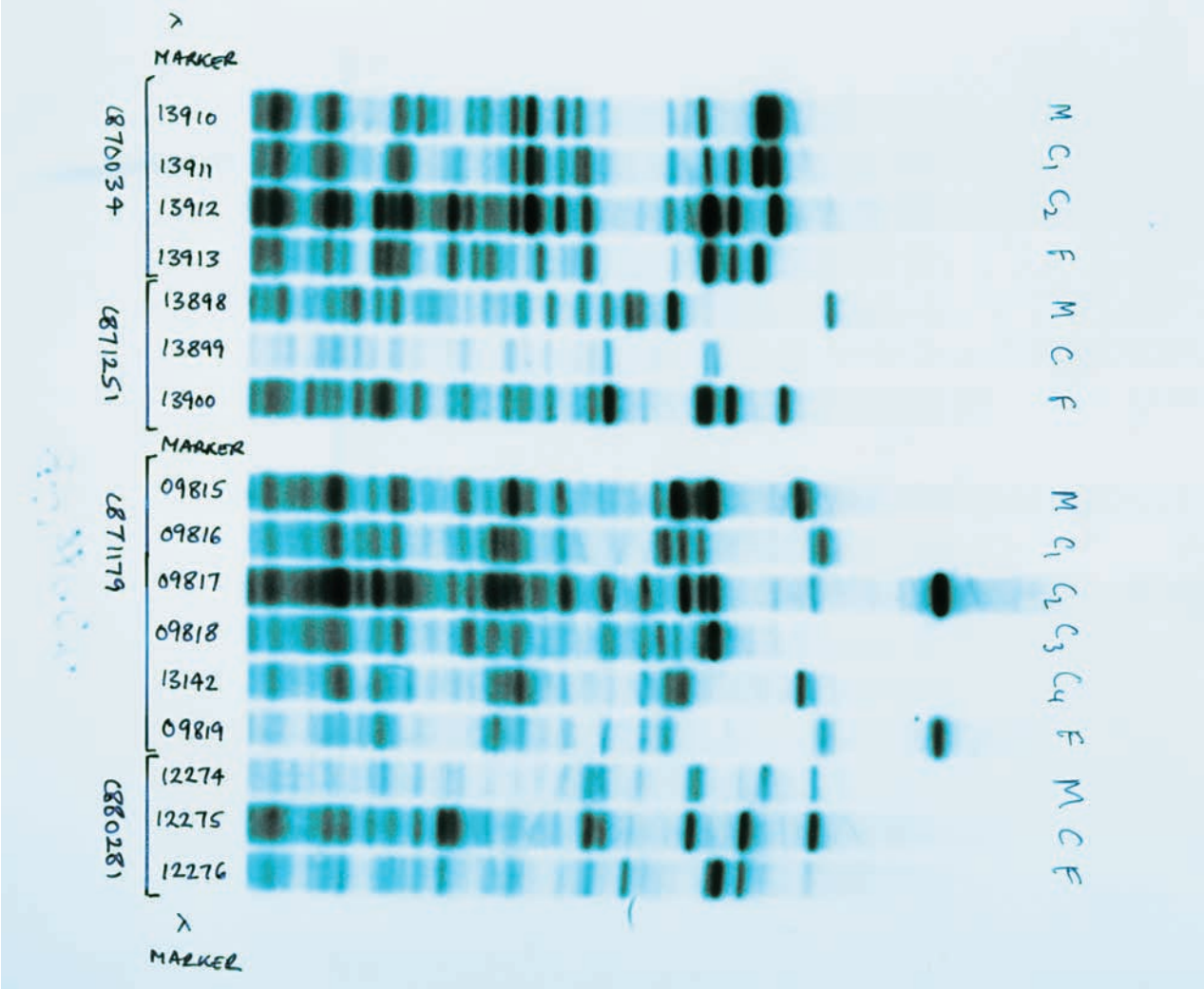
See also ANATOMY.

DNA fingerprinting Humans have 99.9 percent of their genetic makeup in common. Differences in the remaining 0.1 percent of the human genome may be due to naturally occurring alleles (alternate forms of a gene) that change the product of one of the 30,000 genes encoded by the 3 billion nucleotide base pairs of the human genome. Most of the deoxyribonucleic acid (DNA) of the human genome does not appear to encode for anything, and many of those noncoding regions contain repetitive sequences. The differences in these regions are only apparent by DNA testing and, depending on the exact techniques used to examine these sequences, may appear as unique as one's fingerprints. Because of this, medical researchers, forensic biologists, genealogists, and anthropologists can use this information to identify individuals or to determine the relatedness between individuals or groups of individuals. Information gained from DNA fingerprinting can answer questions concerning the structure and migration of human populations, establish paternity, and place a suspect at the scene of a crime.

In 1985 Sir Alec Jeffreys at the University of Leicester invented a technique called DNA fingerprinting, also known as DNA typing, DNA profiling, or DNA testing. Jeffreys and his colleagues were researching a human α-globin gene when they discovered that it contained several adjacent repeats of the same sequence. Further investigation revealed similar repetitive sequences, called minisatellites, elsewhere in the genome. Minisatellites are regions of repetitive DNA consisting of repeated sequences approximately 15 to 100 base pairs in length. Many minisatellites have a GC-rich core sequence and may occur at numerous locations, called loci, scattered throughout the genome. In developing the DNA fingerprinting technique, Jeffreys exploited the existence of these newly discovered minisatellites in the human genome. Variations in the genetic makeup of individuals are called DNA polymorphisms, and they

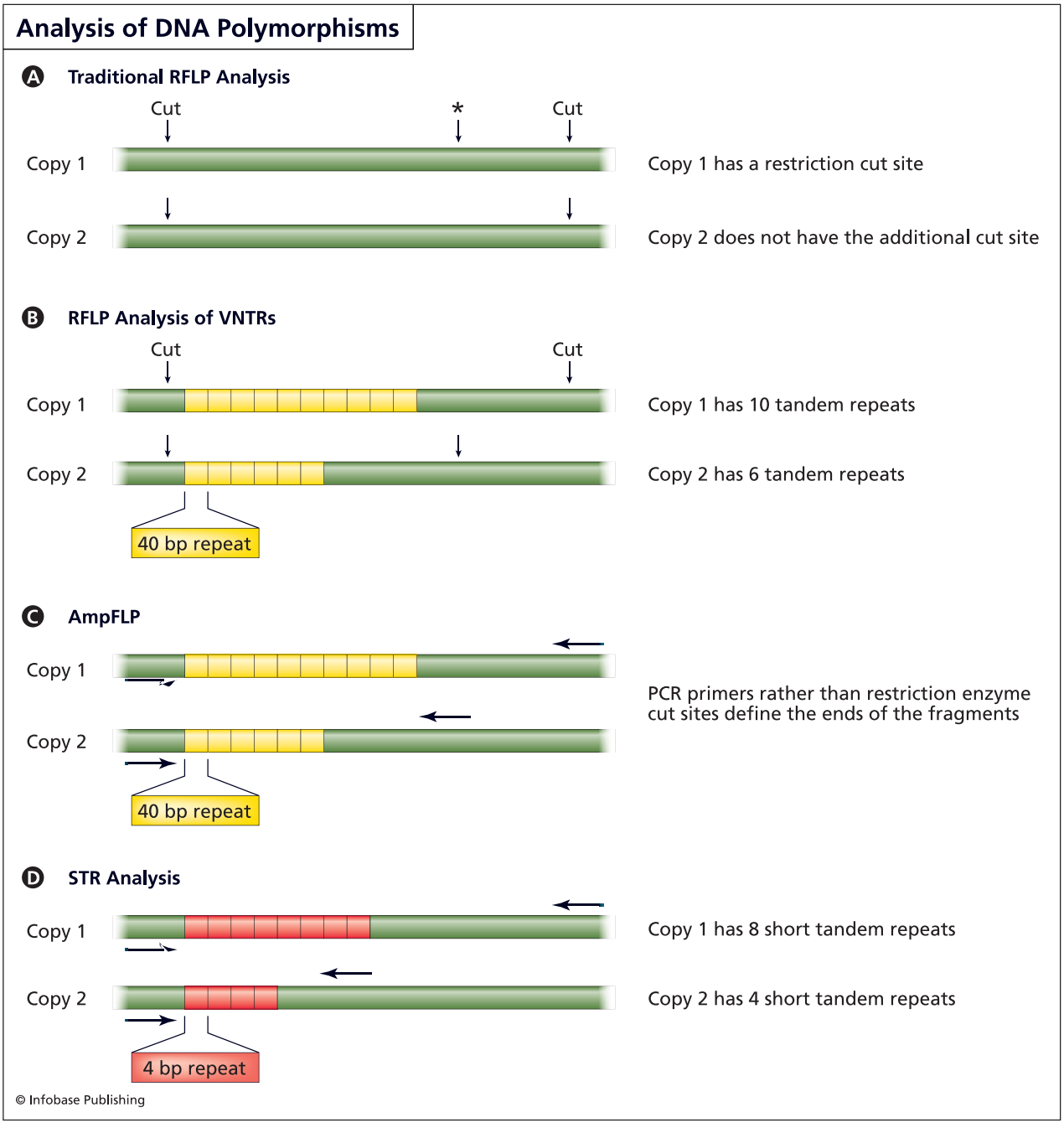
exist as distinct forms within populations. At each minisatellite, the core may repeat a different number of times in different individuals as a result of slippage during DNA replication or of recombination events during meiosis. These polymorphisms make each individual unique.

DNA fingerprinting initially involved restriction fragment length polymorphism (RFLP) analysis. Restriction enzymes cut DNA at locations that contain specific sequences. The digestion releases linear DNA fragments of different lengths, depending on the location and frequency of the recognition sequences in the DNA. Jeffreys used restriction enzymes that did not have a recognition sequence within the repeated sequence of the minisatellite; thus the enzymes cut on either side of the segment containing the repeat. The length of the restriction fragments varies, depending on the number of repeats an individual has at a given locus; accordingly, RFLPs that are polymorphic because of differences in the number of tandem repeats are called variable number tandem repeats (VNTRs). Gel electrophoresis separates the fragments on the basis of size, with the smaller fragments migrating faster than the relatively larger fragments, and therefore farther down a gel in a given period. A procedure called Southern blotting transfers the DNA fragments to a nylon membrane while preserving the banding pattern from the gel. The membrane is treated with a radioactively labeled DNA probe that contains minisatellite sequences complementary to DNA sequences on the membrane. The probe specifically binds by the formation of hydrogen bonds to minisatellite sequences on the membrane, and X-ray film detects the resulting pattern, which is the so-called DNA fingerprint. While RFLP analysis of VNTRs was novel and useful, the technique is time-consuming and required large amounts of good-quality DNA.



DNA fingerprinting can be used to analyze family relationships. This photo shows an autoradiograph of DNA fingerprints from a father (F), mother (M), and several children (C_x). *(David Parker/Photo Researchers, Inc.)*

Kary Mullis, an American biochemist who was working for Cetus Corporation, invented the polymerase chain reaction (PCR) in 1983. This technique generates numerous copies of a specific segment of DNA within a few hours. Oligonucleotide primers, single-stranded pieces of DNA averaging about 20 nucleotides in length, are allowed to hybridize to complementary sequences that flank the site to be

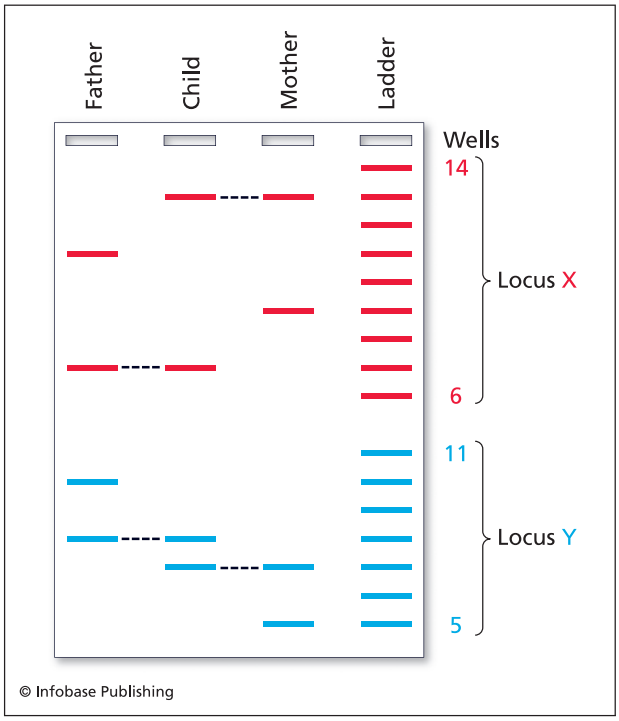


DNA polymorphisms can be detected by several different methods. A) Traditional RFLP analysis reveals the presence or absence of a specific enzyme cut site within a defined restriction fragment. B) Sir Alec Jeffrey's original DNA fingerprinting technique identified RFLPs resulting from differences in the number of tandem repeats in a minisatellite, called VNTRs. C) When PCR performed using specific primers that flank the VNTR amplifies the segment of DNA containing the polymorphism, the technique is called AmpFLP. D) The most common method used for DNA fingerprinting today is STR analysis, which differs from AmpFLP in that microsatellites that contain different numbers of very short repeated sequences, up to five rather than up to 100 base pairs, are examined.

amplified. Then DNA polymerase makes a copy of the template DNA, to which more primers bind during the next round, and the cycle repeats 20 to 30 times. Theoretically, millions of copies of a template can be synthesized within a few hours. PCR was applied to DNA fingerprinting by using primers that amplified polymorphic loci. A major advantage to PCR is the very small amount of DNA is required; thus a tiny sample of blood, semen, saliva, a single hair with the root cells attached, or a few cheek cells is sufficient.

Amplified fragment length polymorphism (AmpFLP) analysis combines the basis of RFLP analysis with PCR. The DNA is digested with one or more restriction enzymes, and then restriction half-site-specific adaptors are ligated to the ends of the restriction fragments. The primers used to amplify the DNA by PCR anneal to sequence of both the restriction site and the attached adaptor segment. Electrophoresis follows PCR and a specific banding pattern results. Biotechnology companies simplified AmpFLP analysis by developing kits that allowed the detection of single-nucleotide polymorphisms (SNPs) by hybridizing PCR products with cards spotted with different probes.

The most common method used in DNA fingerprinting today is short tandem repeat (STR) analysis. The STRs used for this application are four or five nucleotides long, and they occur in tandem, one directly adjacent to the next. DNA sequences that are between two and five nucleotides long and occur as tandem repeats are called microsatellites. As minisatellites do, microsatellites occur throughout the genome, and individuals possess different numbers of the repeated sequences at different locations throughout the genome. For example, the STR named D7S280 is a locus found on the human chromosome 7, and it consists of the tetrameric repeat GATA. Different alleles of D7S280 contain between six and 15 tandem repeats of this tetramer. In STR analysis, PCR amplifies the desired regions containing the repeated sequence by using specifically designed primers, and then the resulting fragments are separated by either gel electrophoresis or capillary electrophoresis, which provides higher resolution. Fluorescent dyes attached to the PCR primers allow detection. Though polymorphisms at a single locus are not uncommon, simultaneous analysis of several loci results in a specific DNA profile for an individual. Greater numbers of examined loci give more statistically discriminating profiles. Because the polymorphic regions of DNA tested during STR analysis are much shorter than in VNTR analysis, the probability is high that sufficient quantities of useful DNA will be present even in minute samples. Spontaneous chemical degradation often reduces the quality of the DNA left behind at a crime scene, but STR analysis only requires very small segments of DNA to be in good shape, meaning readable by DNA polymerase during PCR.



Gel electrophoresis separates the amplified STRs and reveals an individual's genotype. Since people have two copies of each chromosome, each individual has two alleles for each of the two loci shown here. Each allele is represented by one band. In the diagram shown here, the father has the genotype of 11, 7 for locus X and 10, 8 for locus Y. This means, for example, that one of the father's X STR alleles contains 11 repeats, and his other X allele has 7 repeats. The mother has the genotype 13, 9 for X and 7, 5 for Y. The child must have received allele 13 from the mother and 7 from the father for locus X, and 8 from the father and 7 from the mother for locus Y.

In the United States, the Federal Bureau of Investigation (FBI) maintains a database of the DNA profiles of sex offenders, convicted felons, and others who have previously had DNA fingerprinting analysis performed. The database, called *CODIS* for "Combined DNA Index System," allows law enforcement agencies to search stored profiles for a match to DNA from a crime scene even when they do not have a suspect. CODIS profiles contain information from 13 different STRs, and the odds of two individuals' having the same profile are approximately 1 in a billion.

See also BIOMOLECULES; DEOXYRIBONUCLEIC ACID (DNA); ELECTROPHORESIS; GENOMES; POLYMERASE CHAIN REACTION; VARIATION, GENETIC VARIATION.

FURTHER READING

Jeffreys, Alec J., V. Wilson, and S. L. Thein. "Hypervariable Minisatellite Regions in Human DNA." *Nature* 314, no. 6006 (1985): 67–73.

Kobilinsky, Lawrence, Thomas F. Liotti, and Jamel Oeser-Sweat. *DNA: Forensic and Legal Applications*. Hoboken, N.J.: Wiley-Interscience, 2005.

Rudin, Norah, and Keith Inman. *An Introduction to Forensic DNA Analysis*. 2nd ed. Boca Raton, Fla.: CRC Press, 2002.

DNA sequencing

Deoxyribonucleic acid (DNA) serves as the molecular carrier of genetic information. All the genes that instruct cells how to grow, metabolize, multiply, and perform other functions that define life exist on molecules of DNA. The individual building blocks of DNA are nucleotides, subunits that consist of a deoxyribose sugar, a phosphate group, and a nitrogenous base. The nitrogenous bases found in DNA are adenine, cytosine, guanine, and thymine, and the nucleotides are often abbreviated *A, C, G,* and *T,* respectively. The genetic information is contained within the specific order of the four different nucleotides along the length of a DNA molecule, referred to as the sequence. DNA sequencing is the process of determining the composition and order of nucleotides for a segment of DNA, for a gene, for an entire chromosome, or for a whole genome. In the laboratory, molecular biologists sequence DNA molecules by carrying out a set of biochemical reactions called sequencing reactions and then perform gel electrophoresis on the reaction products to analyze, or read, the sequence.

DEVELOPMENT OF THE METHODOLOGY

The earliest DNA sequencing methods depended on the partial hydrolysis of small pieces of DNA and were difficult and time-consuming. In 1975 Frederick Sanger and Alan Coulson at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, developed an entirely different approach that represented a turning point in DNA sequencing technology. Their method, which they called the plus-and-minus method, used making DNA rather than breaking it down as a strategy for determining its construction. The basis of their approach was the tendency of DNA elongation to stop when one of its nucleotide building blocks is in limited concentration. When the polymerization reactions were carried out in four different tubes, each with a different limiting nucleotide, new DNA chains of different lengths were produced. Sanger employed this method to determine the 5,386-nucleotide sequence of the bacteriophage ΦX174, which led to the prediction of the amino acid sequences for the 10 viral proteins. While this method was much improved over the previous techniques, it still had difficulties; thus Sanger continued to explore ways to improve the method.

Meanwhile, in 1977, Allan Maxam and Walter Gilbert published a different method for determining the sequence of a DNA molecule, the chemical degradation method, which quickly became popular. The Maxam and Gilbert method involves radiolabeling purified DNA fragments, then setting up four different reactions and treating each in a manner that results in the differential cleavage of the DNA at one of the four types of nucleotides. After this step, all of the labeled segments in one tube will terminate at the same type of nucleotide; however, many different lengths will be present. Polyacrylamide gel electrophoresis of the four reactions separates the fragments on the basis of size, and exposure of the gel containing the separated radiolabeled bands to a piece of X-ray film allows the researcher to determine the order of nucleotides in the DNA used.

Meanwhile, Sanger and Coulson made a change to the plus-and-minus technique that led to its replacement. The newer method still relied on DNA polymerase but involved the addition of dideoxynucleotides to the reaction mixtures. Once DNA polymerase incorporated a dideoxynucleotide, DNA synthesis stopped at that position. One drawback was the difficulty in creating and purifying DNA template for use in the sequencing reactions; the physical separation methods were messy and inefficient. Joachim Messing and his colleagues at the Waksman Institute of Microbiology, Rutgers, the State University of New Jersey, contributed to the utility of the dideoxy method when they introduced a cloning technique that facilitated the generation of relatively large quantities of purified single-stranded DNA to use as templates in the sequencing reactions. Cloning technology has greatly improved the mechanisms by which researchers obtain the desired DNA for sequencing, and today researchers simply heat the double-stranded DNA to generate single-stranded templates. Though additional modifications to the specific techniques have improved the results, and automation has improved the efficiency, the dideoxy chain termination method remains the basis for DNA sequencing performed today.

Walter Gilbert and Frederick Sanger received the Nobel Prize in chemistry in 1980 "for their contributions concerning the determination of base sequences in nucleic acids." They shared the award with the American biochemist Paul Berg, a pioneer of recombinant DNA technology. Sanger had previously received the Nobel Prize in chemistry in 1958 for his research on the structure of the protein insulin. DNA sequencing technology has come a long way in the last three

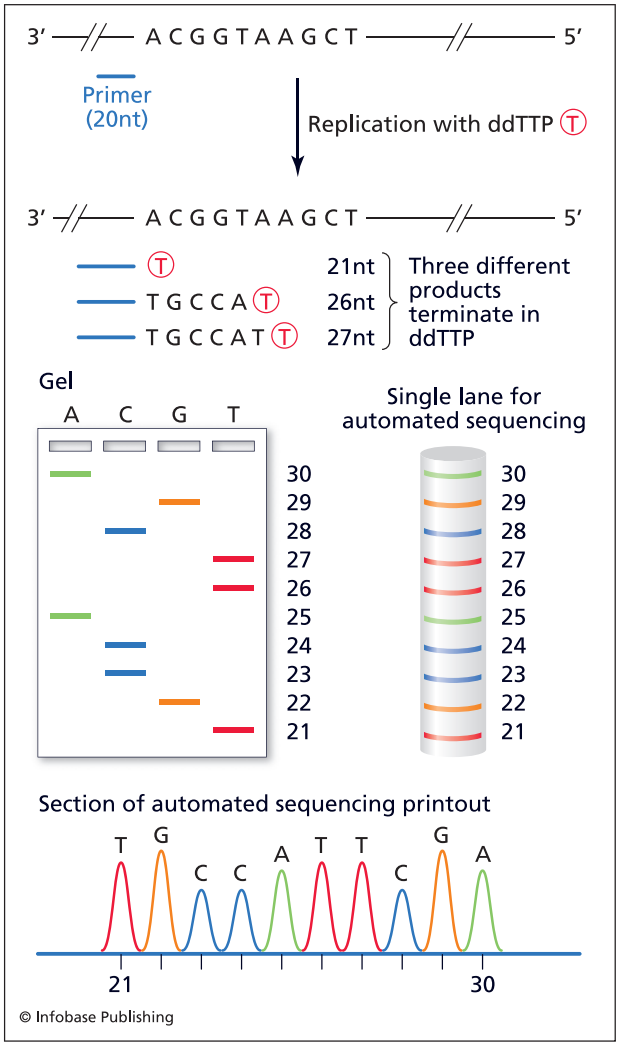
decades. Improvements include the use of automated sequencers that read the sequences and the replacement of radioactivity with fluorescent dyes.

DIDEOXY CHAIN TERMINATION METHOD

Dideoxynucleotides have no 3' hydroxyl (-OH) group attached to the deoxyribose sugar. During DNA synthesis, DNA polymerase forms a phosphodiester bond between the 3' OH of the last nucleotide added to the newly synthesized strand of DNA and the 5' phosphate group of an incoming nucleotide. If DNA polymerase incorporates a dideoxynucleotide rather than a normal deoxynucleotide, the 3' OH will be missing, and DNA chain elongation terminates at that position.

To sequence a fragment of DNA using this method, the researcher sets up four separate reactions. Each reaction contains the DNA template, which has been heated to denature the hydrogen bonds between complementary strands and generate single-stranded templates to be sequenced. The reactions also require short pieces of DNA called primers, because DNA polymerase I cannot add new nucleotides without having a 3' OH already present. (In the cell, a separate enzymatic activity carries out this function to initiate DNA replication.) The primers are about 20 bases long and specifically designed to complement a region of the cloning vector that contains the desired DNA. Each reaction also contains a mixture of the four deoxynucleotide (indicated by the lowercase letter d) subunits from which DNA is synthesized: 2'-deoxyadenosine 5'-triphosphate (dATP), 2'-deoxycytidine 5'-triphosphate (dCTP), 2'-deoxyguanosine 5'-triphosphate (dGTP), and 2'-deoxythymidine 5'-triphosphate (dTTP). A fraction of the dATP contains a radiolabled phosphate group (^{32}P) that facilitates the detection of the DNA after completion of the reactions. Each of the four tubes also contains one dideoxynucleotide (indicated by dd), either ddATP, ddCTP, ddGTP, or ddTTP. Addition of the enzyme allows the reactions to proceed, extending the primers to form a new strand of DNA complementary to the template to which the primer binds. The concentration of the dideoxynucleotide is such that the enzyme incorporates the dideoxynucleotide randomly, so some of the fragments are terminated early on, and others proceed farther before termination. When the DNA polymerase adds a dideoxynucleotide, the reaction stops.

After the reactions are complete, all four tubes contain newly synthesized DNA fragments that have the same 5' end but will terminate in different residues at the 3' end, depending on which dideoxynucleotide was added to that reaction tube. Within a tube, all the fragments will terminate in the same residue but will be of various lengths, depending on how far the enzyme proceeded before adding a chain-terminating dideoxynucleotide rather than a normal deoxynucleotide. Denaturing polyacrylamide gel electrophoresis on the products of the four reactions, run in parallel in adjacent lanes, separates the fragments within each tube according to their length. Laying a piece of X-ray film over the gel will result in the exposure of the film at the positions of the radiolabeled bands in each lane; after developing the film, the pattern will become apparent. Since the smallest fragments will be at the bottom of the gel and the longest fragments will be at the top, one simply starts at the bottom and notes which lane the lowest band is in, then notes which lane the next lowest band is on, and so on.



The dideoxy chain termination method for sequencing utilizes dideoxynucleotides to stop DNA synthesis at specific residues. Denaturing polyacrylamide gel electrophoresis separates the fragments on the basis of length, allowing one to read the ordered sequence of nucleotides of a DNA molecule.

Automated techniques are available for sequencing large amounts of material, such as the human genome, which is more than 3 billion nucleotides. One variation involves tagging the primers with one of four different fluorescent dyes. Each color is specific for the dideoxynucleotide used in a reaction tube. For example, green might indicate A, blue might be C, orange might be G, and pink might be T. The different colors allow all four reactions to be loaded into the same lane on a gel. After running a gel to separate the fragments, a laser scans the gel, and as it passes over each band, a detector reads the color emitted by the fluorescent label and sends the information to a computer that records the data.

See also DEOXYRIBONUCLEIC ACID (DNA); ELECTROPHORESIS; ENZYMES; GENE EXPRESSION; GENOMES; HUMAN GENOME PROJECT; RADIOACTIVITY.

FURTHER READING

Church, George M. "Genomes for All." *Scientific American* 294, no. 1 (January 2006): 47–54.

DOE Joint Genome Institute, U.S. Department of Energy, Office of Science. "How Sequencing Is Done." Available online. URL: http://www.jgi.doe.gov/education/how/. Updated March 15, 2006.

Maxam, Allan M., and Walter Gilbert. "A New Method for Sequencing DNA." *Proceedings of the National Academy of Sciences USA* 74, no. 2 (1977): 560–564.

The Nobel Foundation. "The Nobel Prize in Chemistry 1980." Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1980. Accessed January 24, 2008.

Sanger, Frederick. "Sequences, Sequences, Sequences." *Annual Reviews of Biochemistry* 57 (1988): 1–28.

Sanger, Frederick, and Alan R. Coulson. "A Rapid Method for Determining Sequences in DNA by Primed Synthesis with DNA Polymerase." *Journal of Molecular Biology* 94 (1975): 441–448.

Sanger, Frederick, Steve Nicklen, and Alan R. Coulson. "DNA Sequencing with Chain-Terminating Inhibitors." *Proceedings of the National Academy of Sciences USA* 74, no. 12 (1977): 5,463–5,467.

Dobzhansky, Theodosius (1900–1975) Russian American *Biologist*

Theodosius Dobzhansky was one of the 20th century's most influential evolutionary biologists. Best known for his famous quotation, "Nothing in biology makes sense except in the light of evolution," Dobzhansky was also the first to define and demonstrate clearly the relationship between inheritance as described by the Austrian monk Gregor Mendel and speciation by descent with modification as proposed by the English naturalist Charles Darwin.

BUTTERFLIES AND LADYBIRD BEETLES

Theodosius Dobzhansky was born on January 25, 1900, in Nemirov, Russia, to Grigory Dobzhansky, a high school mathematics teacher, and Sophia Voinarsky, who home-schooled Theodosius until he was nine years old. A German governess taught him her native language, and later in life he became fluent in English and at least three other languages. Theodosius entered his father's gymnasium (high school) in 1909. The following year, Grigory suffered a head injury that left him paralyzed and forced him to quit work. The family moved to Kiev, and Theodosius entered the local gymnasium, where his interest in science grew. When he and a friend found a copy of Darwin's *On the Origin of Species,* they read it together. While the world fought the First World War, he collected butterflies and studied ladybird beetles of the genus *Coccinella.* After collecting them, he dissected them and examined them under the microscope. He learned to distinguish different species and observed their migration patterns.

The army drafted many of Dobzhansky's friends, but because his birthday was in January and the cutoff was December, the army did not call up Dobzhansky. He enrolled at the University of Kiev, where Professor Kushakevich took him under his wing. Kushakevich was a cytologist, a biologist who studies cells. For three years Dobzhansky researched sexual differentiation in snails, but he also continued his studies on *Coccinella.* His first scientific publication, when he was only 18, described a new species of ladybird beetle, *Coccinella luchniki.* Despite the war, scheming to avoid military duty, the Communist takeover of government, the death of both of his parents, and practically starving to death, Dobzhansky managed to graduate with a degree in biology in 1921. Having already worked at the Polytechnic Institute in Kiev the year before, he obtained a position as an instructor in zoology and soon developed an interest in genetics. With a well-equipped laboratory, he published two more papers in the few years he was there.

From a colleague, Dobzhansky became aware of the work of one of the most famous *Drosophila* geneticists of all time, Thomas Hunt Morgan at Columbia University in New York. As it did among many geneticists at the time, Morgan's research focused on the work of Mendel, whose work regarding the manner by which traits are passed from one generation to the next was rediscovered at the turn of the century, after being ignored for almost 35 years. Dobzhansky had heard about his research on gene linkage, the phenomenon whereby more than one characteristic is passed on together. For example, Morgan found that when he mated the offspring of a white-eyed male fly with a female red-

eyed fly, and then crossed their offspring, only males exhibited white eyes—females' eyes were always red. Dobzhansky admired Morgan's work, and when he obtained a collection of mutant *Drosophila,* he excitedly embarked on some studies examining whether genes controlled single traits or multiple traits. The microscopy skills he learned from Kushakevich came in handy. He studied the mutant flies under the microscope and discovered pleiotropy, the phenomenon by which one gene produces a variety of effects or phenotypes. He published his novel findings, and his name attracted the attention of Professor Yuri Filipchencko, the head of the Genetic Department at the University of Leningrad. Filipchencko was looking for an assistant in his lab, and he asked Dobzhansky whether he was interested.

In 1924 Dobzhansky joined Filipchencko's lab, where he continued his studies on pleiotropy in *Drosophila*. Although Filipchencko was a zoologist, at the time the focus of the lab was wheat. Dobzhansky worked in the lab but also continued studying ladybird beetles. During one trip to Central Asia he unexpectedly came across another new species at altitudes where entomologists did not predict ladybird beetles would exist. The same year that he moved to Leningrad, Dobzhansky married an evolutionary biologist, Natalia (Natasha) Sivertzev. In 1933 the couple had one daughter, named Sophie.

The research on pleiotropy impressed Filipchencko, who recommended Dobzhansky for a fellowship from the International Education Board of the Rockefeller Foundation. With this support, Dobzhansky and his wife went to the United States, and in 1927 Dobzhansky joined the lab of none other than his hero, Thomas Hunt Morgan. His wife, Natasha, obtained a position maintaining the fly stocks in Morgan's lab. The following year Morgan accepted a position as head of the new biology division at the California Institute of Technology. Dobzhansky followed. Shortly thereafter he became an American citizen. Cal Tech offered Dobzhansky an assistant professorship in genetics in 1929 and then promoted him to professor in 1936.

CALTECH RESEARCH

Shortly after joining Morgan's group, Dobzhansky came under the tutelage of Alfred H. Sturtevant, one of Morgan's main collaborators, who was responsible for the idea that one could use the data from gene linkage experiments to assign relative positions of genes along the length of chromosomes. While the physical conditions of Morgan's laboratory, one section of which was fondly called the fly room, did not impress Dobzhansky, the quality of Morgan's science did. Dobzhansky was interested in studying genetics in order to understand evolution better. Hermann J. Muller, a former colleague of Morgan, had shown that hereditary mutations, or alterations in the genes, could be induced by X-rays, thus speeding up the process of collecting mutants. (Muller received the 1946 Nobel Prize in physiology or medicine for his discovery.) As Dobzhansky saw it, the mutations were the basis of evolutionary theory. With Sturtevant, he worked on chromosomal translocation, an event involving the breakage of a segment of a chromosome and its attachment elsewhere. If it attached to the same chromosome from which it separated, but in the opposite orientation, the alteration was called an inversion. If it attached to another chromosome, it was called a translocation. He observed the result of these events by removing a female fly's ovaries, fixing them, embedding them in paraffin, slicing them, staining them, and finally examining them under the microscope. Whereas mutations to individual genes cannot be viewed in this manner, such gross structural changes could. The translocation events were rare, but exposure to X-rays increased their incidence. Dobzhansky found that the offspring of irradiated flies had genes on different chromosomes than normal flies. These translocations were the first to be observed under a microscope, and they provided the physical confirmation of the linkage maps generated mathematically from information on the frequencies of combinations of different traits. Morgan's conclusion that genes occurred in linear arrangements along the length of chromosomes was still being challenged; Dobzhansky's cytological maps furthered that hypothesis as well.

In the early 1930s, when most of the Morgan lab was concerned with the newly discovered giant chromosomes found in the salivary glands of *Drosophila* larvae, Dobzhansky's interests took a different turn—toward the evolutionary aspect of genetics. He believed that the changes he observed in chromosomes had some relation to the evolutionary process, and he wanted to determine exactly what. He also began to wonder about the concept of a species. These unresolved questions led Dobzhansky to embark on research concerning the evolutionary history of *Drosophila* populations in North America. At the time, cytologists were aware that different species possessed different numbers and shapes of chromosomes. For example, *Drosophila melanogaster* had four pairs of chromosomes, but other *Drosophila* species had five pairs. Two so-called races, A and B, of *Drosophila pseudoobscura* rarely mated in the wild, and when they did, the male offspring were sterile. He drove to northern California and collected fruit flies by setting out mashed bananas mixed with yeast. To gain insight as to why the male hybrids were sterile, he examined their chromosomes. He published the first of a series of papers about hybrid

sterility in 1933 in the *Proceedings of the National Academy of Sciences*. These studies on the genetic causes of sterility in the flies helped Dobzhansky develop his concept of a species as a reproductively isolated group of like organisms. Scientists today continue to use methods he pioneered and described in these papers, specifically the use of genetic markers to study hybrid sterility.

Though he had been using *Drosophila* as a model system for studying genetics, he still carried out research on ladybird beetles. He combined his new knowledge of cytogenetics with his background in field work in a 1933 paper published in *American Naturalist* describing the geographical variation and evolution in ladybird beetles.

THE MODERN SYNTHESIS OF EVOLUTIONARY THEORY

In fall 1936 Leslie C. Dunn, a geneticist at Columbia University, invited Dobzhansky to return to New York to give a six-week lecture series on evolution. Darwin's theory on the origin of species had inspired Dobzhansky to become a biologist; thus he was enthusiastic, as was his audience. Zoologists, botanists, paleontologists, mathematicians, and geneticists all developed their own theories concerning the evolutionary process as it related to their specific subdiscipline. One of Dobzhansky's greatest contributions to science was integrating all of these theories into one unified, simple biological theory of evolution.

During his lectures, Dobzhansky presented his summary of what came to be known as the modern synthesis of evolutionary theory. In 1859 Darwin had proposed that all life-forms had gradually evolved through the accumulation of minor variations of lower life-forms. Dobzhansky accepted this and the notion that a collection of all the life-forms that ever existed on Earth would exhibit a relatively continuous array of forms. Darwin suggested that ancestral forms diverged again and again; thus more variability exists between extant species and ancestral ones. What Darwin did not explain was the how the changes that led to variation and divergence came about.

Biologists approached resolving this issue in different ways. Some examined evolutionary histories of different types of living organisms; others researched mechanisms of evolution from the genetics perspective. A former colleague of Dobzhansky named Sergei Chetverikov examined genetic similarities and differences in populations of *Drosophila*. J. B. S. Haldane and Sir Ronald A. Fisher (both in England) and Sewall Wright (in Chicago) used theoretical mathematics to describe changes in gene frequencies by creating models of selection, mutation, migration, genetic drift, and inbreeding in the evolutionary process. Dobzhansky was very familiar with the work of all these founders of population genetics, and by the time he delivered his lectures at Columbia in 1936, he had integrated their work into his unified theory of biological evolution. Dobzhansky published an influential book based on these lectures, *Genetics and the Origin of Species* (1937), defining the relationship between the variations studied by geneticists and the theory of evolution. The book was written in plain language and included discussion of diversity, genetic variation and chromosomal rearrangements, mutation as the source of variation, natural selection, the development of reproductive isolation during speciation, the concept of a species, and differences among members of the same species. He provided examples from his own research and from the scientific literature to demonstrate that such genetic mutations were the underlying cause of the differential characteristics upon which natural selection acted. The book was a remarkable success, considered by some to be the most influential book of evolutionary theory of the 20th century. According to Francisco Ayala, the author of Dobzhansky's biographical memoir written for the National Academy of Sciences, the book "had enormous impact on naturalists and experimental biologists, who rapidly embraced the new understanding of the evolutionary process as one of genetic change in populations. Interest in evolutionary studies was greatly stimulated, and contributions to the theory soon began to follow, extending the synthesis of genetics and natural selection to a variety of biological fields." Dobzhansky revised *Genetics and the Origin of Species* twice under the same title (1941, 1951), and a third time under the title *Genetics of the Evolutionary Process* (1970). Modern texts on evolution follow the same pattern laid out by Dobzhansky.

After returning to Caltech, he decided to look at the degree of variation that was naturally present in a population in order to assess the accuracy and usefulness of mathematical modeling. For his population studies, he chose *Drosophila pseudoobscura,* the fly species in which he had examined hybrid sterility. Without technology that has become available through a better understanding of molecular biology, studying variation in a population was difficult. By nature, recessive genes were masked. Detecting their presence in a population required performing testcrosses with homozygous recessive individuals and making deductions based on observations of the unknown population's offspring. To overcome this difficulty, Dobzhansky studied chromosomal rearrangements of this species in collaboration with Sturtevant. By collecting data on the presence and frequency of specific chromosomal inversion events

in different populations from different geographical locations, he reconstructed the evolutionary history of this organism. Wright created mathematical selection models that Dobzhansky then tested by examining wild populations. This line of research led to Dobzhanksy's proposal of the concept and term *isolating mechanisms* to refer to phenomena that prevent different species from exchanging genes, or reproducing. Another important finding derived from these studies was that sterility related somehow to the autosomes, the nonsex chromosomes. Dobzhansky continued his work on *Drosophila pseudoobscura,* in collaboration with Sturtevant and other geneticists, until his death. A series of articles that shared the primary title "The Genetics of Natural Populations" (1938–68) summarized much of his findings related to the geographical and temporal variation of chromosomal arrangements. Others used Dobzhansky's techniques to reconstruct the evolutionary histories of other organisms.

EVOLUTION AS IT RELATES TO HUMANS

In 1940 Dobzhansky left Caltech to accept a professorship in zoology back at Columbia University. His teaching demands were greater, and because of his increased reputation and fame, others sought his expertise more often, leaving Dobzhansky with less time for experimental research. He did continue studying the evolution of *Drosophila* populations but also began to branch into the social sciences and philosophize about the nature of humans, the evolution of the human species, the biological significance of race, and the evolution of human culture.

One public stance taken by Dobzhansky during this stage in his life involved the Soviet agronomist Trofim D. Lysenko, who openly defied classical genetics, including the work of both Mendel and Morgan, by reverting to the belief that acquired characteristics could be inherited. Lysenko claimed that heredity resulted not from genes but from development, and since environmental conditions could affect developmental processes, heredity could also be modified. Josef Stalin succeeded Lenin after he died in 1928, becoming the de facto party leader and dictator of the Soviet Union. Stalin's goal was to advance Russian industry and agriculture rapidly. The Russian government praised Lysenko for his work speeding up the growing season of economically important crops such as wheat. The battle between followers of Lysenko and those of revered scientists such as Mendel and Morgan was mostly political, though also due in part to a misunderstanding of genetics and the faulty assumption that it conflicted with Darwinian evolution, which was by then widely accepted. Because Stalin supported Lysenko's opposition to genetics, Lysenko's power grew. The ignorant man became the leader of Soviet biology, and, as a result, scientific progress in the Soviet Union came to a screeching halt. Many of Dobzhansky's former acquaintances and colleagues were forced from their jobs, were arrested, and faced the threat of death for opposing Lysenko. In 1946 Dobzhansky translated one of Lysenko's books, *Heredity and Its Variability,* into English to expose Lysenko's ridiculous ideas to American scientists, and he wrote several articles criticizing Lysenko's so-called science. Decades passed before Soviet science showed signs of recovery. Dobzhansky had been ignored after leaving his home country, but now Russia declared the renowned "American" geneticist to be an enemy of the people.

Dobzhansky also became involved with the eugenics movement, as an outspoken critic. The term *eugenics,* referring to the science of heredity or "good breeding," emerged in the 1880s and by the 1930s had grown into a movement aiming to enhance or improve the "quality" of the human species by controlled breeding. Dobzhansky saw this movement for what is truly was—a pseudoscientific façade for racial and social prejudice. Political and social leaders with selfish motives tried to manipulate the masses of society into believing that limiting reproduction of a "higher" class or "favored" race to members of the same class or race would improve the quality and purity of that group. They used similar faulty reasoning to suggest that preventing the reproduction of groups of people considered inferior (because of race, behavioral traits, or certain medical conditions) would improve the human species as a whole. Incensed by the racial bigotry fueled by this movement, Dobzhansky spoke out strongly and publicly against it, declaring that no scientific evidence demonstrated a genetic basis of social or personality traits of humans and, furthermore, certainly no evidence of significant differences between racial groups. More genetic variation existed within the human species than any minor differences between races. In 1946, in collaboration with fellow Columbia geneticist Leslie C. Dunn, he published *Heredity, Race, and Society,* a book that explained the scientific differences with respect to individuals, populations, and races.

In the laboratory, Dobzhansky mixed *Drosophila* populations containing specific chromosome types and recorded his observations on changes in the population with respect to those introduced genetic differences. He observed that the populations evolved different genetic characteristics, even when the parental strains, the temperatures, the food supply, and the exposure to light and darkness were the same. Though he was studying fruit flies, he saw the relevance of these studies to the evolution of human populations. These experiments were extended to

attempt the creation of a new species in the laboratory, a successful effort published in *Nature* in 1971 as "Experimentally Created Incipient Species of *Drosophila*." He applied the concept of inherited traits to human nature in a work titled *Mankind Evolving* (1962), which combined knowledge from anthropology, genetics, sociology, and evolutionary biology.

NEVER RETIRED

In 1962 the Rockefeller Institute (now Rockefeller University) in New York City hired Dobzhansky as a professor. For almost a decade, he traveled with his wife on expeditions all over the world, including locations in Brazil, India, and Australia. The purpose of these trips was to find and collect new species of *Drosophila* for study.

In 1969 Natasha suffered a fatal heart attack. Afterward, Dobzhansky tried to keep busy to avoid feeling lonely. During this time he completely revised *Genetics and the Origin of Species* incorporating many new exciting findings such as the discovery of the structure of deoxyribonucleic acid (DNA) and the demonstration that DNA was the molecule of heredity. The new edition, *Genetics of the Evolutionary Process,* published in 1970, was even more successful than his previous editions, as Dobzhansky kept abreast of the scientific literature on the subject and the text reflected decades of progress toward understanding the relationship between genetics and evolution. In 1971 he became professor emeritus at Rockefeller and moved back to California, choosing to serve as an adjunct professor in genetics at the University of California at Davis.

Dobzhansky belonged to the National Academy of Sciences, the American Philosophical Society, and the American Academy of Arts and Sciences. Several foreign scientific organizations elected him to membership as well: the Royal Society of London, the Royal Swedish Academy of Sciences, the Royal Danish Academy of Sciences, the Brazilian Academy of Sciences, the Academia Leopoldina, and the Academia Nazionale dei Lincei. He served as president of the Genetics Society of America (1941), the American Society of Naturalists (1950), the Society for the Study of Evolution (1951), the American Society of Zoologists (1963), the American Teilhard de Chardin Association (1969), and the Behavior Genetics Association (1969). Numerous organizations recognized Dobzhansky's contributions to life science by awarding him medals: The U.S. National Academy of Sciences awarded him the Daniel Giraud Elliot Medal (1946) and the Kimber Genetics Award (1958), the Academia Leopoldina awarded him the Darwin Medal (1959), Yale University awarded him the A. E. Verrill Medal (1966), the American Museum of Natural History awarded him the Gold Medal Award for Distinguished Achievement in Science (1969), and the Franklin Institute awarded him the Benjamin Franklin Medal (1973). In 1964 President Lyndon B. Johnson awarded Dobzhansky the National Medal of Science.

In 1968 physicians had diagnosed Dobzhansky with chronic lymphatic leukemia and gave him only a few months to a few years to live. Seven years later, Dobzhansky died of the effects of lymphatic leukemia, on December 18, 1975, one month shy of his 72nd birthday. He had worked until the day before his death. His students, colleagues, and peers remember him for his generosity, loyalty, exuberance, and willingness to give his time to serve science and teach young scientists. The rest of the scientific community venerated Dobzhansky for his incredible ability to synthesize theories that interwove ideas from numerous fields. Having published nearly 600 papers and books, he was one of the most influential and prolific biologists of the 20th century. Dobzhansky's work continues to manifest its influence in the fields of evolutionary biology, population genetics, and sociology.

See also CHROMOSOMES; EVOLUTION, THEORY OF; MENDEL, GREGOR; MORGAN, THOMAS HUNT.

FURTHER READING

Adams, Mark B., ed. *The Evolution of Theodosius Dobzhansky: Essays on His Life and Thought in Russia and America.* Princeton, N.J.: Princeton University Press, 1994.

Ayala, Francisco J. "Theodosius Dobzhansky (January 25, 1900–December 18, 1975)." In *Biographical Memoirs: National Academy of Sciences.* Vol. 55. Washington, D.C.: National Academy Press, 1985.

Dobzhansky, Theodosius. *Genetics of the Evolutionary Process.* New York: Columbia University Press, 1970.

———. "Nothing in Biology Makes Sense Except in the Light of Evolution." *American Biology Teacher* 35 (March 1973): 125–129.

Land, Barbara. *Evolution of a Scientist: The Two Worlds of Theodosius Dobzhansky.* New York: Thomas Y. Crowell, 1973.

Lewontin, R. C., John A. Moore, William B. Provine, and Bruce Wallace, eds. *Dobzhansky's Genetics of Natural Populations I–XLIII.* New York: Columbia University Press, 1981.

Duve, Christian de (1917–) Belgian *Cell Biologist* The pioneering research of Christian de Duve solidly established cell biology as a subdiscipline of life science. He observed and described the structure and function of two previously undiscovered organelles, the lysosome and the peroxisome. As a result of de Duve's efforts to improve the meth-

odology available for dissecting cells, biologists have identified and elucidated the role of numerous organelles important to cell physiology.

TRAINING IN INSULIN AND BIOCHEMICAL RESEARCH

Christian R. de Duve was born on October 2, 1917, in Thames-Ditton, near London. His parents were Belgian, but the family had escaped to England when the Germans invaded Belgium during World War I. They returned to Belgium in 1920, and Christian grew up in Antwerp.

In 1934 de Duve entered the Catholic University of Louvain, where he planned to study medicine. He obtained research experience in the laboratory of Professor J. P. Bouckaert, a physiologist who studied the role of insulin in glucose uptake. Insulin is a protein hormone that the pancreas synthesizes and releases when blood sugar levels are high, such as after a meal. Cells respond by transporting glucose from the blood into the cells, where they break it down for energy or store it for later use. By the time de Duve graduated with a medical degree in 1941, he had changed his career ambition from practicing medicine to investigating the biochemical mechanism of insulin action.

The Second World War interfered with de Duve's immediate career plans. He served in the army for a while, spent some time in a prison camp, and eventually returned to Louvain. The lack of available supplies hindered research efforts, so de Duve obtained a job at the Cancer Institute and simultaneously enrolled in a program to earn an additional degree in chemical sciences. In 1945 he received the most advanced degree of university level education, *agrégation de l'enseignement supérieur*. His thesis research led to the publication of several articles in the scientific literature and a book titled *Glucose, Insulin, and Diabetes*. He believed that biochemistry was the best approach for determining how insulin worked. To advance his knowledge of biochemistry, de Duve worked for 18 months in the laboratory of Hugo Theorell at the Medical Nobel Institute in Stockholm, Sweden. Theorell received the Nobel Prize in physiology or medicine in 1955 for his studies on oxidation enzymes. Then he went to St. Louis, Missouri, to work with Carl Cori and Gerty Cori, whose research on the metabolic pathways of glycogen metabolism earned them the 1947 Nobel Prize in physiology or medicine. He also collaborated with Earl Sutherland, who received the 1971 Nobel Prize in physiology or medicine for discoveries on the mechanisms of action for hormones. Having received extensive training with such distinguished researchers, de Duve returned to Louvain in December 1947 to make a name for himself.

LYSOSOMES AND PEROXISOMES

De Duve taught physiological chemistry at the medical school and assumed the position of full professor in 1951. After more than 12 years of training and preparation, as a new professor he focused his research efforts on insulin and glucagon action. In addition to making insulin, the pancreas synthesize glucagon, a hormone that promotes the breakdown of the complex carbohydrate glycogen in the liver. Enzymes digest the glycogen into individual molecules of glucose that enter the bloodstream, leading to an increase in blood sugar levels. Thus the action of glucagon is antagonistic to the action of insulin, which acts to decrease blood sugar levels. While examining carbohydrate metabolism in the liver, de Duve became sidetracked by an observation unrelated to insulin action—the latency of the enzyme acid phosphatase. This diversion led to the discovery of two new cell organelles and to the development of better methodology for studying cell components.

Glycogen breakdown occurs by the release of monomers of glucose 1-phosphate, which the enzyme phosphoglucomutase converts to molecules of glucose 6-phosphate. The enzyme glucose 6-phosphatase removes a phosphate group from the molecule glucose 6-phosphate, resulting in the simple sugar glucose. Though both liver and muscle cells store glucose in the form of glycogen, only liver cells express the enzyme glucose 6-phosphatase. Because of this, de Duve thought it played an important role in the effect of insulin on liver cells; specifically, he thought this enzyme blocked the effect of insulin, and he was trying to learn more about it in hopes of gaining insight as to the mechanism of action of insulin on liver cells. The first step was to purify the enzyme for further characterization. Isoelectric precipitation, a standard method for purifying proteins in those days, involves altering the pH of the solution containing the protein so no net charge exists on the protein molecules. At this pH, called the isoelectric point (pI), the amide groups and the carboxyl groups of the amino acids are uncharged, and the protein loses its ability to interact with the water molecules and falls out of solution. The researcher then resolubilizes the precipitated material in a solution with a neutral pH, at which the side chains of the protein can once again readily interact with the solute molecules. When de Duve attempted isoelectric precipitation to purify glucose 6-phosphatase, the enzyme would not redissolve; thus he concluded that it was not simply precipitated, but was associated with some sort of cellular structure. Forced to consider alternate methodology, he decided to purify it by a relatively new technique called cellular fractionation.

Scientists had recently worked out methods for separating cellular components using centrifugation. Albert Claude, a professor at the Rockefeller Institute noted for his studies on animal cells using electron microscopy, had published a procedure for differential centrifugation in the mid-1940s. This technique takes advantage of the fact that cellular components of various sizes and morphologies sediment at different rates when centrifuged. After grinding a tissue such as a mouse or rat liver in a sucrose solution, three successive centrifugations of the homogenate resulted in four different fractions: the nuclear fraction, the mitochondrial fraction, the microsomal fraction (microsomes are fragments of the endoplasmic reticulum), and the remaining supernatant. George Palade, who joined the Rockefeller Institute in 1947, improved upon Claude's methods and described (in collaboration with Keith Porter, who became well known for his electron microscopy work) the structures found in the cell's cytoplasm such as the endoplasmic reticulum, which is a membranous, folded sac that occupies much of the cytoplasm. He also discovered ribosomes, the cellular structures that he demonstrated were responsible for synthesizing proteins, and outlined the pathway by which proteins destined for secretion reached the cell's exterior via the Golgi apparatus, another cytoplasmic structure.

When de Duve utilized differential centrifugation as described in the literature, the glucose 6-phosphatase activity did not separate into a distinct fraction. Activity was distributed such that a portion of the activity was present in the mitochondrial fraction and a portion of the activity was present in the microsomal fraction. They modified the fractionation procedure to generate a small fifth fraction intermediate to the mitochondrial and microsomal fractions. This fraction contained high concentrations of glucose 6-phosphatase.

At the same time, de Duve's group assayed for the distribution of other enzymes, including acid phosphatase, in the cell fractions. At one point when they assayed fractions for acid phosphatase, the enzyme activity was not detectable—it seemed to have disappeared. De Duve assumed they had made a mistake, and they reassayed the fractions after storing them in the refrigerator for five days, at which point the activity had reappeared. This observation intrigued de Duve, and he put his research on insulin action on hold in order to examine it further. What was happening was that the enzyme activity was enclosed within a membrane; thus the enzyme molecules did not have access to the external substrates. The activity was hidden. As the irreversible agglutination that occurred during the attempted isoelectric precipitation suggested, the distributions of the enzymes with specific fractions indicated that the enzymes were associated with specific subcellular particles.

They examined several other enzymes that exhibited similar behavior. These included oxidases, catalase, reductases, phosphatases, nucleases, and others. Latency was observed for some of the enzymes but not others, and one enzyme (urate oxidase) functioned optimally at an alkaline pH rather than an acidic pH as most of the enzymes they had been studying did. These observations suggested that more than one type of subcellular particle contained the enzymes. To separate the types of particles, they turned to density gradient centrifugation. Characterization of the physical properties of the cellular particles in the density gradient fractions revealed three distinct particle populations, with different associated enzymes—now called the mitochondrial, lysosomal, and peroxisomal fractions.

While de Duve was looking at biochemical activities in cellular fractions, others were making advances in the use of electron microscopy. De Duve did not even have a microscope in his lab at the time. In collaboration with Claude in Brussels and Wilhelm Bernhardt in Paris, de Duve was able to examine his fractions. He later acquired his own electron microscope, and, with the assistance of Palade, Henry Beaufay from de Duve's lab became skilled in its use. The lysosomal fractions contained dense bodies surrounded by a membrane, and the peroxisome fractions contained particles referred to as microbodies.

In 1955 de Duve published a paper proposing the existence of these two new types of cellular particles. De Duve's group had studied acid hydrolases. The lysosomes contain these enzymes, which work best at an acidic pH and digest other molecules such as proteins, nucleic acids, and polysaccharides. Because hydrolytic enzymes are capable of attacking and destroying components of the cell's own cytoplasm, he suggested that enclosure in a membrane-bound compartment was necessary to protect the cell from degrading its own parts. When he treated the fraction with substances to dissolve the membrane, the enzymes were released, confirming their enclosure. The activity of the enzymes depends on the acidic conditions maintained within the lysosome; thus if the membrane of the structure ruptures or leaks, the enzymes do not function in the neutral pH of the cytoplasm.

Lysosomes function in intracellular digestion to get rid of old and worn-out cell parts and to destroy foreign substances taken into the cell through phagocytosis. These organelles form from the Golgi complex, another cytoplasmic, membranous structure consisting of sacs and vesicles. Deficiencies of

enzymes found in the lysosomes cause medical conditions that result from the accumulation of undigested material built up in the cells. One such example is Hers disease, a hereditary disease caused by a deficiency in the enzyme glycogen phosphorylase and characterized by glycogen accumulation, liver enlargement, and low blood sugar level.

Peroxisomes help rid the cell of toxic compounds, including toxic by-products of oxygen metabolism. They contain oxidases, enzymes that use molecular oxygen to oxidize organic molecules. One of the peroxisomal enzymes that de Duve studied was urate oxidase, which catalyzes the oxidation of uric acid in most mammals. The name of the organelle is derived from *hydrogen peroxide,* a product made by enzymes within the peroxisome. The enzyme catalase further metabolizes the hydrogen peroxide into water and oxygen. Unlike lysosomes, peroxisomes are self-replicating.

THE NOBEL PRIZE, ICP, AND WRITING

In 1962 the Rockefeller Institute (now Rockefeller University) in New York appointed de Duve professor. He split his time between Rockefeller and Louvain, where he retained his professorship. In 1974 de Duve founded a new institute, the International Institute of Cellular and Molecular Pathology (ICP), at the Louvain medical school in Brussels. The mission of the institute is to facilitate the application of basic knowledge in the cell and molecular sciences.

Christian de Duve was awarded the Nobel Prize in physiology or medicine in 1974, along with Claude and Palade, "for their discoveries concerning the structural and functional organization of a cell." Together, the three are credited with the creation of modern cell biology.

De Duve married the former Janine Herman in 1943, and together they had four children: Thierry, Anne, Françoise, and Alain. He became professor emeritus at the University of Louvain in 1985 and at Rockefeller Institute in 1988. His presidency of the ICP ended in 1991, though he continued to give his time. In 1997 the name was changed to *Christian de Duve Institute of Cellular Pathology* to pay tribute to its founder.

In 1976 de Duve was invited to give the Rockefeller Christmas Lectures (now called the Alfred E. Mirsky Christmas Lectures), a series of four lectures given to a selected audience of 550 high school students from the New York area. In 1984 de Duve published a book, *A Guided Tour of the Living Cell,* originally based on the series of lectures. The book took several years to write, as he was busy with laboratory research at the same time, but it was wildly successful, in part because of its numerous illustrations. After retiring from laboratory research, he had more time on his hands. De Duve decided to write a second, more condensed version about all the structures within a living cell. As he wrote this book, *Blueprint for a Cell* (1991), he felt the last chapter should address the origin of the cell. By the time he finished composing it, the content of what was to be the final chapter not only took up half of his book, but led to a new interest—the origin, evolution, and meaning of life. He has since authored several books exploring this subject, including *Singularities: Landmarks on the Pathways of Life* (2005), *Life Evolving: Molecules, Mind, and Meaning* (2002), and *Vital Dust: Life as a Cosmic Imperative* (1995).

The course of de Duve's career demonstrates the close relationship between biochemistry and modern cell biology. During his biochemical analysis of cell fractions, his laboratory did not even have a microscope, the primary tool for cell biologists at the time. While attempting to correlate certain enzymatic activities with different cell fractions, de Duve discovered a new membrane-bound organelle, the lysosome. The enzymes within the lysosome digested cellular components when released from the confines of the protective membrane. De Duve also discovered the peroxisome, a membrane-bound organelle that contains enzymes that act in oxidative reactions such as the breakdown of hydrogen peroxide. His discovery of these two cellular structures that play such an important role in cell physiology, along with the methods he developed to study cellular components, helped establish the field of modern cell biology. Before de Duve's contributions, cell biology was mostly a descriptive field, but he demonstrated how scientists could bridge the gap of knowledge between the observations concerning the structure of the cell parts and the biochemical functions occurring within them.

See also CELL BIOLOGY; CENTRIFUGATION; EUKARYOTIC CELLS.

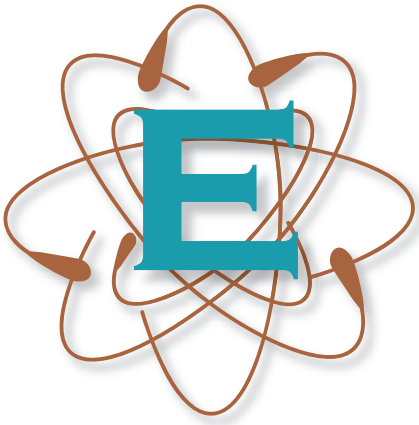
FURTHER READING

Duve, Christian de. *A Guided Tour of the Living Cell.* New York: Scientific American Books, 1984.

———. *Singularities: Landmarks on the Pathways of Life.* New York: Cambridge University Press, 2005.

Duve, Christian R. de, Berton C. Pressman, Robert Gianetto, Robert Wattiaux, and Françoise Appelmans. "Tissue Fractionation Studies. 6, Intracellular Distribution Patterns of Enzymes in Rat-Liver Tissue." *Biochemical Journal* 60 (1955): 604–617.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1974." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1974/. Accessed January 23, 2008.



Earle, Sylvia (1935–) American *Marine Biologist* The pioneering American oceanographer Sylvia Earle was one of the first marine scientists to use scuba as an integral part of her research program and has identified many new marine species. Her area of expertise is phycology, or the study of algae, but she is just as comfortable swimming with 40-ton whales. Having logged 7,000 hours underwater, Earle has been aptly named "Her Deepness."

CHILDHOOD AND EDUCATION

Sylvia Alice Reade Earle was born on August 30, 1935, in Gibbstown, New Jersey, to Lewis Reade Earle, an electrical contractor, and Alice Freas Richie Earle, who both raised her to appreciate the natural world. As the middle child of three siblings, Sylvia enjoyed exploring the pond, creek, and orchard around her family's home, a farm outside Paulsboro, New Jersey. She claims she was called to the sea at age three, when she was knocked over by a wave while wading on the shore. Instead of crying, she laughed and got back on her feet, ready to face more. She has harbored this same fearless attitude throughout her career, while setting deep diving records and advocating public education about the oceans.

When she was 12 years old, the family moved to Dunedin, Florida, into a house with the Gulf of Mexico in the backyard. As a birthday present that year, Sylvia received a gift of swim goggles. She enjoyed reading books by her favorite author, the naturalist William Beebe, and yearned to see the creatures he described. Her first diving experience was with a friend and her friend's father in the Weekiwatchee River. The copper helmet she wore was attached to an air compressor on shore. After 20 minutes the air pump stopped working properly and she had to be rescued, but she had been so enthralled watching a school of fish that she did not let this experience deter her from going under again.

Sylvia enrolled in a summer marine biology course taught by Dr. Harold Humm at Florida State University (FSU) when she was 17 years old. She learned to use scuba gear and loved gliding underwater, following the fishes. After earning her bachelor's degree from FSU in 1955, she applied to and was accepted in several prestigious graduate programs, and she selected Duke University in Durham, North Carolina, where she was awarded a full scholarship and where Dr. Humm was working. As a master's degree candidate, she majored in botany but focused on algae; she became the first person to study the algae in the Gulf of Mexico systematically, amassing a collection with over 20,000 specimens. She received her master's degree in botany in 1956, at only 20 years of age.

That same year she married the zoologist John (Jack) Taylor. They moved to Dunedin, next door to Earle's parents, and set up a makeshift lab complete with specimen cabinets and microscopes in their garage. Their daughter, Elizabeth, was born in 1960 and their son, John (called Richie), in 1962.

In 1964 Earle toured the Indian Ocean on the *Anton Bruun,* for an expedition sponsored by the National Science Foundation. The marine botanist who was scheduled to go had cancelled, and Humm recommended Earle as a replacement on the six-week trip. Though some thought it was bad luck to have a woman on the ship, the 70 members of the all-male crew were impressed with her hard work. She spent as much time as possible exploring underwater and discovered a new species of bright pink algae that reminded her of something Dr. Seuss would invent.

She named it *Hummbrella hydra* in honor of her mentor. Over the next two years, she went on four more expeditions on the *Anton Bruun* and became acquainted with the famous ichthyologist Eugenie Clark.

Researching the role of algae in marine food chains for 10 years familiarized Earle with the far-reaching effects of pollution on aquatic plant life. In 1966 she completed her dissertation, "Phaeophyta of the Eastern Gulf of Mexico." With a Ph.D. from Duke, she assumed a temporary position as resident director of the Cape Haze Marine Laboratories (now the Mote Marine Laboratory), founded by Clark, in Sarasota. The following year Earle accepted simultaneous positions as a research scholar at the Radcliffe Institute and a research fellow at the Farlow Herbarium at Harvard University, which houses algae, fungi, and bryophyte specimens. (Harvard University promoted her to researcher in 1975.) She was particularly interested in ocean ecology, the relationships of plants and animals with each other and with their environment, and studied these relationships by making numerous dives. On one such dive in 1968, Earle dove to 100 feet (30 m) below the surface in the submersible *Deep Diver*; she was four months pregnant with her daughter, Gale, from her second marriage at the time. (She had divorced Taylor and married an ichthyologist from Harvard named Giles Mead.)

TEKTITE II

After seeing an announcement on the bulletin board at Harvard, Earle submitted a proposal for underwater research for Tektite II. Tektite I was a government experiment that put four scientists 50 feet (15 m) underwater in an enclosed habitat near the Virgin Islands for 60 days in 1969. The goals of the Tektite II project were to determine the limitations and practicality of saturation diving and to study undersea habitats, but the National Aeronautics and Space Administration (NASA) was also interested in how people handled living together in tight quarters in an unusual environment. Earle's qualifications and proposal for studying the influence of herbivorous fish on marine plants were impressive. At 1,000 hours, she had more hours of diving experience than all the other applicants, but the navy was not prepared to employ a woman in the project. They decided to hire Earle to lead an all-female team of oceanographers to live in an underwater chamber for two weeks in 1970.

The underwater habitat had carpet, television, bunk beds, showers, and a stove for cooking frozen meals. The upstairs work area had microscopes and a communications panel. A ladder led down to a chamber through which the divers entered the water. Air pressure prevented water from flowing up into the living quarters. NASA psychologists monitored the five scientists constantly and recorded their activities every six minutes. Earle's favorite time to explore was in the predawn darkness, and she especially enjoyed observing the behavior of individual fishes. As part of their experiments, Earle and another team member tested rebreathers instead of scuba tanks. Rebreathers were more expensive and complicated to prepare, but they allowed the divers to stay underwater for four hours rather than one and were quieter, so they could hear the fish grunt and chew on coral.

At the end of two weeks, the five scientists had to spend 19 hours in a decompression chamber to allow their bodies to readjust to normal atmospheric pressure. Earle had documented 154 species of plants, including 26 species never before seen in the Virgin Islands. In addition to confirming that herbivorous fish greatly affected marine plant populations, she

Sylvia Earle, shown here holding a crab while scuba diving off the Florida Keys, has dedicated her life to exploring life under water and educating the public about the oceans. *(Connie Bransilver/Photo Researchers, Inc.)*

learned the sleeping habits of different types of fish and realized that individual fish had food preferences just as people do. The mission stimulated a lot of publicity to which Earle had difficulty adjusting at first, but she decided to use it to her advantage, in order to reach more people with a message about the dangers of ocean pollution. She used her newfound fame to begin writing for *National Geographic* magazine and to produce films that spurred public interest in marine biology. She hoped that greater understanding of the oceans would lead to more positive action toward her goal of protecting them.

STUDIES ON WHALES

In 1976 Earle became a research biologist and curator at the California Academy of Sciences and a fellow in botany at the Natural History Museum of the University of California at Berkeley. Her marriage to Mead had ended, and she had moved to California. She began a project studying humpback whales during winter 1977 in collaboration with Roger Payne and Katy Payne, who were experts on these whales that migrate from Hawaii, where they mate and give birth, to Alaska, where they feed each summer. At the time, most of what was known about whales had been learned from examining carcasses, but Earle knew she could learn much more by studying them in their natural environment. In the 1960s, Roger Payne had set up a microphone in the sea in hopes of recording sounds the whales made and captured scores of songs composed of creaks, grunts, and moans. Biologists have since learned that only males produce the distinct melodies which last up to 20 minutes and can be heard at a distance of 20 miles (32 km). When in the water, the vibrations from the whales' songs made Earle's body vibrate. By following the whales, Earle learned to recognize individual whales by distinctive markings on their faces, flippers, tails, and undersides. Earle collaborated with Payne and the filmmaker Al Giddings to produce a documentary film about the humpbacks, *Gentle Giants of the Pacific,* describing the role the whales play in the oceanic ecosystem.

FREE DIVING AND DEEP OCEAN EXPLORATION

The next adventure involved a Jim suit, which resembled space attire and allowed a person to walk on the ocean floor. Jim suits were typically used for people making repairs to underwater machines or oil rigs; a scientist had never used one for research purposes. Giddings thought it would be remarkable to film Earle outfitted in one strolling along the ocean bottom at 1,000 feet (305 m) for pictures in a National Geographic Society book, *Exploring the Deep Frontier* (1998), and for an upcoming television special. The garments were made of magnesium, a malleable metal that could withstand the intense water pressure at great depths, and had steel pincers as hands. A cable connected the suit's wearer with a submersible that followed behind.

Earle welcomed the challenge, and on October 19, 1979, she set a record for free diving at 1,250 feet (381 m). In the Jim suit, she walked stiffly along the ocean floor and jotted down observations she made while watching the giant seven-foot (2.1-m) rays and long-legged crabs with whom she shared her pedway. She also admired pink sea fans, jellyfish, a cat shark, lantern fish, hatchetfish, and bamboo coral that rippled with gleaming blue rings when nudged. Her two-and-one-half-hour time limit passed quickly, but before returning to the submersible platform for ascension, she planted two flags to mark the historic moment in the ocean floor, a U.S. flag and a National Geographic flag. Since then, more advanced submersibles have replaced the Jim suits for many purposes.

Though Earle was thrilled to have set a new free-diving record, she yearned to go deeper, since the average depth of the ocean is 13,000 feet (3,962 m). In a joint venture in 1982, Earle and the British-born engineer Graham Hawkes (to whom she was married in 1986–89) founded a company called Deep Ocean Technology (later called Deep Ocean Engineering, Inc.) to build state-of-the-art, deep-diving, one-person submersibles. One major obstacle they had to overcome was choosing a material that could withstand the pressure at great depths but was transparent to allow observation. Customers were hard to find, so Hawkes built a large remotely operated vehicle (ROV) that could be used to inspect undersea equipment. After selling one to Shell Oil Company, they started receiving more orders. In 1984 they created *Deep Rover,* a spherical submersible with mechanical arms that held one diver and operated at record-breaking depths of 3,000 feet (914 m). Earle went down in *Deep Rover* at night, an experience she compared to falling into a fireworks display because of the luminescent creatures floating around her. Her mesmerizing experience observing jellies, shrimp, interesting fish, and an octopus was marred by the sight of a soda can on the ocean floor. Today Deep Ocean Engineering continues to design and manufacture ROVs that are sold internationally.

NOAA AND LATER CAREER

President George H. W. Bush appointed Earle chief scientist at the National Oceanic and Atmospheric Administration (NOAA) in 1990. NOAA is the U.S. governmental organization whose mission is to describe and predict changes in the Earth's environment and to conserve and manage the nation's coastal and marine resources. Earle was initially worried

about not being able to voice her opinions in public as an upper-level government representative, but she accepted the opportunity in hopes of changing the stubborn governmental mindset that maintained marine research as a low funding priority. She had become increasingly aggravated at the government's apathy about undersea exploration and the unwillingness to invest money for undersea research.

In 1991, as chief scientist of an underwater research team, Earle investigated the effects and aftermath of 500 million gallons (more than 2 billion liters) of oil that Iraq deliberately dumped into the Persian Gulf during the Gulf War. Her task was to figure out how long it would take the ocean and its inhabitants to recover from the devastating effects. The landscape was completely blackened for miles, and the waters were oily and brown. Seeing the marine organisms covered in black slime reaffirmed her commitment to spreading the message of ocean conservation. She also investigated the effects on the ecosystems in Prince William Sound, Alaska, of the oil spills from the ship the *Exxon Valdez*. She resigned from NOAA after 18 months.

In 1992 Earle founded Deep Ocean Exploration and Research (DOER) with her daughter, Elizabeth Taylor. Located in Alameda, California, DOER has the stated mission designing and developing practical and effective technologies to provide working access to the deep ocean and other challenging environments.

Earle accompanied Japanese scientists on an expedition in 1991, when she descended in a three-person submersible named *Shinkai 6500* to 13,000 feet (almost 4 km), deeper than she had ever been before. The Japanese government asked Earle to lend her expertise for building a remote, then manned, submersible that could dive to 36,000 feet (11 km) in 1993.

In 1995 Earle used her reputation as an expert on the deep ocean to champion conservation by publishing a book, *Sea Change: A Message of the Oceans,* which celebrates the variety and abundance of life below the sea's surface. Along with her fascination, she soberly shared her worries about the future of the oceans in the hands of uninformed humans.

National Geographic named Earle the Explorer in Residence in 1998. From 1998 to 2002 she served as project director for the Sustainable Seas Expeditions, supported by the National Geographic Society, the NOAA, and the Goldman Foundation, to explore and document the marine life and conditions of the 12 U.S. marine sanctuaries. A comprehensive survey of these underwater national parks will allow marine scientists to detect changes to the ecosystems and to make recommendations for the preservation of their health. During this time she also published *Wild Ocean* (1999) and *Atlas of the Ocean* (2001).

In 2006 President Bush established the United States's first National Marine Monument, located in the northwestern Hawaiian Islands, in part because of Earle's persuasiveness. Currently, Earle serves as president of Deep Search International and chair of the Advisory Council for the Harte Research Institute for Gulf of Mexico Studies.

Earle has led more than 60 expeditions and logged more than 7,000 hours underwater. She has earned numerous honors and awards and received 15 honorary degrees from institutions including Florida International University, the Monterey Institute, Duke University, the University of Connecticut, and the University of Rhode Island. She is a member of the American Association of the Academy of Sciences, the California Academy of Sciences, the Marine Technology Society, and the World Academy of Arts and Sciences. She serves on boards and committees for Woods Hole Oceanographic Institute, Mote Marine Laboratory, the World Wildlife Fund, and many more. In 2000 she was inducted into the National Women's Hall of Fame and the U.S. Library of Congress named her a Living Legend, an award presented to people who have made significant contributions to America's diverse cultural, scientific, and social heritage. The sea urchin *Diadema sylvie* and the red alga *Pilina earli* were named in her honor.

Time magazine aptly named Earle the first "hero for the planet" in 1998. Her authorship of more than 150 publications about marine science earned her the right to speak on behalf of the oceans and to educate the public about the oceans and marine life. As an ambassador for the sea, she has strongly and repeatedly expressed the opinion that if people knew about marine life, they would care more about protecting it. Living up to this responsibility, she has made more than 100 television appearances in interviews and in special programs.

See also Beebe, William; Clark, Eugenie; marine biology.

FURTHER READING

Academy of Achievement. "Sylvia Earle, Ph.D." Available online. URL: http://www.achievement.org/autodoc/page/ear0bio-1. Updated January 17, 2008.

Baker, Beth. *Sylvia Earle: Guardian of the Sea.* Minneapolis, Minn.: Lerner Publications, 2001.

Earle, Sylvia A. *Sea Change: A Message of the Oceans.* New York: Ballantine Books, 1995.

Earle, Sylvia A., and Henry Wolcott. *Wild Ocean: America's Parks under the Sea.* Washington, D.C.: National Geographic Society, 1999.

Stanley, Phyllis M. *American Environmental Heroes.* Springfield, N.J.: Enslow, 1996.

eating disorders Eating disorders such as anorexia, bulimia, and binge-eating disorder affect males and females of all ages from all socioeconomic backgrounds, though young females are at the highest risk. Eating disorders are real, treatable medical conditions and are not due to lack of willpower. They are often associated with other illnesses, including depression, substance abuse, or anxiety disorders. The effects of eating disorders can be life-threatening or cause serious damage to one's health. Anorexia nervosa is characterized by extreme weight loss due to self-imposed starvation. People suffering from anorexia have an unrealistic fear of being fat and often see themselves as fat even when dangerously underweight. Bulimia nervosa is characterized by episodes of overeating, followed by an activity to counter the binge, such as vomiting, high doses of laxatives, periods of fasting, or excessive exercising. Binge-eating disorder, also called compulsive eating, is characterized by episodes of uncontrolled, impulsive eating, well beyond the point of becoming full. In contrast to bulimia, the binge is not followed by purging, but after a binge, the person may feel intense shame or feelings of self-loathing. Many people who suffer from eating disorders experience aspects of two or all three of the conditions.

While the symptoms of eating disorders involve obsessions with food, the causes are believed to be a combination of emotional, social, and psychological factors. Food is used to accomplish something other than meeting nutritional needs, such as a means to calm emotions, to cover up one's lack of self-esteem or loneliness, or to symbolize something the person can control. Though in many cases the causes fall within the realm of psychology, in some cases, biochemical or physiological abnormalities also play a role. Hormonal signaling and neural signaling among body tissues affects one's appetite and energy expenditure; thus endocrine or nervous system malfunctions may lead to improper regulation of appetite, which may trigger an eating disorder. Twin studies suggest that anorexia and bulimia have a genetic component, and scientists believe that several genes may interact with the environment to make someone susceptible to eating disorders. Because females are more susceptible than males, and eating disorders most often emerge shortly after the onset of puberty, many scientists suspect that gonadal steroids play a role in the development of these disorders.

In the case of anorexia nervosa, the body responds to starvation by slowing its metabolism to conserve energy. This can lead to a heart rate that is too slow or low blood pressure, and feelings of severe fatigue, weakness, and fainting. The body will eventually break down muscle tissue to utilize the protein stores for energy. Bones will become brittle, skin and hair will become dry, and the hair may start to fall out. A layer of fine hair may develop over the entire body in an effort to preserve body heat, a task that normal metabolism accomplishes in a healthy individual. Dehydration can cause kidney failure.

The health consequences of bulimia differ. One major problem is electrolyte imbalance. As food travels through the digestive tract, the body reabsorbs necessary electrolytes, such as sodium, potassium, and chloride. Induced vomiting prevents the food from ever reaching the intestines, and laxatives move the food through the digestive tract too rapidly for sufficient reabsorption to occur; fluids follow and are lost as well. Electrolyte imbalances can cause heart failure. Over long periods, induced vomiting can cause serious tooth decay from the acidity of the gastric juices, which can also inflame the esophagus. Frequent vomiting can also cause gastric or esophageal rupture. Overuse of laxatives diminishes the digestive tract's ability to maintain regular bowel movements, leading to a dependency on the laxatives to produce bowel movements. The belief that laxatives are effective in controlling weight is erroneous; most of the weight lost by stimulating food to rush through the intestines is water and indigestible fiber that would make its way out on its own eventually. As soon as the person drinks water, the "weight loss" is reversed. If the person does not rehydrate, the effects of dehydration include weakness, blurry vision, fainting, kidney damage, and even death.

The physiological effects of binge eating mirror those of obesity, whether the person suffering from compulsive eating is overweight or not. These risks include high blood pressure, high cholesterol level, high triglyceride levels, Type II diabetes, and disease of the gallbladder.

Females between the ages of 15 and 19 are at the highest risk for developing eating disorders, though every age group of both males and females has seen an increase with each decade since the 1930s. According to the National Institute of Mental Health of the National Institutes of Health,

- Between 0.5 and 3.7 percent of females suffer from anorexia nervosa at some time during their life.
- During their lifetime 1.1 to 4.2 percent suffer from bulimia nervosa.
- Between 2 percent and 5 percent of all Americans suffer from binge-eating disorder within a six-month period.

Experts believe many more cases of eating disorders exist than are reported because of the shame associated with the disorders.

One strategy for prevention and for treatment of eating disorders is to focus on nutritional eating, eating to maintain health. The obsession of dieting is difficult to counter. Instilling healthy eating attitudes and habits is the best means for preventing or treating an eating disorder and for achieving and maintaining a healthy body weight.

See also NUTRITION.

FURTHER READING

National Institute of Mental Health, National Institutes of Health. "Eating Disorders: Facts about Eating Disorders and the Search for Solutions." Available online. URL: http://www.nimh.nih.gov/publicat/eatingdisorders.cfm. Accessed August 2, 2008.

ecology Ecology is the study of interactions and relationships between organisms and their environment. The environment comprises the abiotic, or nonliving, components of the surroundings as well as all the other populations that live in the same area. Abiotic components include factors such as local weather and climate, nearby water bodies, geological structures or features, and the chemical composition of the soil or atmosphere. Learning about these relationships discovers information about the beneficial services ecosystems provide for people and other living organisms. Ecosystem services include vital processes such as purifying water, maintaining ideal atmospheric gas compositions, and pollinating crops. Knowledge gained from ecological studies also reveals how people can utilize the Earth's natural resources more wisely. For example, ecological research led to the discovery that phosphates from laundry detergents and nitrogen from chemical fertilizers cause photosynthetic organisms in lakes and ponds to overgrow, choking waterways and killing fish. The inorganic nutrients also lead to algal blooms in lakes. As the algae and photosynthetic bacteria die, detritivores (organisms that feed on dead and decomposing organic matter) thrive from consuming the organic remains and deplete the oxygen from the waters. This knowledge allowed society to take actions to prevent this negative outcome.

Ecology and evolution are closely related, as the conditions of the environment in which an organism resides select for adaptations that render the organism more suitable for living within that environment. For example, aquatic organisms that live in marine habitats have anatomical and physiological mechanisms that prevent them from losing fluids despite the high solute concentration of the oceans. Bony marine fishes take in water and salt ions through the mouth, excrete excess salt across their gills, and excrete salt ions and very little water from their kidneys. In contrast, freshwater fishes take in water and some salt ions through their mouth and gills, and they excrete large quantities of dilute urine from their kidneys to get rid of excess water. Over time, the different environments have favored selection of the anatomical and physiological adaptations that allowed the organisms to survive in either saltwater or freshwater. This is an example of an animal's interacting with an abiotic environmental factor. Ecologists have also discovered numerous examples of interactions between two or more species that have affected the evolutionary histories of all the involved species. Coevolution occurs when two or more interacting species develop reciprocal adaptations. For example, the pollination of plants provides an exemplary model of the mutual evolutionary influence of plants and pollinators. The flowers of yucca plants are a unique shape and require very tiny pollinators. Yucca moths can enter the flower and lay eggs in it, and then the larvae feed off the seeds in the ovary. The unique shape of the flower limits the evolutionary development of the moth, and because the plant clearly benefits from the moth's ability to enter, its shape is influenced by changes in the moth. Other insects or birds have tongues or beaks that have coevolved with the shape of a flower. The flower shape influences the length of the pollinator's tongue or beak, and the need for pollination by the insect or bird limits the amount of change the plant can undergo.

HISTORY OF ECOLOGY

Ecology as a field of life science initially developed as botanists noticed that the environmental conditions affected the distribution of plant species. The German naturalist Alexander von Humboldt was the first to look specifically for relationships between organisms and their environments. While hiking in Colombia in 1801, he noticed that the types of vegetation changed as the altitude increased. His research led to the emergence of the field of plant geography. In 1866 the German biologist Ernst Haeckel coined the term *ecology,* defined as the comprehensive science of the relationship of an organism to the environment. In 1877 the concept of biological or ecological communities emerged as biologists such as Charles Darwin, Alfred Russel Wallace, and Karl Möbius recognized that organisms depend on and interact with one another. Advancements in chemistry led to the exploration of biogeochemical cycles, the combination of biological, geological, and chemical processes that act to convert elements (e.g., carbon, nitrogen, oxygen, and phosphorus) between inorganic and organic and unavailable and available forms. The Austrian geologist Eduard Suess coined the term *biosphere* in 1875, and the Russian geologist Vladimir Vernadsky elaborated on this concept during the 1920s.

The Danish botanist Eugenius Warming authored the first ecology textbook, *Plantesamfund,* in 1895. This book was translated and published in English in 1909 as *The Oecology of Plants: An Introduction to the Study of Plant Communities*. Warming also taught the first university ecology course.

During the 20th century, ecology gave rise to the environmental and conservation movements that aim to protect nature and its life-forms. The American author Rachel Carson changed the way people viewed nature by demonstrating the potential negative impact that humans have on the ecology of planet Earth. Increased education of the public about human-induced destruction of ecosystems and loss of biodiversity has led to a greater push for ecological research and a better understanding of human ecology.

WHAT ECOLOGISTS DO AND WHY

Ecologists ask questions such as, Where do organisms live? What does the particular environment provide for the organism in terms of food, shelter, and climate? How does the organism affect its surroundings by living there? What unique adaptations does an organism have that allow it to survive and reproduce in the specific conditions of that habitat? To approach these sorts of questions, an ecologist must have a good understanding of many branches of life science, such as anatomy, physiology, evolution, and ethology, as ecology is a multidisciplinary field. Ecologists must also be aware of the general characteristics of different life-forms and appreciate their similarities and differences in order to predict possible interactions among species. A zoologist (one who studies animals), a botanist (one who studies plants), a microbiologist (one who studies microorganisms), or a biologist who specializes in any other life-form may all consider themselves to be ecologists.

An ecologist can specialize in a particular type of ecosystem. For example, a marine ecologist focuses on oceanic ecosystems, such as a kelp forest ecosystem. Tropical ecology, desert ecology, Arctic ecology, forest ecology, and stream ecology are other examples of subfields that focus on a particular type of biome or habitat. Another way to divide ecology into subfields is based on the type of organism studied. A microbial ecologist might research the interactions of different bacterial and protozoan species living in an animal's gut. An insect ecologist may investigate factors contributing to colony collapse disorder (CCD), commonly known as "the disappearing honeybees." A plant ecologist may examine the effect of the introduction of an invasive woody vine into a new geographic region on the native vegetation. Ecologists may also specialize in a particular ecological issue such as maintaining or increasing biodiversity, examining the effects of or ways to prevent acid rain, developing sustainable ecological systems, or restoring ecosystems.

Ecologists can study interactions at numerous levels of biological organization from the individual level up to the biosphere. The subdiscipline concerned with the individual level and how organisms adapt to a particular environment is called behavioral ecology. A behavioral ecologist may examine how an alligator maintains its body temperature within a certain range despite external fluctuations. A population ecologist might explore the factors leading to the steady decline in the numbers of a species of fish. Or at a slightly higher organizational level, the researcher might investigate how the decreasing numbers of fish have affected the populations of their predators, how the numbers relate to the decline in coral reefs in the area, or how the biodiversity of the entire marine community is affected. At the ecosystem level, an ecologist might explore how an increase in atmospheric carbon dioxide levels has affected the flow of energy through trophic levels of the Amazon rain forest. Ecological researchers may also examine how the change in landscaping or geology of a region throughout history has affected the distribution of different life-forms in it, such as in the Arctic. Many ecologists are actively at work trying to figure out the effects of global climate change on the biosphere.

The career opportunities for a trained ecologist vary widely, depending on the specialty. Federal, state, and local governments hire ecologists as consultants for advice on how to reduce negative environmental impacts when planning new developments or industrial projects. Policymakers may consult ecologists for advice on improving conservation efforts or resolving environmental problems. Parks and private nature organizations hire ecologists as resource managers or as developers of educational programs for people of all ages. Academic research institutions hire ecologists to conduct field research or laboratory research and to teach ecology and advise college undergraduate and graduate students. To gain employment as an ecologist one must have a strong background in the life sciences, including zoology, botany, and microbiology, but also in physical and earth science. Chemistry is important for understanding geochemical cycling and how an organism's metabolism affects the surrounding conditions of the soil, the atmosphere, and the hydrosphere. Geology is also relevant since landscaping and geological features, such as altitude, streams or nearby water bodies, and frequency of events such as volcanic eruptions, all affect the type of organisms that live in a certain area. Statistics is useful for determining

how closely a sample represents a population and for determining whether observed trends or patterns are significant. Competence in computer software is also necessary, as are written and oral communication skills for conveying results.

Understanding the relationships between different organisms and the environment is important because a change that might seem minor could have potentially drastic consequences. Ecosystems are composed of a community of interacting populations of living organisms and the physical environment of a certain area. The complex ecological interactions form a web of connections that represent beneficial and seemingly harmful exchanges that together maintain the health of an ecosystem. For example, in 2003 scientists reported a domino effect in North Pacific wildlife resulting from the mass slaughter of whales by commercial whaling. As whales disappeared, killer whales, who feed on other whales, started to feed on other prey. They first turned to harbor seals, then when the harbor seal numbers decreased, the killer whales fed off fur seals, then off sea lions and sea otters. The boom in commercial whaling occurred between 1949 and 1969, when more than 500,000 whales were killed in the Pacific. But the decline in other marine mammals has been more recent, since the turn of the millennium. Sea otters normally prey on sea urchins, keeping their numbers in check. The decrease in the sea otter population caused an explosion in the sea urchin populations. Sea urchins feed on kelp, and as their numbers increased, they destroyed the kelp forest ecosystem in southwestern Alaska. In summary, whaling that took place 50 years ago is responsible for the loss of the kelp forest. An interesting lesson from this incident is that food web interconnectivity is too complex to predict all the consequences of one action—the effects can reach farther and last longer than one might expect. Preserving ecosystems and protecting biodiversity benefit all life-forms on Earth, including people. Better working knowledge of the relationships among different species of organisms and with their environment will help people learn how to protect nature.

See also BIOGEOCHEMICAL CYCLES; BIOGEOGRAPHY; BIOMES, AQUATIC; BIOMES, TERRESTRIAL; BIOSPHERE; CARSON, RACHEL; COMMUNITY ECOLOGY; ECOSYSTEMS; ENVIRONMENTAL SCIENCE; HAECKEL, ERNST; POPULATION ECOLOGY.

FURTHER READING

Ecological Society of America home page. Available online. URL: http://www.esa.org/. Accessed January 25, 2008.

Molles, Manuel C. *Ecology: Concepts and Applications*. 4th ed. New York: McGraw Hill, 2008.

ecosystems An ecosystem consists of all the organisms within a biological community and the physical environment with which they interact. The size of an ecosystem may be as small as one rotting log on a forest floor or as large as the floor of the Atlantic Ocean. On a larger scale, ecosystems function to provide many services such as biogeochemical cycling of nutrients, moving water through the water cycle, maintaining atmospheric conditions, and more. Two critical phenomena that characterize ecosystems are energy flow and nutrient cycling.

PRIMARY PRODUCTION AND ENERGY FLOW

The ultimate source of energy for all ecosystems is the Sun. The energy then flows through the trophic levels of the ecosystem. Living systems must follow natural laws, including the law of conservation of energy, which states that energy cannot be created or destroyed. The energy does, however, become transformed as it moves through the ecosystem.

Photosynthetic organisms such as plants absorb the energy in the form of light, or radiant energy. They convert some of it into chemical energy, but energy transformation is an inefficient process and much of the energy is lost as heat. The primary producers, or autotrophs, of an ecosystem are the organisms that support all the other organisms in an ecosystem by providing energy in a usable form. They constitute in which the first level of an ecosystem's trophic structure: the processes energy and nutrients move through the organisms in the ecosystem. Examples of primary producers include plants in terrestrial ecosystems and phytoplankton in aquatic ecosystems. After using sunlight to generate energy-rich organic compounds, organisms belonging to other trophic levels obtain their energy and nutrients by ingesting the autotrophs, or products synthesized by the autotrophs. These other organisms are called heterotrophs because they cannot synthesize their own organic molecules from inorganic carbon sources. Organisms that consume autotrophs directly are called primary consumers, or herbivores. Secondary consumers eat the herbivores and are called carnivores. If they eat the primary producers (plants) in addition to herbivores (other animals), they are called omnivores. Tertiary consumers eat secondary consumers. Decomposers, or detritivores, ingest the decaying organic material, returning the inorganic components to the ecosystem.

As energy flows through a system of trophic levels, much is lost at each step. The gross primary production (GPP) of an ecosystem is the total primary production. The net primary production (NPP) is the GPP minus the amount of energy that the primary producers use as part of their own metabolism. NPP is therefore an indicator of how much energy is available to other organisms in an ecosystem. Two ways

to express NPP are as new biomass, or dry weight of vegetation, added to the ecosystem per unit area per unit time, or as energy per area per unit time.

Different factors contribute to an ecosystem's primary productivity. Because sunlight provides the ultimate source of energy, its availability affects the productivity of an ecosystem. Geographical regions surrounding the equator are thus generally more productive than other terrestrial ecosystems. Tropical rain forests rank among the highest. Light cannot sufficiently penetrate deeper than several feet (about a meter) of water. Thus the NPP of oceans is rather low; however, they still contribute significantly to Earth's total productivity simply because they cover so much area.

The availability of nutrients also affects primary production. Even if energy is abundant, without nutrients, organisms cannot convert the radiant energy into chemical energy. Limiting nutrients are nutrients that, when added, allow for continued production. Carbon, oxygen, hydrogen, nitrogen, and phosphorus make up 93 to 97 percent of living biomass. Other essential nutrients include potassium, calcium, sulfur, magnesium, and sodium. Photosynthetic organisms and other autotrophs synthesize organic compounds, such as sugars and amino acids, from inorganic sources. Consumers in an ecosystem obtain their nutrients by ingesting the primary producers and other organisms.

When the organisms die, microorganisms decompose the organic matter. As do all living organisms, deritivores require not only the energy easily obtained from digesting the dead and decaying organic material, but also nutrients. Carbon is plentiful, but nitrogen is scarce in dead plant material, thus is limiting to detritivores. Phosphorus is also limiting in most terrestrial and wetland ecosystems; thus agriculture depends on the use of fertilizers containing the correct balance of both nitrogen and phosphates. During the natural process of decomposition, many nutrients return to the environment, where primary producers can once again fix them into organic compounds. Biochemical processes in living organisms also return nutrients to the environment. For example, carbon dioxide is released as a product of cellular respiration, in which organic molecules such as carbohydrates are broken down to release the energy for the organism to use in order to carry out other life processes. Geological processes also act to cycle nutrients through ecosystems. Erosion and weathering of rocks release phosphorus-rich minerals into the soil, where acids can dissolve them, and make the phosphorus available for plants to uptake them in the form of phosphates for incorporation into biomolecules such as nucleic acids. Upwelling carries nutrient-rich waters to the ocean surface, where primary producers can incorporate them in organic material. Thus, biological, chemical, physical, and geological processes all play a role in cycling nutrients through an ecosystem.

Other physical factors that influence the efficiency of primary production in terrestrial ecosystems are temperature and moisture. Warm and moist environments, such as tropical forests, are more productive than drier and colder ecosystems, such as the arctic tundra or vast deserts.

ECOLOGICAL PYRAMIDS

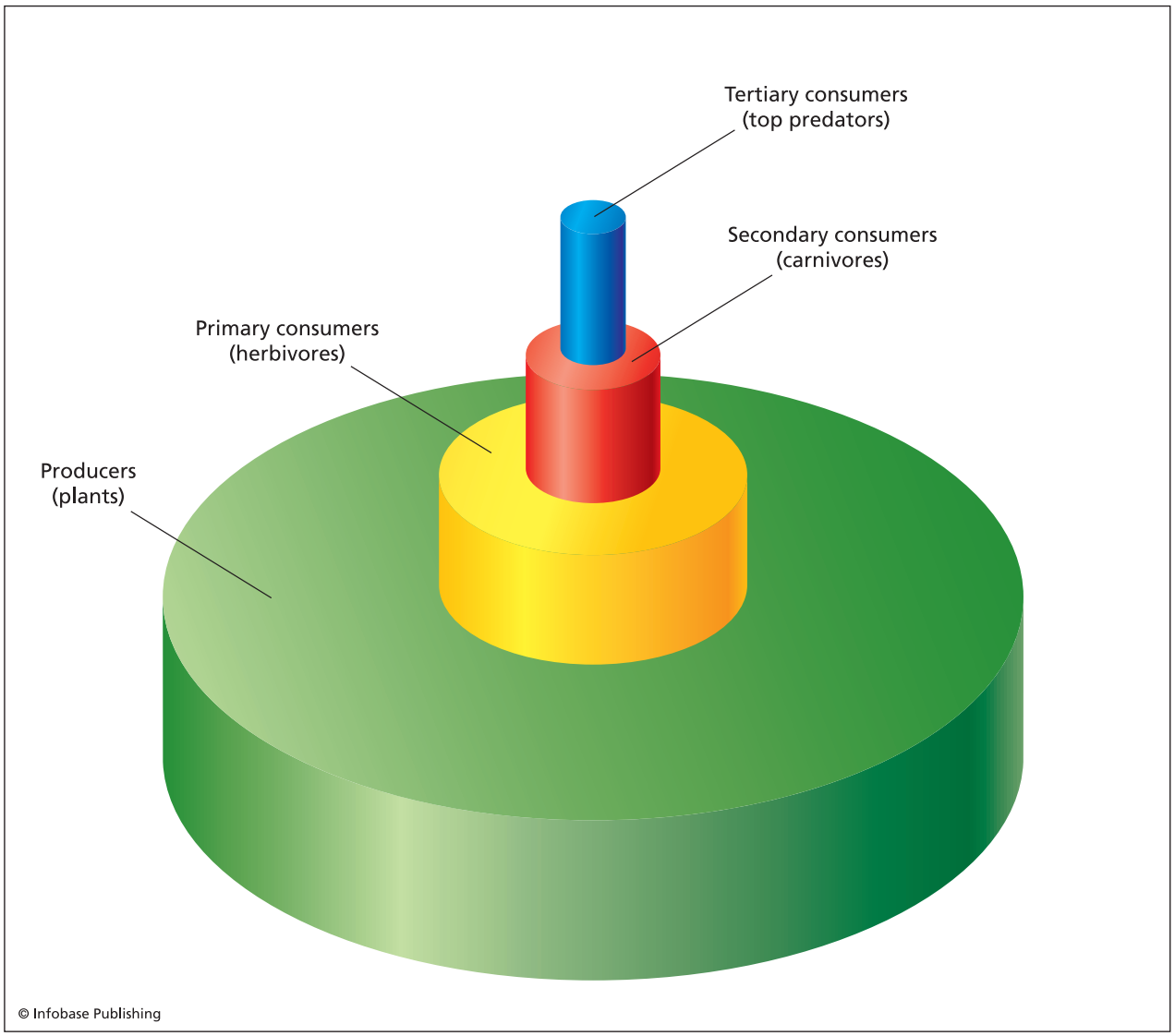
Primary production is the amount of light energy converted into chemical energy by autotrophs in an ecosystem. Secondary production is the amount of chemical energy in a consumer's food that its metabolism converts into new biomass. As primary production can, secondary production can be examined within a defined geographical area and within a given period. As the energy moves from a lower to a higher trophic level, the majority is lost. Between one-fifth and one-sixth of the energy at one level can be transferred to the next. The rest of the biomass or energy in an ecosystem is either not consumed by organisms of the next trophic level, it is not digested, or it is released as heat, which is unusable by other consumers.

Production efficiency measures the amount of energy stored in food that is not used in cellular respiration. Some is used in growth and reproduction to perpetuate biomass, which may be consumed later by another organism. Production efficiency equals the ratio of the net secondary production to the total amount of energy used in growth, reproduction, and cellular respiration, in other words, the assimilation of primary production.

$$\text{production efficiency} = \frac{\text{net secondary production}}{\text{assimilation of primary production}}$$

Of all animals, endotherms, which maintain their body temperature through metabolism, have the lowest production efficiencies (between 1 and 3 percent), as they purposefully burn energy just to keep warm. Insects have a relatively high production efficiency, averaging about 40 percent.

In the early 1940s Raymond Lindeman first proposed the existence of trophic levels as groups of organisms that share a similar role in energy transfer within an ecosystem. He emphasized the complex relationships between organisms in a community and their environment and proposed the concepts of primary producer, primary consumer, secondary consumer, and so on. During his studies on energy flow through ecosystems, he recognized that as energy traveled between trophic levels, much was lost, result-



Ecological pyramids illustrate the concept of progressive loss as one moves up through the trophic levels of an ecosystem. The phenomenon of progressive loss holds true for net production, biomass, available energy, and numbers of individuals.

ing in a pyramid-shaped distribution of energy among trophic levels. Another ecologist, Charles Elton, had previously proposed the pyramid concept, but Lindeman substantiated Elton's claim with data from two lake systems in Wisconsin. As a result of Lindeman's work, ecologists began to study energy patterns and influences on energy flow through ecosystems.

Studies have shown that trophic efficiencies range from 5 to 20 percent, because of energy losses through respiration, energy excreted as unused biomass in feces, and the fact that consumers do not intake all the biomass from a lower trophic level. To illustrate, consider a representative ecosystem in which primary producers only capture 1 percent of the solar energy input. The primary consumers incorporate approximately 4 to 5 percent of the energy they obtain from the primary producers in their own biomass, making it available for organisms at the next trophic level, the secondary consumers. If only 10 percent of that is made available to the next level, pretty soon, very little energy is available for the next level. The major losses that occur as energy moves between trophic levels limits the total number of trophic levels that most ecosystems possess to four or five.

The loss of energy available to consumers at successive trophic levels can be represented by a pyramid of net production. A decrease in the amount of biomass at each trophic level accompanies the loss of available energy. An exception to this generalization

is found in some aquatic ecosystems in which the turnover of phytoplankton is so high that the amount of biomass can never accumulate sufficiently. Predators, tertiary consumers such as hawks or sharks, typically have smaller populations, reflected in a typical pyramid of numbers, because only a fraction of 1 percent of energy flows to this trophic level in an ecosystem.

One may wonder why so much of the biomass and chemical energy made available by primary producers is unused. Scientists have proposed several hypotheses to explain this: 1) Plants have defense mechanisms such as poisonous chemicals to deter herbivores. 2) Nutrients play a larger role in limiting herbivore populations than does energy input. 3) Physical and geological factors help control herbivore populations. 4) Competition keeps the levels of herbivores low. 5) Community dynamics such as predation and parasitic diseases help maintain populations.

See also BIOGEOCHEMICAL CYCLES; COMMUNITY ECOLOGY; POPULATION ECOLOGY.

FURTHER READING

Molles, Manuel C., Jr. *Ecology: Concepts and Applications*. 4th ed. New York: McGraw Hill, 2008.

electrophoresis Electrophoresis is a technique that separates biomolecules according to properties such as size, shape, or charge. Life scientists use electrophoresis both to analyze samples and to purify biomolecules such as proteins and nucleic acids. Samples are placed in a semisolid medium called a gel that is saturated with an ionic buffer solution. An electric current drives the molecules through the medium, which acts as a porous sieve, toward the anode at different rates dependent on size and shape. After running the gel, different mechanisms including staining or radiography allow visualization of the biomolecules. Their position in the gel reveals information about the characteristics of the molecules. If the goal of the electrophoresis is to prepare a substance or reagent for further use, the desired molecules can be extracted from small pieces cut from the gel and subject to purification.

DNA ELECTROPHORESIS

Deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) fragments of different sizes can be separated using either agarose gel electrophoresis or polyacrylamide gel electrophoresis, depending on the approximate molecular weight of the fragment(s) of interest. Agarose is a neutral polysaccharide obtained from the cell walls of algae that is used in a powdered form. When boiled, agarose dissolves in water, and, when cooled, it solidifies into a semisolid gelatinous matrix. Higher concentrations of agarose give firmer gels and are better for separating fragments as small as 100 nucleotide base pairs long, compared to several thousand base pairs for gels made from lower concentrations of agarose. The molten agarose is poured into a rectangular plastic tray with combs positioned to leave indentations called wells at one end of the gel after the gel cools and the comb is removed. Gel trays have a range of sizes and are typically about a few inches wide and slightly longer. Before or sometimes after loading the DNA samples, the agarose gel is submerged in a running buffer that contains a chemical buffer to maintain an optimal pH. DNA electrophoresis requires slightly basic conditions to keep the DNA deprotonated, in other words, to maintain its negative charge.

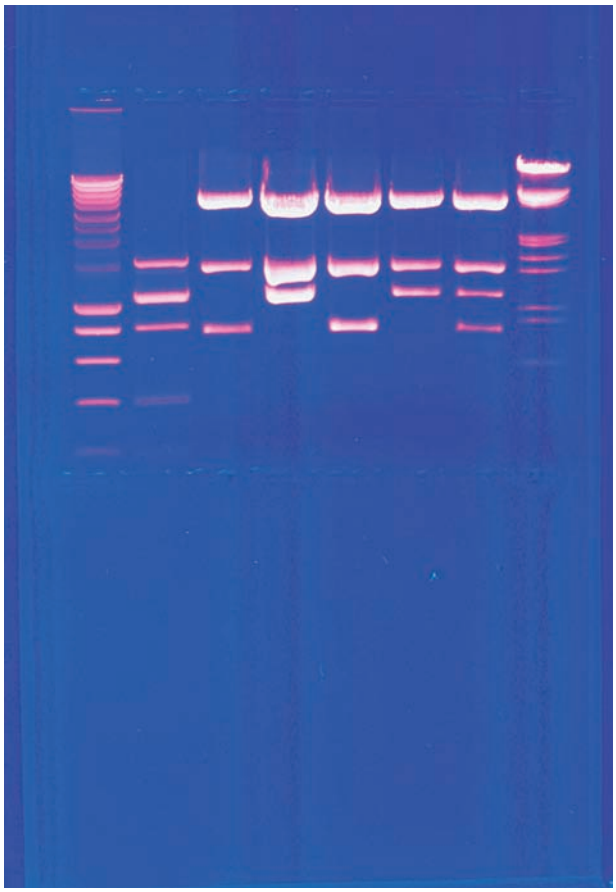
The wells hold only a small amount of sample; typical samples measure between 10 and 25 microliters (one microliter = 10^{-6} liter). Before placing the DNA samples in the wells, loading dye is added. The loading dye usually contains a dense but water soluble component such as glycerol or sucrose to encourage the sample to sink to the bottom of the well. Otherwise, the loaded sample may float freely and mix with the running buffer. The loading dye also contains tracking dyes, such as bromophenol blue and xylene cyanol, that travel at rates comparable to DNA fragments of a specific size; thus one can follow the DNA as it runs through the gel by watching the dye fragments separate.

After the samples are in the wells, a power source supplies the voltage, establishing the flow of an electric current in the tank containing the gel and running buffer. Because the phosphates in the DNA backbone confer a negative charge on it, the DNA will migrate from the wells located near the cathode end toward the positive electrode, the anode. Within a few minutes the dye will appear slightly in front of the wells, indicating movement of the samples into the gel matrix. The complete run is usually shorter than a few hours, depending on the gel size, concentration, and the molecular weights of the fragments being separated. Smaller fragments will travel through a gel at a faster rate than larger fragments. This is because the larger fragments experience more friction as they migrate through the agarose matrix.

Large DNA molecules, those that are between hundreds of thousands and millions of base pairs long, cannot be resolved using a constant current through the gel. Instead, researchers use a technique called pulsed-field gel electrophoresis (PFGE), in which relatively long pulses of current in the forward direction are interrupted by shorter pulses in the

opposite direction. This tightens the bands so they can be viewed as separate entities on the gel rather than appear as smeared streaks.

In order to visualize the DNA, the gel must be stained with ethidium bromide, a chemical that intercalates or inserts itself between the nitrogenous bases of the nucleotides. Exposure to ultraviolet light causes the ethidium bromide to fluoresce a bright orange color. The positions of the DNA fragments are typically recorded by taking a photograph or digital image of the illuminated gel. Running a separate lane with a molecular weight marker that contains fragments of known length parallel with the sample fragments allows one to estimate the size of any fragments present in the sample lanes if they fall within the range of the molecular weight standards. By plotting a curve of the distance migrated versus the log of the molecular weight of the standards, one can read the approximate weight from the curve for any band by measuring the distance it traveled.



DNA fragments fluoresce when stained with ethidium bromide and viewed under ultraviolet light. After electrophoresis, the larger fragments remain closer to the top, while the smaller fragments travel faster and are farther away from the wells. *(Pascal Goetgheluck/ Photo Researchers, Inc.)*

If the purpose of the gel was preparative, the regions of the gel containing the DNA of interest can be excised. Special techniques allow for the extraction and purification of DNA from the fragment. Biotechnology companies sell kits that facilitate this process. Alternatively, one can use agarose that melts at a low temperature, extract the DNA from the molten solution using organic chemicals, and precipitate the DNA from the aqueous solution using salts and alcohol.

The DNA can also be transferred from the gel to a nitrocellulose or nylon membrane in a procedure called Southern blotting that permits the identification of specific DNA fragments. First the DNA is denatured by treatment with a basic solution, so it is single-stranded. During transfer of the DNA from the gel to the membrane, the relative positions of the DNA fragments are preserved. The membrane can then be treated with different radioactive probes, pieces of DNA that will hybridize by forming hydrogen bonds with complementary base pairs if they are present on the membrane. Autoradiography with X-ray film or phosphorimaging reveals the positions of the bands that contain DNA that hybridized with the probe. This characteristic allows a researcher to look for specific DNA sequences within a fragment or sample of DNA.

Agarose gels are quick, cheap, and easy to prepare and run, but the best resolution is limited to fragments about 100 base pairs or longer. Smaller pieces of DNA appear smeared and fuzzy on agarose gels. Polyacrylamide gel electrophoresis (PAGE) is more useful for separating fragments that are as small as a few nucleotides long. Polyacrylamide is a polymer of acrylamide monomers (amides of acrylic acid). Preparation for PAGE is more complicated, as the acrylamide monomers are neurotoxic, and the apparatus is vertical rather than horizontal as in agarose gel electrophoresis. Dyes are still added to the DNA samples before running the gel so the researcher has an idea of how far certain sizes of fragments have run over a given period. Most often, PAGE results are viewed by autoradiography. If the DNA has been radiolabeled, then laying a piece of X-ray film over the gel will expose regions of the film that correspond to the position of the DNA fragments on the gel. Unless the purpose of the PAGE is preparative, the gel is usually dried before exposing it to X-ray film. After the exposed film is developed, dark bands will appear, indicating the location of the DNA fragments on the gel.

PROTEIN ELECTROPHORESIS

Polyacrylamide gel electrophoresis is also useful for analyzing protein samples. As does DNA, most proteins carry a negative charge in a slightly basic

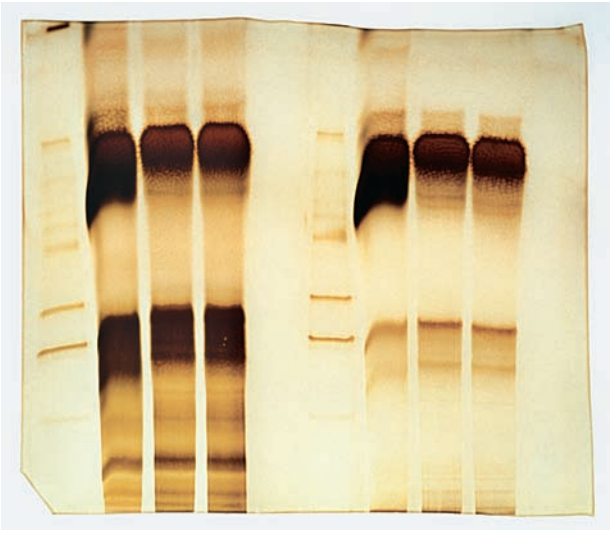
Polyacrylamide gel electrophoresis utilizes vertical apparatuses, and buffer tanks have contact only with the top and the bottom portion of the gel. *(Mauro Fermariello/Photo Researchers, Inc.)*

solution; thus they will migrate through a gel with speed characteristic of their size. Because proteins have complex three-dimensional folded structures held together by hydrophobic interactions, ionic interactions, and hydrogen bonds, it is necessary to add a mild anionic detergent (usually sodium dodecyl sulfate, or simply SDS) to denature the proteins by interfering with those interactions. The SDS also coats the entire polypeptide with negative charges, masking any other charges. Often, a reducing agent is added to disrupt covalent disulfide linkages of different polypeptides of a multisubunit protein. These actions ensure that the rate at which a polypeptide moves through a gel depends only on its size and not its shape or intrinsic charge. In a discontinuous system, a short stacking gel with a very large pore size is layered on top of the resolving gel and contains a different buffer than the resolving gel. This improves the final resolution by compressing the sample before it enters the resolving gel, which then separates the polypeptides on the basis of size. After running a protein gel, staining with a dye such as Coomassie blue or with silver colors the polypeptide bands for direct visualization.

When the mixture of polypeptides is complex, adding a second dimension to the separation procedure provides more resolution. After the sample has been separated by electrophoresis once, the samples are subject to another run at a 90 degree angle to the first, either through a different concentration of polyacrylamide or by use of a buffering system with a different pH. Nondenaturing gels must be used for this, as SDS will mask the native charges, but this means that individual polypeptides cannot be analyzed. In a slightly more complicated version of two-dimensional electrophoresis, the first step separates the molecules according to their native charge. The gel is cast in a tube that has a pH gradient. Application of an electrical field moves the molecules toward the anode until they reach a point in the gradient at which their pH is neutral, then they stop. Their final position along the gradient depends on the number of acidic and basic side chains present in the polypeptide. After this first step, which is called isoelectric focusing, the tube gel is laid on top of a typical slab gel and subjected to typical SDS-PAGE, which then separates the proteins by size.

As DNA can, proteins can be transferred from a gel onto a membrane. In western blot analysis, the proteins are transferred to a nitrocellulose membrane. After being treated with special buffers, the membrane is placed beside the gel, and an electric current forces the polypeptides off the gel onto the membrane.

After the proteins are immobilized onto a membrane, the membrane can be probed with antibodies, proteins naturally made by the immune system



SDS-PAGE separates polypeptides on the basis of their size, with the smaller molecules traveling faster, thus appearing toward the bottom of the gel, while the larger molecules migrate more slowly and stay near the top of the gel. *(R. A. Longuehaye/Photo Researchers, Inc.)*

that specifically recognize and bind to other protein molecules. If the antibodies are radiolabeled, the pattern of the proteins of interest can be visualized by exposure to X-ray film. Otherwise the antibodies can be treated with a series of chemical reagents and fluorescent dyes designed to allow visualization without the use of radioactivity.

One method for detecting protein-DNA interactions is the gel mobility shift assay (GMSA) or the band shift assay. With this technique, one can examine the ability of proteins to bind to segments of DNA containing specific sequences. If a segment of DNA is bound to a protein, its movement through a gel will be much slower than movement by the same segment in the absence of protein. The result is a shift in the position of the band when it is bound to protein; the band will remain closer to the wells of the gel.

See also BIOMOLECULES; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

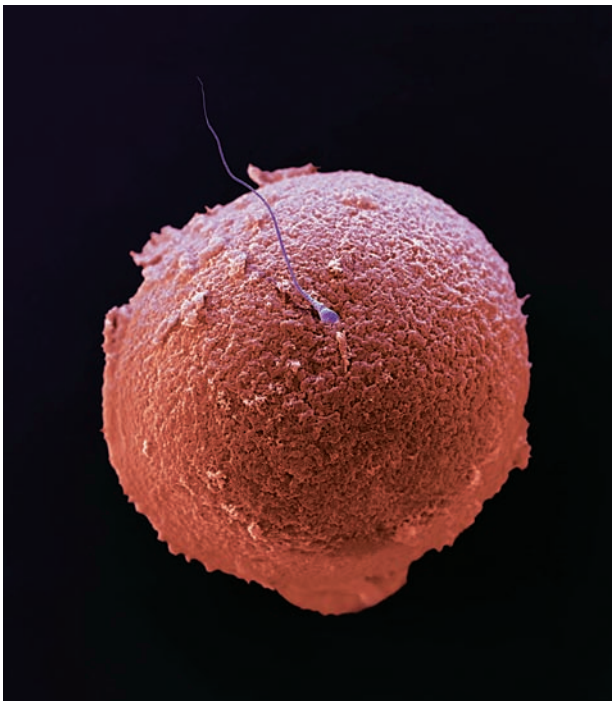
Westermeier, Reiner. *Electrophoresis in Practice: A Guide to Methods and Applications of DNA and Protein Separations*. Weinheim, Germany: Wiley-VCH, 2005.

embryology and early animal development Embryology is the study of embryos and their development. The term *embryo* once encompassed the stages of vertebrate development from the time of conception until birth or hatching, but now it refers only to the earliest stages of animal development. After fertilization, a zygote undergoes a series of rapid divisions, produces the foundation of fundamental tissues, and initiates the formation of organs and organ systems. After the basic body structure is established, the embryo is considered a fetus. In humans, the transition from an embryo to a fetus occurs approximately two months into gestation. The processes involved in early animal development are similar in all species, beginning with cleavage and growth and resulting in the formation of differentiated tissues and organs that perform tasks that contribute to whole-body function.

FERTILIZATION AND CLEAVAGE

Fertilization occurs when two haploid gametes unite to restore the diploid condition and initiate the development of a new individual. Whether this takes place externally (as in marine invertebrates) or internally (most terrestrial animals including mammals), the subsequent events are similar. When a sperm cell from an adult male has contact with an egg cell from an adult female, a sac located at the tip of the sperm head releases digestive enzymes that aid in penetrating the zona pellucida, a matrix surrounding the egg. The plasma membranes of the sperm cell and the egg cell fuse, and all of the sperm except the tail enters into the cytoplasm of the egg. Though many sperm can pass through the corona radiata, the layer external to the zona pellucida that contains cells that nourish the egg, and several sperm can attempt to penetrate the zona pellucida, biochemical mechanisms prevent more than one sperm from passing through. The nucleus of the sperm fuses with the nucleus of the egg, the chromosomes duplicate, and the cell prepares for the first cell division. One variation between species is the stage of meiosis that the egg has reached prior to fertilization. If meiosis is not yet complete, as in humans, fertilization stimulates the completion of meiotic division.

Embryonic development can be divided into three stages: cleavage, gastrulation, and organogenesis. Fertilization initiates the process of cleavage, repeated mitotic divisions in which the cytoplasmic contents of the egg divide into numerous progressively smaller cells called blastomeres, all containing a genetically identical nucleus with one set of chromosomes from each parent. The zygote first divides into two cells, then four cells, then eight, and so on. This can occur very rapidly; for example, a frog zygote produces 37,000 cells in 43 hours, and a fruit fly zygote produces 50,000 cells in 12 hours. The ball of cells resulting from the first five to seven divisions is called a morula. A blastocoel, an intercellular cavity filled



Fertilization occurs when an egg and a sperm unite and their nuclei fuse. *(Eye of Science/Photo Researchers, Inc.)*

with fluid, begins to form, transforming the morula into a blastula, a hollow ball of cells. The process of blastulation usually occurs simultaneously with cleavage, but some embryologists technically consider it part of gastrulation.

The eggs of many animals, not including mammals, show polarity either before or immediately after fertilization. Having polarity means a decided difference exists between opposite ends of the cell or the embryo; for example, the cytoplasmic contents might be distributed unequally. The polarity influences cleavage patterns and persists into the morula and further developmental stages. The sizes of the cells differ, and in different animals the position of the blastocoel also differs.

Some vertebrates and insects have a pronounced yolk, a mass of stored food within the cytoplasm of an egg, positioned at one pole of the egg. The yolk can be larger than the rest of the cytoplasm and displaces other cytoplasmic contents, leading to unequal cell divisions during cleavage, different-sized cells within the morula, and off-center positioning of the blastocoel. At the end of cleavage, the bastula consists of blastomeres with genetically identical nuclei but different cytoplasmic materials. These subtle differences help direct the next stage of embryonic development, gastrulation.

GASTRULATION

Gastrulation involves the movement and rearrangement of the cells in the blastula; different portions of the blastula have different developmental fates. When complete, the result is a cup-shaped embryo with three distinct layers and a primitive gut. The process differs slightly among animal types but generally involves the same mechanisms and results in the foundation of the basic body plan for that species. The three layers are called germ layers and include ectoderm, mesoderm, and endoderm. The ectoderm is the outermost layer, the endoderm lines the digestive tract, and the mesoderm is between the other two. These germ layers develop into the various tissues and organs of the mature animal.

Different mechanisms of cell movement participate in the formation of the primitive gut in different species. In animals such as sea urchins, invagination, the infolding or the simple pushing in of some cells from the surface of the blastula, forms a blind pouch called the archenteron, the primitive gut consisting of endoderm cells. The blastopore, the opening of the archenteron, will become the anus (as occurs in all deuterostomes). Migration of cells called mesenchyme cells accompanies invagination. The mesenchyme cells that will become mesoderm position themselves between the developing archenteron and the outer layer of cells, the ectoderm, of the gastrula. The archenteron eventually reaches ectoderm across the blastocoel and fuses with it, forming the second opening of a digestive tube. The gastrula becomes a ciliated larval form that ultimately develops into an adult sea urchin.

In other animals, such as frogs, a dorsal lip forms where invagination occurs, then continued invagination extends all the way around the blastula, following the inner surface of the ectoderm until the blastopore forms a complete circle. Accompanying invagination is involution, whereby cells roll inward from the surface of the blastula to form endoderm and mesoderm. When gastrulation is complete, the lip of the blastopore surrounds a structure called the yolk plug.

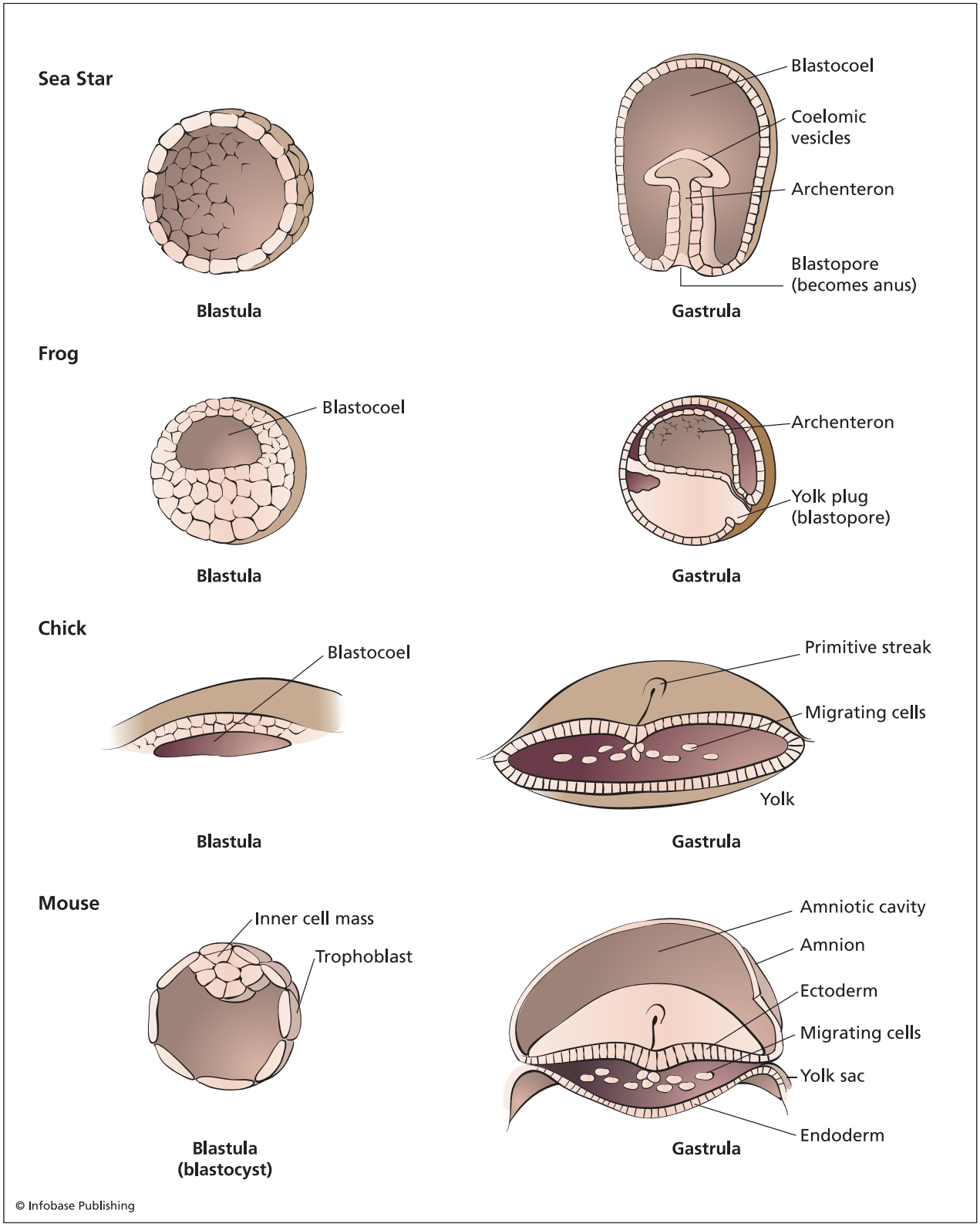
In chicks, blastulation results in a blastoderm rather than a blastula. The blastoderm consists of two layers, the epiblast and hypoblast, covering a large yolk mass. Only the epiblast contributes cells to the developing embryo. Cells move from the epiblast to the middle of the blastoderm, forming a primitive streak that defines the anterior-posterior axis. Some of the migrating cells form endoderm; others move into the blastocoel, located between the epiblast and the hypoblast, and become mesoderm. The remaining epiblast cells form ectoderm.

ORGANOGENESIS

By the end of gastrulation, cells have become associated with one of the three primary germ layers, and their position within the layers determines their fate. During organogenesis, cells of the germ layers undergo numerous morphological and functional changes to develop into various body tissues and organs. Induction directs many of these changes. The process of induction involves chemical agents secreted by some cells that act on their neighboring cells; thus the position of a cell affects the chemical signals it encounters. The chemicals usually alter the expression of specific genes, resulting in the formation of specific tissues, which then organize and cooperate to form organs and organ systems.

In chordates, the first structures to form are the neural tube and the notochord. Dorsal mesoderm condenses to form a notochord, which then signals the ectoderm to form the neural plate. The neural plate rolls up into a tube, the neural tube, which develops into the central nervous system. In vertebrate embryos, cells released from a band of cells called the neural crest travel to different locations and form peripheral nerves, skull bones, and teeth. Dense clusters of mesodermal cells called somites positioned along the notochord give rise to a variety of structures including the ribs, the muscles of the rib cage and back, and the dermis of the dorsal skin. Throughout organogenesis, the structuring of organs and the shaping of the body continue, as does the differentiation of the tissues.

Among vertebrates, the three primary germ layers develop into the same body parts. In general, the ectoderm gives rise to the epidermis, sweat glands, hair follicles, and epithelial lining of the mouth and nasal passages; sense receptors in the epidermis, cornea, and lens of the eye; and the nervous system,



In most animals, gastrulation involves an extensive rearrangement of the cells, converting a roughly spherical blastula into a complex arrangement consisting of three germ layers.

adrenal medulla, tooth enamel, and pituitary glands. The mesoderm develops into the notochord, skeletal system, muscular system, muscular layers of the stomach and intestine, excretory system, circulatory system, lymphatic system, reproductive system, dermis of the skin, lining of the body cavity, and adrenal cortex. The endoderm gives rise to the epithelial linings of the digestive tract and the respiratory system, the linings of the urethra and bladder, the lining of the reproductive system, the liver, pancreas, thymus, thyroid gland, and parathyroid gland. During early cleavage, cells are totipotent, meaning they have the ability to develop into any cell type. The stage at which cells develop unequal potentials varies among species. For example, in amphibians, the developmental potential is restricted after the first cleavage, at the two-cell stage. In contrast, in mammals, totipotency persists until the 16-cell stage. This means that at the eight-cell stage, the blastomeres can be separated and potentially develop into eight equal individuals. After tissue type is determined, cells differentiate further, until their functional potential becomes restricted to one specialized type. In other words, a liver cell cannot change into a thyroid cell.

AMNIOTES

The early development of vertebrates demands an aqueous environment. Animals that live in aquatic environments can lay their eggs directly into their surroundings. Terrestrial vertebrates including reptiles, birds, and mammals require unique adaptations to overcome this obstacle. A fluid-filled sac surrounds the terrestrial vertebrate embryo as it develops either within a shell or in the uterus. Because the membrane that encloses the fluid-filled sac is called the amnion, these animals are called amniotes. Birds and other reptiles have four extraembryonic membranes protecting the embryo inside a shell: the amnion, the yolk sac, the allantois, and the chorion. The amnion encloses the embryo, which is suspended with a cavity filled with fluid that cushions against harsh movements and protects against dehydration. The yolk sac stores the nutrients from the egg. The allantois accepts metabolic waste discharged from the embryo and functions in cooperation with the chorion as a respiratory organ. Gases diffuse from the external environment, through the shell, through the chorion, and between the embryo and allantois.

Mammals are unique because their eggs are much smaller than those of reptiles and birds; they store only minimal nutrients, just enough until the embryo develops a functional placenta, an organ through which nutrients, gases, and metabolic wastes are exchanged between the embryo and the pregnant mother. In humans, cleavage results in a blastocyst, homologous to a blastula, which consists of more than 100 cells seven days after fertilization. An inner cell mass that forms on one end of the blastocyst develops into the embryo, whereas the trophoblast, the cells forming the outer layer of the blastocyst, is responsible for implantation and invasion of the uterus. As implantation ends, gastrulation begins, and the formation of the three primary germ layers and the four extraembryonic membranes is initiated. As in other vertebrates, the chorion functions in gas exchange, and the amnion encloses a fluid-filled cavity. The yolk sac contains no yolk in mammals but acts as the site for blood cell formation. The allantois becomes part of the umbilical cord that transports blood to the placenta, where embryonic (and later, fetal) blood and maternal blood can exchange nutrients and wastes.

See also ANIMAL FORM; CELL COMMUNICATION; CELLULAR REPRODUCTION; HUMAN REPRODUCTION; REPRODUCTION.

FURTHER READING

Bonner, John Tyler. *First Signals: The Evolution of Multicellular Development*. Princeton, N.J.: Princeton University Press, 2000.

Gilbert, Scott F. *Developmental Biology*. 8th ed. Sunderland, Mass.: Sinauer Associates, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

Wilt, Fred H., and Sarah Hake. *Principles of Developmental Biology*. New York: W. W. Norton, 2003.

endangered species Endangered species are populations of organisms that are at risk of becoming extinct throughout all or a significant portion of their range. Threatened species are those that are likely to become endangered in the near future. Many nations have laws to protect endangered and threatened species and to restore their population size. In the United States, the Endangered Species Act (ESA) of 1973 formally defined the terms *endangered* and *threatened,* combined native and foreign species lists, required federal agencies to implement conservation programs, and outlined procedures for the goal of protecting species that fell into these categories. Several amendments to this act (in 1978, 1982, and 1988) have strengthened it to fulfill its main purpose—to protect and prevent extinction of imperiled species by providing programs to conserve them and the ecosystems that support them. The Interior Department's Fish and Wildlife Service (FWS) and the Commerce Department's National Oceanic and Atmospheric Administration (NOAA) Fisheries administer the law, with FWS managing land and freshwater species, and the NOAA Fisheries manag-

ing marine and anadromous species (those that live in the ocean but spawn in freshwater). According to the FWS, as of January 2008, the approximate totals of U.S. and foreign listed endangered and threatened species includes 1,175 animal and 747 plant populations, though some species are counted more than once when more than one distinct population is endangered.

ESA is a U.S. law; thus other nations do not need to follow its regulations. Every country is responsible for enacting its own legislation concerning the protection of endangered and threatened species, killing them, and selling their parts. Protecting endangered species and their habitats is an issue that affects the entire planet, however; thus in order to have a positive impact conservation efforts require global cooperation. In the 1960s growing concern led to worldwide discussions that resulted in the Convention on International Trade in Endangered Species of Fauna and Flora (CITES), an international agreement that entered into force in 1975. CITES regulates the international trade of living or dead endangered and threatened animals and plants and their parts. While the enforcement of such trade restrictions is difficult, the cooperative attitude demonstrated by the 173 participating countries (as of August 2008) is a step in the right direction.

CAUSES OF EXTINCTION

Natural factors that lead to extinction include disease epidemics, lack of genetic variation in a population faced with new environmental challenges, and limited distribution of a species to one or a few geographical areas. Human-induced threats to endangered species include the destruction of the habitats essential to the species' survival, the overexploitation of wildlife for commercial purposes, and the introduction of nonnative species to an environment. Mounting evidence also shows that human activities are affecting the global climate. Estimates of the number of species that could become extinct as a result of global warming by the year 2050 approach 1.25 million.

The destruction of a species's habitat is the leading cause of extinction. A habitat provides the resources for nourishment and energy as well as the optimal physical conditions for an organism to grow and reproduce. Without someplace to live that offers the same, a species will die out. Habitat loss can occur as a result of pollution (such as from chemicals leaching into the soil), a disruption to the balance of an ecosystem that changes the physical environment of the habitat (such as damming a river), or straightforward elimination of the habitat (such as razing for construction and urban development). One serious issue is deforestation, the clearing of forests, which is occurring at a rate of millions of acres per year. Tropical rain forests contain an estimated half of all extant species, but these species are rapidly losing their habitats through clearing for commercial logging or for agricultural purposes, such as for cattle pastures or planting crops. Draining wetlands, areas where water covers the soil, for development purposes or even flooding them for recreational uses is another example of a human activity that contributes to habitat loss. Wetlands are among the most productive ecosystems, serving as home for a variety of microbial, plant, and animal species that perform important ecosystem services, such as cycling nutrients and capturing and storing carbon, an act that moderates climate. An estimated one-third of endangered species live in wetlands, and many other animals depend on wetlands for at least part of their life cycle or for food. Wetlands are also crucial in watersheds, areas that drain to a common waterway, and in controlling erosion of shorelines. Global warming has also caused major changes to habitats, affecting the species that live there. Glaciers are melting and causing sea levels to rise, tundras are shrinking, deserts are growing, and forests and grasslands are gradually moving toward more appropriate climates. Small geographical regions that serve as home to species with precise temperature and humidity requirements are disappearing, as are coral reef systems and tropical cloud forests.

The ESA protects not only organisms at risk for becoming extinct, but also the habitats that support their survival. Legislation regarding the use of protected areas that are inhabited by endangered species and setting aside land and water bodies as nature reserves is one means of preventing further human-induced habitat destruction. The establishment of wildlife corridors, protected patches that physically connect different habitats, also helps by allowing for wildlife movement between habitats so animals can interbreed and maintain genetic variation, helping to maintain functional ecosystems. Actions aimed at reducing human activities associated with increased global warming, such as the burning of fossil fuels, are also an important factor in protecting many threatened and endangered species in the coming decades.

According to the FWS, the introduction of nonnative species to a new environment is the second greatest threat to native species. This may occur either intentionally or unintentionally. Humans first took domesticated cats to Australia in the 17th century. Because the continent's ecosystem had not evolved mechanisms to limit the population, cats rapidly multiplied and now the carnivorous hunters are blamed for the extinction and endangerment of several native small mammals and birds. In another example, ships

from Europe unintentionally carried zebra mussels in their ballast water to the Great Lakes region of North America, where millions of dollars has since been spent to control their population and prevent the decline in native mussel populations. Nonnative species (also called exotic, invasive, or nonindigenous species) may outcompete native species for space or nutrients, affect interspecies dependencies, or change the physical conditions of the ecosystem through their metabolism or behavior. For example, zebra mussels compete with zooplankton for food, disrupting the natural food webs. As filter feeders, the zebra mussels clarify the water, creating advantageous conditions for some photosynthetic organisms, while harming fish such as walleye that prefer turbid water. The large populations of the zebra mussels settle on, suffocate, and starve the native mussel populations.

The overexploitation of animals and plants for commercial purposes also leads to threatening or endangering species. When individuals of a species disappear at a faster rate than they reproduce, the species is at risk of becoming extinct. For example, in the 20th century, whaling was not regulated and many species dropped to endangered status as whalers killed them for their oil and meat. After recognizing this, several nations voluntarily entered into a moratorium on whaling. Some species, such as the gray whale, have recovered, while others remain endangered or threatened as a result. Rhinoceroses are another animal facing possible extinction, not due to habitat loss, but due to poaching. Their horns are used to construct dagger handles that symbolize wealth and status in some Middle Eastern countries, making them profitable enough to worth risking the legal ramifications of selling them on the black market. Plants are often overexploited for their medicinal properties. The first sources of medicines were plants, and today hundreds or even thousands of plants are harvested for their natural products that have therapeutic and medicinal value. Actions to prevent overexploitation of species indigenous to other countries include trade regulations preventing the sale or import of products from endangered species. The perennial herb ginseng is one example of a plant regulated by CITES. Though ginseng is not currently listed as endangered, CITES regulates its trade in order to prevent the overexploitation of this popular herb. Its root has been used for centuries by practitioners of Eastern medicine as a universal medication for fatigue, attrition due to illness, and physical effects of stress. During the past 50 years, Western medicine's use of ginseng root as a mild stimulant and for the relief of indigestion has grown. Its medicinal effectiveness has been disputed, but its popularity led to a demand high enough to warrant oversight by CITES.

WHY SAVE ENDANGERED SPECIES?

Since life first appeared, new species have emerged and others have become extinct as a result of natural geological and biological changes. The five previous mass extinctions on Earth resulted from natural cataclysmic physical events, such as global climate changes, massive lava floods, and dramatic changes in sea level due to glacier formation and melting. Biologists believe the current rate of extinction, which is far higher than any previous mass extinction rates, is the result of activities by the expanding human population. For many, ethical reasons alone are sufficient to justify protection of endangered species. Given that most species evolved after millions of years of natural processes, many question the rights of humans to eliminate them. From another perspective, to what degree is society responsible for protecting and restoring endangered populations? In the text of the ESA, the U.S. Congress cited numerous reasons why people should attempt to restore populations of threatened and endangered species, stating that wildlife and plants are of aesthetic, ecological, educational, historical, recreational, and scientific value.

One scientific reason to protect endangered species is to preserve ecosystems. In addition to the immediate resources that an ecosystem provides, such as timber for construction or fish for food, ecosystems provide services that are not marketed or sold, but whose loss has devastating consequences. Some estimates claim ecosystems provide trillions of dollars worth of services each year. Ecosystems cycle nutrients and water, remove toxins from the soil, filter or purify water, decompose wastes, regulate climate, keep soil fertile, and maintain biodiversity, just to name a few. These fundamental ecological processes support all life-forms on the planet. Because all of the community members in an ecosystem fill unique niches, each species performs an important function. Significant alterations in the size of a particular population can affect the function of the whole ecosystem. This is particularly true for keystone species, species of plants or animals who have a major impact on the structure and therefore the function of an ecosystem. The story of the gray wolf (*Canis lupus*) of Yellowstone National Park demonstrates the far-reaching effects that the decline of one species can have on an entire ecosystem. Believing the gray wolf to be a nuisance that killed livestock and pets and ruined crops, people who lived nearby had hunted and killed off the population by 1926. In 1995 the FWS captured and reintroduced a small population of 14 gray wolves from Canada to the park ecosystem, and 17 more the following year; that population has since grown to more than 1,000 animals, with close to 200 residing in the park itself. Soon after reintroduction of wolves, the elk population began to decline, as

The reintroduction of the gray wolf, *Canis lupus,* to the Yellowstone National Park ecosystem had a tremendous ecological impact. *(Gary Kramer/U.S. Fish and Wildlife Service)*

wolves naturally prey on elk. The reduction in the elk population allowed willow trees that elk fed on to grow taller, particularly those near streams, since the wolves scared the elk away from the streams. More vegetation increased available habitat space for species such as birds, and food for other species, such as beavers. More beavers led to more dams, and therefore more ponds and impoundments, which allowed more shrubs to grow and serve as protection for migratory birds' nests. More shade on the streams from taller trees cooled the water temperatures and provided more areas for trout to brood. Many other species, such as large birds, bears, and other scavenger populations, benefited from eating the leftovers of wolf kills, increasing their populations. Thus, the reintroduction of one predatory keystone species had a tremendous ripple effect.

Many regions depend on nature-based tourist attractions that rely on endangered or threatened species, as do many recreational activities that involve seeking, observing, and photographing wildlife. The National Wildlife Federation estimates that Americans spend $59 billion annually while engaging in such nonconsumptive wildlife recreation. Bird watching is an extremely popular hobby, and regions that boast rare birds are tourist hotspots. People also visit zoos and aquariums specifically to see exhibits with new babies born to endangered animal species and to observe the vast array of biological diversity on Earth.

Preventing the loss of species is also important for the undiscovered benefits they offer. The genetic resources of millions of species have not yet been explored, and once those species are extinct, they are lost forever. Scientists have discovered that organisms produce many substances that are useful and valuable to humans. The fungus *Penicillium notatum* produces penicillin, an antimicrobial chemical that ushered in the age of antibiotics. The soil bacterium *Bacillus thuringiensis* produces a toxin that kills crop-destroying insects. The bark of the Pacific yew tree contains taxol, a drug used to treat ovarian cancer. Perhaps scientists will uncover a solution to global warming or find a cure for Alzheimer's disease in the genes of a seemingly unremarkable organism.

See also BIODIVERSITY; CONSERVATION BIOLOGY; ECOSYSTEMS; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED.

FURTHER READING

Evans, Kim Masters. *Endangered Species: Protecting Biodiversity*. Farmington Hills, Mich.: Thomson Gale, 2006.

NOAA Fisheries Office of Protected Resources. "Endangered Species Act (ESA)." Available online. URL http://www.nmfs.noaa.gov/pr/laws/esa/. Accessed January 19, 2008.

U.S. Fish and Wildlife Service. The Endangered Species Program home page. Available online. URL: http://www.fws.gov/endangered/. Updated January 17, 2008.

endocrine system The purpose of the endocrine system is to coordinate and regulate the functions of body cells, tissues, and organs. The endocrine system regulates major life processes, such as metabolism, immune functions, the production of red blood cells, reproduction, childbirth, and lactation, and controls internal body conditions such as osmolarity, water balance, heart rate, blood pressure, and blood sugar levels. Endocrine glands produce and secrete chemicals called hormones that circulate in the blood and induce functional changes in certain cells. Hormones, also called ligands because they bind to other macromolecules, travel throughout the entire body via circulation, but only target cells, the types of cells that have receptors that specifically recognize the ligand, respond to its presence. While both the nervous system and the endocrine system facilitate communication among different body parts, the nervous system normally responds to stimuli that require an immediate response, such as removing one's hand from a hot stove. The endocrine system generally manages slower or sustained responses that occur over minutes, hours, or longer such as adjusting the

degree of water retention during the production of urine, stimulating cell growth, or initiating sexual maturation during puberty. The intensity of response to a hormone depends on its concentration, in contrast with the all-or-none action potentials that guide the transmission of nervous impulses. Both systems cooperate, however, to coordinate body functions, respond to external and internal stimuli, and maintain homeostasis. For example, neurons that have hormone receptors respond to hormonal signals. Some neurons secrete hormones directly into the circulatory system, and a number of nerves directly innervate certain endocrine glands.

In addition to endocrine signaling, other types of chemical signals communicate information among cells. Neurotransmitters carry signals across a synapse, a junction between a neuron and another neuron or a neuron and an effector cell. Autocrine signals exert local effects on the same type of cells that release them. Paracrine signals affect cells in the immediate vicinity; they are not transported in the blood. Exocrine glands produce and secrete substances such as mucus, sweat, and digestive enzymes, but the secretions travel through ducts to the outside of the body or to cavities that are continuous with the outside of the body. Special chemicals called pheromones leave the body of one individual and influence the behavior of other organisms. Not all chemical signals fall into a single category. For example, some endocrine glands secrete hormones that act locally and at a distance or that function as both neurotransmitters and hormones.

HORMONE SYNTHESIS, TRANSPORT, AND ACTION

Hormones, chemical messengers that generally act over long distances, can be either proteins or lipids. Both are secreted by endocrine glands and travel in blood circulation, but the mechanisms by which they stimulate a response differ. Protein hormones include polypeptide chains and glycoproteins (polypeptides with attached carbohydrate moieties). Growth hormone, insulin, glucagon, and oxytocin are examples of protein hormones. Amines, amino acid derivatives such as epinephrine, melatonin, and thyroid hormones, also function as hormones. Lipid hormones include steroids such as estrogen and testosterone and fatty acid derivatives such as prostaglandins.

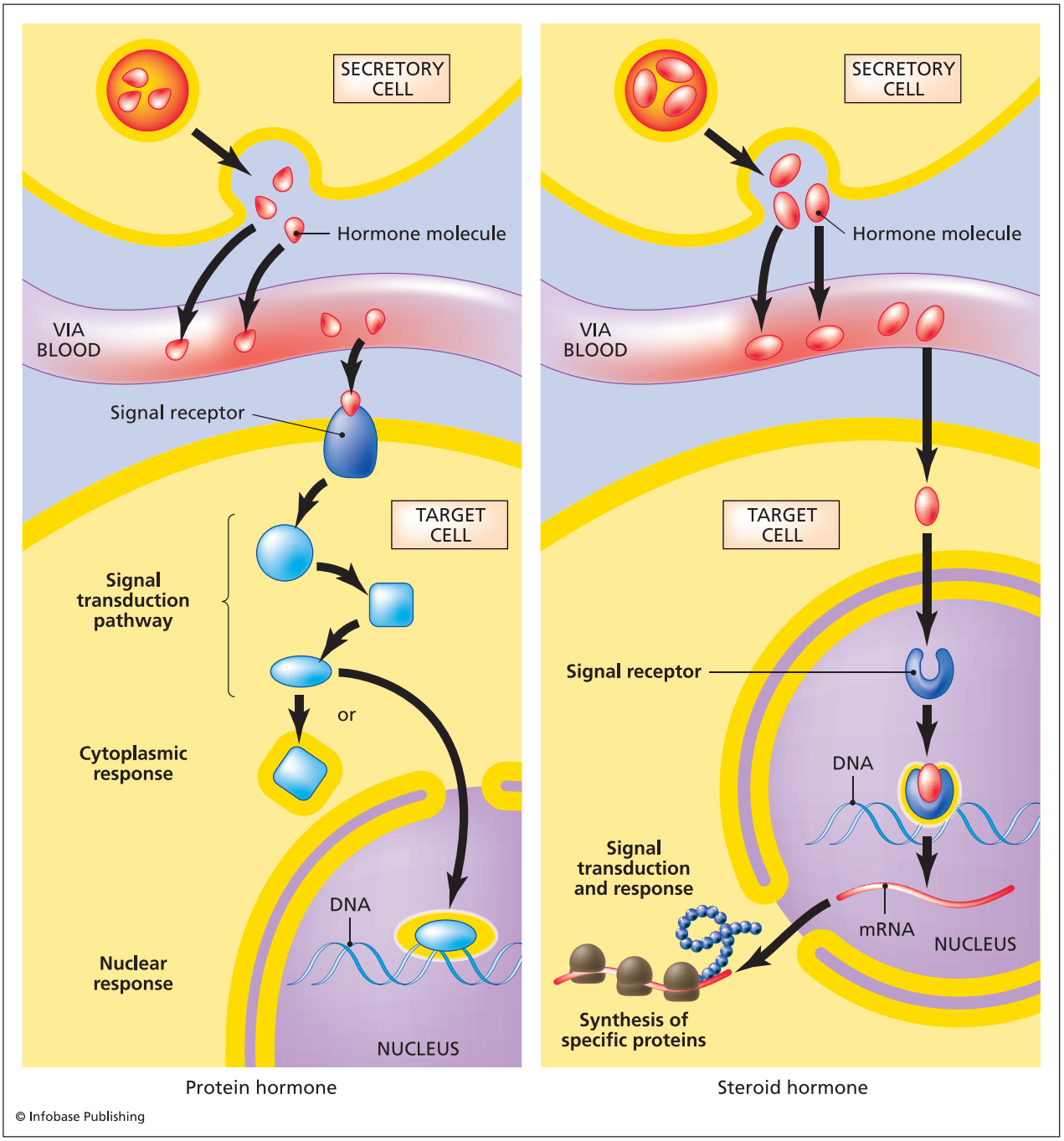
Protein hormones are hydrophilic (water-loving) and therefore are soluble in water. Ribosomes docked on the endoplasmic reticulum (ER) assemble polypeptide chains according to information in the gene that encodes the hormone. During synthesis, the polypeptide is extruded into the lumen (cavity) of the ER and then sent to the Golgi apparatus for processing. As for all secreted proteins, the Golgi packages the protein hormones into vesicles for storage until the cell receives a signal triggering secretion. At that time, the vesicles merge with the cell membrane and release the contents into the extracellular fluids of the interstitium, the space between cells in a tissue. As are protein hormones, amines are stored intracellularly after synthesis until time for secretion.

From the interstitial spaces, protein hormones move into blood circulation by leaky capillary walls. Because they are water-soluble, unique transport methods are not necessary. The hormones enter the capillary beds of various tissues, and when they encounter cells that express receptors that specifically recognize the hormone, they bind to it. Protein hormones and amines cannot diffuse through the phospholipid bilayer of cell membranes. They bind to the receptors located on the exterior surface of the cell membrane and exert an effect without ever entering the target cell. Binding of a ligand to its receptor stimulates a specific physiological response depending on the hormone and the type of target cell. Most protein hormones function by activating second messenger systems that change the activity of proteins present in the cell. Other surface receptor–binding hormones alter the permeability of the cell membranes by either opening or closing specific membrane channels. Because the proteins or membrane channels are already present and waiting to be activated, the effects of protein hormone action occur within minutes.

Second messenger systems mediate signal transduction pathways, mechanisms that carry information from a cell's exterior into the cytoplasm, causing a cellular response. Signal transduction pathways involve a series of changes to intracellular proteins, resulting in a cascade of cellular events. The response varies, depending on the type of target cell activated by the protein hormone. Possible final effects include the induction of the expression of specific genes or the secretion of substances stored in vesicles.

Steroid hormones are lipids and therefore are not soluble in water. Cholesterol is the precursor for all the steroid hormones. Low-density lipoproteins (LDLs) carry cholesterol in circulation to the body cells, including the ones that synthesize steroid hormones, called steroidogenic cells. Specific enzymes catalyze the stepwise conversion of cholesterol into the various steroid hormones including progesterone, aldosterone, cortisol, dehydroepiandrosterone, testosterone, estrone, estradiol, and estriol. The steroidogenic cells do not store steroid hormones; instead the hormones simply diffuse through the cell membrane into the extracellular fluids. The body regulates the levels of circulating steroid hormones by controlling the rate of synthesis rather than controlling the secretion, as in protein hormones.

Because steroid hormones are poorly soluble in water, they circulate in the aqueous plasma bound to



Protein hormones act via surface receptors, whereas steroid hormones bind intracellular receptors.

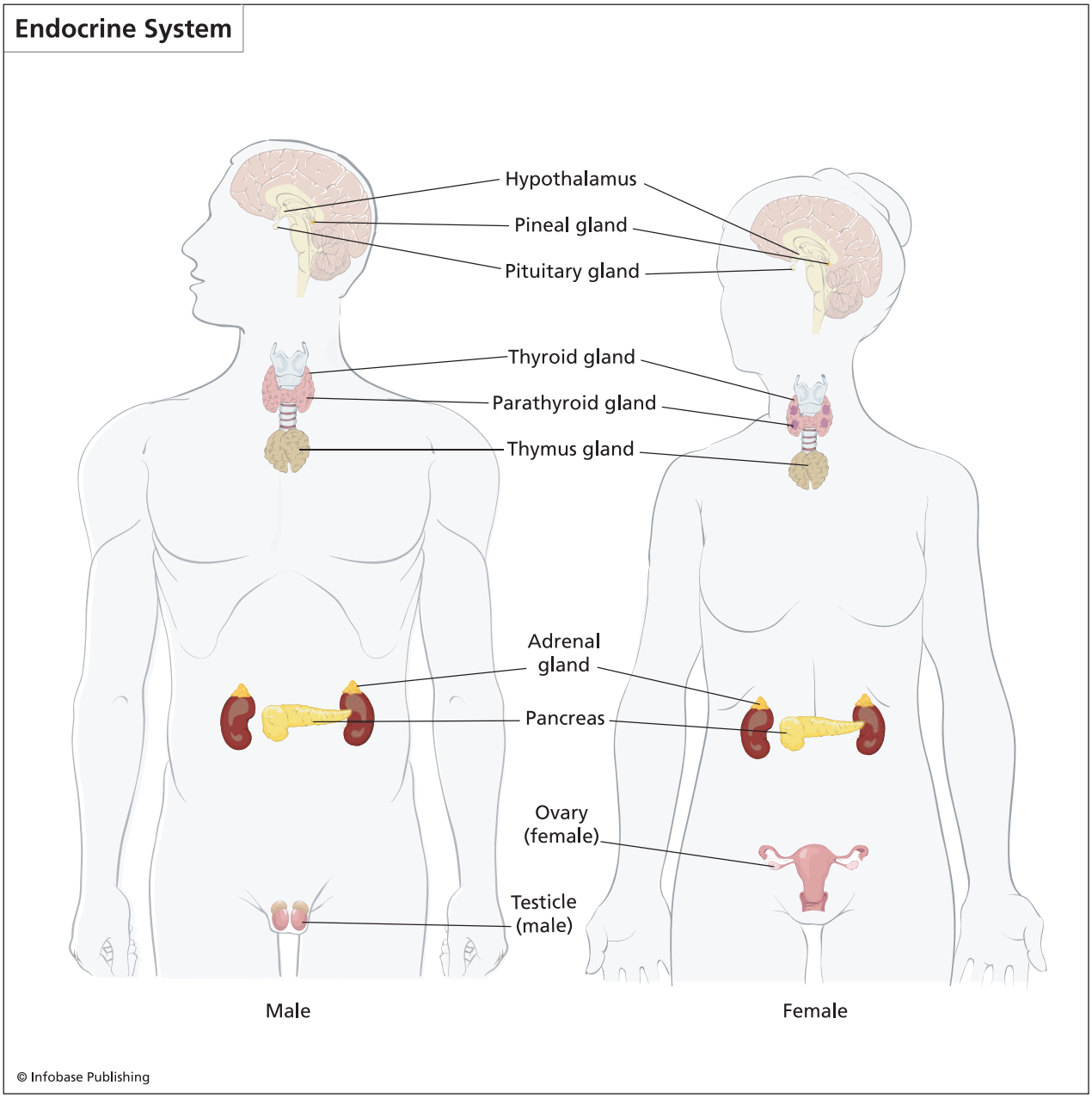
transport proteins. When bound, the hormone is not active, but the bound and unbound forms are in equilibrium, meaning a constant supply of unbound active hormone is readily available. In the unbound form, the steroid hormone can diffuse through capillary walls into tissue beds, and even into the individual cells directly through the cell membranes. This is in contrast to protein and amine hormones, which bind to receptors located on the outer surface of their target cells. Steroid hormone receptors exist inside target cells. If the cells possess the specific receptors that recognize a particular hormone, the receptor and hormone (ligand) bind to form an intracellular complex.

Steroid hormones act by stimulating the expression of certain genes. The protein products of the targeted genes bring about a cellular or physiological response dependent on the function of that protein. Because new protein synthesis is time-consuming, a delay of several hours precedes observable evidence of the steroid hormone's effect.

Steroid hormone receptors are usually in the nucleus but sometimes are found in the cytoplasm. The steroid hormone diffuses from the bloodstream, through the cell membrane, and into the nucleus, where it finds and binds its receptor, forming a complex. If the receptor is in the cytoplasm, the complex can diffuse into the nucleus from the cytoplasm. Most steroid receptors are transcription factors, regulatory proteins that bind to deoxyribonucleic acid (DNA) and stimulate gene expression. Only certain genes possess the specific DNA sequences, called hormone response elements (HREs), to which the complex binds and activates gene expression. Activation leads to the synthesis of the protein encoded by that gene. The same hormone can stimulate the expression of different genes in different cell types depending on the presence of other tissue-specific transcription factors.

VERTEBRATE ENDOCRINE SYSTEM

Endocrine glands are organs located throughout the body that produce and secrete hormones into the extracellular fluid surrounding cells. Hormone secretion is the main function of some glands, while other organs contain cells that synthesize and secrete hormones but also have other important functions. For



The endocrine glands are located throughout the human body.

example, the main function of the stomach is to churn food and begin the process of chemical digestion, but certain cells in the stomach also secrete gastrin, a protein hormone that helps control appetite. The testes produce the male gametes, spermatozoa; the Leydig cells inside the seminiferous tubules of the testes produce the steroid hormone testosterone.

Located in the ventral portion of the forebrain, the hypothalamus plays a major role in the coordination of many endocrine and nervous system activities. The pituitary is a lima bean–shaped gland that is located at the base of the hypothalamus and consists of two regions: the anterior and the posterior pituitary. The main functions of the hypothalamus include maintaining homeostasis, controlling secretion of hormones from the posterior pituitary, and releasing chemical factors that regulate the anterior pituitary. The brain relays information about both internal and external environmental conditions to the hypothalamus, and the hypothalamus reacts by giving chemical commands to the pituitary gland. Specialized neurosecretory cells of the hypothalamus synthesize two hormones, antidiuretic hormone (ADH) and oxytocin, which are stored in the posterior pituitary, an extension of the hypothalamus consisting of neural tissue, also called the neurohypophysis. The anterior pituitary, also called the adenohypophysis, consists of endocrine cells rather than neural tissue and functions in the synthesis of numerous hormones. Four of the anterior pituitary hormones are tropic hormones, meaning they stimulate activity in other endocrine glands: follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH). The anterior pituitary synthesizes and secretes the tropic hormones in response to hypothalamic secretions. The tropic hormones stimulate another endocrine gland to produce yet another hormone that initiates an appropriate physiological change in response to the information originally received by the hypothalamus. The table below, "Human Endocrine Glands and Their Hormones," summarizes the role of several important human endocrine glands.

HUMAN ENDOCRINE GLANDS AND THEIR HORMONES

Gland	Hormone	Main Action
hypothalamus	releasing hormones for growth hormone, thyroid hormone, adrenocorticotropic hormone, the gonadotropins (FSH and LH), and prolactin	stimulates the secretion of the named hormones
	inhibiting hormones for growth hormone and prolactin	inhibits the secretion of growth hormone and prolactin
anterior pituitary gland	adrenocorticotropic hormone (ACTH)	increases glucocorticoid secretion from adrenal cortex
	follicle-stimulating hormone (FSH)	stimulates follicle development in the ovaries of females, stimulates sperm cell production in the testes of males
	luteinizing hormone (LH)	stimulates ovulation and estrogen and progesterone production by the ovaries in females, supports sperm cell production in the testes of males
	prolactin	stimulates milk production in mammary glands of females, increases sensitivity of follicles to FSH and LH
	growth hormone (GH), also called somatotropin	stimulates tissue growth and the necessary metabolic processes for growth
	thyroid-stimulating hormone (TSH)	increases thyroid hormone secretion by the thyroid gland
	melanocyte-stimulating hormone (MSH)	stimulates the melanocytes in the skin to produce melanin pigments, making skin darker

(continues)

(continued)

Gland	Hormone	Main Action
posterior pituitary gland	antidiuretic hormone (ADH)*	promotes water retention in kidney tubules, decreases urine volume
	oxytocin*	increases uterine contractions during childbirth, stimulates milk release in mammary glands
thyroid	triiodothyronine (T_4) and thyroxine (T_3)	increases metabolism, necessary for normal growth and maturation
	calcitonin	inhibits bone breakdown, decreases blood calcium levels
parathyroid glands	parathyroid hormone (PTH)	increases bone breakdown by osteoclasts; stimulates bone breakdown; stimulates calcium reabsorption in kidneys and small intestine, leading to an increase in blood calcium levels; increases vitamin D synthesis
pancreas	glucagon	stimulates liver cells to break down glycogen stores and release glucose into blood
	insulin	stimulates uptake of glucose from blood
	somatostatin	inhibits insulin and glucagon secretion
adrenal glands (medulla)	epinephrine and norepinephrine	increases heart rate and blood pressure, increases blood flow to skeletal muscles and to heart, reduces blood flow to visceral organs and skin, increases blood glucose levels
adrenal glands (cortex)	glucocorticoids (cortisol)	stimulates protein and fat catabolism, increases glucose production, decreases inflammatory response, inhibits immune response
	mineralocorticoids (aldosterone)	increases sodium and potassium reabsorption, increases water reabsorption, increases hydrogen ion excretion
	androgens and estrogens	stimulates the development of some secondary sexual characteristics, but effect is mostly masked by ovarian and testicular steroid hormones
testes	testosterone	aids in sperm production, stimulates development and maintenance of male secondary sexual characteristics, maintains testicular function
	inhibin	inhibits FHS secretion from anterior pituitary gland
ovaries	estrogens	promotes the development and maintenance of female secondary sexual characteristics including the menstrual cycle
	progesterone	stimulates development of the uterine and mammary glands, regulates menstrual cycle, maintains pregnancy
	inhibin	inhibits FSH secretion from the anterior pituitary gland
	relaxin	loosens the connective tissue in the pelvis in preparation for childbirth
pineal gland	melatonin	inhibits gonadotropin-releasing hormone activity, possibly regulates sleep cycles

*These hormones are produced by neurosecretory cells of the hypothalamus but are stored in and released from axon terminals in the posterior pituitary.

See also ANATOMY; ANIMAL FORM; BIOLOGICAL MEMBRANES; BIOMOLECULES; CELL COMMUNICATION; CIRCULATORY SYSTEM; EUKARYOTIC CELLS; GENE EXPRESSION; HUMAN REPRODUCTION; INVERTEBRATES; NERVOUS SYSTEM; PHYSIOLOGY; VERTEBRATES.

FURTHER READING

The Facts On File Encyclopedia of Health and Medicine. Vol. 3. New York: Facts On File, 2006.

Larsen, P. Reed, Henry M. Kronenberg, Shlomo Melmed, and Kenneth S. Polonsky, *Williams Textbook of Endocrinology*. 10th ed. Philadelphia: W. B. Saunders, 2002.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 1. Detroit: U*X*L, 2000.

Norris, David. *Vertebrate Endocrinology*. 4th ed. San Diego: Academic Press, 2006.

Petit, William A., Jr., and Christine Adamec. *The Encyclopedia of Endocrine Diseases and Disorders*. New York: Facts On File, 2005.

Pfaff, Donald W., Ian Phillips, and Robert T. Rubin. *Principles of Hormone/Behavior Relations*. San Diego: Academic Press, 2004.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

environmental concerns, human-induced Human beings are part of the global biological community. As all other life-forms do on the planet, humans interact with their environment. Characteristics of the biosphere influence the way humans live, and conversely, humans affect the environment in which they live. The Earth supplies materials, water, food, and energy humans need not only for basic survival but also for the sake of convenience and entertainment. While the degree to which humans are responsible for leaving the planet in the same condition as they found it is a matter of ethics, the effect that human activities have on the Earth's ecosystems is a matter of science.

Change is natural. Life-forms evolve in response to specific environmental factors, but the environment also changes. At this moment geological processes are eroding mountain ranges and laying new crust on the ocean floor. The chemical composition of the atmosphere and the physical conditions on Earth's surface are vastly different from those when the planet formed 4.5 billion years ago. As an integral part of the planet's ecosystems, natural biological phenomena, such as metabolic processes that participate in the biogeochemical cycling of nutrients or photosynthesis that generates and releases oxygen into the atmosphere, contribute to the chemical and physical conditions of the biosphere. Many of the recent changes to the Earth's ecosystems, however, are the result of artificial human-induced processes. Whether through negligence, ignorance, or apathy, human activities have challenged the Earth's ecosystems beyond their ability to recover without a concerted cooperative effort from society.

A few serious current environmental concerns include the degradation of land, a diminishing clean water supply, depletion of nonrenewable energy sources, and increased pollution. Many environmental issues are interconnected; for example, in order to plant more crops farmers may clear forests, destroying habitats and leading to decreased biodiversity and removing vegetation that helps remove carbon dioxide, which is considered a form of air pollution, from the atmosphere. If the farmer uses artificial fertilizer, excess may run off and pollute the water supply. The severity of all human-induced environmental concerns is compounded by the continually expanding world population and the accompanying increased demand for food, space, and resources. The fields of ecology, environmental science, and conservation biology all aim to understand the causes and effects of the changes occurring in the biosphere so the knowledge can be applied to reduce the negative impact human activities are having on the biological health of the planet and to develop strategies for achieving a sustainable society.

LAND DEGRADATION

Land degradation is a decrease in a land's ability to absorb, store, and recycle water, energy, and nutrients. Natural phenomena such as hurricanes and earthquakes can destroy regions of land, but human activities can have a greater effect. The forest and grassland biomes provide society with food and with land for growing crops, grazing cattle and sheep, providing fiber for clothing and wood that is used for numerous purposes including construction, fuel, and papermaking. In addition to these and other material resources, forests and grasslands perform numerous ecosystem services, such as fixing carbon and producing oxygen via photosynthesis, recycling chemical elements, preventing soil erosion, regulating climate, and providing shade and shelter for thousands of species of animals, plants, and microorganisms. The biological health of these lands is deteriorating. The Millenium Ecosystem Assessment (coordinated by the United Nations Environment Programme) reports

(continues on page 298)

THE EFFECTS OF GLOBAL CHANGE ON TROPICAL FORESTS

by Kenneth J. Feeley, Ph.D.
Center for Tropical Forest Science, Harvard University Arnold Arboretum

The Earth's environment has changed a lot over the past several decades. Some of these changes are very clear and striking. For example, the concentration of carbon dioxide (CO_2) in the atmosphere has increased by more than 30 percent over the past 150 years and continues to increase steadily by almost 0.5 percent each year. However, most changes are more complicated. Human activities have altered temperature, rainfall, cloud cover, and many other environmental factors, but the rate and even the direction of change can vary widely from region to region. In other words, some parts of Earth are getting hotter while some are getting colder, some parts of Earth are becoming drier while others are becoming wetter, and still other parts of Earth do not seem to be affected much at all. Despite this confusing complexity, scientists now have a fairly detailed understanding of how environments are changing worldwide and which factors are likely driving these changes. They are even able to make some fairly accurate predictions about how the environment is likely to change in the near future, but much more remains unknown. Perhaps most importantly, little is known about the various effects that these changes to Earth's environment are going to have on its living creatures, including human beings.

The limits of ecological knowledge are perhaps best exemplified in tropical rain forests. Tropical rain forests are amazingly rich and complex ecosystems. Within a one-hectare patch of rain forest (approximately 2.5 acres, or a little less than twice the size of an American football field) one may find close to 1,000 different species of trees—more species of trees than in all of the United States and Canada combined. This bewildering diversity is what makes rain forests so beautiful and so much fun to visit, but it is also one of the primary reasons rain forests are so difficult to study and even more difficult to comprehend. Understanding the complex interactions among the multitudes of organisms inhabiting tropical forests and their rapidly changing environment is absolutely critical. Not only do tropical rain forests support most of the world's plant and animal species, but also they provide many important services to people everywhere. For example, tropical plants supply many types of food and provide much of the wood used in constructing houses and furniture. In addition, the forests play a vital role in regulating Earth's atmosphere and climate. Therefore, disturbances in tropical forests can have implications worldwide.

Ecologists recognize the importance of tropical forests and are actively working to determine how they are responding to changes in CO_2, temperature, rainfall, and other environmental factors. One hypothesis is that trees will benefit from several aspects of global change. Through photosynthesis, plants use CO_2 to synthesize carbohydrates that are then used in the production of wood, leaves, roots, fruits, and seeds. Therefore, as the concentration of atmospheric CO_2 increases, the rate of photosynthesis in plants may also potentially increase, allowing them to grow faster and larger. This would have important implications for the global environment since bigger, faster-growing trees will uptake and sequester lots of carbon from the atmosphere and potentially help to offset some of the carbon emitted from cars, air conditioners, and power plants.

The idea that plants will respond to higher concentrations of CO_2 with faster growth does not apply exclusively to tropical rain forests. In the United States, ecologists have conducted some impressive studies in which they experimentally increased the concentration of atmospheric CO_2 within patches of pine forest and then examined how fast the trees grew compared to control patches of forest where CO_2 was kept at natural ambient levels. In these studies, the pine trees and other plants responded to increased concentrations of CO_2 by growing faster and also by producing more seeds earlier in life. However, many of the effects were only temporary. The amount of nutrients in the soils could not sustain the accelerated growth, and after several years the trees returned to approximately their normal growth rates. Likewise, in a similar experiment conducted in Switzerland, researchers did not find any long-lasting effects of elevated CO_2 on the growth rates of mature deciduous trees.

Unfortunately, conducting these sorts of large-scale experiments in tropical rain forests is generally not feasible—not only because the forests are so incredibly diverse, but also because they are often too remote and the conditions too rough to allow scientists to set up and maintain all of the necessary equipment. Instead, tropical ecologists investigating the effects of global change have relied primarily on long-term records from tree inventory plots, patches of forest where researchers measure the diameter of every tree at periodic intervals (for example, every year or every five years). The investigators can then calculate tree growth rates by comparing the sizes of individual trees over time. Given enough measurements, they can then determine whether growth rates have changed over time and examine how these changes correspond to any concurrent changes in environmental conditions.

Using an extensive network of tree plots situated throughout the Amazonian rain forests of Brazil, Peru, and Ecuador, ecologists found that some very significant changes have occurred in these forests over the past several decades. Specifically, Amazonian trees appear to be growing significantly faster now than when the studies were initiated 20–30 years ago. Likewise, the total amount of wood, or aboveground biomass, in

these forests has increased dramatically over time. These findings are consistent with the predictions of the hypothesized increase in plant productivity due to elevated CO_2.

Studies from tropical rain forests in other parts of the world have produced conflicting results. In Costa Rica, researchers working with a few select species of trees found that contrary to the predictions of so-called carbon fertilization, growth rates actually decreased significantly through time. Likewise, in collaboration with scientists from the Center for Tropical Forest Science and the Smithsonian Tropical Research Institute, this author conducted an extensive set of studies looking at the growth rates of almost a million individual trees representing more than 1,000 different species growing in the rain forests of Panama and Malaysia. This study demonstrated that growth rates have decreased rapidly over the past two decades across the vast majority of species.

Why are trees in some parts of the world growing slower? Why is the widespread increase in CO_2 levels not stimulating tree growth in Central America and Southeast Asia as it is in the Amazon? One possible explanation for the unexpected slowdown is temperature. In Costa Rica, Panama, and Malaysia, the declines in tree growth rates were strongly associated with higher temperatures. Ask most gardeners and they will probably claim that plants benefit from higher temperatures. This is certainly true, but only to a point. In some parts of the Tropics, temperatures are already so high that further increases can cause net productivity to diminish and growth rates to decline. In the Amazon, temperatures have been relatively more stable over the past several decades, so for trees in these forests the benefits of additional carbon may still be outweighing the negative effects of elevated temperatures.

While elevated temperatures can explain the observed patterns, scientists must always be willing to consider alternative explanations and weigh them against the available evidence. For example, changes in cloud cover may also be responsible for the observed results. The number of cloudy days did increase in both Panama and Malaysia, and therefore the decrease in growth may be due to less sunlight. Or maybe the amount of rain has changed? Or perhaps trees in these forests are utilizing the extra carbon to produce more flowers and seeds rather than to grow bigger? The truth is that ecologists will never know the answer with 100 percent certainty. Ecology is a tremendously complex science and involves very few certainties, especially in the extraordinarily diverse Tropics. However, rather than allowing uncertainty to discourage further research, scientists should use it as motivation for their ongoing persistent efforts to gain a better understanding of the relationships within ecosystems.

Tropical ecologists continue to examine the response of rain forests to global change. Using new and improved measuring techniques, they are investigating the patterns of growth and productivity in different types of forests in distant parts of the world. They are looking at patterns of fruit and seed production, how the composition of species within forests is changing through time, and what is happening under the ground with the trees' roots and surrounding soils.

While much additional work is clearly necessary, scientists have already learned a great deal. For example, they now know that tropical forests are not all growing faster in response to increasing CO_2, meaning that society cannot count on them to buffer against ever-increasing carbon emissions. Scientists have learned, in fact, that some forests are actually growing slower, potentially in response to elevated temperatures.

Slower tree growth in tropical rain forests will have very important implications for both the global environment and the global economy. Tropical forests support the majority of terrestrial animal species, all of which depend either directly or indirectly on plant productivity as a source of energy. Consequently, decreased growth will reduce the amount of energy available, thus potentially reducing the number of animal species that these ecosystems can support and thereby diminishing global biodiversity. Tropical forests are also an extremely valuable source of timber. Slower growth may result in decreased standing stocks of timber available for logging. In addition, the rate of forest recovery following logging may decrease, thus lengthening the time that loggers must wait before reharvesting patches of forest. In order for loggers to maintain current yields, they will have to increase either the intensity of the logging or the area of forest that they log. More intensive and more widespread logging does not bode well for tropical forests since logging may negatively impact diversity either directly or indirectly through fragmentation, edge creation, and/or other synergistic effects such as the increased risk of accidental fires. Likewise, an overall decrease in productivity could necessitate an increase in the intensity or extent of extraction for nontimber products. In some cases (such as brazil nuts), harvests are already at or exceeding sustainable levels, and any increase in harvesting is likely to have negative impacts.

To make matters worse, the potential exists for slowing tropical tree growth rates to create a dangerous series of feedbacks. For example, reductions in tree growth may result in reduced rates of carbon uptake from the atmosphere, which, coupled with the extra emissions of CO_2 from the associated increases in logging and deforestation, could accelerate the increase of atmospheric CO_2 and global warming, causing even further reductions in tree growth, and so on and so on.

The world is changing. Most of these changes are extremely complex and can vary greatly from place to place. In some places, the changes may appear positive in the short term, for example, resulting in warmer winters and earlier-blooming flowers. But one must always remember that the living world comprises a delicate web of interactions and that disturbances even in places as seemingly remote as Southeast Asia, the Amazon, or the Congo

(continues)

(continued)

can have important effects for all living creatures, including people. Scientists may never fully understand all the implications of changes in Earth's environment, but they must continue striving to learn as much as possible while trying to minimize human-induced contributions to global change.

FURTHER READING

Clark, D. A. "Detecting Tropical Forests' Responses to Global Climatic and Atmospheric Change: Current Challenges and a Way Forward." *Biotropica* 39 (2007): 4–19.

Clark, D. A., S. C. Piper, C. D. Keeling, and D. B. Clark. "Tropical Rain Forest Tree Growth and Atmospheric Carbon Dynamics Linked to Interannual Temperature Variation During 1984–2000." *Proceedings of the National Academy of Sciences USA* 100 (2003): 5,852–5,857.

Feeley, K. J., S. J. Wright, M. N. Nur Supardi, A. R. Kassim, and S. J. Davies. "Decelerating Growth in Tropical Forest Trees." *Ecology Letters* 10 (2007): 461–469.

Körner, C., R. Asshoff, O. Bignucolo, S. Hattenschwiler, S. G. Keel, S. Pelaez-Riedl, S. Pepin, R. T. W. Siegwolf, and G. Zotz. "Carbon Flux and Growth in Mature Deciduous Forest Trees Exposed to Elevated CO_2." *Science* 309 (2005): 1,360–1,362.

Laurance, W. F., A. A. Oliveira, S. G. Laurance, R. Condit, H. E. M. Nascimento, A. C. Sanchez-Thorin, T. E. Lovejoy, A. Andrade, S. D'Angelo, J. E. Ribeiro, and C. W. Dick. "Pervasive Alteration of Tree Communities in Undisturbed Amazonian Forests." *Nature* 428 (2004): 171–175.

Lewis, S. L., O. L. Phillips, T. R. Baker, J. Lloyd, Y. Malhi, S. Almeida, N. Higuchi, W. F. Laurance, D. A. Neill, J. N. M. Silva, J. Terborgh, A. T. Lezama, R. V. Martinez, S. Brown, J. Chave, C. Kuebler, P. N. Vargas, and B. Vinceti. "Concerted Changes in Tropical Forest Structure and Dynamics: Evidence from 50 South American Long-Term Plots." *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 359 (2004): 421–436.

Oren, R., D. S. Ellsworth, K. H. Johnsen, N. Phillips, B. E. Ewers, C. Maier, K. V. R. Schafer, H. McCarthy, G. Hendrey, S. G. McNulty, and G. G. Katul. "Soil Fertility Limits Carbon Sequestration by Forest Ecosystems in a CO_2-Enriched Atmosphere." *Nature* 411 (2001): 469–472.

(continued from page 295)

that dry lands make up 41 percent of the planet's land surfaces, and up to 20 percent of that has become unusable as a result of desertification, the conversion of land into desert. The soil of degraded land is barren, and its conversion into a functional desert biome may take millions of years. Desertification can result from changes in climate, but the increased desertification initiated during the last few hundred years is due to improper land management practices—mainly overgrazing and poor soil practices. In the absence of vegetation that once prevented the soil from eroding, the wind and water wash it away, leaving behind barren, infertile land that cannot be used for growing crops or for growing grass to feed herds. The dust clouds and sandstorms affect neighboring areas as well and are a particular problem in China, Japan, South Korea, and Mongolia.

Archaeological evidence from the Fertile Crescent suggests that even thousands of years ago, people cut down forests to graze cattle on common grasslands. Though grasses can recover from grazing, when too many cattle graze on common grounds, they eat up the metabolic reserves in addition to the top half of the blades of grass, leaving behind insufficient photosynthetic tissue to support the root system. As a result the grasses die, and the previously rich grasslands and forests transform into barren desert.

The trend continues today; farmers have replaced lively ecosystems consisting of grasses, shrubs, and trees with crops of corn, soybeans, wheat, oats, and alfalfa. As of 2005 191 governments worldwide had joined the United Nations Convention to Combat Desertification (UNCCD) with the goal of combating desertification and protecting drylands. Affected countries are developing national, regional, and subregional programs to reverse land degradation. Countries such as those in Africa are the most affected, since two-thirds of the continent is desert or drylands, but the UNCCD reports that even in the United States, desertification affects more than 30 percent of the land, a problem exacerbated by recent severe droughts. In the United States, the Bureau of Land Management, part of the U.S. Department of the Interior, manages 258 million surface acres of public land and 700 million acres of subsurface mineral estate. The bureau tries to balance the use and conservation efforts on these lands by overseeing mineral mining operations, managing commercial forests and woodlands for timber, administering grazing permits, controlling recreational use, protecting wild animal herds, and overseeing other activities and uses.

Tropical rain forests contain an estimated 50 to 80 percent of the world's terrestrial species of plants and animals, though they only cover 6 percent of the land surface. Despite its ecological importance, this biome is rapidly shrinking. Human activities such as harvesting the wood for firewood, construction timber, paper, and plywood and clearing the land for agricultural purposes are degrading the Amazon, which contains more than half of the world's remaining rain forest. In 2006 Britaldo Soares-Filho at the Universidade Federal de Minas Gerais in Brazil and

others published the results of a computer simulation that predicted that 40 percent of the Amazon forests will be gone by 2050 if current practices continue (Soares-Filho et al., 2006). Degradation of a forest's canopy reduces precipitation and can lead to the eventual conversion of forest into grassland. Efforts to reduce deforestation are under way. Logging proponents suggest selective logging as one solution, but environmentalists believe that this would lead to a cycle of degradation and land clearing. In addition to logging, people are destroying tropical rain forests to use the land for growing crops. Because of the rapid nutrient recycling within the rain forest ecosystem, and because of the frequent rains, the soil is nutrient-poor. Only the upper superficial layer contains nutrients to support crop growth, so though farming on land that was previously rain forest terrain may initially be successful, the soil soon becomes depleted, and with the vegetation gone, the wind and rains erode the soil, making it unusable.

Another endeavor that essentially vandalizes functional land is oil prospecting. In addition to the Arctic National Wildlife Refuge (ANWR), the Alaskan tundra contains the largest portion (23 million acres) of unprotected wildlife in the nation, part of the National Petroleum Reserve-Alaska (NPRA). Biologists claim that the lush NPRA is critical to the ecological health of the area, which serves as home to caribou, geese, grizzly bears, and bowhead and beluga whales, but the richness in hydrocarbons make the land valuable to the oil industry. Drill pads and pipelines drive away the animals, which must look elsewhere for food. Not only is the disappearance of undisturbed lands an issue, but hundreds of oil spills occur there every year and the amount of pollutants pumped into the air exceeds that of major urban areas.

WATER RESOURCES

All life-forms require water. The availability of a clean water supply determines the distribution of different types of living organisms, migratory patterns of animals, and the locations of cities and human populations. Physical and geological factors drive water through the hydrological cycle, in which water evaporates from water bodies, soil, and plant leaves and rises into the atmosphere, where it affects weather patterns and forms clouds, which the winds move around until they deposit their moisture over the Earth in the form of precipitation. No matter

This aerial photo depicts the stark reality of deforestation by soybean farmers in Terra do Meio, Para, Brazil, in 2004. *(AP Images)*

where the precipitation falls, the water eventually returns to the oceans. The oceans comprise 97 percent of the Earth's water, and of the remaining 3 percent freshwater supply, most exists in the form of glaciers or is inaccessible below the Earth's surface. An estimated 0.003 percent is available for use by humans, and the Food and Agriculture Organization of the United Nations estimates that, globally, 70 percent of that is used for agricultural purposes (for crop and livestock production), 20 percent for industries, and 10 percent for domestic use. Though the quantity of freshwater that falls as rain should be sufficient to meet the world's needs, the distribution of water resources is unequal. *Water for Life Decade (2005–2015),* a booklet published by the United Nations Department of Public Information, states that Asia holds more than 60 percent of the world's population but only 36 percent of the world's river runoff. In contrast, South America has 26 percent of the runoff but only 6 percent of the world's population. Continued population growth in addition to economic growth will worsen the situation. The World Health Organization estimates that 1 billion of the 6 billion-plus population currently do not have access to freshwater, and the United Nations predicts that by 2025, 5 billion of the world's projected 7.9 billion people will not have access to clean water. Inefficient use of water, contamination, and destruction of wetlands, estuaries, and coastlines threaten the already limited supply.

Global warming also contributes to the problem, as many regions depend on snowmelts for freshwater, and the freshwater stored in glaciers melts into the sea. While one might think that warming snow and glaciers more quickly would relieve the problem of water shortage, many glaciers will recede or disappear to the point where certain areas that rely on them will have no source. Also, the shift in the timing of the melting to an earlier point in the spring creates problems later in the summer when the rivers run low.

The saltwater content of the oceans prohibits its use for agricultural or domestic uses. Technological advances have improved desalination processes, the removal of salts from seawater or brackish groundwater. Large desalination plants can process between 25 and 50 million gallons per day; in comparison, in the United States the per capita water withdrawals averaged 1,430 gallons (5,413 L) per day in 2000 (the most recent year for which the United States Geological Survey has published data on estimated water use). Desalination plants utilize a lot of energy, the uptake of seawater kills local marine life, and the resulting hypersaline wastewater presents another problem. Rerouting water supplies may relieve a shortage in one area but then cause a shortage elsewhere. Even if the area from which water is rerouted is not populated, rerouting the supply can cause ecological destabilization.

In addition to excessive water withdrawal, the introduction of chemical pollution and the construction of dams negatively impact the surface waters. Not only do lakes, ponds, rivers, and bays serve as sources of water, but the habitats they provide are crucial to organisms that play an important role in the food chain of many ecosystems and in the food supply for humans. Wetland ecosystems, both inland and along coastlines, are home to numerous species, some of which are endangered. They also help regulate stream flow, control flooding, collect sediment to prevent it from entering streams, and remove fertilizer chemicals from surface runoff before they enter river systems. Despite this, the area covered by wetlands globally has declined as they are being filled in for urban growth and farming. The worst losses have been in the state of California, Australia, and New Zealand, which have all lost approximately 90 percent of their wetlands. Estuaries, the zones where freshwater rivers flow into the sea, are also at risk. Pollution from sewage treatment plants and industries, oil spills, and erosion sediment chokes out the marine life, and excessive inland water withdrawal has reduced the flow to some estuaries, causing them to dry up completely.

NONRENEWABLE ENERGY RESOURCES

Society's dependence on nonrenewable fuels has led to a multitude of problems. Depletion of the resources is in itself an issue, since most of them require millions of years to produce them, but the manner in which they are harvested from the Earth, are transported, and are used has contributed to air pollution that has led to acid rain and an increase in greenhouse gases, chemical pollution of both lands and waters, and the destruction of habitats and ecosystems. People making decisions related to the use of energy resources must consider not only their renewability or nonrenewability but also their availability and their environmental impact. (Economics, politics, and ethics also play a major role.) Industrial nations fulfill about 90 percent of their energy demands using nonrenewable resources, and less developed countries about 59 percent. According to information posted in September 2007 by the Energy Information Administration, which compiles official energy statistics for the U.S. government, the United States is a major offender, making up less than 5 percent of the world's population, but using more than one-fifth of its primary energy. In 2005 the United States used 22 percent of the world's primary energy. Canada, which has about 0.6 percent of the world's population, uses about 3 percent of its energy. The

per capita use is double that of other developed countries including Japan and those in Western Europe.

Renewable forms of energy include hydroelectric, geothermal, solar, and wind. These natural resources are virtually unlimited; they are present in abundance. Society simply needs to commit to utilizing them. With respect to energy, biomass is defined as anything made by a living organism that can be used for energy, such as wood, cow manure, seaweed, crops, or other plant material. Though indiscriminate use of biomass can cause other environmental concerns, biomass is also considered a renewable energy resource. For example, wood can be regenerated by growing new trees, but destroying forests to obtain wood for burning to heat houses leads to other serious problems, such as land degradation and habitat destruction, that contribute to decreased biodiversity.

Nonrenewable energy resources include oil, coal, natural gas, and nuclear power. These resources cannot be regenerated once they are gone, or, if they can, the process would take hundreds of millions of years. Although natural biological and geological processes produced these resources, and although the same processes may still be occurring today, the processes occur at such a slow rate that these resources cannot be replenished for sustained use. *Fossil fuel* is the name given to any fuel created in the Earth from plant or animal remains. Oil, natural gas, and coal are all fossil fuels.

Crude oil, also called petroleum, is an oily liquid made of hydrocarbons. More than 300 million years ago, phytoplankton called diatoms that lived in the sea died, coated the seafloor, became buried, and were converted to oil by the intense pressure and heat. Gasoline and diesel fuel used mainly for transportation purposes are made from crude oil. Combustion of these products during use emits large quantities of pollutants including the greenhouse gas carbon dioxide and sulfur dioxide and nitrogen dioxide, both of which convert to acidic compounds once in the atmosphere. These pollutants lead to numerous other environmental problems, discussed later. Commonly occurring offshore leaks and accidental spills pollute the water, kill marine and coastal lifeforms, and destroy habitats. At the current rate of usage, the world's supply of crude oil will be depleted within 50 years.

Natural gas consists mostly of the flammable gas methane (CH_4), but also of other small hydrocarbons, and it is used to heat homes, heat water, and cook food. The advantage of natural gas is that is burns cleanly and contains very few contaminants, but if it leaks, methane is a powerful greenhouse gas. Gas companies pump natural gas from underground, often near petroleum sources. Though it has no odor and is invisible, gas companies often add a chemical that gives natural gas a bad odor so people know when a leak has occurred. Explosions sometimes occur during extraction or transportation through pipelines.

The solid, black, combustible, rocklike organic substance called coal is the most abundant fossil fuel, and therefore the cheapest. During the Carboniferous, the geological period that occurred between 354 and 290 million years ago, many large leafy plants covered the Earth, and algae filled the seas. After they died, their remains sank to the bottom of swamps and the ocean, where they became peat. Sediment buried the peat and transformed into heavy layers of rock. The pressure forced all the water out of the organic remains and, under conditions of high temperatures, converted them into coal over millions of years. Coal can be mined from the Earth's surface or from underground. In surface mining, bulldozers scrape off the terrain that lies over the coal seam, the horizontal layer containing the coal, so it can be extracted. This type of mining destroys the landscape. Exposed areas erode, and rain washes the sediment away, filling streams, with potential for flooding. Underground mining is hazardous; fires, explosions, and cave-ins are not uncommon and can harm or trap workers, and breathing the coal dust can damage the lungs. Environmentally, cave-ins can lead to cracking or sinking of the land surface, making land unusable for farming. Even when abandoned, coal mines can leak sulfuric acid, a by-product of bacterial metabolism of iron pyrite released from rocks. The sulfuric acid then leaches heavy metals from the rocks, and the acid mine drainage kills plants and animals and corrodes structures. Once extracted from the Earth, the majority of coal is burned to generate electricity or heat. The combustion of coal creates tons of hazardous solid waste in the form of fly ash and bottom ash and releases tons of particulate matter, sulfur oxides, nitrogen oxides, carbon monoxide, and carbon dioxide into the atmosphere, leading to acid rain and contributing to global warming.

To access fossil fuels, geologists must first locate them—not an easy task since they exist trapped underneath rock formations. After extraction, the fossil fuel must be transported by pipelines, over water bodies, or across land to processing plants for cleaning or refinement. The products are then distributed by pipelines, tankers, barges, trucks, or trains to storage depots located both aboveground and underground. Electric, gas, or petroleum industries either transmit the product to customers, use it to generate electricity, or sell it to other companies or to consumers. The numerous steps necessary for the production and use of energy resources themselves require energy, use land and other natural resources, generate pollution, and can cause damage to habitats.

Nuclear energy is another less common energy source. The main fuel for nuclear power plants is the naturally occurring radioactive isotope uranium 235. Radioactive isotopes are atoms that are unstable because they have an excess of neutrons in the atomic nucleus, causing them to emit radiation. Different forms of radiation differ in their ability to penetrate substances, but all of the types of radiation emitted from radioactive isotopes can damage biomolecules, such as deoxyribonucleic acid (DNA) and proteins. Because of this, exposure to radiation can kill cells or cause genetic mutations that can lead to cancer. Fission is the splitting of atoms, accompanied by the release of enormous amounts of energy. When uranium 235 atoms split, they release neutrons that bombard other uranium atoms, splitting them and causing a chain reaction. When these chain reactions occur under controlled conditions in a nuclear reactor, the energy released can be captured and used to perform work. The advantage of nuclear energy is that it does not produce air pollution, and since a small amount of uranium creates such large quantities of energy, less disruption to the environment is necessary for mining. Two major drawbacks are reactor safety and waste disposal. The sources must be contained by special mechanisms. Spills, accidents, and even slow leaks of radioactive material have serious long-term effects on all living organisms and on the surrounding environment. Sources of radioactivity lose their power naturally by spontaneous decay, but this can take hundreds or tens of thousands of years before the source decays to nonhazardous levels.

Researchers are actively seeking alternate energy sources and better methods for providing energy. For example, biofuels are derived from biomass, which contains stored energy. Some crops, such as corn or flaxseed, are grown specifically for conversion into biofuels such as ethanol or anaerobic digestion into biogases. Some automobiles in production today can run on ethanol. Such solutions are not simple, however, since growing crops to use as a substrate for fermentation in alcohol production requires irrigation, contributing to the water shortage crisis; uses land resources; and also requires processing (which also requires large quantities of water, possibly beyond that which water plants can possibly handle), transported, and distributed. Scientists must continue to investigate creative ways to meet society's future energy needs.

POLLUTION

Pollution is the biological, chemical, or physical alteration of the water, land, or air that is harmful to living organisms. Ocean waters and surface waters, such as lakes, rivers, streams, and underground aquifers, can be affected by pollution. Water pollution in different countries takes different forms. Common pollutants found in surface waters of developing countries include human and animal wastes, pathogenic microorganisms, pesticides, and sediment. Water pollution in industrial nations includes all of these and toxic metals (such as mercury), organic chemicals, pharmaceuticals, and acids. Human activities that contribute to water pollution are agriculture, mining, construction, drilling, waste disposal, dam construction, salting of roads and driveways to melt ice, and fertilizing of lawns. Even hot water is considered a pollutant. Thermal pollution most often results when an electric power plant dumps hot waters that have been used to cool liquids or machinery into lakes, rivers, or oceans. In addition to harming aquatic life that cannot tolerate the warmer water, the warmer temperatures lower the amount of dissolved oxygen. The effects of water pollution follow the path of the waters as they trickle through soil, filter through rock beds, and flow down streams and river systems, and into oceans.

When excess inorganic nutrients such as nitrates and phosphates or raw sewage pollute lakes or ponds, algae and aquatic plants overgrow. As they die and fall to the bottom of the water body, bacteria and other microorganisms decompose the remains. As they do so, they use up all the dissolved oxygen in the water, suffocating the other aquatic life in the lake or pond. The anaerobic bacteria thrive, and the by-products of their metabolic processes typically include methane and the foul-smelling gas hydrogen sulfide. This phenomenon is called eutrophication and can happen naturally, but human activities can accelerate this process.

Though contaminants from land activities pollute the ocean, the oceans also suffer from other types of pollution such as oil spills. Almost half of the more than 3.5 million tons (3.2 million metric tons) of oil that enter the oceans every year is from natural offshore deposits. More than half of the rest is from oil dumped into inland water systems, and oil spills or breakages in pipelines or wells account for the rest, though this source receives the most media attention. A significant portion (about 25 percent) of spilled oil, that containing the most toxic components, evaporates and becomes air pollution within a few months. Bacteria naturally metabolize approximately 60 percent of the oil, and much of the rest sinks to the ocean floor. The thick oil or the emulsions it forms when mixed with water physically smothers some marine life near the surface. Marine animals have contact with the surface, where the oil floats, are most affected: marine mammals, reptiles, birds, and animals that live along the shoreline. In estuaries, beaches, and coastlines, the oil kills marine invertebrates and birds that inhale and ingest it. The

oil that sinks to the bottom kills benthic life or accumulates in the tissues of filter feeders such as clams and mussels. Sometimes the harsh cleanup techniques cause at least as much environmental damage as the oil itself. The extent of the biological damage caused by an oil spill depends on the particular habitat, the physical and chemical conditions before the spill, and the season of year.

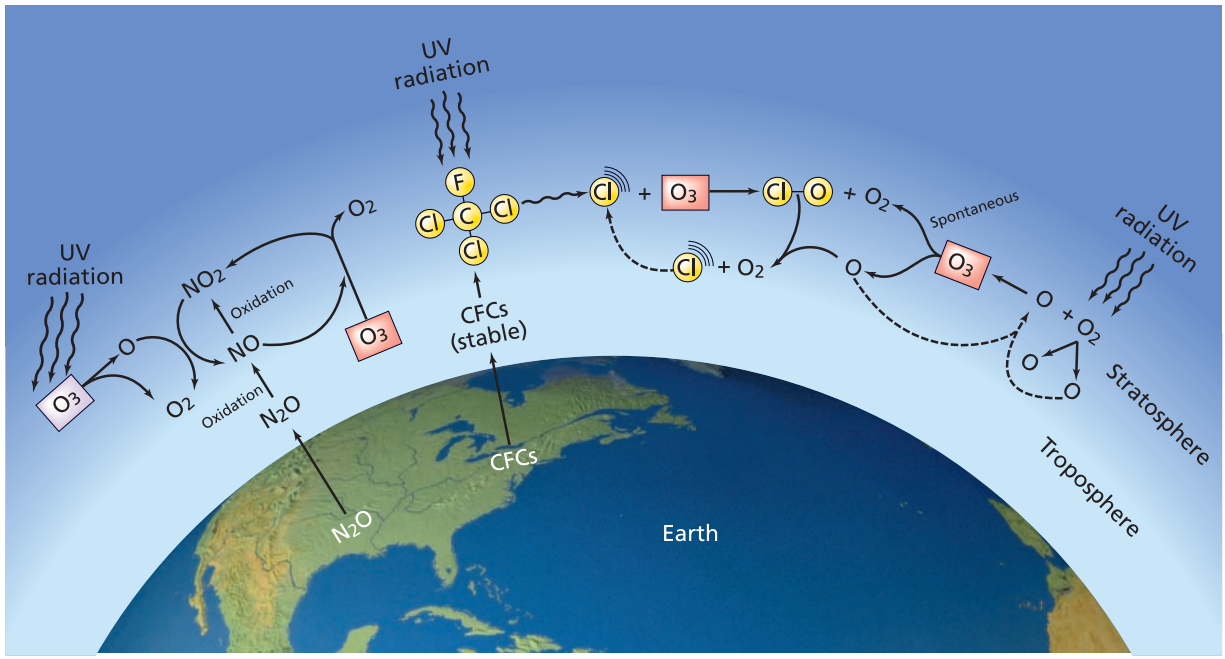
Hazardous wastes need to be disposed of in a manner that will not pollute the groundwater supply. This is often accomplished by storing the waste in underground steel tanks, but over time the tanks corrode and begin to leak into the surrounding earth. Radioactive waste generated by nuclear power plants and nuclear weapons facilities has a long life. Finding perpetual waste storage sites for hazardous and radioactive wastes is especially difficult because people do not want them close to their own cities or communities. Care must be taken to ensure the site is not located near places at risk of earthquakes or volcanoes. A solution that works for some wastes is detoxification, converting the toxic waste into a nontoxic form that allows for disposal in a landfill. Certain plants will uptake and store toxic products; this process is called phytoremediation. The plants, which are often associated with bacteria or fungi, act as biological sponges, absorbing chemicals or toxic elements from the soil or from water bodies.

Air consists of approximately 78 percent nitrogen, 21 percent oxygen, 0.04 percent carbon dioxide, and other trace components present in even smaller amounts. Many air pollutants have natural sources, such as sulfur oxides from volcanic activity, dust from wind storms, methane and hydrogen sulfide from decaying plants, or carbon monoxide, carbon dioxide, nitrogen oxide, and particulates from forest fires. Anthropogenic pollutants are those derived from human sources or activities. Though natural sources contribute more pollution to the environment than anthropogenic pollutants, the latter cause more significant, lasting damage. The U.S. Environmental Protection Agency identifies six major anthropogenic air pollutants as criteria air pollutants, those for which they have set national air quality standards: nitrogen dioxide, ozone, sulfur dioxide, particulate matter (PM), carbon monoxide, and lead. Except ozone and PM, all of these are directly emitted into the environment from three main sources: transportation, fuel combustion, and industrial processes. Ozone forms when nitrogen oxides react with volatile organic compounds (VOCs), and PM can be either directly emitted or formed during chemical reactions in the atmosphere. The effects of exposure to these pollutants on human health include headaches, dizziness, cardiovascular problems, respiratory illnesses, and eye irritation. Fewer studies on the effects of these pollutants on nonhuman species have been performed, but some are known to be hazardous to plants. Lichens, symbiotic associations of fungi and photosynthetic organisms, are especially sensitive to air pollution, and scientists use them as an indicator of the air quality in a particular area. Contaminants in the air also cause severe damage to metals, buildings, and other structures.

In addition to having local effects on the environment and on human health, air pollutants harm global health. Ozone gas naturally present in the stratosphere, part of the upper atmosphere located more than six miles (10 km) above Earth's surface, screens out much of the Sun's dangerous ultraviolet radiation. The ozone molecules absorb the ultraviolet radiation, temporarily split, and then spontaneously reform ozone. Life on Earth would not be possible without this protection from the ozone layer, and even diminished quantities of ozone cause eye cataracts and skin cancer. In the 1970s scientists discovered that some synthetic chemicals destroy the ozone layer and have been researching and monitoring ozone levels ever since. According to the *Scientific Assessment of Ozone Depletion: 2002,* the most recent assessment carried out by the United Nations Environmental Programme and the World Meteorological Organization, the global average for the period 1997–2001 averaged about 3 percent lower than pre-1980 values, with most thinning occurring in the midlatitude and polar regions. A so-called ozone hole, a large region with localized thinning, exists over the Antarctic and experiences up to a 70 percent decrease in ozone each October, though the levels generally restore themselves by natural processes during the Antarctic winter.

Chlorofluorocarbons (CFCs), once thought to be inert, are the main anthropogenic threat to the ozone layer. Spray cans contain CFCs such as Freon as a propellant, and refrigerators, freezers, and air conditioners use Freon to chill materials. Other CFCs are used in the production of Styrofoam or for cleaning electronic or other types of equipment. Scientists discovered that ultraviolet radiation in the stratosphere causes dissociation of the CFCs, forming a very reactive chlorine free radical. The chlorine free radical reacts with ozone to form chlorine oxide and molecular oxygen. Chlorine oxide also reacts with free oxygen atoms released when ultraviolet radiation breaks down ozone; this results in the formation of more chlorine free radicals and molecular oxygen. One single chlorine free radical can break down 100,000 molecules of ozone.

Jets that fly in the stratosphere also contribute to ozone depletion, though not to the same degree as CFCs. They release nitric oxide gas, which reacts



Certain chemical compounds released into the atmosphere cause the breakdown of ozone molecules.

with ozone to form nitrogen dioxide and molecular oxygen.

The depletion of ozone results in a diminished capacity for the stratosphere to block ultraviolet radiation from reaching the Earth's surface. In addition to the hazards this causes to human health, ecosystems could be destroyed as ultraviolet radiation can harm or kill algae, phytoplankton, and plants, the producers within biological communities. In 1987 many countries participated in the development of the United Nations Montreal Protocol, with the goal of addressing this global concern by controlling the levels of ozone-depleting chemicals manufactured and released into the environment. In 1992 nations around the world gathered again and formed the Copenhagen Amendments, with the aim of accelerating the phasing out of CFCs and other ozone-depleting chemicals. Today most CFCs have been replaced with hydrochlorofluorocarbons (HCFCs), which cause less damage, and researchers continue to look for other chemicals that can perform the same functions as CFCs without causing any harm to the ozone layer.

Some pollutants cause acids to be deposited in rain and snow or as dry, dustlike particles or as gases that settle on surfaces and turn into acids when combined with water. Even in the absence of pollution, rainwater is slightly acidic from atmospheric carbon dioxide, but atmospheric contaminants such as sulfur oxides and nitrogen oxides make precipitation even more acidic. While some natural events release these compounds into the atmosphere, the major anthropogenic source is the combustion of fossil fuels for transportation or electric power plants. Acid deposition causes acidification of lakes, which kills aquatic life not only through the decreased pH levels, but also through the increased amounts of metals dissolved from rocks and soils. Not only is aquatic life harmed; life-forms that feed off the aquatic organisms accumulate concentrated levels of the metals in their bodies. Acid rain also causes the destruction of forests and the communities they support by directly damaging the leaves, decreasing photosynthetic capabilities, dissolving nutrients in the soil so they wash away, and dissolving toxic substances that may damage structures involved in water transport.

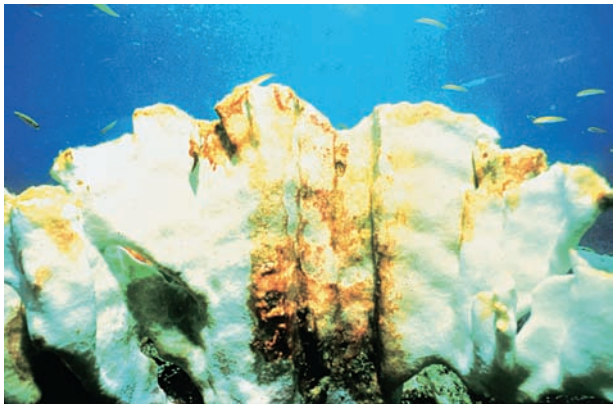
GLOBAL CLIMATE CHANGE

According to an article, "Global Temperature Change," authored by James Hansen and others and published in *Proceedings of the National Academy of Sciences* in September 2007, the average Earth surface temperature has increased 1.4°F (0.8°C) since the late 19th century, and 1.1°F (0.6°C) in just the past three decades. On the basis of computer modeling, the Intergovernmental Panel on Climate Change (IPCC) predicts the average global surface temperature will continue to rise 2.0°F–11.5°F (1.1°C–6.4°C) during the 21st century. This worldwide phenomenon

Emissions from manufacturing plants and oil refineries can lead to acid rain, which can have a devastating effect on trees and other life-forms. *(Simon Fraser/Photo Researchers, Inc.)*

is called global warming, and scientists attribute it to anthropogenic air pollution. Certain chemical compounds present in the atmosphere are referred to as greenhouse gases because they trap heat in the lower atmosphere. The land, water, air, and life on Earth absorb radiation from the Sun and radiate it back as heat. Instead of moving back into outer space, however, the greenhouse gases reflect the heat back toward the Earth, increasing the surface temperature. Without this natural greenhouse effect, the temperature of the Earth would be too low for many current life-forms to survive—0°F (-18°C) compared to its actual temperature of about 57°F (14°C). Naturally occurring greenhouse gases include water vapor, carbon dioxide, methane, and nitrous oxide. Human activities have increased the levels of these compounds, enhancing the greenhouse effect, in addition to adding anthropogenic compounds including CFCs, HCFCs, and others, compounding the problem. Burning fossil fuels has disrupted the normal carbon cycle by releasing carbon that was stored underneath the Earth's surface back into the atmosphere. Coal mining and combustion and the use of natural gas and chemical fertilizers have increased the levels of sulfur oxides, nitrous oxide, carbon dioxide, carbon monoxide, and methane released into the atmosphere. Though CFC levels are declining because of the Montreal Protocol and the Copenhagen Amendments, some will remain for at least the next 100 years. The increase in greenhouse gases has also affected rainfall patterns and the severity of storms.

Climate changes will have a tremendous ecological impact. Ecosystems function in a manner dependent on the presence and interactions of life-forms that have evolved through adaptations that are uniquely suited to particular conditions. The rapid changes in temperature, precipitation, and habitats could devastate ecosystems. In Earth's history, mass extinctions of 50–90 percent of species have accompanied global temperature changes of 5°C. Some effects are already apparent, such as the bleaching of coral reefs. Photosynthetic algae called zooxanthellae that live in symbiotic associations within the corals provide organic nutrition for the corals, and



One adverse effect of increased surface water temperatures is the destruction of zooxanthellae that inhabit coral tissues, which leads to the bleaching of coral reefs, as demonstrated by this photo taken in the Florida Keys. *(National Oceanic & Atmospheric Administration/Department of Commerce)*

the corals provide a protected habitat and a supply of carbon dioxide for the autotrophic zooxanthellae. The increased temperatures and violent storms can kill the zooxanthellae of coral reefs, and since these organisms confer the variety of bright colors on the corals, their death causes bleaching of the corals. If the loss of zooxanthellae persists, or if another population of zooxanthellae does not take over, the coral also will die. Coral bleaching can also result from other anthropogenic effects, such as overexploitation, increased sedimentation, chemical pollution in the water, and solar irradiation of shallower coral colonies.

Another significant concern related to the increased surface temperatures is the associated rise in sea levels due to melting glaciers and ice in the Antarctic. Over the past 50 years, the sea level has risen by four to six inches (10 to 12.5 cm), and the IPCC predicts that the sea level will rise by an additional 20 inches (50 cm) by the year 2100. This would not only affect coastal cities, but flood wetlands, draw in salt water that could destroy crops and ruin fertile soils, and lead to worse damage from storms and hurricanes.

SUSTAINABILITY

The best way to solve environmental problems is to prevent them. For all the concerns mentioned and for concerns not addressed here, education is the first step. People need to know the far-reaching impact of their actions. The collective action of many individuals can reverse dangerous trends, for example, reusing and recycling materials, making efficient decisions about energy use, using less water, and buying environmentally friendly products. Society needs to work together to find and implement sustainable solutions, ways to meet needs without harming the environment. Achieving this will require a change in attitude. Many systems society uses today—such as agriculture, energy production, transportation systems—worked well when the world population was small but now have environmental consequences that will affect the Earth for generations.

Society needs to use its resources more efficiently and lessen its dependence on fossil fuels. In the United States, recently ranked at the bottom of the list of industrialized nations for environmental performance (Esty et al. 2008), the EPA has enacted legislation regarding air and water pollution, the use and sale of toxic chemicals released into the environment, hazardous waste, oil spills, and more. Laws will stimulate positive change, but ethical and economic factors will affect attitudes and motivation, which will result in actions that protect the environment longer term.

See also BIODIVERSITY; CONSERVATION BIOLOGY; ECOLOGY; ENVIRONMENTAL SCIENCE.

FURTHER READING

Bourne, Joel K., Jr. "Fall of the Wild." *National Geographic* 209, no. 5 (May 2006): 42–77.

Energy Information Administration. U.S. Department of Energy. "International Total Primary Energy Consumption and Energy Intensity." Available online. URL: http://www.eia.doe.gov/emeu/international/energyconsumption.html. Accessed January 21, 2008.

Esty, Daniel C., Marc A. Levy, Christine H. Kim, Alex de Sherbinin, Tanja Srebotnjak, and Valentina Mara. *2008 Environmental Performance Index*. New Haven, Conn.: Yale Center for Environmental Law and Policy, 2008. Available online. URL: http://epi.yale.edu/Home. Accessed January 30, 2008.

Hansen, James, Makiko Sato, Reto Ruedy, Ken Lo, David W. Lea, and Martin Medina-Elizade. "Global Temperature Change." *Proceedings of the National Academy of Sciences* 103, no. 39 (September 26, 2007): 14,288–14,293.

Intergovernmental Panel on Climate Change. *IPCC Fourth Assessment Report. Climate Change 2007: Synthesis Report*. Released on November 17, 2007. Available online. URL: http://www.ipcc.ch/ipccreports/ar4-syr.htm. Accessed January 21, 2008.

National Oceanic and Atmospheric Administration. "Global Warming Frequently Asked Questions." Available online. URL: http://www.ncdc.noaa.gov/oa/climate/globalwarming.html. Updated March 29, 2007.

Soares-Filho, Britaldo Silveira, Daniel Curtis Nepstad, Lisa M. Curran, Gustavo Coutinho Cerqueira, Ricardo Alexandrino Garcia, Claudia Azevedo Ramos, Eliane Voll, Alice McDonald, Paul Lefebvre, and Peter Schlesinger. "Modelling Conservation in the Amazon Basin." *Nature* 440, no. 7083 (March 23, 2006): 520–523.

United Nations Department of Public Information. *Water for Life Decade (2005–2015)*. Available online. URL: http://www.un.org/waterforlifedecade. Accessed August 18, 2008.

U.S. Environmental Protection Agency. "The Science of Ozone Depletion." Available online. URL: http://www.epa.gov/ozone/science/index.html. Updated August 20, 2008.

Wallace, Scott. "Last of the Amazon." *National Geographic* 211, no. 1 (January 2007): 40–71.

Environmental Protection Agency The U.S. Environmental Protection Agency (EPA) is an independent regulatory federal agency whose mission is to protect human health and the environment. The president of the United States appoints an administrator to lead the agency, which employs more than 18,000 people at the headquarters in Washington, D.C.; 10 regional offices; and one dozen labs across the country. The EPA is involved in all aspects of environmental science research, education, and

MAJOR ENVIRONMENTAL LAWS

Law	Summary
Clean Air Act (CAA), 1970	regulates air emissions from area, stationary, and mobile sources
Clean Water Act (CWA), as amended 1977	regulates discharges of pollutants into U.S. waters
Emergency Planning and Community Right to Know Act (EPCRA), 1986	helps local communities protect public health, safety, and the environment from chemical hazards
Endangered Species Act, 1973	provides a program for the conservation of threatened and endangered plants and animals and the habitats in which they are found
Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), 1996	provides federal control of pesticide distribution, sale, and use
Freedom of Information Act (FOIA), 1966	allows any person to make requests for government information without requiring identification or explanation
National Environmental Policy Act, 1969	assures that all branches of the government consider the environment prior to undertaking any major federal action that significantly affects it
Occupational Safety and Health Act (OSHA), 1970	ensures that employers provide a place of employment free of recognized hazards to safety and health
Oil Pollution Act (OPA), 1990	strengthens EPA's ability to prevent and respond to catastrophic oil spills
Pollution Prevention Act (PPA), 1990	focuses industry, government, and public attention on reducing the amount of pollution through cost-effective changes in production, operation, and raw materials use
Resource Conservation and Recovery Act (RCRA), 1976	gives the EPA authority to control hazardous waste generation, transportation, treatment, storage, and disposal
Safe Drinking Water Act (SDWA), 1974	protects the quality of drinking water in the United States
Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or Superfund), 1980	provides a federal "Superfund" to clean up uncontrolled or abandoned hazardous waste sites as well as accidents, spills, and other emergency releases of pollutants and contaminants into the environment
Superfund Amendments and Reauthorization Act, 1986	reauthorizes CERCLA to continue cleanup activities around the country
Toxic Substances Control Act (TSCA), 1976	gives the EPA the ability to track the 75,000 industrial chemicals currently produced or imported into the United States, screen the chemicals, require reporting or testing of certain chemicals, and ban the manufacture and import of chemicals that pose an unreasonable risk

assessment. They develop and enforce regulations, provide financial support for state environmental programs, perform environmental research, sponsor voluntary partnerships and programs, and educate the public to inspire personal responsibility for the environment. Examples of topics that the EPA investigates include acid rain, hazardous waste, asbestos, mold in homes, ozone, radon gas, recycling, wetlands, and many more.

In response to the public demand for a healthier and cleaner environment, stimulated in part by Rachel Carson's writing of *Silent Spring* (1962), which educated people about the potential dangers of indiscriminate pesticide use, President Richard M. Nixon proposed and Congress approved the formation of the EPA in 1970. In order to regulate environmental issues more efficiently, the EPA consolidated several preexisting programs coordinated by the Department of the Interior; the Department of Health, Education, and Welfare; the Department of Agriculture; the Federal Radiation Council; and the President's Council on Environmental Quality.

At least 15 major environmental laws form the legal basis for many of the EPA's programs. These are summarized in the table on page 307.

See also CARSON, RACHEL; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED; ENVIRONMENTAL SCIENCE.

FURTHER READING

U.S. Environmental Protection Agency home page. Available online. URL: http://www.epa.gov. Updated January 18, 2008.

environmental science Environmental science is an interdisciplinary field concerned with the interaction of the natural components of the environment, including the living and nonliving matter and the forces that act on them. In particular, environmental science focuses on the interconnected issues of the human population, natural resources, and pollution. The long-term goal is to achieve sustainability—using and managing the Earth's resources efficiently without depleting or irreparably damaging them. Understanding the environment will help society to use its resources better, minimize or prevent any detrimental impact, and solve environmental problems. Current environmental problems include degradation of the land, overuse of natural resources such as water, air and water pollution, acid rain, depletion of ozone in the stratosphere, production of greenhouse gases in the atmosphere, generation of nuclear waste, and contamination of the seas by oil spills. Many governments have recognized these alarming negative environmental trends and taken steps toward reversing them by developing programs to monitor such trends and to encourage sustainable economic development and sustainable societies. Sustainable development focuses on meeting current needs without compromising the ability of future generations to meet theirs. Measures to improve sustainability include recycling, using renewable energy resources, restoring that which has been used or damaged, and managing population growth.

Almost every other natural science is related to environmental science in some way, and one can approach studies of the environment from any of these perspectives. From the perspective of life science, preserving the atmosphere, the chemistry, and the geology of the environment is necessary in order to sustain healthy living conditions for all of the planet's life-forms. Thus ecology, the study of the relationships and interactions between organisms and their environment, is an important subdiscipline of environmental science. Other subdisciplines include atmospheric science, environmental chemistry, and geoscience. Atmospheric science is the study of the atmosphere. Atmospheric scientists, also called meteorologists, study the physical and chemical properties of the atmosphere. Emissions from automobiles and from industries pollute the air and contribute to the greenhouse gases in the atmosphere, a growing environmental concern. Environmental chemistry is the study of chemicals and chemical reactions that occur in the environment, both naturally, as the way excess rainfall can leach positively charged ions such as calcium and magnesium from the soil, making it more acidic and affecting the biological availability of nutrients, and as a result of human activities, such as the disposal of hazardous wastes. Environmental science can also be studied from a geoscience perspective, as the natural geological processes such as weathering, erosion, climatic phenomena, earthquakes, and volcanic activities all affect landscape structures and the chemical and physical conditions of an ecosystem.

HUMANS AND THE ENVIRONMENT

People are simply part of nature, despite the fact that they exhibit higher intelligence, communicate using language, live in artificially constructed buildings, and depend on numerous technological advancements and inventions to live every day. Many people would have a tough time surviving isolated from the conveniences of modern society—without clothing, tools, electronics, housing, running water, or readily available food. One might think it would be near impossible to depend on nature so completely to provide sustenance and fulfill every other need. In reality, all of society already completely depends on nature. Everything a person sees, touches, makes, and uses is

derived from components the environment provides. Though a colorful house with vinyl siding, glass windows, and a concrete driveway might look artificial if placed in the middle of an undisturbed forest or desert, all the building materials, the tools and machinery used to clear the land and construct the house, and the energy that was supplied to perform the work of assembling it all originated in the earth. Not only do food, fibers for clothing, and wood for lumber originate in the environment, but people also depend on natural resources for everything from computer chips to vaccinations whether they recognize it or not. Beyond material needs, Earth's ecosystems provide many services that maintain the conditions necessary to support life on this planet. For example, ecosystems remove carbon from the atmosphere and incorporate it in organic molecules used as food, they produce oxygen necessary for respiration through photosynthesis, they decompose organic matter and return the chemical nutrients to the environment, and they move water through the hydrologic cycle. The inhibition of any of these functions alone would result in the extinction of most of the Earth's life-forms.

Because people cannot be separated from nature, their actions affect nature. When people take in and use natural resources, such as air, water, minerals, plants, fuels, and animals, to meet their needs, they return them to the environment in a different form or alter the environment in the process. This holds true even for natural processes such as breathing: the air people exhale contains more carbon dioxide and less oxygen than the air they inhale. Burning fossil fuels for transportation or for generating of electricity releases particulates, sulfur oxides, nitrogen oxides, carbon monoxide, and carbon dioxide into the environment. In order to grow sufficient quantities of food, farmers must plant increasing acreage of crops for which they clear and cultivate grasslands and forests. The soil becomes depleted of its nutrients, the addition of fertilizer becomes necessary, and the fertilizer runs off, leaches into the soil, and is carried away into the water supply. The ecosystems are destroyed, the biomes altered, and resources ruined.

The goal of environmental science is sustainable development—to management and use of resources in ways that preserve the natural environment. People cannot withdraw from the biological community, but knowledge gained from environmental science can be used to protect the physical environment while meeting people's needs. When environmental issues first emerged into the limelight in the 1960s and 1970s, the initial approach was to treat symptoms. For example, when the dangers of the pesticide DDT became apparent, the public demanded the prohibition of its use. While admirable, this approach will not solve environmental issues in the long term. Without working to develop alternative strategies for pest control, will replacement chemicals be just as dangerous? Will farmers simply use more land to grow more crops to make up for those destroyed by pests? Modern environmental science aims for longer-term solutions by taking a more comprehensive approach that focuses on the root causes of the problem. This is analogous to an individual's adopting a healthier lifestyle that involves nutritious food choices, regular exercise, and weight control rather than treating the symptoms of heart disease or diabetes, diseases associated with obesity. Instead of alleviating the symptoms, or even curing the disease after ill effects occur, the goal is to prevent the disease. For example, consider the effect of an increasing human population on many environmental issues. Decreasing the population growth rate would translate into a smaller population that would require less food and therefore less land to grow the food. Reduced quantities of nonrenewable energy resources would be needed to transport the food and for transportation in general.

In addition to stabilizing the population, other objectives that society must meet in order to achieve sustainability include planning the use of landscapes for purposes based on ecologically sound principles, such as using nutrient-rich soil for agricultural purposes; using resources wisely—for example, not wasting water by watering the lawn in the heat of the day when most of it will quickly evaporate; using renewable clean energy sources such as solar power or wind power; recycling goods and using recycled goods in manufacturing; restoring habitats and ecosystems that human activities have destroyed; and preventing any further damage to the environment. In order to meet these objectives, one must also explore the biological, cultural, psychological, and economic influences on society's current attitudes and behavior. Thus the current need for environmental scientists with a broad-based background is as great as for those trained for more specialized research.

CAREERS IN ENVIRONMENTAL SCIENCE

The training necessary to pursue a career in environmental science depends on the type of career one desires. Knowledge of how to utilize the scientific method is crucial for identifying problems, collecting information about a subject, objectively analyzing data, and designing strategies for preventing and solving environmental problems. A student should consider the aspect of environmental science that he or she finds most interesting—for example, the Earth's resources and the natural biogeochemical means for recycling them, the effect of human activities on biodiversity, the development of new

technologies that reduce waste, the mechanisms by which living systems break down toxic chemicals, or other aspects. A future environmental scientist must obtain a strong background in the key principles of environmental science: how organisms interact with biotic and abiotic factors in their environment, the functions of an ecosystem, the unique qualities of different biomes, human impact on the environment, and how living organisms respond to changes in environmental conditions. One might choose to specialize in environmental aspects of a specific subject, such as biology, chemistry, physics, or Earth science, or focus on a specific issue, such as surface water pollution, chemical pesticides, deforestation, biofuels, sustainable transportation systems, and so on. Postgraduate studies or additional training prepares a scientist to become a principal investigator at a research facility, to obtain an academic appointment with a research institution, or to attain a higher-level position with more responsibilities.

Environmental scientists work in a variety of jobs in a variety of atmospheres—at a desk in an office, in a laboratory, in a greenhouse, or outdoors in fields or streams. Governmental agencies might hire an environmental scientist to monitor a specific trend, such as the annual water consumption in a particular area, or to serve as chief naturalist at a national park. A water treatment facility might need an environmental scientist to find ways to eliminate sewage overflows and reduce discharges. An environmental scientist might work at an academic research institution and study the effect of decreasing wetlands on the population of different amphibian species and how that might affect the food web of that community. The agricultural industry employs environmental scientists to develop new crop strains that naturally resist pests in order to decrease the need for chemical pesticides that can adversely affect other species or add to the problem of chemical pollution. An environmental scientist working for a commercial industry might assay gaseous emissions and recommend methods for decreasing the levels released into the atmosphere. A conservation organization might hire an environmental scientist as an expert consultant to testify concerning the likelihood that chemicals released into the soil are harming a neighboring prairie that serves as a habitat for an endangered wildflower species in a trial against a local manufacturing plant.

Knowledge gained through studies in the environmental sciences is applied to enact legislation aimed at preserving the long-term well-being of the planet. Because some of the root causes of current environmental issues are relevant to politics, economics, religious beliefs, ethics, and personal attitudes, environmental scientists often work closely with social scientists to develop and implement sustainable solutions.

See also ECOLOGY; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED.

FURTHER READING

Chiras, Daniel D. *Environmental Science.* 7th ed. Sudbury, Mass.: Jones and Bartlett, 2006.

enzymes Biological catalysts called enzymes speed up the rate of chemical reactions that take place in living cells. Most enzymes are proteins, but some ribonucleic acids (RNAs) have catalytic activity. Enzymes are not consumed or altered during a chemical reaction, and they are specific for the substrates upon which they act. Chemical reactions will only proceed if they are energetically favorable: in other words, an overall decrease in free energy must accompany the reaction. Enzymes cannot force a reaction to proceed if it is energetically unfavorable, but they do lower the activation energy of a reaction, that is, the energy needed to raise a molecule to its transition state so as to undergo a particular reaction. Without a high energy barrier, the velocity of the reaction increases. One molecule of enzyme may catalyze thousands or tens of thousands of biochemical reactions per second.

Enzymes are often named for the type of biochemical reaction they catalyze, and their names often end in *-ase*. For example, oxidoreductases catalyze oxidation-reduction reactions, and transferases catalyze the transfer of certain groups of atoms (such as methyl groups) from one molecule to another. Hydrolases are enzymes that cleave water molecules, often in conjunction with the breakdown of another molecule. Isomerases facilitate the conversion of isomers between forms. Lyases cleave covalent linkages between carbon and another atom, and ligases assist in the formation of bonds between carbon and another atom.

ENZYME FUNCTION

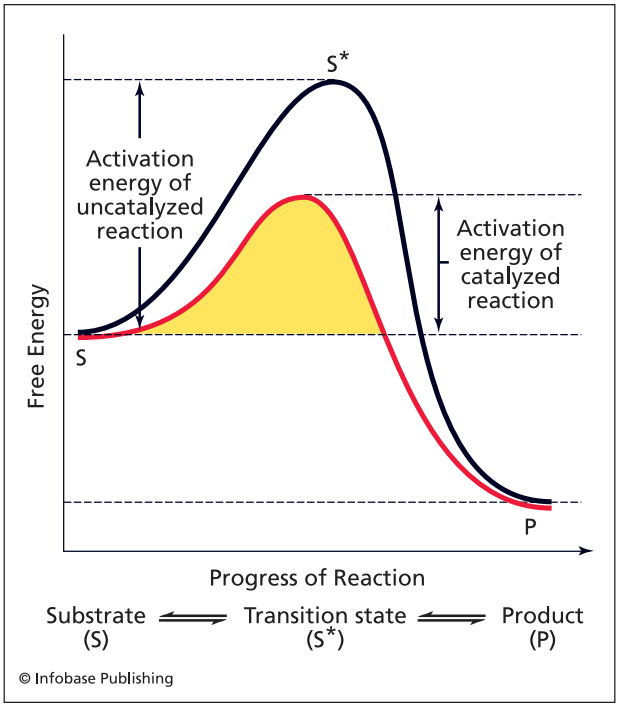
During a chemical reaction, a substrate is converted to a product. Chemists sometimes add a catalyst to speed up the reaction. Catalysts increase the reaction rate but are unchanged during the reaction, meaning after the reaction has occurred, the same amount is present as before the reaction took place. Because of this, only small amounts are necessary. Common catalysts include pure elements such as platinum, compounds such as manganese dioxide, and ions such as copper ions. Heat can also increase the velocity of a reaction. The conditions of living systems with respect to temperature, ionic strength, and concentration of trace elements are very particu-

lar, however, and thus common chemical catalysts are not appropriate, effective, or safe. Though all reactions, including biochemical reactions that occur within living organisms, must be spontaneous, they may proceed at rates so slow that no product would be synthesized during the organism's lifetime. Biological catalysts called enzymes facilitate the progress of biochemical reactions, effectively increasing the rate by 1,000 to 100 million times the rate without a catalyst present. The enzyme does not alter the equilibrium of the reaction, just the rate at which the reaction proceeds.

Enzymes work by lowering the activation energy of a biochemical reaction. The activation energy is the energy required to move a substrate (S) from its original form to a higher-energy transition state (S*). The transition form is unstable and soon converts to product (P), which has a lower energy state than both the original substrate and the transition state in a spontaneous reaction. The activation energy serves as a barrier that must be overcome in order for the reaction to proceed.

$$S \rightleftharpoons S^* \rightarrow P$$

Enzymes (E) work by first binding their substrates to form an enzyme-substrate (ES) complex, providing a surface upon which the reaction can take place; then the substrate converts to product. Formation of ES facilitates the transformation of substrate to its higher-energy transition state, so product forms more quickly.



Enzymes work by lowering the activation energy required for the substrate to achieve the transition state.

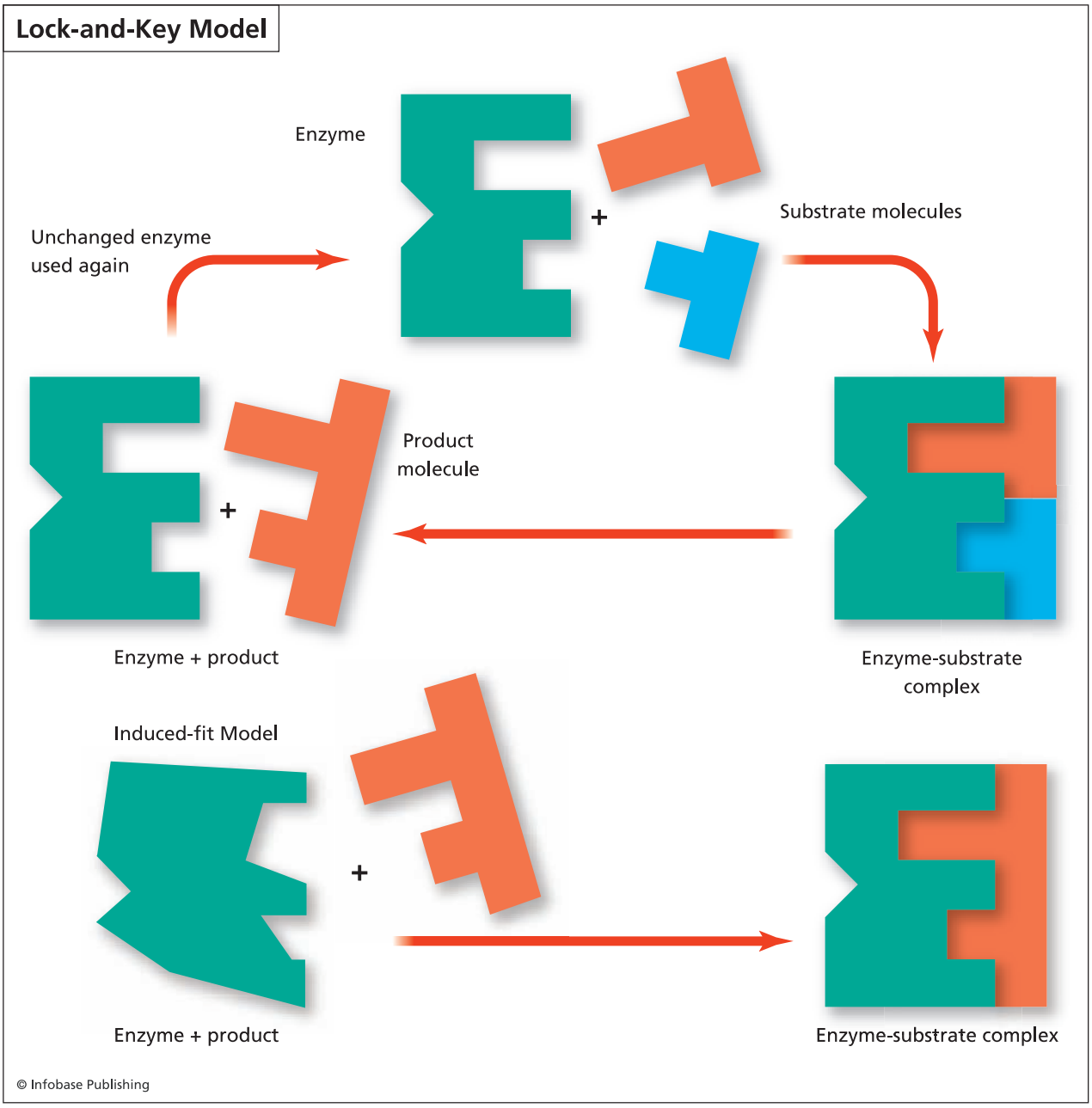
Enzymes are highly specific for the substrate(s) they bind. Substrates interact with one particular region of the enzyme called the active site. Multiple weak bonds such as hydrogen bonds, ionic bonds, and hydrophobic interactions confer specificity to the interaction between the active site of the enzyme and the substrate. The simplest model for the interaction of an enzyme with its substrate is the lock-and-key model, in which the lock represents the enzyme and the key represents the substrate. A more accurate representation is the induced fit model, in which the enzyme and the substrate change conformation slightly as they approach one another. In the more flexible induced fit model, mild distortion of the enzyme and substrate allows the substrate to begin its transformation to the transition state more quickly, further accelerating the progress of the reaction.

Depending on the type of chemical reaction, the formation of the enzyme-substrate complex either puts strain on covalent linkages that must break or holds two substrates in the proper position and orientation to facilitate the formation of the transition state. Once the unstable transition state forms, either the bonds break or new covalent linkages form, creating product.

Some enzymes require additional nonprotein components called cofactors. For example, the enzyme that hydrolyzes the last amino acid in a polypeptide chain, carboxypeptidase A, requires a zinc ion at the active site. When the cofactor is an organic molecule, it is referred to as a coenzyme and is recycled between enzymatic reactions. Many coenzymes are derivatives of vitamins; for example, nicotinamide adenine dinucleotide (NAD^+) is derived from niacin, tetrahydrofolate is derived from folate, thiamine pyrophosphate is derived from thiamine, and flavin adenine dinucleotide is derived from riboflavin.

ENZYME KINETICS

Enzyme kinetics refers to studies examining the rate (V) at which an enzyme catalyzes change from substrate to product. In 1913 the German biochemist Leonor Michaelis and the Canadian medical scientist Maud Menten proposed a model explaining the kinetic behavior of many enzymes. Most enzymes (E) follow the Michaelis-Menten model, in which the substrate concentration plotted against the initial reaction velocity assumes a hyperbolic curve. The reaction rate, or the number of substrate molecules converted to product per unit of time, increases as substrate concentration increases until the maximal



In the lock-and-key model for the interaction of enzyme with substrate, the active site of the unbound enzyme is complementary to the shape of the substrate, whereas in the induced fit model, the enzyme changes shape as the substrate binds.

velocity (V_{max}) is reached. Michaelis and Menten suggested that formation of the enzyme-substrate (ES) complex was reversible or that it could lead to product formation, as depicted by the following equation:

$$E + S \rightleftharpoons ES \rightarrow E + P$$

If k_1 is the rate constant for the formation of ES, k_{-1} is the rate constant for the reverse reaction, and k_2 is the rate constant for the breakdown of ES into product, then K_m is the Michaelis constant, given by

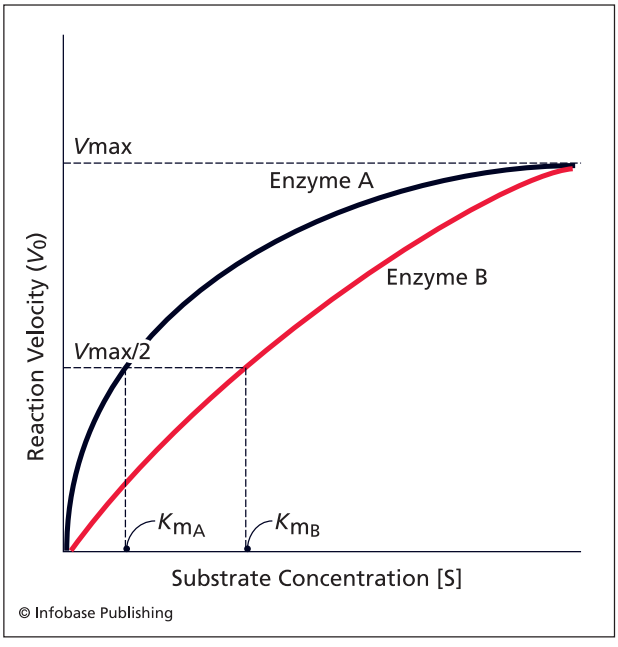
$$K_m = \frac{(k_{-1} + k_2)}{k_1}$$

One can determine K_m and V_{max} by measuring the rate of catalysis at different substrate concentrations ([S]). The reaction velocity (V) is represented by

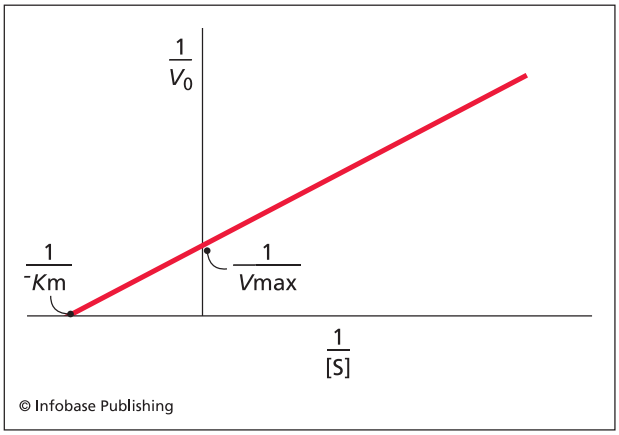
$$V = \frac{v_{max}[S]}{K_m + [S]}$$

when three assumptions are made: 1) the concentration of substrate is much greater than the concentration of enzyme ([S] > [E]); thus the reaction rate will directly depend on the enzyme concentration; 2) the concentration of the enzyme-substrate complex remains constant; and 3) V is measured immediately after the enzyme and substrate are combined so that the amount of product present is negligible.

K_m is an important aspect of the Michaelis-Menten model. As the substrate concentration at which half of the active sites are filled, K_m serves as a measure of the characteristic affinity of an enzyme for its substrate, the relative attractive force between the two. If K_m is large, then the enzyme has a low affinity for its substrate; if K_m is small, then the enzyme has a high affinity for its substrate. If the reaction velocity is plotted as a function of substrate concentration, then one can determine K_m by taking the substrate concentration that correlates with half of V_{max}. When V_{max} is difficult to determine because of a gradual slope of the curve at high substrate concentrations, a Lineweaver-Burke plot (named after its discoverers, Hans Lineweaver and Dean Burke) can help to determine K_m. A double reciprocal of the Michaelis-Menten equation forms the equation for a Lineweaver-Burke plot.



Enzymes that follow Michaelis-Menten kinetics exhibit a hyperbolic curve when the reaction velocity is plotted as a function of the substrate concentration. In this diagram, which compares two enzymes with a similar V_{max}, enzyme A has a higher affinity for its substrate, as reflected by its lower K_m, than enzyme B.



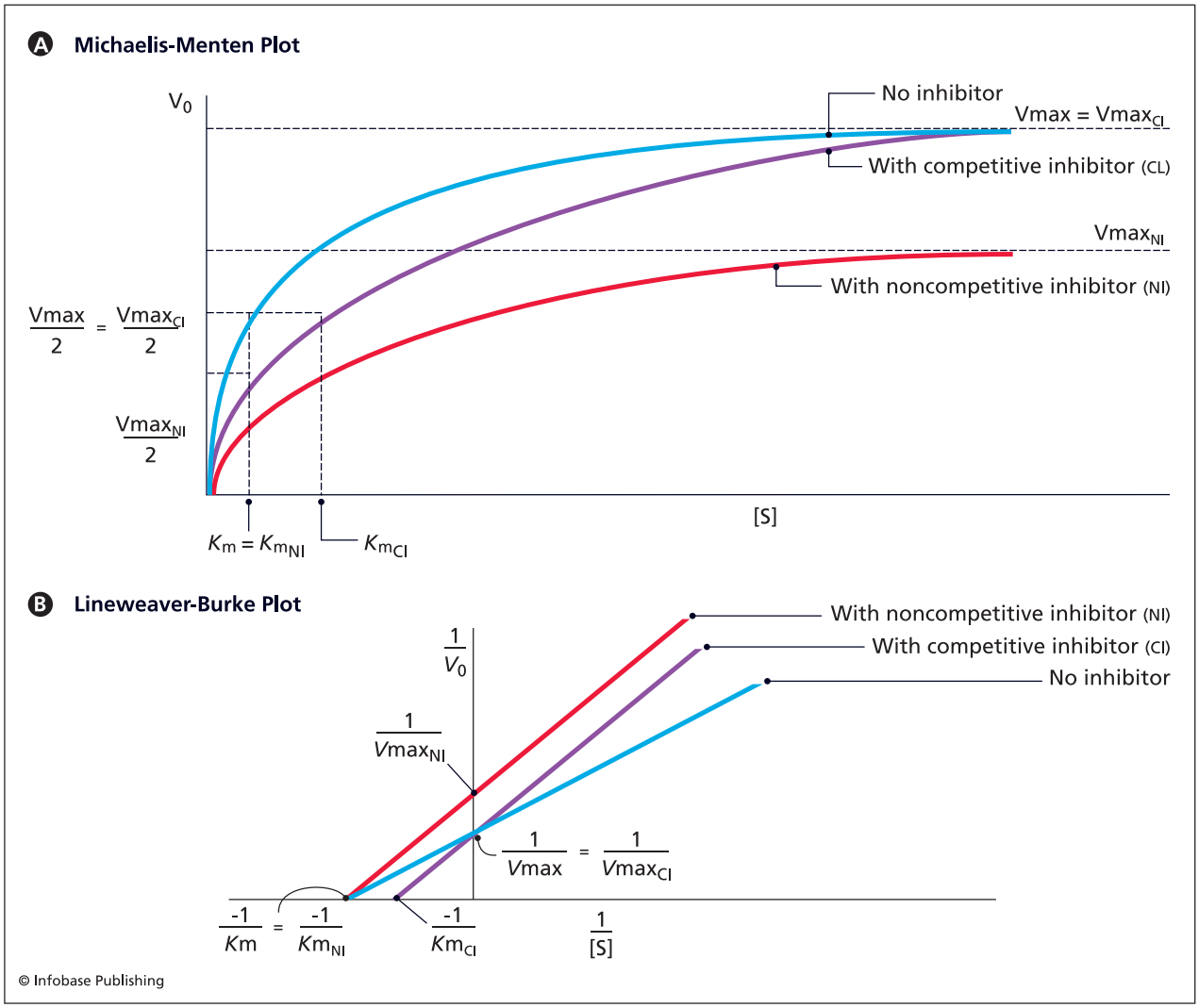
A double reciprocal plot, also called a Lineweaver-Burke diagram, is useful for determining kinetic parameters such as V_{max} and K_m.

$$\frac{1}{V} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

On a Lineweaver-Burke plot, the intercept of the x axis equals $-1/K_m$, and the intercept of the y axis equals $1/V_{max}$.

Allosteric enzymes do not follow Michaelis-Menten kinetics. When the reaction velocity is plotted as a function of the substrate concentration, the result is a sigmoidal (S-shaped) curve rather than a hyperbolic curve. In these cases, the enzyme has more than one active site. After substrate binds to one active site, the affinity of the enzyme for additional substrates increases. The first binding event causes a slight conformational change that facilitates binding at the other sites.

In addition to substrate concentration, other factors also affect the velocity of a reaction. Enzymes function most efficiently within a specific range of temperatures. As the temperature increases up to the optimal temperature, the velocity of the reaction will slowly increase. At temperatures higher than the optimal temperature, the velocity will drop off, first gradually, then more sharply as the enzyme becomes denatured. Enzymes also typically function within an optimal pH range. Ionic interactions often play a role at the active site, and, depending on the pH, ionizable side chains may exist in their protonated forms or deprotonated forms, which affect their ability to participate in ionic bonds. Deviation from the optimal pH is also important because in highly acidic or highly basic conditions, the enzyme itself might denature and therefore lose functionality.



As seen in the Michaelis-Menten and Lineweaver-Burke plots of inhibition, competitive inhibitors decrease K_m but not V_{max}, and noncompetitive inhibitors decrease V_{max} but not K_m.

REGULATION OF ENZYMES

Cells must be able to control the chemical reactions occurring within them in order to respond appropriately to changed conditions, such as different stages during development or in a life cycle, in the presence or absence of various nutritional sources, at different temperatures or altered water availability. In multicellular organisms, cells are organized into tissue types and organs that carry out unique functions, and the proteins each cell type expresses give the cell its specificity. Evolution has selected for cells to be energy efficient, and thus cells typically only make the proteins that they might actually use. Thus one method of controlling which chemical reactions a cell carries out is controlling the expression of an enzyme. This includes whether or not the enzyme is synthesized at all as well as how much is made. If more enzyme is present, more substrate will be converted into product.

Making a protein takes a lot of time, so when the cell needs to be able to start converting substrate to product without delay, a better method of control is to regulate the activity of the enzyme. In this case, the enzyme is already present in the cell in sufficient quantities, but something else needs to happen before it gains function. One common modification used by cells to regulate enzyme activity is phosphorylation. The addition or removal of a phosphoryl (PO_3) group to one or more amino acid side chains can either activate or deactivate an enzyme. Some enzymes are secreted as zymogens, inactive precursors that become functional after the cleavage of one or more peptide bonds.

Allosteric regulation is a type of regulation in which small molecules bind to the enzyme at a site other than the active site, causing the enzyme to change its conformation slightly and affecting its

activity. Inhibitors are molecules that reduce the activity of an enzyme after binding. Feedback inhibition is a common regulatory mechanism for stopping the synthesis of a biomolecule that is already present at sufficient levels. In feedback inhibition, a product of a metabolic pathway binds to and allosterically inhibits the activity of an enzyme involved in its own synthesis. In this manner, the molecule self-regulates its production. As the metabolic pathway proceeds, the product accumulates. When the concentration reaches sufficient levels, the product "feeds back" to bind an enzyme that participates in an early step of the metabolic pathway, halting its further production until the concentration of the product decreases to the point where quantities are not great enough to bind and inhibit the enzyme, lifting the inhibition.

Inhibitors can be classified as either competitive or noncompetitive. A competitive inhibitor is a molecule that binds at the enzyme's active site. The inhibitor and the substrate compete for binding to the active site; if one occupies the active site, then the other cannot bind there. If both are present, the relative affinities of the enzyme for the two molecules and the concentrations of each will determine which "wins" the competition. One hallmark of competitive inhibition is the ability to overcome it by the addition of more substrate. In noncompetitive inhibition, the inhibitor binds to a region of the enzyme other than the active site. Upon binding of the inhibitor at a regulatory site, the enzyme shifts its conformation, adopting a slightly different structure that interferes with its ability to function. Because the inhibitor and the substrate do not compete for the same site on the enzyme, increasing the substrate concentration cannot overcome noncompetitive inhibition.

A Lineweaver-Burke plot helps to distinguish between competitive and noncompetitive inhibition. In competitive inhibition, the y intercept ($1/V$ at a substrate concentration of 0) is the same whether the inhibitor is present or not; this is because V_{max} remains unchanged. The slope of the line (K_m/V_{max}), however, increases, because the K_m increases in the presence of a competitive inhibitor. Remember that K_m is the substrate concentration at which half of the maximal velocity is obtained. The increase in K_m reflects the fact that the inhibitor and the substrate compete for binding to the same site and that the addition of more substrate can overcome the inhibition to achieve the maximal velocity. V_{max} does not change; it just requires more substrate to attain it when inhibitor is present. A plot of data measured in the presence of a noncompetitive inhibitor will have a higher y intercept, reflecting a decrease in the value of V_{max}. The slope also increases, but this change is due to a decrease in V_{max}, rather than an increase in K_m, as in competitive inhibition. K_m remains unchanged because the enzyme's ability to bind the substrate does not change in this situation. No matter how much additional substrate is added, the inhibitor can still bind to the regulatory site and reduce the enzyme's activity; thus V_{max} is lower in the presence of noncompetitive inhibitor.

See also BIOCHEMICAL REACTIONS; BIOCHEMISTRY; BIOENERGETICS; BIOMOLECULES; CHEMICAL BASIS OF LIFE.

FURTHER READING

Berg, Jeremy M., John L. Tymoczko, and Lubert Stryer. *Biochemistry*. 6th ed. New York: W. H. Freeman, 2006.

Champe, Pamela C., Richard A. Harvey, and Denise R. Ferrier. *Lippincott's Illustrated Reviews: Biochemistry*. Philadelphia: Lippincott, Williams & Wilkins, 2005.

Copeland, Robert A. *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*. 2nd ed. New York: John Wiley & Sons, 2000.

Purich, Daniel L. *The Enzyme Reference: A Comprehensive Guidebook to Enzyme Nomenclature, Reactions, and Methods*. San Diego: Academic Press, 2002.

epilepsy Epilepsy is a chronic, noncontagious neurological condition characterized by recurrent seizures, disruptive electrical discharges in the brain. Seizures occur when nerve cells in the brain send out abnormal bursts of electrochemical impulses, causing a range of symptoms from barely noticeable moments of unresponsiveness to violent convulsions. Approximately 0.5–2 percent of all people will experience epilepsy during their lifetime, but it occurs most commonly in young children or the elderly. More than 2 million Americans have epilepsy, and up to 200,000 new cases are diagnosed in the United States each year. Medication successfully treats the majority of cases, allowing people who have epilepsy to live relatively normal lives.

In ancient Greece and Rome, many people believed that seizures were caused by the possession of evil spirits and that the spirits could move from one person to another by touch. Until the 15th century, religious leaders vainly performed spiritual or magical rituals to heal the affected. Society shunned people who had epilepsy and isolated them in institutions. During the 1800s, the English neurologist John Hughlings Jackson proposed that seizures resulted from abnormal discharges in the brain, and although society's acceptance of the illness improved, misunderstandings persisted. In the early 1900s, out of fear that the disease was contagious or was inherited,

people with epilepsy were prohibited from some public places or from marrying. A few very rare forms of epilepsy do have a genetic component, but epilepsy is never contagious; nor does it cause mental retardation or mental illness.

BIOLOGY OF A SEIZURE

The brain, one of the most complex and least understood organs of the human body, is responsible for movement, sensory perception, language, physiological processes, emotions, memory, and learning. As the control center of the nervous system the brain has the major function of receiving information from inside and outside the body, processing the input, and coordinating body movements and functions. Cells called neurons form networks that carry signals throughout the body. Specialized cells have the ability to sense environmental stimuli, such as a musical melody or the prick of a rose thorn, and to transform the input into a nervous impulse that a neuron transmits to the brain, which directs the appropriate response. For example, sound waves from a ringing telephone will cause the eardrum to vibrate, an action that disturbs fluid in the inner ear. The motion of the fluid jiggles tiny hairs, a mechanical motion that results in the formation of electrical impulses within auditory neurons (neurons involved in hearing). The impulses travel along the length of the neurons until they reach the portion of the brain involved in auditory responses. The brain processes the information and responds by sending out new impulses to motor neurons (neurons involved in muscle movement). The motor neurons carry the signals to the muscles needed to walk to the ringing phone and pick it up.

A seizure results when nerve cells in the brain fire a surge of uncontrolled electrical impulses. The highly coordinated, precise communications system among neurons becomes disordered commotion. The effect of the misfiring depends on the size and function of the affected area of the brain, and the episodes are characterized by unique feelings, sounds, smells, tastes, and sights. If the confused electrochemical activity occurs within a restricted portion of the brain responsible for sight, then the person might experience temporary blurred vision. If the seizure involves a large portion of the brain, the person might lose consciousness and suffer violent convulsions. Other epileptics (people who have epilepsy) report feeling a tingling sensation or appear to stare into space for a brief period, conscious but unaware of their surroundings. Seizure duration usually varies from a few seconds to several minutes. Though an isolated seizure does not damage the brain, seizures lasting longer than five minutes or immediate repeated seizures can be dangerous.

Neurologists categorize seizures into two broad categories, partial and generalized seizures, based on the degree to which the brain is affected. A person remains conscious during a partial seizure, the more common type, which affects only part of the brain (the focus) and can be simple or complex. The symptoms of a partial seizure depend on the specific part of the brain that is affected: auditory, visual, voluntary muscular movement, and so on. During a complex partial seizure, the person remains conscious but loses the ability to interact with other people. He or she might be aware of the surroundings but is unable to respond to them. The person might mumble, twitch, stare, wander around, perform repetitive or other seemingly deliberate movements, exhibit other unusual behavior, or simply seem confused, but these actions are beyond his or her control. These seizures are the most common type in teenagers and adults, and they last only a minute or two. Some epileptics have only one type of seizure; others have more than one type.

Generalized seizures affect the entire brain and include absence, tonic clonic, atonic, and myoclonic seizures. Absence seizures, also called petit mal seizures, are most common in children and cause someone to have a blank expression and be unresponsive for a few seconds. People may mistakenly assume the child is just not paying attention. Many children outgrow these seizures before reaching adulthood. The most dramatic type of seizure, tonic clonic seizures, also called grand mal seizures, are characterized by a brief initial tonic phase when the person's body extends and stiffens, followed by a clonic phase during which the person's muscles repeatedly contract for a period of one or two minutes. The person may also cry out or foam at the mouth. Atonic seizures cause a person's muscles suddenly to relax, causing the head to droop and the body to drop to the ground if unsupported. Myoclonic seizures are characterized by jerking muscles. Someone might be sitting at the table reading the paper when suddenly his or her arms flail up. They last only a second or two and frequently occur upon waking.

CAUSES OF EPILEPSY

Though epilepsy is more common in people younger than 20 years old or older than 65 years old, people of any age, gender, or race can develop epilepsy, which encompasses a variety of conditions that result in recurrent, unprovoked seizures. More than 70 percent of all epilepsy cases are idiopathic, meaning no obvious structural or functional abnormality can be found. Several known factors that provoke seizures include head injury, tumors, strokes, infections such as encephalitis or meningitis, high fever, toxic chemicals, drug overdose, low blood sugar level, and

genetic factors. Approximately 4 percent of children experience febrile seizures, induced by high fevers, but they usually outgrow them. A few women have seizures related to hormone level fluctuations during their menstrual cycles. Seizure frequency tends to increase during pregnancy of women who have epilepsy, probably the result of a reduction in dosage or stoppage of their medication or their body's absorbing or metabolizing the drug at a different rate.

When a person has a seizure, that does not mean he or she has epilepsy. Epilepsy is a condition in which a person has a tendency for recurrent, unprovoked seizures. In the presence of a medical explanation, such as a traumatic head injury sustained during a high-speed automobile accident, the epilepsy is said to be symptomatic. The reason for the brain's susceptibility to seizures is explained. Oxygen deprivation, such as might be sustained by a baby during a difficult childbirth, can disturb the intricate electrical system in the brain, as can developmental defects, neurodegenerative diseases such as Alzheimer's disease, and strokes.

DIAGNOSIS AND TREATMENT

Most people who have seizures are otherwise healthy, and because seizures occur at random, the physician usually cannot get observe them, making diagnosis difficult. A doctor will consider the events and symptoms preceding, during, and following a seizure as well as the patient's medical history to determine whether that person has epilepsy. The brains of patients with epilepsy often exhibit distinctive activity patterns, as shown by an electroencephalograph (EEG), a machine capable of detecting, amplifying, and recording brain electrical patterns (brain waves). The test is painless and only lasts about 30 minutes. During the procedure, electrodes connected to the EEG are pasted to the patient's forehead, temples, and scalp. Sometimes the technician will ask the patient to perform a task to stimulate brain activity, such as rapid breathing, or the technician may provide a stimulus, such as flashing lights, which trigger seizures in some patients. The tracings from an EEG showing normal brain activity are smooth and the peaks are rounded, whereas sharper peaks indicate erratic electrical activity typical of a seizure. The pattern of spikes and waves in the resulting electroencephalogram helps the physician determine the type of seizure and the area of the brain that is affected. Multiple EEGs might be necessary if the patient's brain exhibits normal brain waves between seizures. Because fatigue increases the likelihood of seizures, sometimes the doctor will request the patient stay up all night before the EEG to increase the chance of being able to record the erratic brain activity.

If the EEG shows abnormal activity, further testing may be performed to gain information about the

During an electroencephalogram, electrodes placed on the patient's scalp detect electrical activity in the brain. A normal diagram is shown in the screen. *(AJPhoto/Photo Researchers, Inc.)*

anatomy of the brain and to determine the appropriate treatment. Computed tomography (CT), magnetic resonance imaging (MRI), and positron emission topography (PET) are imaging methods that allow a physician to search for possible injuries, tumors, pooled blood, or scars that could be affecting electrochemical activity in the brain and that might be indicative of other dangerous conditions. The quickest method, a CT scan, uses X-rays to produce three-dimensional images of the body's internal structures, such as the brain. MRIs use magnets and radio waves to achieve a more detailed, multicolored, digital image on a computer screen. A more recent advancement in medical technology, the PET scan, allows for observation of metabolic activity in the brain in real time. The prior administration of radioactively labeled glucose, a type of sugar molecule that the brain breaks down for energy, reveals which areas of the brain are utilizing the glucose and are therefore active during monitoring. Areas that are damaged will not utilize the sugar effectively and will appear a different color on the PET computer screen.

Each individual case of epilepsy is unique, and patients and doctors implement a variety of treatment strategies. The goal of any treatment plan is to help the patient reduce the number of seizures while limiting any negative side effects. Although there is no cure, medications successfully control seizures in the majority of epilepsy cases. Having regular sleep, reducing stress, avoiding alcohol, and eating special diets can also reduce the quantity of seizures. Surgery or implantation of an electrical device is helpful in other cases. One rare seizure disorder results from a deficiency of vitamin B_6, and taking vitamin supplements successfully treats this form of epilepsy.

The U.S. Food and Drug Administration (FDA) has approved about two dozen different medications for treating epilepsy, most of which are taken orally and work by providing some control over communication among neurons. Two common antiepileptic drugs (AEDs) are phenobarbital and phenytoin. Because many AEDs are sedatives, drugs that decrease the activity of the central nervous system, common side effects of AEDs include drowsiness, lack of energy, clumsiness, and difficulty concentrating. Sometimes a patient must try several drugs before finding the best one. Half of the patients who do not have any seizures for several years while on medication can stop taking their medication and remain seizure-free. Under a doctor's supervision, the patient takes decreasing dosages over time.

Approximately 20 percent of patients continue to have seizures while on medications. If their quality of life is significantly decreased, brain surgery is another possible form of treatment. The surgeon either removes brain tissue (resection) or, if the focus is in a region that would prevent it from being removed safely, the surgeon may functionally isolate it by cutting the nerve fibers that connect it to other parts of the brain (disconnection) in order to prevent seizures from spreading. The most common resection surgery, a temporal lobectomy, in which a surgeon removes the damaged portion of the brain where the seizures originate, eliminates partial seizures in more than 60 percent of patients and reduces the number significantly in even more. Two less common operations for epilepsy are a corpus callosotomy and a hemispherectomy. In a corpus callosotomy, performed to treat severe generalized seizures, the surgeon severs the corpus collosum, the bundle of nerve fibers that connect the two sides of the brain. This procedure does not stop seizures but restricts them to one side of the brain. In a hemispherectomy, one-half of the brain is removed. Because this procedure can lead to partial paralysis or loss of some motor function, this rare surgery is only performed for extreme cases in which the brain is already severely damaged. Young children recover best from a hemispherectomy; thus the surgery is rarely performed in children older than 13 years old. Patients usually are discharged from the hospital one week after brain surgery and can return to normal activities within a few months. Most still require AEDs after surgery.

A strict ketogenic diet that is high in fats (as in cream and vegetable oil) and low in carbohydrates helps some people control their seizures. This controversial diet is so named because it causes the body to produce ketones, products that result from utilizing fats rather than carbohydrates for energy. This type of diet is dangerous, must only be initiated under a doctor's supervision, and requires a lot of effort to maintain. Side effects include nausea and vomiting, and the patient must take nutritional supplements to prevent deficiencies.

Since FDA approval in 1997, the implantation of devices that periodically shock the vagus nerve, a nerve believed to be connected to the part of the brain where seizures originate, has decreased the incidence of seizures in a number of patients. A battery-operated pulse generator is placed inside the chest and connected by electrodes threaded underneath the skin to the vagus nerve, located in the neck. Timed periodic electrical impulses reduce the number of seizures by about 20–40 percent, but the mechanism is not understood.

In some cases, epileptics can predict an oncoming seizure. The warning, or aura, resembles a tickling of one's senses and can be in the form of a specific smell, sound, or tingling feeling. The aura is caused by a small partial seizure and is often followed by a larger seizure. Further stimulation of the senses may ward off the oncoming seizure. If the person has a vagus

nerve stimulator, holding a special magnet over the implanted device to stimulate it can sometimes halt the oncoming seizure or lessen its severity. Being able to predict a seizure is useful even if the seizure cannot be prevented. The person can get to a safe environment, set aside items that might cause harm, or notify someone for help.

LIVING WITH EPILEPSY

The impact of epilepsy on someone's daily life varies greatly among individuals. Some people with epilepsy require treatment for the rest of their lives, but others completely stop having seizures over time. The majority of patients diagnosed during childhood outgrow their seizures naturally before reaching adulthood. A large number of people who have epilepsy can still attend school, have fulfilling careers, play musical instruments, participate in sports, have families, and drive cars (if they have been seizure-free for a period, from three to 12 months, defined by each state's department of motor vehicles), though changes to one's lifestyle are often necessary. For safety reasons, if seizures become uncontrolled, people who have epilepsy should not work at jobs involving dangerous machinery, jobs that one performs at great heights, or jobs that require driving.

Because no one can predict when a seizure may occur, certain situations could be particularly dangerous for someone who has a seizure disorder. People who experience seizures that cause them to fall to the ground must wear helmets to prevent traumatic head injury. Activities that require complete constant attention, such as mountain climbing or scuba diving, can be fatal if an untimely seizure occurs. All 50 states have laws restricting people with uncontrolled seizures from driving, but most permit a patient with epilepsy to drive if he or she has been seizure-free for a set period—commonly six months, with many requiring three or 12 months depending on the state. Other countries, including Canada and the United Kingdom, permit driving only after a 12-month seizure-free interval. Some countries, such as Australia and Ireland, require two years with no seizures before licensing.

Certain adaptations might be necessary for a person who has seizures and lives alone. The goal is to prevent possible harm if a seizure occurs during certain activities. For example, carpeting hardwood floors will make falls less injurious, and using plastic instead of breakable dishes can prevent accidental cuts if the person is holding them when a seizure begins. Other safety precautions may include abstaining from drugs and alcohol, not swimming alone, wearing a helmet when swimming or playing contact sports, and informing associates and peers what to do or not to do in case of a seizure.

Fallacies prevalent before the disease was better understood have been disproved, but because behavioral symptoms often accompany the physical symptoms, some people wrongly assume that people who have epilepsy are mentally ill or dangerous. Educating others about the illness can help remove the stigma that results from misconceptions and that can be just as traumatic as the seizures themselves. Awareness programs strive to educate the public to generate support for research that could lead to improved methods for diagnosis and strategies for treating for epilepsy. Neuroscientists are constantly seeking more effective AEDs that cause fewer side effects. One possible technological advance includes a device that could be implanted into the brain of someone who has epilepsy to detect an oncoming seizure and send electrical impulses to the brain to avert the seizure. A better understanding of normal brain function and neuron interactions will help advance neurological research toward a cure for epilepsy.

See also NERVOUS SYSTEM; SENSATION.

FURTHER READING

American Epilepsy Society home page. Available online: URL: http://www.aesnet.org. Accessed January 14, 2008.

Epilepsy Foundation home page. Available online. URL: http://www.epilepsyfoundation.org. Accessed January 14, 2008.

National Institute of Neurological Disorders and Stroke. "NINDS Epilepsy Information Page." Available online. URL: http://www.ninds.nih.gov/disorders/epilepsy/epilepsy.htm. Updated October 6, 2008.

Wilner, Andrew N. *Epilepsy: 199 Answers: A Doctor Responds to His Patients' Questions*. New York: Demos, 2003.

ethology Ethology is the scientific study of animal behavior, particularly in natural conditions. The goal of this comparative branch of zoology is to understand how and why animals do what they do. Ethologists must have a solid grasp of anatomy, physiology, and evolution to carry out their research. Because many behaviors affect an animal's ability to survive to reproductive age and pass on its genes to the next generation, natural selection acts on behaviors. The fields of ethology, animal behavior, and behavioral ecology are all closely related. An ethologist generally focuses on innate behaviors, those that have strong genetic components, whereas the focus of someone traditionally referred to as an animal behaviorist resembles the modern field of comparative psychology, which focuses more on learned behaviors or cognitive abilities of animals. The field of behavioral ecology emerged from ethology and

concentrates on the role of the environment in shaping modifications to behavioral adaptations. Studies in ethology and behavioral ecology have advanced the field of comparative animal psychology.

Many human behaviors are cultural, meaning they are learned from previous generations; honoring certain religious beliefs and having respect for elders are examples of cultural behaviors. Ethology is mainly concerned with behaviors that have biological components, such as mechanisms animals use to seek and obtain food, communication with other members of the species, migration patterns, and choice of mates. Some behaviors are learned, such as forming a cognitive map of one's immediate surrounding environment, and others are innate, such as a spider's knowing how to build a web characteristic of its species despite having never seen one.

Research performed by the German zoologist Karl von Frisch, the Austrian naturalist Konrad Lorenz, and the Dutch-British biologist Nikolaas Tinbergen formed the basis of the field of modern ethology. In the early 20th century, animal behaviors were explained either as controlled by inexplicable extranatural instincts outside the scope of zoology or as learned; both explanations fell outside the scope of zoology. Frisch, Lorenz, and Tinbergen demonstrated that certain behavioral characteristics contributed to the survival of animals in the same way physiological and anatomical modifications did and therefore resulted from natural selection. While their research specifically addressed behaviors in bees, fish, and birds, the basic principles apply to all animals, including humans. Their efforts birthed the field of ethology and earned Frisch, Lorenz, and Tinbergen the 1973 Nobel Prize in physiology or medicine "for their discoveries concerning organization and elicitation of individual social behavior patterns."

Ethologists combine two different approaches to the study of animal behavior to achieve a more comprehensive understanding: they ask both proximate and ultimate questions. Proximate questions address the mechanisms of different behaviors—determining the trigger of a response, defining the genetic components, and describing the anatomical and physiological mechanisms for carrying out the behavior. In other words, how does it work? Ultimate questions address why animals exhibit the behavior or how it took its current form. What is the evolutionary significance and how did the behavior develop in a species over time? Tinbergen suggested ethology address four central issues, comprising both proximate and ultimate questions, in order to understand a behavior: function, causation, development, and evolutionary history. The first two are proximate questions and the latter two are ultimate questions. The function of a behavior refers to the way the behavior contributes to an animal's fitness (success in passing on one's genes to the next generation). Causation includes examination of the mechanisms: genetic components, the stimuli that elicit the response, the morphological features that enable the response, and the physiological mechanisms. Developmental issues encompass how the development and maturation of an animal affect the behavior, and which early experiences are required for the animal to exhibit the behavior. Understanding the evolutionary history involves a phylogenetic comparison (a comparison of similar behaviors in related species) in order to outline the origin and modification of the behavioral adaptation over time.

Classical ethology focused on topics such as communication among animals and imprinting. Animals communicate by a variety of mechanisms including visual, chemical, mechanical, and auditory. Von Frisch studied the complicated dance performed by worker bees to communicate the distance, direction, and quality of a feeding source. Other bees observing this dance can interpret its meaning to locate the food. When animals mark their territory by urinating, they leave behind a chemical signal that communicates the boundaries of their claimed territory to other members of their species. Male spiders, who are much smaller than their female counterparts, must approach a female in order to mate, but because of the male's size, the female often mistakes a potential mate for food. To prevent this, the male might vibrate or twitch the web in a particular pattern, a form of mechanical communication to let the female know that he is approaching and that he is a mate rather than a food source (though sometimes he serves as both—a phenomenon known as sexual cannibalism). Auditory communication is also really a form of mechanical communication because information is transmitted through vibrations in the air, detected by special sense organs in the recipient. An example of auditory communication is exhibited by chickadees, whose alarm call communicates information about the size of a potential predator and how rapidly it is moving by the pitch and length of vocalizations they emit. Imprinting occurs when, during the early development of a social animal, a specific event takes place and establishes a behavior pattern. The most famous example of imprinting is the recognition of the first moving object a duckling sees as its mother. In the natural world, the object usually is the mother, but ethologists have demonstrated imprinting on cardboard boxes and on human beings as well.

Other important topics that ethologists research include fixed action patterns, food and feeding behavior, defensive behaviors, movement and migration patterns, courtship and mating behaviors, and paren-

tal care. Modern ethology has branched into topics such as playful behavior, honesty and dishonesty in signaling systems, and emotions in animals. Juveniles, and adults to a lesser degree, both exhibit playful behavior, such as chasing and sparring, actions that ethologists believe promote social bonding, reinforce social hierarchies, help improve survival skills by improving locomotor functions, and prepare young animals for sexual, predatory, and defensive behaviors. Deciphering the neuroendocrine basis for behaviors is a popular modern approach to ethology, one that molecular biological techniques have advanced.

The specialty of human ethology, or human behavior, originated with the British naturalist Charles Darwin's text *The Expression of the Emotions in Man and Animals* (1872), in which he examined how human beings and other animals showed emotions such as fear, anger, and pleasure, emphasizing the shared evolution of humans and other animals. One approach to studying human ethology is comparative: examining the behaviors of humans alongside those of chimpanzees and other related species; comparing typical behavior with the behavior of individuals with congenital defects, particularly those with sensory deficits; and comparing human behaviors across cultures, since behaviors exhibited by individuals in a wide variety of cultures are probably biological rather than cultural in nature. The overlapping subfield of evolutionary psychology specifically addresses the evolutionary significance of psychological characteristics such as memory, perception, and language, mostly in humans, though other animals are not excluded. Whereas ethology is concerned with the study of all animal behaviors, sociobiology focuses on the evolution of social behaviors in animals.

Attaining a better understanding of normal or appropriate animal behavior will help zoologists to identify when behavior exhibited by an animal is a result of injury or fear and to decipher the body language animals display to communicate how they feel. Knowing which behaviors are instinctual, which behaviors are learned, and which behaviors are crucial for survival will help in conservation efforts. People whose occupations involve direct interaction with animals—veterinarians, ecologists, zookeepers, forest rangers, wildlife biologists, and conservation biologists—benefit from studying animal behavior but so does the rest of society. Many people keep animals in their homes, voting members of communities affect legislation passed to protect animals and their environments, and as animals themselves, humans can develop a better appreciation of their own behaviors and actions and recognize their relationship to the rest of the living world.

See also ANIMAL BEHAVIOR; ANIMAL COGNITION AND LEARNING; ECOLOGY; FRISCH, KARL VON; LORENZ, KONRAD; SOCIAL BEHAVIOR OF ANIMALS; SOCIOBIOLOGY; TINBERGEN, NIKOLAAS; ZOOLOGY.

FURTHER READING

Barnett, S. A. *Modern Ethology: The Science of Animal Behavior*. New York: Oxford University Press, 1981.

Dugatkin, Lee Alan. *Principles of Animal Behavior*. New York: W. W. Norton, 2003.

Hinde, Robert A. *Ethology: Its Nature and Relations with Other Sciences*. New York: Oxford University Press, 1982.

Lorenz, Konrad Z. *The Foundations of Ethology: The Principle Ideas and Discoveries in Animal Behavior*. Translated by Konrad Z. Lorenz and Robert Warren Kickert. New York: Simon & Schuster, 1981

McFarland, David. *Animal Behavior: Psychobiology, Ethology, and Evolution*. 3rd ed. Harlow, England: Longman, 1998.

Eukarya

According to modern biological classification schemes, all living organisms belong to one of three major domains of life: Archaea, Bacteria, or Eukarya. Microfossil evidence suggests that life first appeared about 3.6 billion years ago. The first life-forms were anaerobic and prokaryotic, then oxygenic photosynthesis emerged about 2.5 billion years ago. Phylogenies based on molecular analyses indicate that the universal common ancestor diverged into two lineages. The Bacteria lineage diverged first, and the other subsequently split into two lineages, Archaea and Eukarya. Fossil evidence suggests that eukaryotic cells first emerged approximately 2.1–1.6 billion years ago. The emergence of eukaryotic cells was a significant event, as the complexity of their structure allowed for the evolution of sexual reproduction and multicellularity, two milestones in the history of life leading to the diversity of modern day life-forms.

EUKARYA AS A DOMAIN

Organisms belonging to the domain Eukarya have in common cell structures that are absent in prokaryotes, most notably, a nucleus and a cytoskeleton. Biologists believe that the membrane-bound nuclear compartment resulted from an infolding of the plasma membrane that surrounded the genetic material contained in a region called the nucleoid in prokaryotic cells. The nuclear envelope encloses the deoxyribonucleic acid (DNA), protecting it from damage and allowing for another step in the regulation of gene expression, which conserves energy. Additional invaginations of the plasma membrane led to the membrane-bound endoplasmic reticulum,

the site for the synthesis of lipids and some proteins, and the Golgi apparatus, where protein modification, sorting, and packaging occur. The endosymbiotic theory, popularized by Lynn Margulis, a professor of geosciences at the University of Massachusetts, purports that subcellular structures characteristic of eukaryotic cells evolved as a consequence of mutually symbiotic relationships of prokaryotic cells. Smaller cells lived inside other cells and took on specialized functions, and eventually the organisms depended on each other. As the smaller cell lost its ability to carry on independent life functions, it evolved into an organelle within the larger cell. Mitochondria, organelles responsible for cellular respiration, and plastids, organelles that perform photosynthesis, are believed to have developed in this manner.

In 1969 Robert Whittaker proposed a five-kingdom classification system. One kingdom, Monera, consisted of prokaryotic organisms, and the remaining four—Protista, Fungi, Plantae, Animalia—were composed of eukaryotic organisms, characterized by nucleated cells. In 1990, after spending more than two decades conducting labor-intensive research, the American microbiologist Carl Woese proposed a taxonomic category above kingdom. He argued that phylogenies based on molecular evidence supported three different major lineages. He found that the distinction between members of Bacteria and members of his newly proposed group of prokaryotic organisms, Archaebacteria (now known as Archaea), was more significant than the differences between eukaryotic kingdoms. Thus, he placed all four of the eukaryotic kingdoms within a single domain, Eukarya.

Classification schemes continue to evolve as biologists learn more about the phylogenies of different life-forms, including eukaryotic categories. Molecular evidence is revealing that relationships defined on the basis of gross structural appearances differ from those based on DNA sequence comparisons. Molecular data suggest that some organisms once thought to be closely related on the basis of outward appearance are more distantly related, and organisms that look different may have a more recent common ancestor than once assumed. These new data have led to the proposal of several new kingdoms, consisting of organisms once grouped within the traditional four eukaryotic kingdoms. Many biologists continue to employ Whittaker's five-kingdom system, and this encyclopedia often also uses these traditional descriptions because taxonomists are still analyzing data and working to reach a consensus on an improved phylogenetic framework. One must remember that these groupings are used for the sake of convenience and familiarity rather than a demonstrated evolutionary history.

Traditionally, the domain Eukarya includes the four kingdoms Protista, Fungi, Plantae, and Animalia. Protista represents the most ancient eukaryotic kingdom, containing unicellular, colonial, and multicellular organisms that did not fit into Fungi, Plantae, or Animalia for various reasons. The plantlike, autotrophic protists termed *algae* include species as diverse as unicellular diatoms and multicellular kelp. Protozoa are unicellular, heterotrophic animallike protists. Protists have undergone the most drastic changes in classification. Once considered a single kingdom, these organisms are being redistributed into as many as 18 new kingdoms, with some being placed in one of the other three traditional eukaryotic kingdoms. While Protista is no longer considered a valid taxon, the term *protist* is still useful for referring to eukaryotic organisms consisting of a single cell or a few cells.

KINGDOMS FORMERLY INCLUDED IN PROTISTA

The protists consist of approximately 60 different lineages, and comparison of the sequences for the small ribosomal ribonucleic acid (RNA) subunit reveals patterns concerning the evolution of these members of Eukarya. The earliest lineages include the present-day parasites diplomonads (such as *Giardia lamblia*), microsporidia (which causes the silkworm disease pébrine), and parabasalids (represented by *Trichomonas vaginalis*, which lives in the female vagina). These three proposed kingdoms all lack the membranous structures making up the Golgi apparatus, peroxisomes (organelles that contain enzymes that catalyze oxidative reactions), and developed mitochondria. They are adapted to anaerobic environments, and their mitochondria do not have their own DNA, electron transport chains, or enzymes for the citric acid cycle. They do have simple cytoskeletal components and a few membrane-bound vesicles.

An increase in the complexity of membranous intracellular structures, such as those making up the Golgi apparatus in addition to mitochondria and chloroplasts, led to the emergence of new lineages. The myxomycotes are the plasmodial slime molds, which were formerly considered a type of fungus. These protists exist in three different forms during their life cycle. The plasmodial form resembles a patch of wet slime and consists of one enormous fused cell containing thousands of diploid nuclei. In dry conditions, a second form develops—a tall, stalk-like structure containing haploid spores that germinate into the third amoebalike form. The haploid amoebalike cells can fuse with other cells in sexual reproduction and then multiply to form the feeding plasmodial form again.

Euglenozoans are flagellated protozoans that comprise two main groups: the kinetoplastids and

the euglenoids. Kinetoplastids are characterized by a kinetoplast, a granule containing a mass of DNA, and include free-living and parasitic species, such as *Trypanosoma,* a blood parasite that causes sleeping sickness. Euglenids, such as *Euglena,* have chloroplasts and are photoysnthetic.

Naegleria make up a separate kingdom. These organisms can exist as an amoebalike form or have a pair of flagella depending on the environmental conditions.

Amoebozoans are characterized by lobe-shaped pseudopodia. Most are free-living, but some, such as *Entamoeba histolytica,* which causes dysentery, are parasitic.

Cellular slime molds, belonging to the tentative kingdom Acrasiomycota, have multicellular fruiting bodies made of separate cells. They exist as unicellular amoebalike cells when organic material is abundant but can form a multicellular sluglike aggregate that migrates as a unit. When it settles, the cells form a stalk with an asexual fruiting body that contains haploid spores that can develop into new amoebalike cells.

Red seaweeds, or rhodophytes, were the first multicellular, photosynthetic organisms. The 4,000-plus species are all marine, reproduce sexually, and possess the photosynthetic pigments chlorophyll a and phycobilins. They are structurally diverse, and some become calcified, like corals do.

The next three types of protists—dinoflagellates, ciliates, and apicomplexans—all belong to a taxon named alveolates, but their exact relationships remain unresolved. They have in common sacs called alveoli, whose function is unclear, under the plasma membrane. The dinoflagellates are mostly marine, planktonic organisms, covered by a hard shell called a test, and motile by a single flagellum. Some are photosynthetic, having chlorophyll a, variants of chlorophyll c, carotenes, and xanthins. Dinoflagellates are responsible for red tide, some produce toxins poisonous to invertebrates and fishes (and humans if they ingest contaminated seafood), and some are bioluminescent. The ciliates are characterized by cilia and two nuclei: one large nucleus for gene expression and a smaller nucleus that functions in sexual reproduction. One example of a ciliate is *Paramecium.* Apicomplexans are sporozoite-forming parasites that have complex life cycles requiring more than one host species. An example is *Plasmodium,* the causative agent for malaria.

The stramenopiles is a clade consisting of six closely related tentative kingdoms. They have in common tiny, hollow hairs that often occur on a flagellum or are derived from organisms that had such hairs. The slime nets (Labyrinthulids) are mostly marine and form colonies on marine plants and seaweed.

TENTATIVE KINGDOMS FORMERLY BELONGING TO PROTISTA

Tentative Kingdom	Examples
Diplomonads	*Giardia lamblia, Giardia intestinalis*
Microsporida	*Nosema*
Parabasalids	*Trichomonas vaginalis, Trichomonas foetus*
Myxomycota	*Echinostelium, Physarum polycephalum*
Euglenozoa	*Euglena viridis, Peranema, Trypanosoma brucei, Trypanosoma cruzi*
Naegleria	*Naegleria*
Amoebozoa	*Entamoeba histolytica*
Acrasiomycota	*Dictyostelium discoideum*
Rhodophyta	*Polysiphonia, Porphyra, Palmaria palmata*
Ciliates	*Paramecium, Stentor*
Dinoflagellates	*Gonyaulax, Pfiesteria shumwayae*
Apicomplexa	*Plasmodium, Toxoplasma, Coccidia*
Labyrinthulids	*Labyrinthula*
Oomycota	*Phytophthora infestans*
Xanthophyta	*Ophiocylium*
Chrysophyta	*Ochromonas, Dinobryon*
Phaeophyta	*Fucus, Sargassum, Postelsia, Macrocystis, Laminaria*
Diatoms	*Thalassiosira*
Foraminiferans	*Globigerina*
Radiolarians	*Actinomma*

They are unique in that they form trails of a mucus-like polysaccharide that their cells move along to find food. The oomycotes, commonly known as water molds, resemble fungi in that they consist of thread-like hyphae and secrete enzymes to digest organic substances then take in the nutrients. Oomycotes were once grouped with fungi, but their similarities are now known to result from convergent evolution.

Their cell walls contain cellulose. Xanthophytes are freshwater photosynthetic protists having various xanthins and chlorophylls a, c, and e. Chrysophytes, commonly known as golden algae, may be unicellular or live in colonies. Some form elaborate skeletons made of silica. The brown seaweeds, kingdom Phaeophyta, include about 1,500 mostly marine species. Some of these grow to be more than 300 feet (about 100 m) long and are called kelps. Though they do not contain true tissues, many brown seaweeds (also called brown algae) have thalli and holdfasts. A thallus is the plantlike body of a seaweed. Leaflike blades extend from a stemlike stipe, and the rootlike holdfast is for gripping and anchoring the alga. Brown seaweeds contain chlorophylls a and c and the pigment xanthophyll. Some multicellular algae undergo an alternation of generations similar to that in plants. Diatoms have shells called tests consisting of two siliceous halves; these shells form diatomaceous earth, which is mined to serve as a filter medium. The diverse diatoms make up a major portion of phytoplankton. Most are photosynthetic and contain chlorophylls a and c, carotenes, and xanthins.

The phylogenies of foraminiferans and radiolarians are not as well understood. Both are protists that have threadlike pseudopods. Foraminiferans have porous calcified tests, or shells. The pseudopods extend through the pores to function in motility and feeding. Radiolarians are marine protists with fused siliceous tests. Their threadlike pseudopods are called axopodia, and they are surrounded by microtubules. When smaller microorganisms land on the axopodia, the radiolarian phagocytoses it, then carries it into the main part of the cell by cytoplasmic streaming down the axopodia.

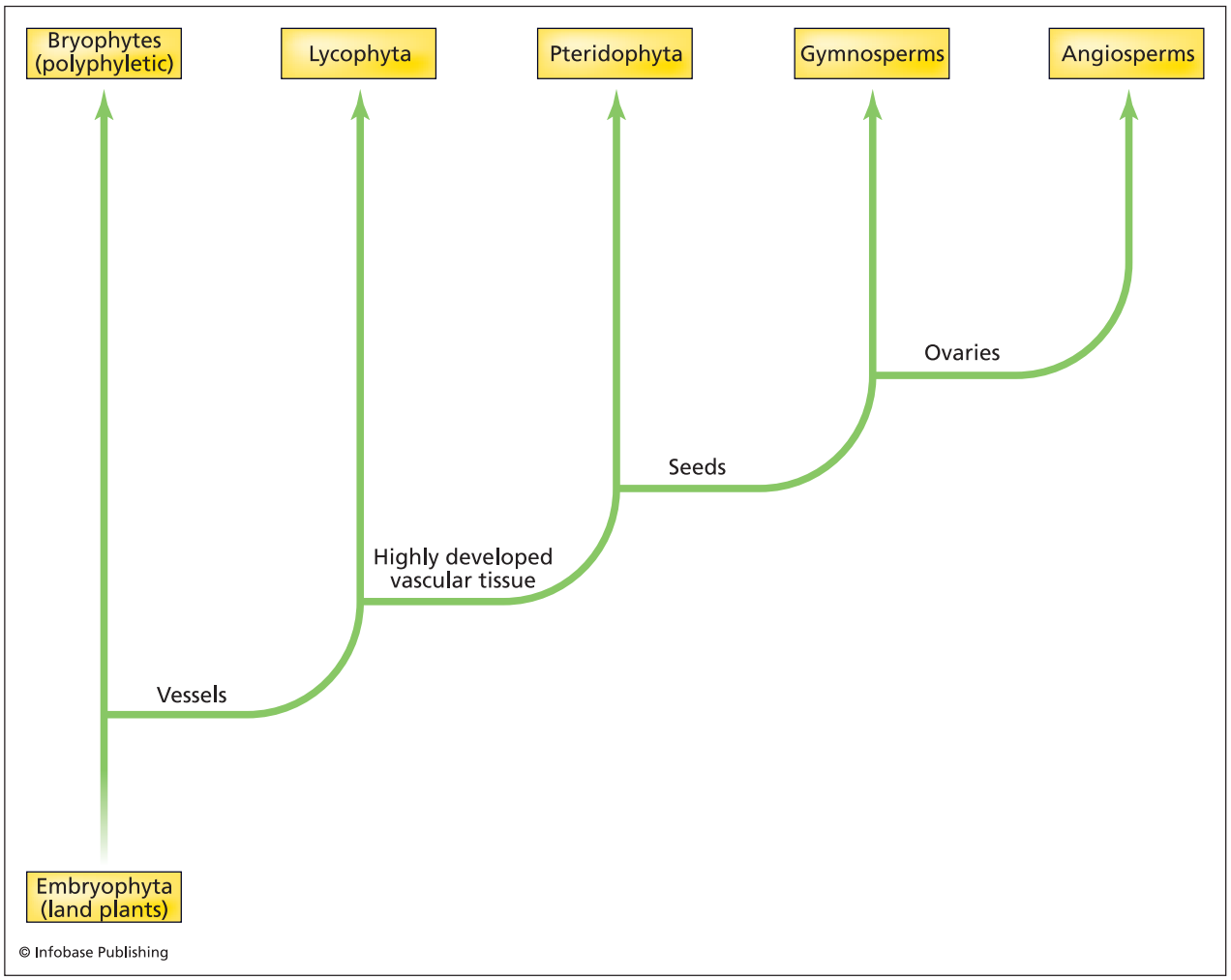
KINGDOM FUNGI

The kingdom Fungi includes eukaryotic, heterotrophic multicellular organisms that have cell walls. They mostly feed on dead or decaying organic matter by secreting digestive enzymes and then absorbing the smaller organic compounds. Biologists used to think fungi were plants because they were immobile, had structures that resembled roots, and had cell walls. Even after biologists recognized Fungi as a distinct kingdom, they included plasmodial slime molds, cellular slime molds, slime nets, and oomycotes as members. More recent molecular evidence suggests that each of these groups also warrants its own kingdom. Distinguishing features of true fungi are cell walls composed of chitin, a nitrogen-containing polysaccharide, and a unique biochemical pathway for the metabolism of lysine, one of the 20 amino acids that cells use to build proteins. One surprising finding from comparisons of small ribosomal RNA between fungi and other kingdoms of Eukarya is that fungi may be more closely related to animals than plants.

Fungi are traditionally categorized in terms of the anatomy of their sexual reproductive structures. The major fungal phyla include Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. The chytrids, believed to be the most ancestral form, are aquatic, do not have septa dividing the filamentous hyphae, and include both saprobes that feed on decaying matter and parasites that feed off living hosts. They produce flagellated gametes that fuse during sexual reproduction to form a zygote that can remain inactive in unfavorable conditions. The polyphyletic phylum Zygomycota includes nonseptate fungi that sexually reproduce by forming zygospores. When hyphae of opposite mating types meet and fuse, a tough zygosporangium forms. Inside, the nuclei fuse and meiosis occurs. Germination leads to the formation of a sporangium that releases genetically variable haploid spores that grow into new organisms. Ascomycotes, also known as sac fungi, reproduce sexually by growing specialized hyphae that have spores called conidia on the tips. Conidia can fuse with hyphae of the opposite mating type, leading to the formation of an ascus, inside of which the parental nuclei fuse. Meiosis follows, forming four genetically variable nuclei. One round of mitosis then creates eight ascospores that disperse and germinate. Basidiomycotes include mushrooms and bracket or shelf fungi. When two hyphae of opposite mating types fuse, dikaryotic mycelia form and grow. (*Dikaryotic* means that the two haploid nuclei remain separate inside each cell of the mycelia.) Under certain environmental conditions, basidiocarps develop, and their gills contain dikaryotic cells called basidia. The nuclei fuse and undergo meiosis, forming four haploid nuclei. Four basidiospores develop, each containing a single nucleus. When mature, basidiospores disperse and germinate to form haploid mycelia. A fifth group, called Deuteromycota, or more appropriately the "imperfect fungi," is not a true clade, but rather a group of fungi whose sexual state is not yet known or may not reproduce sexually at all.

KINGDOM PLANTAE

The remaining two kingdoms of the domain Eukarya—Plantae and Animalia—once were thought to encompass all life but are now known to describe a very small portion of it. The kingdom Plantae includes immobile, multicellular, photosynthetic eukaryotic organisms that have cell walls made primarily of cellulose. Botanists agree that plants evolved from a green algal ancestor, but they have not reached a general consensus of whether or not green algae should indeed be placed in the kingdom Plantae, or whether



Major structural innovations in the history of land plants include vessels for transporting water and nutrients, seeds, and ovaries to enclose the seeds.

they warrant their own kingdom. Characteristics shared by green algae and land plants include the same type of chlorophyll, the presence of carotenoids as accessory pigments, and starch storage inside the chloroplasts. Many green algae also have cellulose as the main component of their cell walls.

The first major transition in the evolutionary journey from green algae to flowering plants is multicellularity, which developed independently in different lineages. Green algae include unicellular, colonial, and multicellular forms, but land plants are derived from a different photosynthetic protist ancestor. The next major transition, occurring approximately 460 million years ago, was the colonization of land, a move that required numerous adaptations. The common terrestrial ancestor was probably a form of green alga that formed a symbiosis with fungi. This evolved into the Embryophyta lineage, the land plants. The term *bryophyte* informally refers to the nonvascular lineages that include the earliest land plants, but Bryophyta (with a capital *B*) is a clade of bryophytes consisting of mosses. Vascular plants have organized systems for transporting water and nutrients throughout the plant. The living vascular plants evolved from an ancestor in common with the mosses. This branch diverged into Lycophyta and Tracheophyta. Lycophyta consists of the club mosses and their relatives. The tracheophyte lineage diverged into groups. One group led to the evolution of Pteridophyta, the ferns and horsetails, which as do all other plants up to this point, reproduce by spores. The other branch developed an innovative adaptation approximately 360 million years ago that led to widespread success, the seed. Seed-bearing plants surround their embryos with a supply of nutrients inside a tough, protective covering, and thus they do not depend on water to reproduce. Two major divisions of seed plants are gymnosperms, whose seeds are not enclosed by chambers, and angiosperms, whose seeds develop inside structures called ovaries.

KINGDOM ANIMALIA

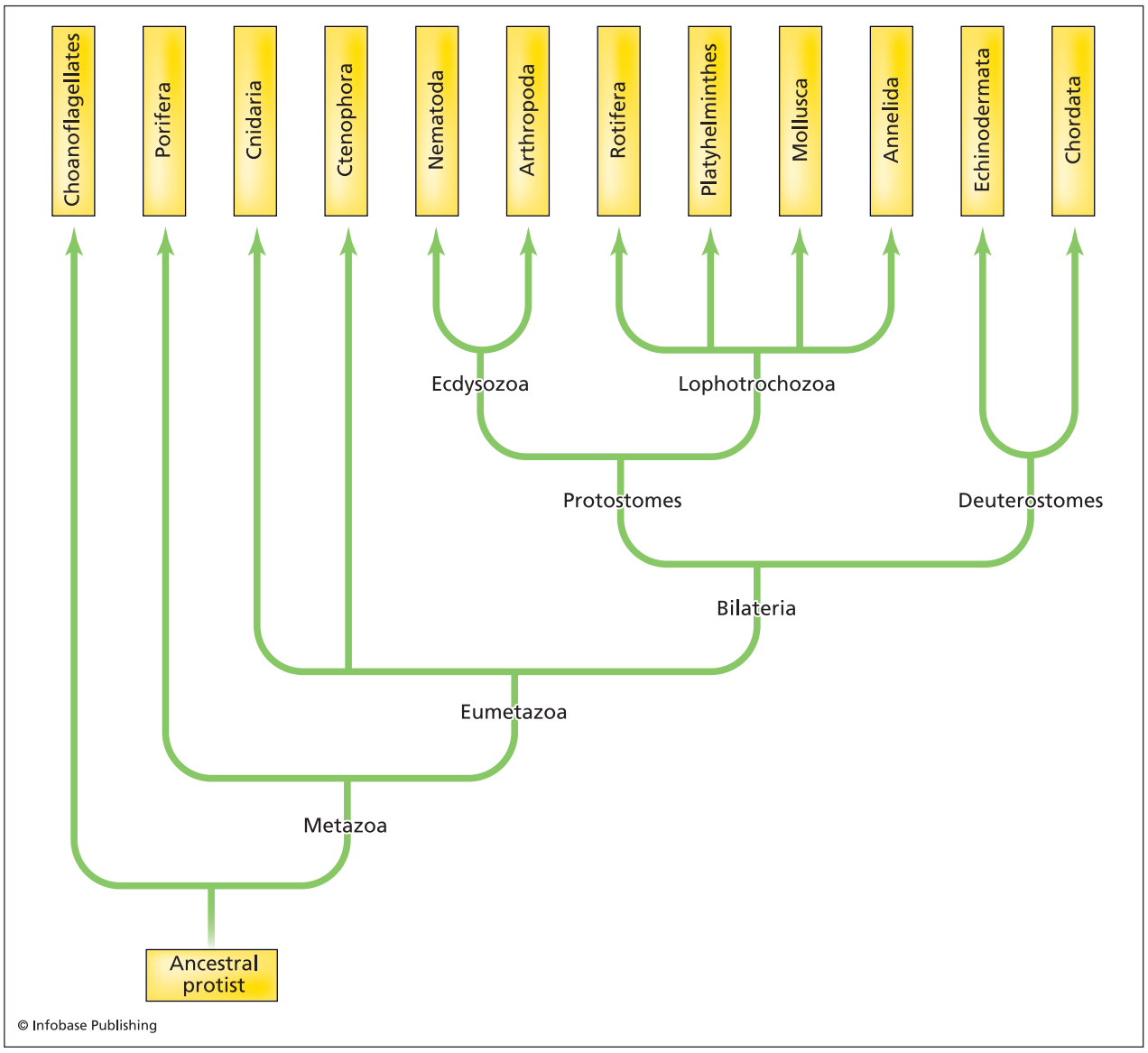
The kingdom Animalia includes multicellular, heterotrophic eukaryotic organisms that have no cell walls, are capable of motility during at least one stage of their life cycle, and digest their food internally. Animal life cycles are characterized by haploid gametes that fuse during sexual reproduction to form a diploid zygote that undergoes numerous rounds of mitotic division and a complicated differentiation and development stage to form the complete, mature multicellular adult. More than 35 phyla of animals with similar evolutionary traits exist, with the majority of animals belonging to 11: Porifera, Cnidaria, Ctenophora, Platyhelminthes, Nematoda, Rotifera, Mollusca, Annelida, Arthropoda, Echinodermata, and Chordata.

Animals developed from a common ancestral protist that diverged into the modern-day choanoflagellates and the lineage that evolved into all animals. The monophyletic clade including extant and extinct animal lineages is called Metazoa, animals with bodies composed of differentiated tissues and a digestive cavity lined with specialized cells. Whether or not choanoflagellates should be included in the animal kingdom is debatable. Choanoflagellates are sometimes unicellular and sometimes colonial. Each cell has a collar of microvilli at one end, with a single, protruding cilium that beats to move water toward the circle of microvilli. The collar catches microscopic pieces of food for ingestion by the cell. Sponges, organisms belonging to the phylum Porifera, contain very similarly structured cells called choanocytes. Porifera are said to be parazoan, meaning they do have specialized cells, but they are not organized into true tissues separated from other tissues by membranes—the cells retain some of their independence. The remaining metazoans are eumetazoans, meaning their cells are organized into true tissues that cooperate with other body tissues and combine with other tissues to form organs.

The common ancestral eumetazoan diverged into three lineages, the phyla Cnidaria and Ctenophora and the Bilateria. Cnidaria includes sea anemones, jellyfish, and corals, and Ctenophora includes the comb jellies. Members of both phyla exhibit radial symmetry and are diploblastic, meaning they have only two germ layers, the endoderm and the ectoderm. During the blastula stage of early animal development, the embryo resembles a hollow ball of cells. The next stage, gastrulation, involves the invagination of one side to form a gastrula defined by two cell layers. The inner layer becomes the endoderm, and the outer layer becomes the ectoderm. The third branch, Bilateria, consists of animals that develop from three cell layers; they are said to be tripoblastic. The third layer is called the mesoderm, and it offers a new degree of complexity.

The bilaterians branch into two clades that have different patterns of early development: the protostomes and the deuterostomes. After fertilization, the zygote undergoes a series of mitotic cell divisions called cleavage, resulting in a multicelled embryo. Protostomes undergo a spiral cleavage, in which the layers of cells look twisted, whereas deuterostomes undergo radial cleavage, resulting in cells neatly stacked one over another. In addition, the cleavage in protostomes is determinate, meaning the fate of specific cells in the early embryo is already determined. If a cell is removed, it cannot develop into a complete organism. In deuterostomes, cleavage is indeterminate, so each cell taken from an early embryo, say, at the four-cell stage, can develops into a complete individual. During gastrulation, when one side invaginates, the indentation forms the blastopore, a structure involved in formation of the gut. In protostomes, the blastopore extends to the other end of the embryo, the original site of blastopore formation develops into the mouth, and the anus forms at the other end of the embryo. In deuterostomes, however, the blastopore becomes the anus, and the mouth develops from another opening. The larvae that form from protostomes and deuterostomes also differ, especially in the arrangement of the cilia. Protostome larvae are called trochophores, and deuterostome larvae are called tornarias. Last, the arrangement of the nervous system differs between the two. While zoologists are still working on the phylogenetic details within the two major branches of protostomes and deuterostomes, they agree which phyla belong to which group. The protostomes include molluscs, annelids, rotifers, platyhelminthes, arthropods, and nematodes. Echinoderms and chordates are deuterostomes.

Recent molecular evidence suggests that the protostome ancestor diverged into two major sister branches, the Ecdysozoa and the Lophotrochozoa. The molecular studies group phyla differently than traditional placement based on morphologies does. The ecdysozoans secrete exoskeletons, tough outer coverings that the animal outgrows and sheds in a process called ecdysis. Though some animals in other phyla do molt, sequence comparisons of ribosomal RNA genes and the *Hox* genes (which play a role in animal development) link the nematodes and arthropods by a common ecdysozoan ancestor and group the rotifers, platyhelminthes, mollusks, and annelids together in the lophotrochozoan group. Traditionally, the arthropods have been most closely linked with the annelids because their body plans share similar characteristics, particularly with respect to segmentation and appendages. If the molecular evidence accurately reflects the evolutionary history, then segmentation arose separately in these taxa. Zoologists



Major developments leading to the current diversity of members of the kingdom Animalia include specialized tissues, bilateral symmetry, and unique developmental processes.

need to examine molecular data from more species from different phyla before drawing reliable conclusions regarding this classification scheme.

Beyond the divisions created by the milestones mentioned so far—multicellularity, true tissues, three rather than two germ layers, and radial versus bilateral symmetry—other innovations upon which classification is based include cephalization, presence of a coelom, and segmentation. Cephalization is the concentration of nerves and sensory organs in an anterior head. A coelom is a space between the body wall and the gut that results when the mesoderm forms a layer of tissue that wraps around the internal body organs. Animals with a coelom (annelids, mollusks, arthropods, echinoderms, and chordates) are called coelomates, and animals without one (poriferans, cnidarians, ctenophorans, and platyhelminthes) are called aceolomates. Pseudocoelomates are animals with a body cavity that is only partially lined with tissue derived from mesoderm (nematodes and rotifers). The coelom allows for freer movement, more complex structure, and larger body size. Segmentation is a body plan divided into repeated segments, a system of organization that underlies the body plans of all coelomates except mollusks. In animals such as earthworms, the segments are identical, with all containing the same structures, or have different structures and perform specialized functions, as in the head, thorax, and abdomen of insects. The presence or absence of these different body plan innovations

does not necessarily imply common ancestry, as several features, such as coeloms and segmentation, have evolved independently.

Informally, animals are largely divided into two main groups, the invertebrates and the vertebrates, based on the absence or presence of a backbone. More detailed discussion of the organisms in the animal kingdom in this encyclopedia follows this format. Though this popular manner of dividing the animal kingdom seems to imply equal degrees of significance of the two groups, one must remember that vertebrates are only a subphylum of Chordata, one of the 35 or so recognized phyla within the kingdom Animalia.

See also ALGAE; ARCHAEA; BACTERIA (EUBACTERIA); BIOLOGICAL CLASSIFICATION; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; EUKARYOTIC CELLS; FUNGI; HISTORY OF LIFE; INVERTEBRATES; PLANT DIVERSITY; PROTOZOA; SLIME MOLDS; VERTEBRATES.

FURTHER READING

Adl, Sina M. et al. "The New Higher Level Classification of Eukaryotes with Emphasis on the Taxonomy of Protists." *The Journal of Eukaryotic Microbiology* 52, no. 5 (October 2005): 399–451.

Maddison, D. R., and K. S. Schulz, eds. *The Tree of Life Web Project*. Available online. URL: www.tolweb.org/tree/. Accessed January 25, 2008.

Margulis, Lynn, and Karlene V. Schwartz. *Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth*. 3rd ed. New York: Henry Holt, 1998.

Tudge, Colin. *The Variety of Life: A Survey and a Celebration of All the Creatures That Have Ever Lived*. New York: Oxford University Press, 2000.

eukaryotic cells

Organisms and the cells they comprise can be broadly classified as either one of two major types based on structural complexity: prokaryotic or eukaryotic. Prokaryotic organisms include the unicellular archaea and bacteria, neither of which contains nuclei or other membrane-bound organelles. Eukaryotic organisms can be either unicellular, as in the case of protozoa or yeasts, or multicellular, as in the case of plants and animals. The cells that make up eukaryotic organisms share many common structures, most notably, a nucleus to house the deoxyribonucleic acid and other organelles as part of their endomembrane system.

Eukaryotic cells are generally larger than prokaryotic cells, but certain constraints limit their maximal size. Smaller cells have a larger surface area to volume ratio, and therefore can more efficiently exchange nutrients and waste products across the cell membrane. As cell size increases, this ratio decreases to the point of no longer being able to meet the cell's needs. Eukaryotic cells typically range from 0.0004 to 0.004 inch (10 to 100 μm).

CELL WALL AND CELL MEMBRANE

Whether eukaryotic or prokaryotic, all cells are enclosed by a cell membrane, also called a plasma membrane, that acts as a barrier between the cell and the external environment. The basic structure of the cell membrane is a phospholipid bilayer containing numerous embedded proteins. The main function of the cell membrane is to keep the cellular contents enclosed while preventing unwanted materials from entering and allowing waste materials to exit. Because the interior of the bilayer is hydrophobic, polar or charged substances cannot easily cross. Specialized channels in the membrane allow materials to move in and out in a controlled manner. Receptor molecules located on the exterior surface receive signals via the binding of specific ligands in a process called signal transduction, by which cells communicate with the environment and other cells within a multicellular organism. (The structure and function of cell membranes are covered more fully in the entry on biological membranes.) The characteristics of the cytoplasm, the entire contents bounded by the cell membrane excluding the nucleus and other membrane-bound organelles, can differ greatly from the external environment as a result of the selectivity of the cell membrane for the substances it allows to pass through. Because of this, eukaryotic cells can live in a wide variety of environments.

Plant cells, fungal cells, and some protist cells also have a cell wall external to the cell membrane. Whereas the main function of the cell membrane is to allow or prevent selected substances from passing into or out of the cell, the major functions of the cell wall are protection and structural support. The composition varies, depending on the organism. Plant cell walls are constructed from cellulose, fungal cell walls are made from chitin or cellulose and mixed glycans, and algal cell walls contain polysaccharides such as cellulose, pectin, and mannans and minerals such as silicon dioxide and calcium carbonate.

NUCLEUS

The presence of a nucleus distinguishes eukaryotic cells from prokaryotic cells. Bound by a double membrane called the nuclear envelope, the nucleus holds the cell's chromosomes. About 7.87×10^{-7} to 1.58×10^{-6} inch (20 to 40 nm) separate the two membranes, and pores of approximately 3.97×10^{-6} inch (100 nm) in diameter span the envelope, connecting the interior of the nucleus with the cytoplasm. A nuclear lamina made of protein filaments lines the envelope and gives it support. Because an organism's

genetic information is contained within the chromosomes, the nucleus is referred to as the control center of the cell. The directions for all cellular activities ultimately originate in the nucleus. DNA replication occurs here, as does transcription, the first stage of gene expression and protein synthesis. The nucleolus is a specialized region of the nucleus where ribosomal ribonucleic acid is synthesized.

ENDOMEMBRANE SYSTEM

Eukaryotic cells have an endomembrane system, an elaborate arrangement of internal membranes that compartmentalize cellular functions and play a role in protein processing. These membranes are all related either because they are physically connected or because they transfer tiny vesicles to one another. The plasma membrane and the nuclear envelope are considered part of the endomembrane system.

The endoplasmic reticulum (ER) is continuous with the nuclear envelope and extends outward from it into the cytoplasm. Consisting of approximately half of the cell's total membranes, the ER is organized into connecting tubules and folded stacks called cisternae. The ER serves as the site for both protein and lipid synthesis, though regions that accomplish the two tasks are separate. Ribosomes perform the job of protein synthesis, and, depending on the type of protein being made, a ribosome can do this while floating freely in the cytoplasm or while bound to the cytoplasmic surface of the ER. Generally, proteins that are destined to remain in the cytoplasm are synthesized by free ribosomes, whereas proteins that will be embedded in a membrane, secreted out of the cell, or compartmentalized within the cell are synthesized by ribosomes sitting on the ER. As the ribosomes make the proteins, the polypeptide chains cross the membrane into the ER. Small chains of carbohydrates called oligosaccharides are attached to secretory proteins. Portions of the ER that are covered in ribosomes are termed rough ER (RER) because of the grainy appearance they give the membranes. Other parts of the ER are called smooth (SER) because they are not covered in ribosomes and appear smooth. SER is the site for lipid synthesis, for the detoxification and breakdown of toxic substances, and for carbohydrate metabolism in some tissues.

After making secretory or membrane proteins, parts of the ER containing them pinch off, forming transport vesicles that travel to the Golgi apparatus, another network of flattened, membrane-bound sacs located throughout the cell. The membranes of the transport vesicles fuse with the membrane of the Golgi apparatus, releasing the contents into the Golgi's interior. Enzymes inside the Golgi modify the oligosaccharide chains that were added to the proteins in the ER by removing some of the carbohydrates and replacing others. Different cisterna of the Golgi apparatus perform different tasks and the polypeptides bud off from one cisterna and then fuse with the next, where additional modifications such as the addition of phosphate groups occurs. The Golgi then sorts and delivers the processed proteins to their final destination. Again, vesicles bud off and deliver proteins that are to be secreted or that will become part of the cell membrane to the boundary of the cytoplasm. Other proteins, such as the cell's digestive enzymes, stay packaged in vesicles that remain in the cytoplasm.

Lysosomes are spherical sacs filled with digestive enzymes for breaking down materials taken into the cell by phagocytosis, for recycling a cell's own organic matter, or for carrying out programmed cell death during certain stages of normal development. The pH within lysosomes is slightly acidic, and the enzymes they carry function optimally at a pH lower than in the cytoplasm of the cell.

As are lysosomes, vacuoles are spherically shaped membrane-bound sacs within the cytoplasm. Vacuoles are larger, however, and are used for storage of different substances. Food vacuoles are formed when a cell takes in food particles by phagocytosis. After fusion with a lysosome, enzymes digest the food into smaller molecules that can be used for the cell's metabolic or energy needs. Contractile vacuoles allow certain types of protists to live in freshwater environments by collecting and pumping out excess water that has entered the cell by osmosis. Plant cells have central vacuoles that make up about 80 percent of the cell and perform functions including storage, waste removal, protection, and growth.

OTHER ORGANELLES

Most eukaryotic cells contain numerous mitochondria, organelles involved in metabolism. Bound by a double membrane, mitochondria contain extensive infoldings of the inner membrane called cristae that increase the surface area on which processes of cellular respiration occur. The inner membrane and the intermembrane space between the two membranes contain many enzymes and molecules that play an active role in the harvest of energy from organic compounds. The matrix, the material located within the innermost compartment of mitochondria, contains its own ribosomes and DNA that encodes several mitochondrial specific proteins. Other proteins are imported from the cytoplasm.

Chloroplasts also contain their own ribosomes and DNA. Related to amyloplasts, which store starch in plant cells, and chromoplasts, which give fruits and flowers color, chloroplasts contain chlorophyll, the green pigment involved in photosynthesis. As are mitochondria, chloroplasts are enclosed by a

double membrane and contain an internal membranous network in order to increase the productivity of the organelle, whose function is to carry out photosynthesis, the conversion of light energy into organic compounds. Resembling stacks of pancakes, structures called grana consist of individual flattened disks of membranes called thylakoids. Chlorophyll, the pigment that absorbs wavelengths of electromagnetic radiation in the blue and red ranges, is embedded in the thylakoid membranes. (Green light is not absorbed—it is reflected, giving plants a green appearance.) The reactions of photosynthesis that involve the conversion of light energy into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) and that produce molecular oxygen, called the light reactions, occur in and on the thylakoids. The dark reactions, which use the energy stored in the form of ATP and NADPH, function to produce organic compounds from carbon dioxide and occur in the stroma, the region of the chloroplast bound by the inner membrane.

Another membrane-bound organelle found in eukaryotic cells is the peroxisome. Roughly spherical in shape, peroxisomes contain enzymes that add hydrogen atoms to oxygen atoms to produce hydrogen peroxide (H_2O_2), which is then broken down into water and molecular oxygen. Peroxisomes function in the metabolism of fatty acids and in liver cell, to detoxify alcohol or other poisons, by removing the hydrogen atoms and transferring them to oxygen.

Ribosomes are the site of protein synthesis. Not bound by a membrane, these structures give a dotted appearance to the cytoplasm of cells and when attached to the endoplasmic reticulum make it appear rough. In eukaryotic cells, the ribosomes comprise two subunits, a 60S subunit and a 40S subunit, that combine to form an 80S complex. Each subunit contains numerous polypeptides and ribonucleic acid.

CYTOSKELETON

Eukaryotic cells have an internal framework that provides cellular shape and support, acts as an anchor for organelles and a track for their movement, and assists in cellular motility. The cytoskeleton is made of three main components: microtubules, microfilaments, and intermediate filaments. Dimers of the proteins α-tubulin and β-tubulin assemble into long, hollow microtubules that grow out of a centrosome, a region near the nucleus. A pair of centrioles made from nine triplets of microtubules sits in the centrosome and functions in cell division. The major function of microtubules is to maintain cell shape. Microtubules also act to pull apart the chromosomes during mitosis so that each daughter cell receives one complete set of genetic information. Organelles and molecules inside a cell use microtubules and microfilaments as tracks along which they can move to different locations within a cell. For example, a vesicle containing secretory proteins can be transported along a microtubule from the Golgi apparatus to the cell membrane for fusion and release.

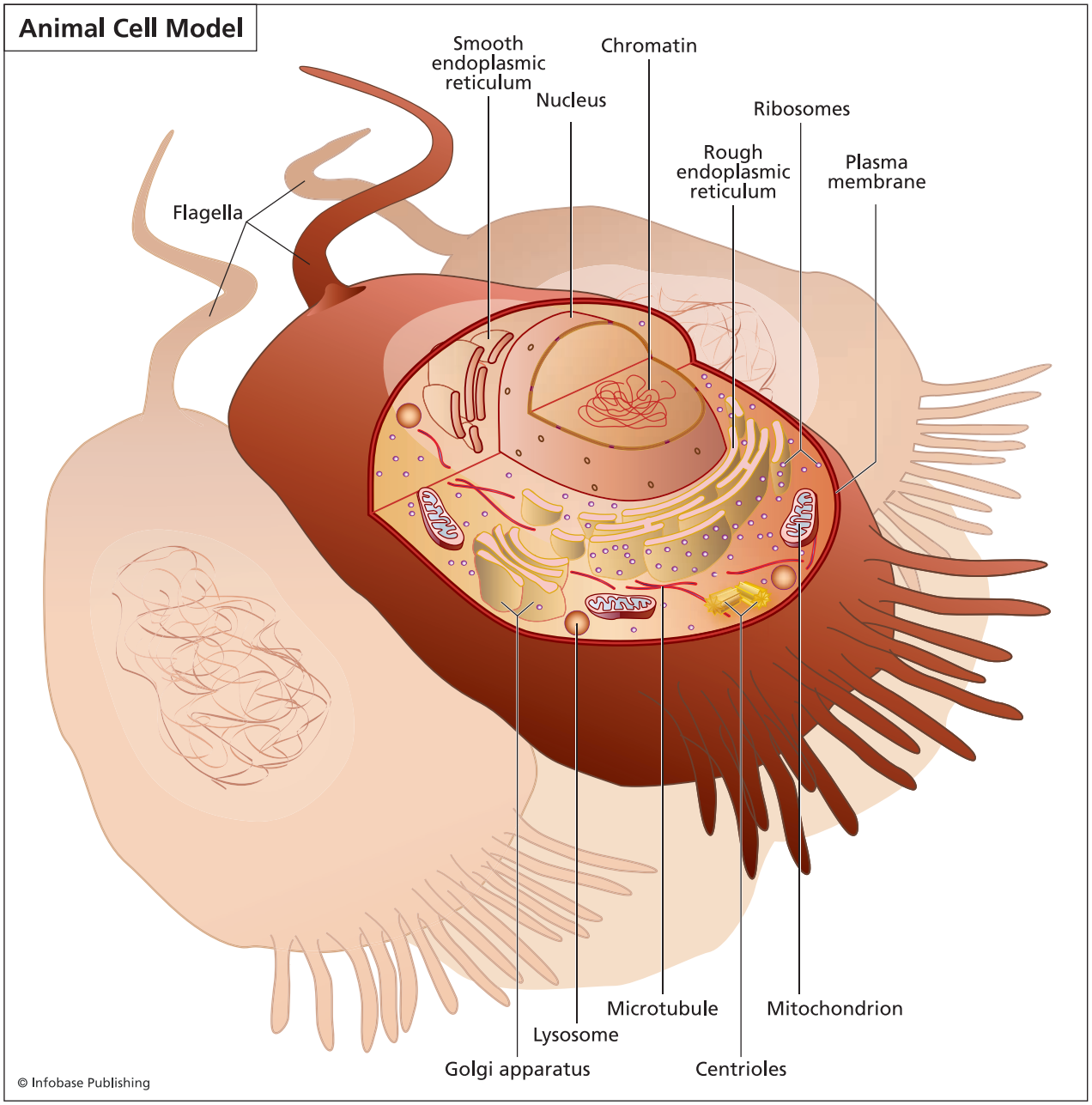
Microfilaments consist of two chains made of the globular protein actin, and they line the inner side of the plasma membrane. Bundles of microfilaments form microvilli, microscopic protrusions from cells whose job is transporting substances across membranes. The microvilli effectively increase the surface area across which transport occurs. Microfilaments also play a major role in muscle contraction by sliding along myosin filaments. Actin-myosin bundles also form cleavage burrows that divide a mitotic cell into two daughters, form pseudopodia that drive amoeboid movement, and function in cytoplasmic streaming, a process that acts to distribute materials within plant cells. Both microtubules and microfilaments constantly disassemble and reassemble within a cell.

The more stable intermediate filaments are constructed from different types of keratins and have different functions. Some form a network that anchors the position of cellular organelles. The nuclear lamina lines the nuclear envelope and is made of another type of intermediate filament. The many different types of intermediate filaments form a framework that reinforces the shape of the cell.

EXTERNAL STRUCTURES

Appendages involved in locomotion include cilia and flagella, both of which are made from microtubules. Cilia are smaller bristlelike structures that cover a cell and can beat in unison to propel unicellular organisms through water. They are approximately 9.84×10^{-6} inch (0.25 μm) in diameter and range from 7.87×10^{-5} to 7.87×10^{-4} inch (2 to 20 μm) in length. When they occur on the surface of stationary cells, such as on the lining of the respiratory tract, their beating moves fluids such as mucus along the surface of the tissue. Flagella are much longer than cilia, measuring between 3.94×10^{-4} and 7.87×10^{-3} inch (10 and 200 μm) in length. Usually only one or a few flagella are found on a single cell. Cilia move using a back-and-forth motion, while flagella undulate in a wavelike motion. Anchored to the cell by a structure similar to a centriole called a basal body, both cilia and flagella possess a common core of microtubules arranged in a "9 + 2" arrangement. When viewed in cross section, nine microtubule doublets form a circle surrounding a pair of singlets.

Most eukaryotic cells have a glycocalyx, a carbohydrate-rich coating that covers the outer surface of the cell. The glycocalyx can form a capsule or a slime layer and functions in protection, adherence



This diagram of a typical animal cell illustrates the most common eukaryotic cellular structures.

of the cell to other surfaces, and reception of signals from the environment.

ENDOSYMBIOTIC THEORY

Endosymbiosis is an association in which one organism dwells within the body of another. The endosymbiotic theory explains the origin of certain organelles of eukaryotic cells by proposing that smaller prokaryotic cells that had been engulfed by larger prokaryotic cells became permanent intracellular structures, eventually lost their ability to survive independently, and developed into an integral part of the larger cell. The smaller cells not only survived after being engulfed by the larger cell but became established in the host cytoplasm and carried out essential metabolic functions. In 1966 Lynn Margulis, the main proponent of the endosymbiotic theory, published a landmark paper titled "The Origin of Mitosing Eukaryotic Cells" that drew on 19th-century ideas and on microbial observations and brought this now largely accepted theory to the limelight.

A proposed scheme for the evolution of eukaryotic cells begins with a portion of a prokaryotic cell membrane folding inward to surround the nucleoid

region, resulting in a nucleus bound by an envelope consisting of a double membrane enclosing the genetic material. The cell engulfs a smaller bacterium that respires aerobically, and the smaller cell becomes established as an endosymbiont, living in a mutualistic relationship with its larger partner. The smaller cell multiplies within the host, creating several intracellular "machines" that evolve to perform aerobic respiration quite efficiently in an environment where other survival needs are met by the host cell. The host cell becomes dependent upon the early mitochondria to supply its energy needs. Meanwhile, the cell membrane forms numerous infoldings that increase the ancestral eukaryotic cell's ability to synthesize and process proteins and ultimately develop into endoplasmic reticulum. Algal and plant cells developed by the engulfment of photosynthetic bacteria that resembled cyanobacteria. They survived inside the larger cell, harvested energy from light, and converted it onto chemical energy stored in organic compounds, eventually developing into chloroplasts. Flagella and cilia possibly developed from symbiotic relationships of ancestral eukaryotic cells with spiral bacteria. The existence of circular chromosomes and 70S ribosomes inside mitochondria and chloroplasts in addition to other overwhelming structural similarities between prokaryotes and these organelles support the endosymbiotic theory.

See also ARCHAEA; BACTERIA (EUBACTERIA); BIOLOGICAL MEMBRANES; BIOMOLECULES; CELL BIOLOGY; CELLULAR METABOLISM; CELLULAR REPRODUCTION; CHROMOSOMES; EUKARYA; GENE EXPRESSION; MARGULIS, LYNN; MOLECULAR BIOLOGY; PHOTOSYNTHESIS; PROKARYOTIC CELLS.

FURTHER READING

Alberts, Bruce, Dennis Bray, Karen Hopkin, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Essential Cell Biology*. 2nd ed. New York: Garland Science, 2004.

Panno, Joseph. *The Cell: Evolution of the First Organism*. New York: Facts On File, 2004.

Sullivan, James A. "Cells Alive!" Available online. URL: http://www.cellsalive.com/. Accessed January 14, 2008.

evolution, theory of The theory of evolution is a cornerstone of modern life science. Biological or organic evolution is the change over time within a population in the proportions of individuals with genetically different characteristics. Evolutionary theory purports that currently existing organisms originated from preexisting forms that have been modified in successive generations by the combined action of several processes: genetic variation, the phenotypic expression of genetic characteristics, natural selection, gene flow, genetic drift, and inheritance. Evolutionary changes can be examined at different levels and in different time frames. Microevolution encompasses the changes in the gene frequencies within a limited population or between populations of the same species, often over short periods. Macroevolution refers to major evolutionary changes that occur above the species level, usually over periods of millions of years. Taxonomic groupings are based on macroevolutionary developments.

GENETIC VARIATION AND POPULATIONS

A population is a group of individuals belonging to the same species. Though members of the same species share the same number and types of genes, each individual has a unique set of forms of those genes. The genotype, or genetic composition of an individual, determines the characteristics that individual will have, both those common to all members of the species and those unique to the individual. Environmental factors can also influence the expression of certain traits to varying degrees. The differences among members of the same species are called variations. At the molecular level, genetic variation of individuals includes the existence of different alleles (forms of a gene) occurring at a specific gene locus and different combinations of genes. Parents pass on their genes to offspring through reproduction.

In asexual reproduction, the parent passes on the entire genome to the next generation. Mutations to the genes are the only source of genetic variation. In sexual reproduction, each parent contributes half of his or her genome to the offspring; thus the offspring contain a mixture of genetic material. During the production of gametes (eggs and sperm), meiosis, a specialized form of cell division that halves the genetic material in the sex cells, randomly divides the genetic material into equal quantities. Because the genetic material is partitioned randomly, as described by the law of independent assortment, the resulting cells contain numerous different combinations of alleles. This is similar to shuffling and dealing two hands of 26 cards each from a single deck. Crossing over, or recombination, when pairs of homologous chromosomes exchange segments with one another during meiosis, contributes additional genetic variation to the gametes by altering the associations of alleles located on a single chromosome. Though meiosis maintains genetic variation among individual members of a sexually reproducing population and provides a foundation for evolutionary change, it does not, by itself, change the gene frequencies from generation to generation within the population. Other processes must also take place in order to drive evolution: natural selection, mutation, nonrandom mating, gene flow, or genetic drift.

Because an individual's genotype does not change during one's lifetime, an individual cannot evolve, in the biological sense. Individuals must pass on their genes to create new individuals through reproduction. Populations, groups of members of the same species that live in the same geographic area and interbreed, are the smallest units of evolutionary change. Some populations are well defined by distinct geographic boundaries, such as mountain ranges or islands, and only breed within the population. Other populations are not completely isolated. The gene pool of a population comprises the entire collection of genes present in a given population at one time, including all the alleles at all the gene loci of all the individuals in the population. Some alleles are fixed, meaning they are present in every individual at a given gene locus, whereas others vary. Allele frequency is a measure of the relative proportion of a particular allele in the population. (Fixed alleles have frequencies of 100 percent.) Stable gene pools do not change over time; while individuals exhibit genetic variation due to meiosis, the allele frequencies are stably maintained from generation to generation. No mutation, natural selection, genetic drift, or gene flow into or out of the population occurs, and mating is completely random. When these conditions are met, the gene pool will stabilize and the population is said to be in Hardy-Weinberg equilibrium. In nature the five conditions are rarely met, and as a result, populations evolve.

MECHANISMS OF EVOLUTION

Mutations to genes can occur spontaneously because of errors during replication of the deoxyribonucleic acid (DNA). Exposure to certain chemicals and environmental conditions can also cause genetic mutations. As mentioned previously, sexual reproduction increases genetic variability by altering the associations of certain alleles with one another through independent assortment, recombination, and random fertilization. Sexual reproduction alone does not change allele frequencies in a population; it simply alters the combinations that occur in individuals. New genetic mutations can alter allele frequencies, but the change from one generation to the next is minimal. When natural selection acts on mutations, however, evolution can occur.

Genetic variation can lead to phenotypic differences in individuals. If the phenotype associated with a specific genotype increases an individual's fitness, or reproductive success, then individuals with the fitter phenotype will produce more offspring than individuals with alternate phenotypes. Since offspring receive genes from their parents, the next generation of the population will contain a higher frequency of the alleles responsible for the favorable phenotype, since those individuals produced more offspring. This process, called natural selection, depends on the environmental conditions. Phenotypes that are advantageous in one environment might not be in a different environment. To illustrate, consider a few of the many modifications to desert plants that help them withstand long periods of drought. For example, the roots of desert plants do not penetrate deep into the soil but remain close to the surface, where they can absorb water before it evaporates. Cacti have no leaves to prevent water loss through transpiration; instead the stems of cacti carry out photosynthesis. These same characteristics would not confer an advantage if the plants were grown in a moist environment.

Adaptations are traits that increase fitness, the driving force for natural selection. The level of fitness associated with an allele can only be ascertained by comparison with alternate alleles. Traits that increase the survival rate of a species contribute to an animal's fitness, but selection will only favor such traits insofar as survival improves the reproductive success of the organism. Consider that some species commit suicide in order to reproduce. The male Australian red-back spider allows a female to cannibalize him during the mating process. Natural selection has favored this adaptive behavioral trait because it increases the male individual's reproductive success even though it kills him. Individual fitness, the number of offspring produced per individual over a lifetime that survive and go on to reproduce, is typically stronger than selection at the group or species level. Another factor affecting reproductive success is sexual selection, variation in the ability to obtain a mate for reproduction. The existence of preferences for certain mates leads to nonrandom mating. Certain characteristics may increase an organism's reproductive success by increasing its ability to attract a mate, sometimes even at the expense of a decreased survival rate. A male peacock's feathers increase his attractiveness to females and therefore increase his reproductive success, even though the same feathers hinder his movement, making it more difficult to escape from possible predators. Some social animals exhibit altruistic behavior, in which they appear to sacrifice their own reproductive success for the success of a relative. For example, many bird species help rear young born to others. One might think that natural selection would disfavor genetic variation that leads to such altruistic behavior, but the phenomenon of kin selection helps explain this. The young are often related and thus share the same genes. By helping to defend and feed the young, the nonparental caretaking birds are ensuring the reproductive success of others sharing the same genes: in other words, the allele for this apparently altruistic behavior gains higher fitness by being passed on through relatives.

(continues on page 335)

ADAPTATION OR NOT: HOW TO TELL THE DIFFERENCE

by James Wagner, Ph.D.
Transylvania University

In 1835 Charles Darwin was only 26 years old, and he was already on the fourth year of his five-year voyage around world on the H.M.S. *Beagle.* At this point in the voyage the *Beagle* had sailed into a small chain of equatorial islands about 500 miles (805 km) off the west coast of South America. These islands, known as the Galápagos Islands, were inhabited by a variety of unusual animals, such as the large Galápagos tortoises and the seaweed-eating marine iguanas. However, there were also a number of familiar animals, most of which were various varieties of common birds and plants.

Contrary to popular belief, while visiting the Galápagos Islands Darwin did not have a "Eureka!" moment when he envisaged his concept of evolution. (It was actually not until eight years later, comfortable at his English home in the country, that Darwin concluded, quite reluctantly, that species evolve.) In fact, the 26-year-old Darwin found the small chain of islands to be fascinating but no more so than the jungles and pampas of South America where he had spent the past four years exploring. In his book *Voyage of the Beagle,* Darwin's first published work, he recounts an unusual observation he made while on the Galápagos Islands.

> *In Charles Island, which had then been colonized about six years, I saw a boy sitting by a well with a switch in his hand, with which he killed the doves and finches as they came to drink. He had already procured a little heap of them for his dinner; and he said that he had constantly been in the habit of waiting by this well for the same purpose.*

Darwin then remarks that the birds of the islands had not yet "learnt that man is a more dangerous animal than the tortoise or the *Amblyrhynchus* (marine iguana), disregard him in the same manner as in England shy birds, such as magpies, disregard the cows and horses grazing in our fields." An avid bird hunter, Darwin showed great restraint when he recounts a time he pushed a hawk off a branch using the muzzle of his gun.

When Darwin later developed his idea of "evolution by natural selection" he noted that those aspects of the organism that influence its survival and reproduction will become prevalent in the population because those individuals who lack these traits will disappear from the population. In terms of wild birds, Darwin noticed that it was not that birds were learning to be afraid of humans as much as that humans were killing those birds that were not already afraid of humans. Over time only those birds that had an innate fear of humans survived to reproduce, a trait they passed on to their descendants, leading to the cautious nature of most birds today.

Darwin called those traits that influence an organism's survival and reproduction adaptations. Darwin recognized that adaptations could be physical traits like sharp teeth or poisonous flesh or physiological traits like the ability to digest wood or an immune system that fights infections. Adaptations could also be behavioral traits such as exhibiting caution around humans, a trait that many of the Charles Island birds originally lacked.

Early evolutionary biologists (and many nature shows on television today) viewed organisms as an assemblage of adaptive traits. If the organism has a trait, that trait must have a function and therefore it must be an adaptation. In 1979 two famous evolutionary biologists, Stephen J. Gould and Richard C. Lewontin, challenged this view, which they called the adaptive program, in their paper "The Spandrels of San Marco and the Panglossian Paradigm." Gould and Lewontin argued that biologists frequently explained the existence of all traits of an organism as if they were adaptations optimized by evolution. In contrast, Gould and Lewontin proposed that some traits are just by-products of building an organism and are not adaptive per se. Or if they are adaptive, they may not be perfectly adaptive because not all traits can be optimally created since trade-offs must occur. Although the paper had some critics, it was influential in inducing biologists to broaden their view of the structure and form of organisms and allow for nonadaptive explanations to be considered as part of the process of evolution.

One can apply Gould and Lewontin's idea of trade-offs to the bird example. In the cautious bird scenario, imagine that those birds that were absolutely terrified of humans and always flew away whenever they saw a person would have a low probability of ever being shot and therefore enjoy a high survival rate. But although their mortality rate from hunting would be low, the birds' constant avoidance of people could prevent them from finding food and acceptable nest sites, which may be near people. For birds in people's backyards, a trade-off exists between being cautious of the boy with a stick and tolerating him to a degree in order to land at the bird feeder. Gould and Lewontin argued that for most adaptations perfection is not to be expected because of these types of trade-offs.

The other major contribution made by Gould and Lewontin was provoking scientists to question the basic assumption that all traits must be adaptations. For example, in humans on the underside of the wrist are veins that reside just below a thin layer of skin. These are the blue median antebrachial veins that return the deoxygenated blood from the hand and lower forearm back toward the heart and lungs to be reoxygenated. Having a circulatory system consisting of veins and arteries is clearly adaptive (imag-

ine trying to survive without one), but is having *blue* veins an adaptation? Human veins appear blue because the shorter, blue wavelengths of light more readily reflect off the shallow veins, thereby giving them a bluish tint. The blue color does not result, as many believe, from the blood's changing color from blue to red when it is oxygenated. Human blood is always red; it just shifts from dark red in the absence of oxygen to bright red when it is oxygenated. So is having blue veins an adaptation per se? And how does one determine whether blue veins are an adaptation?

To approach this question, an evolutionary biologist would posit how the color of veins influences the organism's ability to survive or reproduce. In terms of the blue veins it would seem that having blue veins is a nonadaptive by-product of the interaction among the reflective properties of different wavelengths of light, the depth of the veins in the skin, and the collagen from which the veins are constructed. If the veins more readily reflected yellow light than blue, it is hard to imagine how having yellow veins would reduce your survival.

One could argue, however, that in some cultures the appearance of the blue veins may act as an indication of beauty since their visibility indicates clarity of skin, which itself is an indication of health. Having blue veins is the norm, and though having yellow veins would not negatively affect one's health, it might affect the ability to find a mate, a circumstance that could reduce one's ability to reproduce. In this imaginary scenario, the blue color of veins was originally a nonadaptive trait that later became adaptive because it could be used to signal some other trait—such as an individual's health.

One of Darwin's brilliant insights in developing his idea of adaptation was that some traits may improve an organism's ability to survive while other traits may improve an organism's ability to reproduce. In both situations, those adaptive traits should increase in the population unless they work in opposition to each other. Darwin recognized that adaptive traits favored in sexual selection may in fact be opposed by natural selection. The classic example of the male peacock's tail is a case in point. The large conspicuous tail of the male peacock reduces the male's ability to avoid capture by tigers, their natural enemies. So in terms of natural selection this trait seems to be maladaptive since the tails can increase male mortality. But research has shown that those males with the largest tail feathers, most brightly colored tails, and tails with the most eyespots have more successful matings than their less endowed male neighbors. Thus, sexual selection favors tail feathers, while natural selection opposes it. This exemplifies one of the challenges in determining whether a trait is really an adaptation since one must consider the trait in the contexts of both natural and sexual selection. In addition, some traits are only adaptive for one sex of the species but both sexes possess the trait. Nipples in male humans exemplify that phenomenon. For female humans the nipple is part of the mammary structure, a specialized gland system used in the production and delivery of milk to their young. However, both males and females are built from the same body type, which is simply modified by hormones during development. Therefore, males possess nonfunctional nipples, making their nipples a nonadaptive trait.

The task of determining whether a trait in an organism is an adaptation or a nonadaptive trait presents a challenge for biologists. Humans naturally desire to explain the world around them, and it is easy to propose an adaptive explanation for why an organism looks or behaves the way it does, but one must be cautious and ask, What is the adaptive advantage of the trait? And what is the evidence for that advantage?

FURTHER READING

Cronin, Helena, and John Maynard Smith. *The Ant and the Peacock: Altruism and Sexual Selection from Darwin to Today.* New York: Cambridge University Press, 1991.

Darwin, Charles. *Voyage of the Beagle.* New York: P. F. Collier & Son, 1909.

Gould, Stephen J., and Richard C. Lewontin. "The Spandrels of San Marco and the Panglossian Paradigm." *Proceedings of the Royal Society* 205 (1979): 581–598.

(continued from page 333)

Another instigator of evolution is genetic drift, a fluctuation in allele frequency due to random chance. The likelihood of genetic drift increases in smaller populations. The bottleneck effect occurs after much of a population dies as a result of a radical change in the environmental conditions, such as caused by a natural disaster or severe climate change. The gene composition of the few individuals that survive the disaster and reproduce becomes amplified in the next few generations. The founder effect occurs when a few individuals become isolated from a larger population and establish a new population. This situation also leads to the reduction of genetic variability due to genetic drift.

Gene flow is the movement of genes into or out of a population, as when individuals migrate. They leave one population and enter another population, taking their genes with them. This increases the variability of the new population and reduces the degree of genetic differences between populations.

MACROEVOLUTION

The microevolutionary genetic changes caused by mutation, selection, nonrandom mating, genetic drift, and gene flow lead to adaptations within a population or species, but macroevolutionary changes lead to the creation of new species and new lineages. Speciation, the creation of new species, is a cornerstone of evolutionary theory and must not only explain changes to

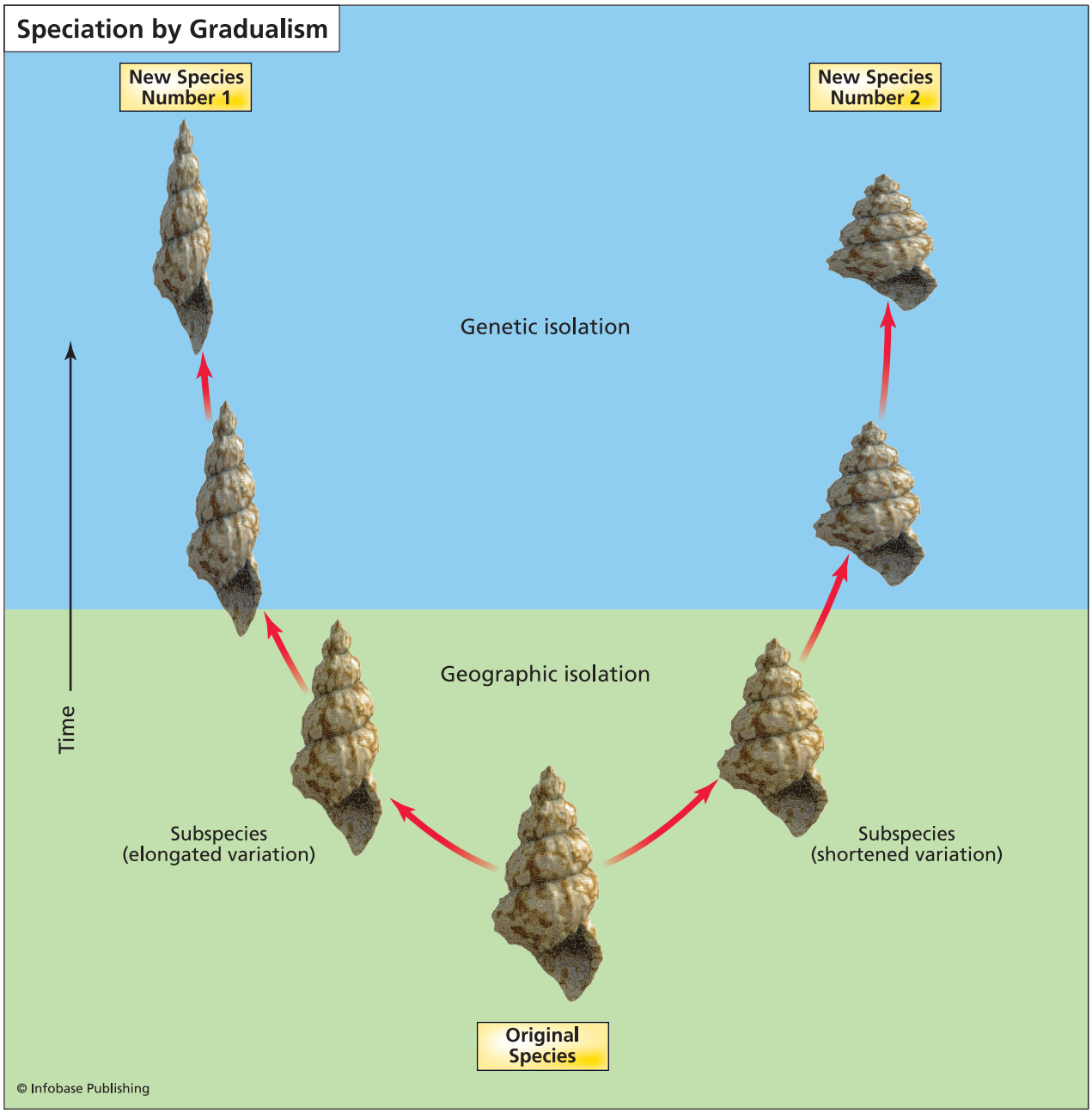
a limited gene pool, but also account for the current and past biological diversity. Biological species are defined as groups of similar organisms that have the ability to interbreed. Other definitions of the species concept exist: morphological species share similar body size, shape, and function; phylogenetic species are clusters of organisms that share parental ancestry and descent; paleontological species are groups of organisms with distinct morphological characteristics as evidenced by the fossil record; and ecological species are groups that share the same niche in a community. Since sexual reproduction plays a key role in variation and adaptation, the concept of biological species is most useful when considering the process of speciation, which can occur by two patterns: anagenesis or cladogenesis. Anagenesis occurs when genetic variation accumulates, resulting in the eventual origin of a new species. Cladogenesis occurs when heritable changes result in the branching off of a new species from a parent lineage. The parent species may or may not also change.

The development of new species depends on reproductive isolation, when barriers prevent two different species from reproducing to create viable, fertile offspring. The preventative mechanisms can be classified as either prezygotic or postzygotic. Prezygotic barriers include factors that hinder one species's sperm from fertilizing another species's eggs. When the habitats are geographically separated, such as when fish occupy two different lakes or a mountain range separates two populations of squirrels, the two populations rarely if ever encounter each other in order to mate. Some species do not mate because of temporal isolation, when the two species mate at different times of the day or year, or behavioral isolation, when differences in courtship or mating behaviors prevent two species from coming together. An attempt at mating by two species does not ensure reproductive success. Structural differences or incompatibility of sperm and egg may prevent successful fertilization. Postzygotic barriers prevent the development of the zygote into a viable, fertile offspring when fertilization occurs. The zygote might not complete development, or, if it does, the offspring or the offspring of the next generation might not be fertile. In these cases, even though new organisms are produced, without the production of viable, fertile offspring, the new phenotypes cannot continue to reproduce; thus the two original species remain reproductively separated.

Speciation can occur after geographic isolation, a process called allopatric speciation, or within the same geographic boundaries, called sympatric speciation. Neither of these processes is directed; speciation occurs as a by-product of the reproductive isolation. Allopatric speciation, which appears to be more common, may result when one population of a species migrates to a different area or when a lake recedes and forms two separate isolated lakes. Whatever the physical means for separating part of a population from the rest of the population, after that separation, the populations are reproductively isolated, and the gene pools can diverge. Then selection, mutations, drift, and other mechanisms can result in the eventual inability of the two species to interbreed, even if placed back together physically. Sympatric speciation can occur when populations geographically overlap by changes in chromosomal makeup, by nonrandom mating preferences, or by a change in the environmental conditions of the habitat. Meiosis occasionally fails to reduce the number of chromosomes during cell division, resulting in the production of a polyploidy individual, one that contains more than the typical number of sets of chromosomes. Many crop plants contain four sets of chromosomes rather than two. A polyploidy plant initially may be reproductively isolated but still able to reproduce asexually, then develop the ability to reproduce sexually in future generations.

Macroevolution results from the accumulation of numerous speciation events. Major alterations in morphology, novel adaptations, and new lineages evolve over time. The tempo by which evolution occurs varies. In gradualism, species diverge slowly through the acquisition of new adaptations that accumulate and that can affect major change over long periods. The noted geologists James Hutton and Charles Lyell greatly influenced Darwin's ideas of biological evolution, which incorporated the notion of gradualism into biological processes. Hutton first proposed that the formation of geological features could be explained by the slow but continuous action of geological processes that acted in the past and still act today. Lyell popularized this idea by generalizing it into his theory of uniformitarianism. The premise of Darwinian evolution was that the gradual accumulation of numerous variations over long periods resulted in the creation of new species.

In 1972 Stephen Jay Gould and Niles Eldredge published their hypothesis of punctuated equilibrium, suggesting that throughout evolutionary history, long periods of stasis (no change) were interrupted by short periods of rapid change. They proposed that small subpopulations on the periphery underwent major transitions, then invaded the habitat of the ancestral population. Their paper sparked considerable controversy, as biologists long held their belief in gradualism. Punctuated equilibrium explains the absence of fossil evidence for transitional forms in some lineages, since transitional fossils would not be present in the region into which new forms migrated to join their ancestral population. Transitional fossils



Reproductive isolation may be achieved by geographic separation, resulting in a gradual accumulation of slight variations, eventually leading to the formation of distinct species.

may exist but would be in the peripheral location where speciation occurred. Today biologists consider both gradualism and punctuated equilibrium as valid hypotheses. Periods of stasis may result from stable environmental conditions, during which no selection pressure acts to drive the evolution of adaptations. Intrinsic constraints such as the existence of previous adaptations may also explain the observance of long periods of stasis.

The opposing process of speciation is extinction, resulting in the loss of an entire species and playing a major role in the shaping of evolutionary history. Extinction occurs when reproductively isolated species fail to produce sufficient numbers to maintain the species. One species in the community may compete for food or resources with another species to the point of exclusion, leading one of the species to extinction. An abrupt change in environmental conditions can kill large numbers of the species, making it difficult for the species to regain its footing. In recent years, human activities have had a profound impact on many species, both directly, by activities such as hunting certain animals for food or for their products, and indirectly, such as by destroying

habitats or organisms. Mass extinctions wipe out several groups at one time, leaving many unfilled ecological niches. Surviving species serve as common ancestors that evolve by radiation to fill those niches. Two well-known mass extinctions occurred at the end of the Permian (about 250 million years ago), when plate tectonics moved all the Earth's continents together, leading to the extinction of more than 95 percent of all marine species and 70 percent of all terrestrial species, and at the Cretaceous/Tertiary boundary (about 65 million years ago), when a large asteroid impacted the Earth, leading to the extinction of the dinosaurs.

EVIDENCE FOR EVOLUTION

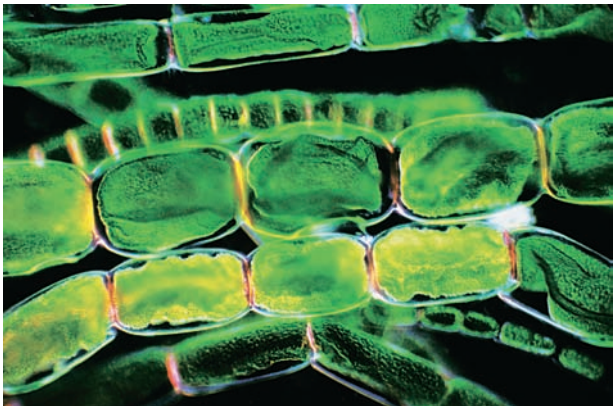
Biologists can directly test and observe microevolution through the study of population genetics. Macroevolution is inferred from numerous observations and data collected from comparative biochemical and genetic analyses, developmental biology, biogeographical studies, comparative anatomy, and fossil evidence.

Archaeans, bacteria, fungi, protists, plants, and animals carry out many common biochemical processes. The basic cellular process of respiration, by which cells extract energy from molecules to make adenosine triphosphate (ATP), includes the same stages and many similar molecules and enzymes across domains of life. Photosynthesis in plants resembles that of algae and photosynthetic bacteria, though some of the structures involved differ. Molecular processes such as deoxyribonucleic acid (DNA) replication, transcription, and translation occur by very similar mechanisms, with slight adjustments to the enzymes and machinery. These and other biochemical processes support a common ancestor that performed similar tasks.

Comparison of the DNA sequence of the genomes of different organisms provides powerful support for the theory of evolution in addition to a lot of information about lineages and the history of life. Genes that encode proteins contain codons that specify certain amino acids. Natural selection acts on phenotypic characteristics, determined by the function of the protein, which is determined by the sequence of amino acids. Evolutionary theory predicts that the amino acid sequence of proteins that play important basic roles in survival and reproduction would be conserved, that is, exhibit minimal differences between species. Furthermore, closely related species share more sequence similarity than more distantly related species. For example, the sequence similarity between protein coding regions of human and chimpanzee genes approaches 100 percent, of humans and mice 99 percent, of humans and fruit flies 60 percent, and of humans and roundworms 35 percent. Silent mutations are changes to the DNA sequence that result in a different three-nucleotide codon but encode the same amino acid; the sequence has changed, but the protein has not. Because selection acts to preserve function rather than a specific genotype, silent mutations occur more frequently than mutations that alter the amino acid sequence of a protein, which often are deleterious. Other molecular evidence supporting evolution involves introns and pseudogenes. Introns are noncoding regions found within eukaryotic genes. As expected, they contain a higher percentage of differences in nucleotide sequence than the coding regions, as do pseudogenes, genes that that have become inactivated by mutation and no longer encode functional proteins. A phylogenetic tree generated by a computer based on DNA sequence similarities looks practically identical to one generated from fossil evidence and studies of comparative anatomy.

Developmental biology also supports the theory of evolution. Closely related organisms follow similar developmental pathways. Distinguishing related organisms at early developmental stages is difficult, because modifications of the earlier stages of developmental pathways have a greater impact on an organism's overall development. Such changes have a tremendous impact on the formation of the basic body structure and therefore may be more deleterious; thus most modifications occur at later stages of development. Because of this, the developmental pattern of an organism often reflects its evolutionary history, and one can draw inferences about the ancestry of a species by comparing its development with that of other species. The earlier differences in the developmental patterns of two species emerge, the more distantly related the species are. For example, after cleavage, vertebrate embryos consist of a cluster of cells. In amphibians, the cluster of cells forms a roughly spherical ball, whereas in reptiles and mammals, the shape is more disklike. The altered shape is due to an adaptation not present in amphibians but that appeared in the ancestor common to reptiles and mammals, the amniotic egg. Reptiles, however, including the lineage that developed into birds, have heavy yolks, which cause the embryos to spread out over the top of the yolk, being more extended. Mammals and reptiles diverged, and though modern mammals no longer have yolks, the embryo retains a shape more similar to the closely related reptiles than to the more distantly related amphibians.

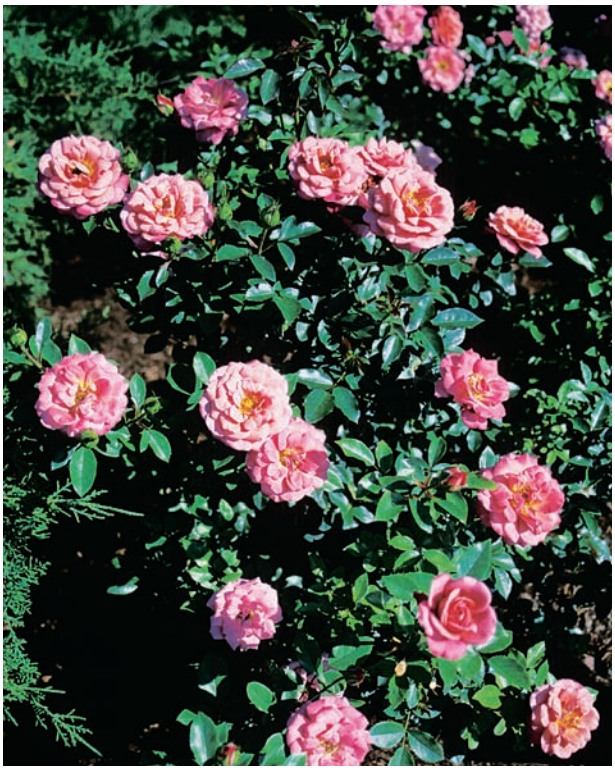
Comparative anatomy studies of both extant and past life-forms provide additional evidence for evolution. Because natural selection can only act on preexisting traits, one would expect closely related species to share many morphological features. Related species that have major structural differences must have



Algae. *(James M. Bell/Photo Researchers, Inc.)*

Gymnosperm *(John J. Mosesso/National Biological Information Infrastructure)*

Moss *(tln, 2007, used under license from Shutterstock, Inc.)*



Angiosperm *(John J. Mosesso/National Biological Information Infrastructure)*

Ferns *(National Oceanic & Atmospheric Administration/Department of Commerce)*

The fact that closely related organisms share more common anatomical features than distantly related organisms supports evolutionary theory. Of the pictured organisms, angiosperms are most closely related to gymnosperms, then ferns, then mosses, then algae.

a more distant common ancestor than species with relatively minor differences in anatomy. To illustrate this, consider some of the structural evidence used to reconstruct the evolution of angiosperms (flowering plants). Biologists believe that modern land plants, including angiosperms, originated from an ancestral form of green alga. As are plants, algae are eukaryotic, can be multicellular, and have cellulose in their cell walls. As land plants evolved, they developed unique structural adaptations that helped them survive on land, such as roots that stabilize the plant and absorb water from the soil. The earliest land plants, called bryophytes, originated about 475 million years ago, and like algae, were nonvascular. About 425 million years ago, plants began to develop specialized systems for transporting water and nutrients throughout the body of the plant, giving rise to vascular plants. Seeds first appeared approximately 360 million years ago. The anatomy of a seed includes a tough protective coating surrounding the plant embryo and nutrition to fuel early development. Gymnosperms (plants that produce naked seeds) and angiosperms diverged from an ancestral vascular seed plant. One unique morphological adaptation in angiosperms is the presence of structures called ovaries, chambers where seeds develop. As evolutionary theory predicts, angiosperm species share most anatomical features with other angiosperm species, then with gymnosperms. Comparing the morphologies of angiosperms and ferns, a type of seedless vascular plant, reveals fewer shared characteristics, and comparison with mosses, a type of bryophyte, even fewer. Though a handful of structural similarities exist between angiosperms and algae, they are mostly limited to cellular anatomy, because they are more distantly related.

The fossil record also supports evolution. Fossils include remnants, impressions, or traces of organisms from past geological ages that have been preserved in the Earth's crust. Recent or young fossils closely resemble extant species, whereas older fossils show less similarity to current life-forms. Examination of fossils sheds light on evolutionary lineages and gives information regarding the time frame for the existence of ancestral forms. Radioactive dating methods reveal when a particular layer of rock formed, and one can infer the age of the fossils found within that rock. Transitional fossils, those that demonstrate transitions between ancestral lineages and modern life-forms, have been particularly useful in constructing phylogenetic trees. A paleobiologist can follow the evolution of certain traits by studying a series of progressively younger fossils within a lineage. Geological studies give information about the conditions on Earth at the time the fossilized organisms lived, and fossils of other organisms that lived in the same region at the same time help evolutionary biologists to understand the ecological features of the organism's habitat and therefore to make sense of the evolutionary processes that led to certain adaptations.

The fact that closely related organisms are often in physical proximity to one another supports evolution, not only extant organisms, but fossil evidence of past life-forms. Biogeography is the study of the geographical distribution patterns of organisms. One would expect knowledge of geographical changes, such as continental drift, rise or fall in sea level, or island formations, to explain the development of unique adaptations of organisms found in those areas, speciation, and extinction events. For example, the isolation of a particular species of bird on an island would select for a beak shape suited to feed off that island's resources. When combined with knowledge of plate tectonics, biogeographical data reveal important patterns regarding the movement or migration between large landmasses at different periods in Earth's history. Conversely, fossil evidence of certain types of fauna or flora in a region gives information about the past climate in that area.

COMMON MISCONCEPTIONS

Many misconceptions concerning evolution are prevalent, not only in general society, but even in biological science literature. One false notion is that the process of evolution is directed toward improvement. This is only the case with respect to a given environment, the conditions of which define "better," and in relation to the other members of the same species living in that environment. An adaptation that once benefited an organism might not in a different environment, and it might constrain future developments or even cause problems under a different set of living conditions. For example, humans have wisdom teeth, a third set of molars that were important in grinding plant tissue that served as the main part of the ancestral human diet. The wisdom teeth allowed for more efficient extraction of the nutrition from the cellulose-laden plant tissue. Today, humans are not as dependent on raw plant material for nutrition and thus do not need the extra set of molars. The jaw size has decreased over time, to the point where the jaw is too small to accommodate the wisdom teeth in some people's jaws. Because of this, impaction often occurs, and extraction is sometimes necessary.

Evolution does not mold "perfect" organisms. Genetic variation results from several random mechanisms: mutations, independent assortment and crossing over during meiosis, random fertilization, and genetic drift. Natural selection acts on these, favoring the best of the available "choices," but adaptations do not appear *because* they will better

suit an organism for a particular environment. Selection is only an effect resulting from improved fitness. When variation occurs, it can only modify existing traits. Furthermore, many adaptations are helpful in one aspect, but price is paid elsewhere. For example, endotherms generate body heat through metabolic activity. While this allows one to survive in a wider range of temperatures, endotherms must find and take in more energy in the form of food to fuel their internal heating mechanisms.

Another misconception regarding evolution is summarized by the phrase *survival of the fittest*. Survival is important, but evolution only occurs when successful reproduction accompanies survival. In an evolutionary sense, fitness is a measure of the reproductive success of an organism. Characteristics that are often mistakenly associated with fitness, such as being the strongest, toughest, fastest, or largest, only improve an individual's fitness if they improve that individual's ability to pass on his or her genes to future generations. For example, taller aerial hyphae in fungi might seem advantageous, as increased height would improve dispersal of the spores, but selection limits the height because of the potential for damage to the stalk before the spores are mature and ready for dispersal.

People often associate evolution with the origin of life or, more specifically, with the origin of the human species. The biological process of evolution can only occur in living, reproducing organisms. How life first appeared is a separate issue. Some religions explain the origin of life as the work of a divine being, and some specifically believe that a supernatural being created all life, including humans, in their currently existing forms. Because creationism combines the topics of the origin of life and the creation of individual species, many people assume evolution, the scientific explanation for the existence of the diverse number of species living on the Earth, also encompasses the origin of life. Related to this is the fallacy that humans evolved from apes. Scientific evidence supports that humans and apes share a common ancestor, but extant apes differ from the original common ancestor, as do humans.

See also Darwin, Charles; Dobzhansky, Theodosius; evolutionary biology; gene expression; Gould, Stephen Jay; history of life; human evolution; origin of life; point mutations; reproduction; variation, genetic variation.

FURTHER READING

Futuyma, Douglas J. *Evolution.* Sunderland, Mass.: Sinauer, 2005.

Jastrow, Robert, ed. *The Essential Darwin.* Boston: Little, Brown, 1984.

Rice, Stanley A. *Encyclopedia of Evolution.* New York: Facts On File, 2006.

Ridley, Mark. *Evolution.* 3rd ed. Malden, Mass.: Blackwell, 2003.

Russell, Michael, Andrew Pomiankowski, George Turner, Paul Rainery, and Robin Dunbar. "Evolution: Five Big Questions." *New Scientist* 178 (2003): 32–39.

"The TalkOrigins Archive: Exploring the Creation/Evolution Controversy." Available online. URL: http://www.talkorigins.org/. Accessed January 21, 2008.

University of California Museum of Paleontology. "Understanding Evolution." Available online. URL: http://evolution.berkeley.edu. Accessed January 21, 2008.

evolutionary biology Evolutionary biology is a comprehensive branch of biology that examines the origin of species and how they change over time. The main theme or the central dogma of evolutionary biology is that natural selection shapes the genetic evolution of a population living in a particular environment. This in turn influences the anatomies, physiologies, and behavior of the species over time. The major goals of evolutionary biology are to reveal the history of life on Earth and to understand the fundamental process of evolution. Evolutionary biologists study both the diversity and the similarity among organisms in order to understand better the many different mechanisms by which different species carry out life's processes, the reasons they behave in certain ways, the methods through which they reproduce, and the origin and relatedness of different species. Whereas disciplines such as anatomy, physiology, cell biology, and molecular genetics seek to understand how organisms work, evolutionary biology attempts to explain why. Evolution, or change by descent with modification, unifies all the biological disciplines and gives them deeper significance.

Biologists began recognizing evolutionary biology as its own discipline in the late to mid-20th century as a result of modern evolutionary synthesis. Until the 19th century, society generally accepted that the world existed as it was originally created. Charles Darwin formally described his theory of evolution by natural selection in his seminal book *On the Origin of Species,* published in 1859. He suggested that natural selection caused adaptation, the modification of an organism that renders it more fit for survival and reproduction in a specific environment. Living organisms were dynamic; they changed, as did the Earth they inhabited. After the Newtonian revolution in physics of the 17th century, physical scientists sought mechanistic rather than divine or supernatural causes to explain natural phenomena. As a result of Darwin's work, biologists followed suit. In 1865

the Austrian monk Gregor Mendel published the results of his studies on inheritance in pea plants, laying the foundation for the field of classical genetics. He suggested that individuals possessed pairs of units of inheritance, now called genes. Each parent contributed one gene for each trait, and the combination of genes in the gametes was completely random. Biologists did not recognize the significance of Mendel's work until the early 1900s, when his original paper was rediscovered. Once biologists described the process of random genetic mutation and began to understand how this led to variation among individuals and populations, leaders in the emerging field of evolutionary biology began to formulate the modern evolutionary synthesis.

Also referred to as neo-Darwinism, the modern synthesis combines the concepts of genes as the units of inheritance with evolution by natural selection. Genetic variation resulting from mutation, independent assortment and crossing over during meiosis, random fertilization during sexual reproduction, and other genetic phenomena provides the material for natural selection. Those traits that consistently increase the fitness of individuals are considered adaptive, and selection will maintain them at ascending levels of hierarchal organization: the individual, the family, the population, and the species.

Because evolutionary biology is so comprehensive, scientists approach it from a variety of specialties. Evolutionary biologists often specialize in the study of one particular type of organism such as ornithology, the study of birds, or in different branches of biology such as evolutionary ecology or molecular evolution. The focus may be on mechanisms versus general patterns or over short periods (microoevolution) versus long periods (macroevolution). In an attempt to answer both proximate (how) and ultimate (why) questions regarding evolutionary adaptations, evolutionary biologists carry out their investigations both in the laboratory and in the field, and their research often relates to and incorporates knowledge from other fields. For example, population genetics is the study of allele frequencies and distribution within a population. Alleles are alternate forms of a gene. Mutations can result in the creation of new alleles that may or may not persist in the population. Natural selection is one factor that causes allele frequencies to shift when the encoded characteristic affects an organism's fitness. Ecology, the study of the interrelationships of organisms and their environments, is closely related to evolutionary biology, as the interactions with one's environment are what defines whether or not natural selection will favor a particular characteristic. Evolutionary ecology examines how relationships among species affect their evolution, adaptations associated with diet or foraging, the numbers and distributions of organisms, lengths of life spans, ways adaptations affect communities, and other ecological features of evolution. Many evolutionary biologists are paleontologists, scientists who study the history of life on Earth by examining fossil evidence preserved in the Earth's crust. Geological research also provides information about the conditions on Earth, such as climate and atmospheric composition, for evolutionary biologists investigating past life-forms. Computational biology complements lab and field studies by allowing evolutionary biologists to measure changes in the deoxyribonucleic acid (DNA) of a species and to compare DNA among species, facilitating the construction of phylogenies, or evolutionary histories. Advances in bioinformatics have led to the development of genetic algorithms that model evolution. Other subdisciplines of evolutionary biology include behavioral ecology, evolutionary physiology and morphology, human evolution, systematics, and molecular evolution.

One current topic of active research in evolutionary biology is how variations become fixed within a species. In other words, over long periods, what maintains traits that once varied? A better understanding regarding molecular control of development should help biologists solve this problem. A related issue is how the fixed traits constrain the evolution of other variations. Time, another constraint for evolutionary change, raises another puzzle. Laboratory research indicates that genetic changes can occur rapidly under strong selection pressure, but in the natural setting, evolution occurs much more slowly. Another ongoing objective is to reach a better understanding of the concept of species—variations within a species are often continuous, whereas more significant differences exist between species. What factors, other than sexual reproduction, prevent a more continuous pattern of variation between related species?

Evolutionary biology provides a useful perspective for understanding life and relationships among organisms. Past life histories explain why organisms look and function the way they do. In addition to providing a framework upon which all other life sciences can be studied, understanding evolutionary biology is important for many applications. Agriculturists employ artificial selection, the process of choosing parents with a specific characteristic for breeding purposes, to improve crops or domesticated animals. As natural selection does, artificial selection works by the same principles to increase the occurrence of a particular desirable characteristic (or decrease the occurrence of an undesirable one) with the only difference being that humans rather than the environment selects which characteristics are favorable. Also, the problem of resistance to pesticides has

increased in recent years, and knowing how to slow this evolutionary process would benefit agriculture. Similarly, bacteria have become increasingly resistant to antibiotics. Viruses mutate rapidly, leading to the development of new strains that can spread rapidly. Thus, knowledge of the evolutionary process benefits the field of medicine. Principles of evolution can also be used in conservation efforts, to try to revive endangered species and preserve biodiversity.

Evolutionary biologists work for many types of companies and institutions. Museums with biological specimen collections, academic institutions, conservation agencies, environmental consulting firms, medical research facilities, the U.S. Department of Agriculture, and resource management agencies in fields such as forestry, fisheries, and wildlife all employ evolutionary biologists.

See also Darwin, Charles; Dobzhansky, Theodosius; evolution, theory of; Gould, Stephen Jay; history of life; Mendel, Gregor; origin of life; variation, genetic variation.

FURTHER READING

Futuyma, Douglas J. *Evolution.* Sunderland, Mass.: Sinauer, 2005.

Ridley, Mark. *Evolution.* 3rd ed. Malden, Mass.: Blackwell, 2003.

excretory system The major functions of the excretory system are the removal of nitrogenous wastes from the body and osmoregulation. Nitrogenous wastes are the waste products from the metabolism of proteins and nucleic acids. The amino groups ($-NH_2$) from catabolized amino acids that are not used to synthesize new molecules form ammonia (NH_3), a highly reactive and toxic substance that dissolves in an aqueous environment to form ammonium ions (NH_4^+) and hydroxide ions (OH^-). Most animals convert the ammonia to either urea or uric acid, both of which are less toxic but still must not be allowed to build up in the body. The excretory system safely eliminates them. Osmoregulation is the regulation of osmotic pressure, the pressure associated with the diffusion of water. Animals that live in marine or freshwater habitats have special adaptations for adjusting to their environments, so their body cells do not dehydrate or uptake too much water and undergo lysis.

DIVERSITY OF EXCRETORY MECHANISMS

Animals use several different mechanisms to rid their bodies of ammonia formed during the metabolism of amino acids and nucleic acids. The availability of water in a particular habitat greatly influences the type of nitrogenous waste an organism excretes. Animals that live in aquatic environments usually excrete nitrogenous wastes as ammonia. Ammonia is very soluble and can easily diffuse through most membranes into the surrounding water, where it can quickly diffuse to tolerable levels. In aquatic invertebrates, ammonia diffuses directly into the water from all of the body cells. In fish, nitrogenous waste leaves in the form of ammonium ions that exit the body through the gills, and some leaves through the kidneys. In large water bodies, the amount is insignificant, but in a fish tank, the water must be changed to prevent buildup of ammonium ions to lethal levels.

Without access to sufficient quantities of water to dilute ammonia to tolerable levels, terrestrial animals must first convert nitrogenous waste to a less toxic form, then excrete it as a diluted solution. Because marine animals such as sharks, bony fishes, amphibians, and turtles already lose large quantities of water via osmosis, many also do not have sufficient amounts of water to dilute ammonia to tolerable levels and utilize the same strategy. Vertebrate livers convert ammonia to urea by combining it with carbon dioxide. Because urea is 100,000 times less toxic than ammonia, the circulatory system can safely transport and store it prior to elimination without requiring large amounts of water for dilution. The disadvantage of excreting nitrogenous wastes as urea is that the conversion is an energetically expensive process.

The conversion of ammonia to uric acid requires even more energy input; however, animals such as snails, insects, reptiles, and birds that do not have access to sufficient water excrete nitrogenous wastes in this form. Uric acid is insoluble in water, and thus the animals can excrete it as a paste without having to sacrifice much water.

Most systems eliminate nitrogenous waste products as urine, a multistep process involving filtration, reabsorption, secretion, and excretion. The first step is filtration, the passage of fluids through selectively permeable membranes resulting in the formation of a filtrate that consists of water, salts, glucose, amino acids, nitrogenous waste products, and other toxins. The body then selectively reabsorbs valuable nutrients including some of the salts, glucose, and amino acids by active transport. Excess salts and other substances are secreted into the filtrate, the water content of the filtrate is adjusted to maintain the osmotic balance of the bodily fluids, and the urine exits the body.

The mechanisms by which organisms with different anatomies accomplish urine production and excretion differ among animal groups and their various habitats. Members of the phylum Platyhelminthes (flatworms such as planaria) have simple excretory systems composed of protonephridia that function

in osmoregulation and the removal of nitrogenous wastes in freshwater species. Metabolic waste products diffuse either out of the body directly or into a gastrovascular cavity and then out of a mouth. Protonephridia consist of networks of branched tubules that terminate in flame bulbs. Vibrating cilia that project from the end of the flame cell into the tubule seem to flicker like candle flames, hence the name. The beating of cilia inside the tube creates a current that draws body fluids into the tubules and sends them out through a nephridiopore in the body wall. The perforations through which fluids enter are too small to permit the passage of large molecules such as proteins. The tubules reabsorb important solutes before excreting the urine into the external environment. Rotifers, some annelids, mollusk larvae, and lancelets also have protonephridia.

Most annelids (segmented worms such as earthworms) have excretory organs called metanephridia. Each body segment contains a pair of metanephridia that are surrounded by capillaries. Cilia near the opening of a metanephridium move fluid from the coelom through a structure called the nephrostome into the tubule. As fluid passes through the metanephridium, solutes are reabsorbed into the blood capillaries. Nitrogenous wastes continue to move through the metanephridium, eventually leaving the body through a nephridiopore. Organisms that live in hypotonic (low-solute) environments excrete very dilute urine to rid the body of excess water taken in by osmosis.

Some mollusks and some arthropods also have metanephridia, but most insects and terrestrial arthropods have Malpighian tubes that function in nitrogenous waste removal and osmoregulation. These thin fingerlike structures extend outward from the digestive tract and are in direct contact with hemolymph, the fluid that circulates in an open circulatory system. Cells of the Malpighian tubes transport substances including nitrogenous waste products, salt, and water from the hemolymph into the lumen of the tubes. The contents of the Malpighian tubes drain into the digestive tract and then the rectum, where the valuable solutes are reabsorbed. Water follows the solutes by osmosis, but the insoluble uric acid is left behind and eliminated with feces. This excretion strategy conserves water very efficiently and was instrumental in the adaptation of insects to land and dry environments.

ANATOMY OF THE MAMMALIAN EXCRETORY SYSTEM

The kidneys are the main excretory organs in vertebrate animals. In addition to excreting wastes, they produce some hormones and help regulate water balance, osmolarity, ion balance, and pH levels. Occurring in pairs and situated in the lower back, the mammalian kidneys contain complex systems of tubules and numerous capillaries. Each human kidney has about 1 million nephrons, the functional units of the kidney. After the kidneys filter the blood to remove wastes and excess water and salts, ureters transport the urine to the bladder for storage. When the bladder is full, the urine passes through the urethra to the exterior of the body. In females the urethra exits near the vaginal opening, and in males the penis contains the urethra.

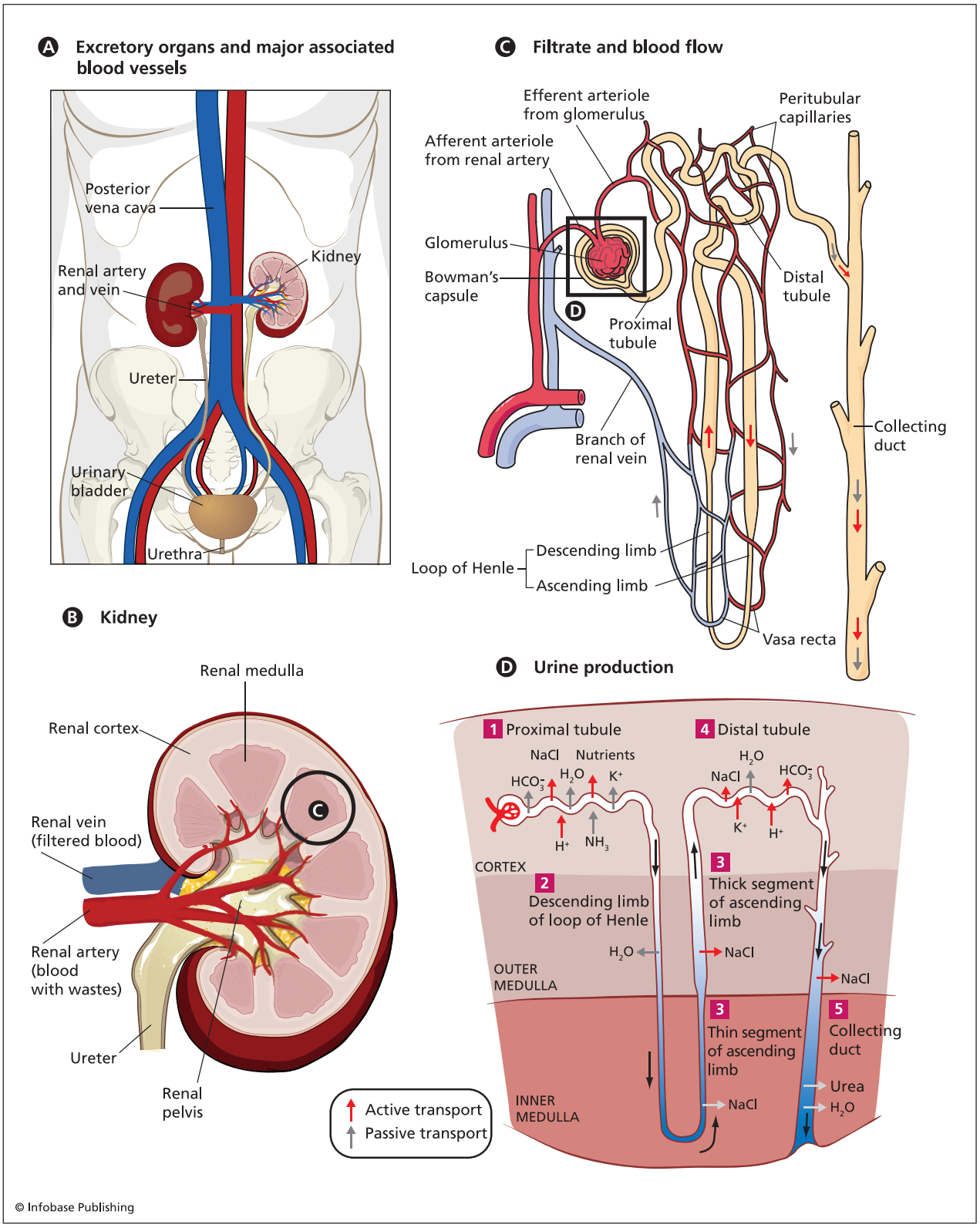
The mammalian kidneys consist of an outer renal cortex that surrounds the outer and inner renal medulla. The nephron consists of several distinct regions that perform different functions in the production of urine. The Bowman's capsule envelopes the glomerulus, the site of filtration. In mammals and birds, most nephrons (cortical nephrons) exist in the cortex and do not extend into the medulla. In about 20 percent of nephrons (juxtamedullary nephrons), the proximal tubule carries the filtrate from the cortex into the descending limb of the loop of Henle situated in the inner medulla. The ascending limb of the loop of Henle carries the filtrate through the medulla back into the distal tubule, which drains into a collecting duct. Nephrons with this structure allow for the production of concentrated urine to conserve water and are only found in mammals and birds. Cortical nephrons have much shorter loops of Henle, and nephrons of other vertebrates do not have loops of Henle at all. Several nephrons empty into a single collecting duct that leads to the renal pelvis and then the ureter.

Renal arteries supply blood to each kidney, and renal veins carry away the filtered blood. In the cortex, renal arteries diverge into arterioles that branch out and associate closely with each individual nephron. As the blood enters a nephron through an afferent arteriole, it first flows into a ball of capillaries called the glomerulus. As the blood leaves the glomerulus, the capillaries converge into an efferent arteriole, which branches out again into peritubular capillaries that surround the tubules of the nephron and into the vasa recta that extends down into the inner medulla region. The blood vessels are bathed in interstitial fluid, as are the nephron tubules.

NEPHRON FUNCTION

The high pressure of blood in the afferent arterioles forces fluid through the porous capillaries of the glomerulus and across a filtration membrane into the lumen of the Bowman's capsule. The filtrate in the Bowman's capsule contains water, salts, bicarbonate ions (HCO_3^-), hydrogen ions (H^+), urea, glucose, amino acids, and certain drugs. Blood cells and large molecules such as proteins cannot penetrate and remain in the blood vessels. Reabsorption and secretion both occur through a transport epithelium

in the proximal tubule via a combination of active transport, cotransport, facilitated diffusion, and simple diffusion. Transport epithelial cells help maintain blood pH levels by secreting excess H^+ into the filtrate in addition to synthesizing ammonia, which passively diffuses into the filtrate, preventing it from becoming



The kidneys are the main excretory organ in humans. The functional unit of the kidney is the nephron, a long tubule that performs the functions of filtration, secretion, and reabsorption in urine production.

too acidic. Sodium ions (Na^+) and chloride ions (Cl^-) from the filtrate diffuse into the transport epithelial cells, which actively transport the Na^+ into the interstitial fluid. Cl^- ions follow to balance the charge gradient, and water follows by osmosis. The salts and water then move back into blood circulation via the peritubular capillaries. The proximal tubule is also the site for reabsoprtion of HCO_3^-, potassium ions (K^+), and nutrients such as sugars and amino acids. Some drugs or other toxins pass from the peritubular capillaries to the interstitial fluid, and the transport epithelium secretes them into the urine.

As the filtrate travels down the descending loop of Henle, reabsoprtion of water continues. The concentration of solutes in the interstitial fluid increases gradually from the cortex to the inner medulla of the kidney. The transport epithelium of the descending loop of Henle is not permeable to salts; thus only water moves out, resulting in significant concentration of the filtrate. In contrast, the ascending loop of Henle is permeable to salt but not water. In the lower portion of the ascending loop, salts diffuse out of the filtrate into the interstitial fluid. This helps maintain the solute concentration gradient between the cortex and the medulla. By the time the filtrate reaches the upper portion of the ascending loop, the transport epithelium must actively transport salts into the interstitial fluid. Because the ascending loop of Henle is not permeable to water, the result is dilution of the filtrate. The distal loop plays an important role in regulating pH levels and salt concentrations. The transport epithelium secretes H^+ and K^+ into the filtrate and reabsorbs HCO_3^-, Na^+, and Cl^-. Water moves out by osmosis as the filtrate travels through the distal tube and down the collecting duct, concentrating the filtrate once again. Under hormonal influences, the transport epithelium of the collecting duct actively reabsorbs varying amounts of Na^+ and Cl^-, depending on how hydrated or dehydrated a person is. As the filtrate approaches the end of the collecting duct, the transport epithelium becomes permeable to urea. Though most urea is destined for excretion, its concentration is so high in the filtrate at this point that some diffuses out, contributing to the high solute concentration in the medulla. The high levels of solute in the medulla allow mammals to conserve water very efficiently, resulting in the production of hyperosmotic urine, urine that is much more concentrated than body fluids. Urine drains from the collecting duct, into the renal pelvis, and down the ureters to the bladder.

The average human bladder can hold approximately one pint (close to 500 mL) of urine. As the volume increases, the bladder wall stretches, resulting in micturition, or emptying of the bladder. Stretch receptors communicate the information that the bladder is full to the nervous system, which responds by sending signals that relax the skeletal muscles around the urinary sphincter, opening it and allowing the fluid to drain out. Contractions of smooth muscle surrounding the bladder help force the urine out. In infants, micturition is a reflex, but as one grows older, the ability to inhibit micturition voluntarily develops.

See also ANATOMY; ANIMAL FORM; BIOLOGICAL MEMBRANES; CIRCULATORY SYSTEM; HOMEOSTASIS; INVERTEBRATES; PHYSIOLOGY; VERTEBRATES.

FURTHER READING

Alexander, Ivy L., ed. *Urinary Tract and Kidney Diseases and Disorders Sourcebook*. 2nd ed. Detroit: Omnigraphics, 2005.

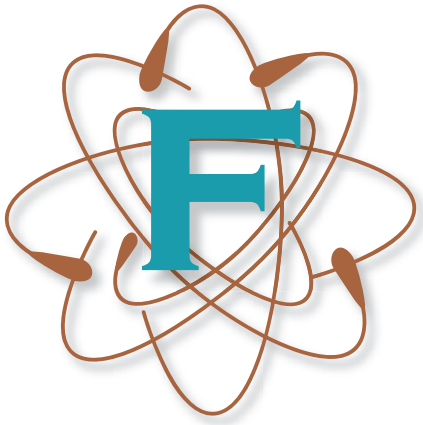
Field, Michael J., Carol A. Pollock, and David C. Harris. *The Renal System*. London: Churchill Livingstone, 2001.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 2. Detroit: U*X*L, 2000.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Sherwood, Lauralee, Hillar Klandorf, and Paul Yancey. *Animal Physiology: From Genes to Organisms*. Belmont, Calif.: Thomson/Brooks/Cole, 2005.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.



Fleming, Sir Alexander (1881–1955) Scottish *Bacteriologist* Throughout history, sudden outbreaks of disease periodically decimated populations. Though the 19th century introduced the discovery of vaccines to prevent certain illnesses caused by infectious microorganisms, medicine had no way to fight the plague, influenza, smallpox, gonorrhea, tuberculosis, malaria, yellow fever, and other infectious diseases once someone was already sick until a Scottish-born bacteriologist, Sir Alexander Fleming, discovered the antibacterial properties of a substance produced by the mold *Penicillium notatum*. This breakthrough led to a revolution in medicine, stimulating the discovery of several lifesaving antimicrobial compounds that can be used to destroy pathogenic (disease-causing) microbes after they have infected a host.

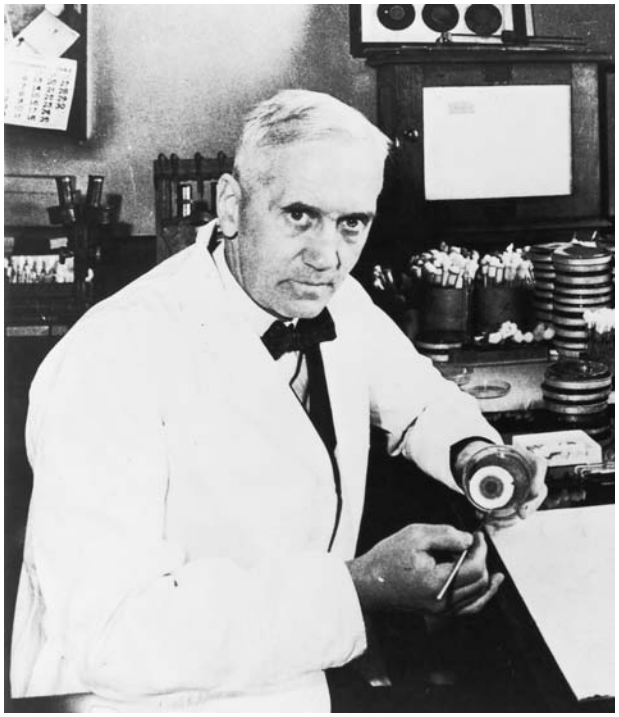
CHILDHOOD AND MEDICAL TRAINING

Alexander Fleming was born to Hugh Fleming and his second wife, Grace Morton Fleming, on August 6, 1881, in Lochfield, in Ayrshire, Scotland. Nicknamed Alec, he was his father's seventh of eight children and grew up on an 800-acre farm, where he spent his younger days tending the family's sheep, playing in the barns, and fishing in the river. Surrounded by nature, he developed keen observational skills while learning to hunt for peewit eggs and rabbits with his bare hands. He started attending school when he was five, and his father died when he was seven.

At age 13 Alec went to London to live with one of his older brothers, who was an eye doctor. Alec took business classes at the Regent Street Polytechnic Institute for two years and by age 16 had passed all of his exams. Not particularly interested in any specific career, he took a job as a junior clerk in a shipping office, where his duties included hand copying records, bookkeeping, and keeping track of all the cargo and passengers on the ships. In 1900 he joined the London Scottish Regiment, but the Boer War between the United Kingdom and the southern African colonies ended before he was sent overseas. For enjoyment, Alec played on the regiment's water polo team and entered shooting competitions, which he often won. He remained a member of the regiment until 1914. His shipping job bored him, so when his uncle left him an inheritance, he decided to spend it studying medicine, as his brother had.

Fleming was almost 20, older than most embarking on the path to a medical career, but he hired a private tutor and, in less than one year, passed his exams ahead of all the other British candidates. In October 1901 Fleming entered St. Mary's Hospital Medical School on a scholarship. He chose St. Mary's over the other 11 London medical schools because he had once played against them in water polo. He became enthralled by his studies of anatomy and physiology and excelled with minimal effort while also participating in the school's water polo team, drama society, debate team, and rifle club. In 1906 Fleming received his Conjoint Board Diploma, which granted him permission to practice general medicine, but at the suggestion of one of his teammates, he joined the inoculation department as a junior assistant so he would be eligible to participate as a school team member in an upcoming national rifle competition.

The inoculation department was headed by Almroth Wright, a staunch believer in vaccine therapy. Wright had been influenced by the French chemist



Sir Alexander Fleming is famous for his studies on the antibacterial properties of penicillin and lysozyme. *(Library of Congress)*

Louis Pasteur's work on vaccines. Vaccines stimulate the body's immune system to produce antibodies against disease-causing microbes by the introduction of weakened or killed microbes or parts of the microbes into the body. Antibodies are proteins produced by white blood cells that defend against or help prevent diseases. Some vaccines called toxoids stimulate production of antibodies against a poison produced by a microorganism. Wright was convinced that all infectious diseases could be cured by antibodies either made by the patient or by the introduction of serum from another person. His department extensively examined how vaccinations worked and studied phagocytes. Found in body tissues and fluids, phagocytes are cells that are capable of ingesting and destroying harmful substances or disease-causing microbes. In 1908 Fleming passed his final medical examinations and was awarded the Gold Medal of the University of London. Though Fleming was interested in department's research, he decided to take the exam necessary to specialize in surgery. He passed the surgical examinations in 1909 but continued to work for Wright and developed a good reputation.

One of Fleming's earliest medical accomplishments was the development of a diagnostic test for syphilis, a potentially fatal sexually transmitted disease. The German bacteriologist Paul Ehrlich identified a compound effective in treating syphilis, salvarsan, in 1910. Fleming became an expert in intravenously administering salvarsan to treat syphilis. Intravenous injections were uncommon at the time, and many doctors did not know how to give them.

In 1914 Fleming and several other members of Wright's team joined the Royal Army Medical Corps and established a research center in Boulogne, France. He had to pass through the patient wards on the way to his laboratory, and the surgeons often showed him severe cases of septicemia, tetanus, and other infectious diseases. Fleming was disturbed by the astounding number of infections suffered by wounded soldiers and by the apparent ineffectiveness of the antiseptics used to treat them. He was particularly horrified by the deadly gas gangrene, which caused high fevers, brownish pus at the infection site, and the production of gas below the skin. In these cases, amputation of the infected limbs was necessary in order to save the patient's life. Fleming researched the effect of antiseptics such as carbolic acid, boric acid, and hydrogen peroxide on wounds and found that in the case of deep wounds, they actually did more harm than good because the chemicals killed the white blood cells that naturally fight off infection and did not penetrate deep enough into the wound to be effective. Wright and Fleming encouraged rinsing wounds with only saline solution and letting the body combat bacterial infection naturally, but most ignored their recommendations.

While on leave in 1915, Fleming married an Irish nurse, Sarah Marion McElroy, whom he called Sareen. In 1921 they bought a country house that they called The Dhoon, where they spent weekends together. In 1924 they had one cherished son, Robert, who later became a physician. Sareen died in 1949, and in 1953 Fleming married a Greek bacteriologist, Dr. Amalia Coutsouris-Voureka, who had begun to work at St. Mary's in 1946.

BACTERIOLOGY AND LYSOZYME RESEARCH

In January 1918 Fleming returned to London and to his studies of bacteriology. His experiences treating wounded soldiers with severe infections motivated him to search for an effective antiseptic. The British physician Joseph Lister founded antiseptic surgery, in which the environment, medical instruments, and the surgeon's hands were sterilized before surgery. This practice required instruments to be soaked in carbolic acid to kill any contaminating microorganisms and greatly reduced infection rates after surgeries; however, the acid damaged living tissues. Fleming wanted to find an antiseptic that would not cause harm to the patient's tissues but would kill potential microbial invaders. Meanwhile, Wright appointed Fleming assistant director of the inocula-

tion department, which was renamed the Pathology and Research Department.

Eager to obtain cultures of a wide variety of bacteria, Fleming collected many unusual specimens and grew them in the laboratory in petri dishes containing artificial media. One interesting sample was obtained from his nasal mucus, collected during a recent cold. The plate had many golden yellow colonies of the bacteria that he later named *Micrococcus lysodeikticus*. In 1921, while preparing to dispose of the culture dish, he examined it once more and noticed the bacterial colonies immediately surrounding the mucus itself appeared dissolved. He wondered whether the mucus contained an antibacterial agent.

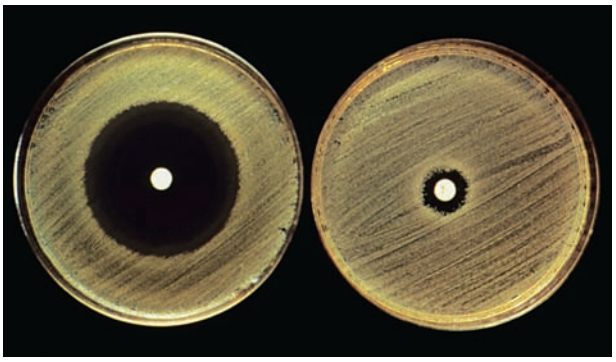
Further investigation showed that mucus did indeed contain a substance that naturally killed bacteria. He named it *lysozyme,* since it lysed, or broke open, the bacterial cells. Lysozyme acts by punching holes in the cell wall that encircles bacterial cells. With the integrity of the cell disrupted, the cellular contents leaked out and the cells perished. After examining many other bodily fluids, Fleming also found lysozyme in tears (collected by squeezing lemon juice into his own eyes and the eyes of others), saliva, blood serum, pus, and egg whites. Lysozyme obviously was not harmful to the host's living tissues, or to the host's own immune system components, unlike chemical antiseptics. The substance was harmful only to the invading bacteria, acting as a first line of defense to prevent them from colonizing the body. He tested microorganisms that were virulent to different degrees and, not surprisingly, found that the microbes that were most susceptible to lysozyme were the least dangerous. Fleming thought this made sense, since if they were not susceptible to lysozyme, they would be more likely to invade the body and cause infection. Fleming was not successful in preparing concentrated extracts of lysozyme, but later others were able to crystallize the bacteriolytic enzyme, which has become an important tool for microbiologists.

DISCOVERY OF PENICILLIN

Fleming was noted for his procrastination in cleaning up his old culture dishes. This habit resulted in one of the most important medical breakthroughs of the 20th century. A plate with staphylococci had become contaminated with a fuzzy-looking mold later identified as *Penicillium notatum*. Staphylococci are spherical bacteria that grow in clusters that resemble bunches of grapes and may cause infections by entering breaks in the skin, leading to pimples, boils, or a skin disease called impetigo. Though bacteria were found throughout the plate, there were no colonies in the immediate vicinity of the mold; instead, there was a clear halo surrounding the mold growth. Fleming recognized what none of his colleagues noticed when he showed them the petri dish—that the mold must have been secreting an antibacterial substance. He named it *penicillin*.

He systematically investigated his observation, first by culturing the mold, growing it in the lab, and then trying to duplicate the bactericidal action. He inoculated a plate of agar with the mold at the center and then streaked different bacterial cultures like radii of the circular petri dish. After incubation, some bacteria grew near the mold, while others did not. The mold thrived on a broth containing meat extract, which he poured into sterile bottles and then inoculated with tiny pieces of the mold. He filtered some of the broth in which the mold grew and applied it to plates containing healthy staphylococcal cultures. He performed a series of dilutions to determine the strength necessary to destroy the bacteria and looked for negative effects on living tissue. To see whether penicillin was effective in killing other types of bacteria, he added it to plates of several other species. Penicillin proved effective against bacteria that caused pneumonia, syphilis, gonorrhea, diphtheria, and scarlet fever, but not against microorganisms that caused influenza, whooping cough, typhoid, dysentery, or other intestinal infections. Injection of penicillin into mice and rabbits caused no ill effects.

In 1929 Fleming, now a professor of bacteriology at the University of London, reported that penicillin did not harm white blood cells and elicited no negative responses in laboratory animals. In "On the Antibacterial Action of Cultures of a *Penicillium,* with Special Reference to Their Use in the Isolation of *B. influenzae,*" published in the *British Journal of Experimental Pathology,* Fleming described not only the possible use of penicillin as an injectable antiseptic agent, but



Penicillin diffuses from the center pellet of the *Staphylococcus aureus* cultures. The antibiotic inhibits growth of the culture on the left, while the bacterial strain shown on the right demonstrates resistance to the drug, as evidenced by a smaller zone of inhibition. *(John Durham/Photo Researchers, Inc.)*

also a unique application of penicillin, namely, in the establishment of pure cultures of other bacteria. Because penicillin was very effective against some bacteria and not at all against others, it could be added to the media used to grow the penicillin-resistant cultures to ensure pure cultures, that is, cultures containing only the desired bacteria.

Fleming and his assistants tried to develop a method for the extraction and concentration of penicillin so they could attempt clinical trials; however, it kept losing its potency. By 1932 Fleming stopped actively researching penicillin, but he maintained a culture of *Penicillium* in the laboratory at all times and generously provided specimens to scientists who requested it. In 1935 the German physician Gerhard Domagk announced the identification of the sulfonamide prontosil as a cure for systemic streptococcal infections, stimulating others to search for additional "magic bullets." Fleming switched his focus to the antibacterial properties of sulfonamides, a new class of chemically related drugs found to be effective in preventing the multiplication of some types of bacteria, but he maintained his hope that penicillin would one day be an effective treatment for fatal bacterial infections.

FLOREY AND CHAIN'S WORK

The Australian pathologist Howard Florey and the German biochemist Ernst Chain at Oxford University spent time in the late 1930s characterizing lysozyme. In researching antibacterial substances, they came across Fleming's journal article describing penicillin. By 1940 they successfully developed a procedure involving lyophilization (freeze-drying) and dissolution in methanol for purifying stable penicillin in quantities large enough to test on animals. After a few preliminary investigations, Florey injected 50 white mice with lethal doses of virulent streptococci. He then injected penicillin into 25 of those mice at three-hour intervals for two days and nights; the other 25 were not treated. Within 16 hours, the 25 untreated mice were all dead, but 24 of the treated mice survived. These results were published in "Penicillin as a Chemotherapeutic Agent" in *Lancet* in 1940. Fleming was thrilled when he read the article and soon traveled to Oxford, where the researchers congratulated one another and exchanged information.

The Oxford team performed a series of experiments to determine the best mode of treatment and the optimal dosages. The next step was a human trial, but they needed 3,000 times more penicillin than they used in the mice trial; thus they geared up their production methods. They converted every bit of free space to grow massive amounts of *Penicillium,* creating a makeshift factory in the pathology building and using a variety of everyday objects to extract and concentrate the penicillin juice until they had enough.

Their first patient was Albert Alexander, a policeman who had scratched his face on a rosebush and, as a result, developed severe staphylococcal infections covering his head and potentially fatal blood poisoning. Sulfonamides were not effective, and without treatment he was sure to die. On February 12, 1941, the team began a series of injections, and the patient showed marked improvement. Unfortunately, the bacteria began multiplying again, and this time they did not have any more penicillin to give him, and he died. Slowly, they produced enough penicillin to test on several more humans, and in all cases it proved effective.

In 1942 Harry Lambert, the director of an optical lens–making business owned by Fleming's older brothers, was dying at St. Mary's Hospital of meningitis, an infection of the membranes that surround the brain and spinal cord. Fleming removed a sample of fluid from Lambert's spinal column and examined it under the microscope. He found streptococci. Though doctors had administered sulfonamides to Lambert, he showed no improvement. Fleming wrote to Florey to ask for some penicillin, which Florey provided. Every three hours for one day, Fleming injected penicillin into Lambert, and for the first time in six weeks, Lambert's body temperature returned to normal. When Lambert became feverish again the following week, Fleming took another sample of fluid from his spinal column and once again found streptococci. After consulting Florey, Fleming decided to inject penicillin directly into Lambert's spinal column. After several injections, Lambert miraculously improved and within one month he was completely recovered.

The Oxford team knew they needed help synthesizing the large quantities of penicillin necessary to treat humans but could not find anybody in Britain willing to help them. An agricultural research laboratory in Peoria, Illinois, agreed to grow the mold and extract large quantities of penicillin; however, once the word spread about the miraculous infection-fighting properties the mold juice possessed, all of the product was devoted to treating war casualties. In 1944 production had finally ramped enough for civilians to benefit from treatment with penicillin.

By this time, news of the wonder drug was widespread and Fleming became famous. Several large pharmaceutical firms began researching penicillin production methods. The most respected scientific society in Great Britain, the Royal Society of London, elected Alexander Fleming to membership in 1943, and the following year he was knighted. In 1945 Fleming was awarded the Nobel Prize in physiology or medicine, shared with Chain and Florey, for

the discovery of penicillin and its curative effect in various infectious diseases. Other numerous honors and medals were showered upon Fleming such as the Moxon Medal from the Royal College of Physicians (1945), the Honorary Gold Medal of the Royal College of Surgeons (1946), the Gold Medal of the Royal Society of Medicine (1947), and the Medal for Merit from the United States of America (1947). He spent much of his time traveling around the world making appearances, giving speeches, and accepting almost 30 honorary degrees from European and American universities.

FLEMING'S ACCOMPLISHMENTS

Sir Alexander Fleming died after a heart attack on March 11, 1955, in London and was buried in St. Paul's Cathedral. He had never received any money for his hard work and discovery; he donated it all to St. Mary's for research. Today, the Wright-Fleming Institute at the Imperial College London, named in tribute to Fleming and his mentor, houses scientists dedicated to researching human bacterial and viral infections. Often serendipity is credited for the discovery that launched a medical revolution, but one must remember the man who took notice, believed, and pursued what he observed.

Fleming devoted his life to helping humanity by trying to figure out how to fight infections. He did not invent penicillin and, in fact, was not the first to discover it, as others had earlier noticed that mold had some antibacterial properties, but Fleming was the first to recognize its broad significance and to draw attention to it. Since Fleming discovered the antibiotic penicillin, hundreds of other chemicals naturally produced by microorganisms have been found to have antibacterial properties. Interestingly, Fleming prophetically warned against the improper use of antibiotics, predicting it would lead to antibiotic resistance. Insufficient doses, failure to complete the entire course of an antibiotic treatment, and the widespread use of antibiotics to promote growth in cattle and farm animals, as well as the use of antibiotics to treat colds or other nonbacterial maladies, have resulted in a sharp increase in the number of strains of bacteria that are resistant to a wide variety of antibiotics.

See also ANTIMICROBIAL DRUGS; BACTERIA (EUBACTERIA); MICROBIOLOGY.

FURTHER READING

Horvitz, Leslie Alan. *Eureka! Scientific Breakthroughs That Changed the World*. New York: John Wiley & Sons, 2002.

Macfarlane, Gwyn. *Alexander Fleming: The Man and the Myth*. Cambridge, Mass.: Harvard University Press, 1984.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1945." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1945/. Accessed January 21, 2008.

forensic biology

Forensic science is the application of the natural sciences and scientific principles to legal matters. Most often this involves the collection and scientific analysis of physical evidence, as from a crime scene. Forensic science encompasses numerous subdisciplines including forensic biology, forensic chemistry, forensic pathology, forensic toxicology, forensic odontology (dentistry), forensic anthropology, and forensic physics. Forensic biology refers to the application of biological knowledge and methods to matters of the law.

Forensic investigators work as part of a team. A forensic biologist handles the biological evidence that might aid in the legal investigation. A forensic chemist analyzes and interprets nonbiological evidence, such as flammable substances in arson cases, soil composition, or flecks of paint. Someone trained in forensic physics can gain useful information from blood splatter patterns and ballistics finding about how a crime was committed. Forensic drug chemists and toxicologists look for signs pointing to the involvement of alcohol or drugs such as marijuana, cocaine, or heroin by examining blood and urine. Team members who specialize in forensic pathology are also trained to recognize evidence of drug use or poisoning as well as other information gathered from body tissues that can help determine whether the death was caused by an accident, suicide, or homicide. A forensic pathologist can establish how much time has passed since death by changes in body temperature, rigor mortis (the stiffening of muscles following death), liver mortis (the pooling of blood in blood vessels), the degree of decomposition, and the degree to which stomach contents have been digested. Forensic anthropology is a branch of physical anthropology, the study of the physical evolution and structure of human beings. A forensic anthropologist inspects and takes measurements of skeletal remains in order to determine the gender, age, race, and stature of the victim. Because experiences or lifestyle habits can also leave characteristic marks or signs on bones or bone fragments, the forensic anthropologist may be able to draw conclusions about whether or not the person had previously broken any bones, was active or sedentary, made repetitive movements that might be associated with a particular occupation, or was malnourished. The anthropologist can make deductions concerning the time of death, the cause of death, and movements that occurred post mortem as well as perform facial

reconstructions from a skull that may help others recognize a missing person. Trace specialists analyze pieces of evidence that are by nature very small in size or quantity, such as carpet or clothing fibers, a strand of animal hair, or a sliver of wood. Forensic scientists collaborate with other investigators trained to analyze other forms of evidence including computers, documents, explosives, firearms, indicators of arson, or impressions or markings made by shoes, tires, or tools. Experts in criminal psychology, personality profiling, and psychophysical detection of deception (use of lie detector tests) also help solve crimes.

Forensic biologists work both at the crime scene and in a crime lab. At the crime scene they collect biological evidence and sometimes perform simple tests, such as determining whether a stain contains blood or not, in order to make sure they obtain samples from anything present that might assist in solving the case. They must follow carefully designed procedures to ensure samples of evidence do not become contaminated with other organic substances or chemicals that might damage the biological material. Storage in plastic bags or sterile containers helps protect and preserve the biological samples, which might include insects, plants, bodily fluids, hairs, or clothing that belonged to the victim or the person who committed the crime. After returning to the lab, forensic biologists process and analyze the samples, prepare a written report summarizing the findings, and occasionally appear in court to testify as impartial expert witnesses in criminal or civil matters.

Forensic botany is a specialty of forensic biology in which the scientist uses knowledge of not only plants as the name suggests but also fungi, algae, and other unicellular plantlike microorganisms to aid an investigation. Biological samples retrieved from the victim or the crime scene provide valuable information. Botanical, fungal, and microscopic specimens can indicate a particular geographic location determined through their knowledge of where the species normally grows or the season of death, indicated by the presence or absence of different structures such as spores or flowers. Forensic botanists also assist in cases that involve drugs or poisons, since these substances are plant products.

Another branch of forensic biology is forensic entomology, the application of knowledge regarding insects to legal investigations. In civil cases, expertise in forensic entomology can aid the prosecution of a property management company for an insect infestation or a food distributor for contamination. In criminal cases, entomologists can help establish the time and location of a crime by applying knowledge of insect life cycles and the succession of types of organisms involved in decomposition of a body post mortem. Female flies lay eggs on an exposed corpse within minutes of death. The eggs hatch into maggots that pass through three stages called instars before maturing into adult flies. From specimens taken from the body and knowledge of the physical conditions where the body was found, forensic entomologists can accurately estimate how long the body was in a particular location. As a body decomposes, other insects such as ants, wasps, and beetles feed off it and the maggots. Information concerning the particular species and stages of development of insects present on a body may indicate postmortem movement of the body or reveal information about the conditions (e.g., temperature, moisture) preceding death. In unusual cases, contents of the stomach of a blood-sucking insect or chemicals present in a maggot that has been feeding off a decomposing body can provide biological evidence about the crime.

Scavengers are animals that eat the remains of other organisms. Vertebrate zoologists who study these types of animals and their behavior are valuable to investigations in which the remains are scattered. Familiarity with the relatively consistent patterns of soft tissue modification and disarticulation and the effects of weather on these patterns allows one to estimate the length of time the body has been exposed. Typically, scavengers remove only soft tissue within the first few weeks, followed by destruction of the abdomen, internal organs, and dismemberment of the arms, and then legs. After several months, scavengers will have disarticulated the entire skeleton, saving the vertebral column for last and leaving only the skull behind. Because animals drag bones away from bodies in predictable patterns, knowledge of animal behavior is helpful. Rodents, cats, and dogs leave distinct tooth markings on the surface of the bones. More insects will be present in warmer temperatures and discourage scavengers from feeding off the corpse. In contrast, fewer insects will be present in colder temperatures, and therefore there will be more tissue damage and animals will start removing the bones within a shorter time span. Because flies cannot lay eggs on a submerged body, water slows the process, as do any coverings, clothing, or partial burial. Aquatic creatures such as crabs, sea louses, and some fish feed on decaying corpses.

Different forms of biological specimens (tissue, blood, bone, hair, saliva, semen, or urine) can link a person to another person, to a piece of physical evidence, or to a specific physical location. The investigator must carefully follow detailed protocols for collecting, documenting, storing, and preserving the sample if it is to be used as legal evidence. Microscopic analysis of a single strand of hair can reveal whether or not it was taken from a human or an animal, whether the person had certain diseases, and which part of the body it came from. Historically, the

analysis of blood samples involved determination of the blood type based on different proteins located on the surface of the red blood cells, the identification of different variants of isozymes found in the serum, or the presence of other specific antigenic markers. Since the 1980s, however, forensic biologists routinely analyze any deoxyribonucleic acid (DNA) left behind in tissue samples, bodily fluids, or hair roots. Because the nucleus is the cellular compartment that contains the DNA, biological samples that do not contain nucleated cells, such as sweat, tears, or hair shafts without attached root cells, cannot be analyzed by standard methods. DNA profiling (also called DNA fingerprinting or DNA typing) enables the specific identification of individuals because of the uniqueness of an individual's DNA. During sample collection, the investigator must use prescribed precautionary measures to ensure the biological material does not become contaminated, as the techniques used to analyze the DNA are very sensitive. Sometimes factors beyond the investigator's control such as weather conditions, exposure to radiation from the Sun, chemicals, or other organic material that was already present may destroy a sample. These factors can degrade the DNA or chemically alter it, preventing it from being used. DNA fingerprinting is performed when ample material is available and in fair condition. The scientist compares the resulting pattern, or DNA fingerprint, to the DNA fingerprints of any known suspects. If there are no suspects, the sample can be compared to a database of DNA profiles maintained by the Federal Bureau of Investigation. This database, called CODIS (for Combined DNA Index System), contains DNA profiles of numerous convicted felons, DNA profiles from unidentified suspects from other investigations, and other individuals whose DNA has been previously analyzed. When sample quantity or quality limits standard DNA analysis, forensic biologists can use mitochondrial DNA analysis. Mitochondria are cellular structures that contain DNA and are inherited through maternal lineages. One advantage of mitochondrial DNA analysis is that it is particularly sensitive because a single cell contains numerous copies; thus mitochondrial DNA analysis can be performed on old skeletal remains or on hair shafts without roots.

Though forensic investigations have come to rely on DNA analysis to establish the identity of a suspect, investigators still also use traditional methods. Individuals, even identical twins, have unique fingerprints. Forensic investigators can collect fingerprints from all sorts of objects, create digital images from them, and use a computer to compare the prints with those stored in a national database. Bite marks are also unique, and preparing casts or molds from indentations or impressions left on a victim may help establish a suspect's identity.

Training for a career in forensic biology requires a bachelor's degree and a strong background in biology, chemistry, and statistical analysis. Technical training includes microscopy, proficiency in the use of computers, and methods in biotechnology, particularly with respect to DNA manipulation and analysis. Employers often provide additional specialized training for their forensic scientists because technology advances rapidly in this field. Forensic biologists work for law enforcement agencies, for other governmental agencies such as the Postal Inspection Service, and in laboratories of colleges and universities. In order to obtain a position in a reputable crime lab, a forensic investigator must pass a background check and random drug testing. Because of the environment in which they work, investigators must receive training to work safely with hazardous chemicals. Crime lab directors often have doctoral degrees.

See also DNA FINGERPRINTING.

FURTHER READING

American Academy of Forensic Sciences home page. Available online. URL: http://www.aafs.org. Accessed January 25, 2008.

Hunter, William. *DNA Analysis*. Philadelphia: Mason Crest, 2006.

Walker, Maryalice. *Entomology and Palynology: Evidence from the Natural World*. Philadelphia: Mason Crest, 2006.

Franklin, Rosalind (1920–1958) British *Biophysicist*

Rosalind Franklin was an expert X-ray crystallographer whose experimental data were instrumental in solving the structure of deoxyribonucleic acid (DNA), though she did not receive proper recognition for her role during her lifetime.

ATTENDS CAMBRIDGE

Rosalind Franklin was born on July 25, 1920, to Jewish socialist parents who lived in London. As a schoolgirl she excelled at arithmetic and science and played hockey, cricket, and tennis. She decided to become a scientist when she was 12 years old, and at age 17 she earned the highest score on the Cambridge entrance examination in chemistry. In October 1938 she entered Newnham College on scholarship. At the time, Cambridge did not accept women as members of the university but did allow them to attend lectures. She took classes in chemistry, physics, mathematics, and mineralogy; joined a mathematics society; and became interested in X-ray crystallography, a method that uses X-rays to reveal the structure of crystals. Crystals form from the regular arrangement

of the atoms inside, and when X-rays are directed at the crystal, the atoms cause the X-rays to scatter, creating patterns of spots on X-ray film. Mathematical analysis allows one to determine the position of atoms within a molecule from the spacing and the intensity of the spots on the film.

By her third year at Cambridge, many of the male faculty and students had joined the war effort. Her studies focused on the structure of atoms and molecules. She performed well enough on her exams to earn a college scholarship for another year and, more importantly, a research grant from the Department of Scientific and Industrial Research. She began graduate studies in gas-phase chromatography, but her social life was lacking, and she did not get along with her colleagues or her professor. To escape, in August 1942, she took a job as an assistant research officer for the British Coal Utilisation Research Association studying the physical and chemical properties of coal and charcoal. She submitted a thesis to Cambridge University on this work, and she received a doctorate in physical chemistry in 1945. The following year she moved to Paris to work for the Laboratoire Central des Services Chimiques de l'État, where she continued studying carbon and became proficient in X-ray crystallography. Her reputation as a skilled crystallographer grew as she published several papers with important industrial applications. One significant finding made by Franklin was the discovery of a class of carbons that can never be transformed into graphite by heating.

STUDIES NUCLEIC ACID AT KING'S COLLEGE

In 1951 Franklin joined the Biophysics Unit of the Medical Research Council at King's College of the University of London. The original purpose of her appointment was to study protein structure, but just before she arrived, the direction of the laboratory changed. The head of the physics and biophysics unit at King's College, Professor J. T. Randall, explained that he thought the examination of some nucleic acid fibers obtained from the Swiss scientist Rudolf Signer was of greater immediate importance. This change of plans led to a misunderstanding of Franklin's role at the lab. She thought the X-ray diffraction work on the fibers was to be her own project, whereas Maurice Wilkins, a biophysicist at King's who had been working on nucleic acids for years, thought she was going to assist him.

Wilkins and Franklin initially were cordial as they worked separately studying the DNA fibers, but their quiet bitterness grew into bare tolerance of one another, and then into open hostility. They shared a common research goal of solving the structure of DNA, but they did not share information. Though they might have made a formidable team in the race to solve the structure of DNA, their bitter relationship hindered any progress. Franklin did work closely with Raymond Gosling, a graduate student who had been assigned to her. One of Franklin's achievements while working at King's was the identification of two different forms of DNA, an A form and a B form. The A form converted into the B form when the humidity was increased, as the phosphate groups absorbed the water molecules, causing the fiber to become longer and thinner. To facilitate peace, in October 1951 Randall suggested that Franklin focus her research efforts on the A form and Wilkins on the B form. As part of this agreement, Franklin also was given the DNA that had been supplied by Signer and the better camera equipment. She used it to determine that the phosphates were positioned on the outside of the molecule and to obtain several more beautiful X-ray photographs of DNA.

When James Watson learned that Linus Pauling was publishing a model for DNA structure in February 1953, even though it was wrong, he rushed to King's College to share the information with Franklin and Wilkins in hopes of collaborating. But Franklin thought their modeling approach was elementary and wanted no part of it. She believed that model building was only appropriate after collecting sufficient hard evidence from X-ray diffraction studies. A little more than a year earlier, Watson and Francis Crick had invited her and Wilkins to see a DNA model they believed to be the answer, but the metal plate and stick arrangement was completely wrong and only confirmed her belief that model building was useless without first having experimental data. According to Watson, Franklin also denied the existence of any evidence that DNA was helical, but her personal laboratory notes divulge she believed otherwise. In fact, it was one of her X-ray photographs that showed the clear central X pattern that suggested a helical form for B DNA. Gosling had shown this photograph to Wilkins, who in turn showed it to Watson after his confrontation with Franklin. Wilkins also divulged figures obtained from the photograph, namely, the repeat length of the helix, 34.4 angstroms.

This information stimulated Watson to resume model building seriously. Within a few weeks, Franklin learned that Watson and Crick planned to publish a model for DNA structure. She and Gosling quickly adapted a paper they had already written but not yet submitted for publication to accompany Watson and Crick's paper. Franklin and Gosling's paper, titled "Molecular Configuration in Sodium Thymonucleate," appeared in the same issue of *Nature* and provided strong experimental evidence for the double-helical model with an exterior phosphate backbone. She was unaware that her data had provided the impetus for the final solution of the struc-

ture of DNA by Watson and Crick and certainly had never been properly consulted on its use. Wilkins and his colleagues Alex Stokes and Herbert R. Wilson also published an accompanying paper presenting additional X-ray diffraction evidence for the model proposed by Watson and Crick. In July 1953, Franklin and Gosling published another paper in *Nature* detailing the differences between two different forms of DNA, "Evidence for 2-Chain Helix in Crystalline Structure of Sodium Deoxyribonucleate."

Despite her scientific successes in a field in which she had only been a part for two years, Franklin was not happy at King's. She believed that her coworkers were not serious researchers and was frustrated that she had no academic appointment. After the DNA reports were published, Franklin transferred to John Desmond Bernal's laboratory at Birkbeck College, another college of the University of London, where she remained for five years until her death. By December 1953 she was immersed in studies of tobacco mosaic virus (TMV) structure. She made remarkable advances in the field of viral structure, including the discovery that the ribonucleic acid of TMV was embedded in the inner groove of the helix formed by the protein subunits. She published many papers on the structures of several plant viruses and was admired for her technical competence and expertise. Her reputation was demonstrated when Sir Lawrence Bragg, head of the Cavendish Laboratory at the University of Cambridge, asked Franklin to create virus models for the general public to view at the Brussels World Fair. One of her collaborators and close friends from Birkbeck, Aaron Klug, later won the Nobel Prize in chemistry in 1982 "for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid-protein complexes." In his Nobel lecture, he acknowledged Franklin, crediting her with introducing him to viral research and setting an example of going after the difficult problems of science.

Despite Watson's negative portrayal of Franklin as stubborn, confrontational, and uncooperative in his personal narrative, *The Double Helix,* which described his perception of the events leading to the discovery of the structure of DNA, she had productive and meaningful collaborative engagements with her scientific teams at Birkbeck College and in Paris. She was not only a serious scientist, but an avid mountain hiker and an expert cook and enjoyed playing with the children of her friends and relatives. Because Franklin tragically died of ovarian cancer on April 16, 1958, she was not eligible for nomination for the 1962 Nobel Prize for her role in the discovery of DNA structure. After her death, Bernal described Franklin's photographs as "among the most amazing beautiful X-ray photographs of any substance ever taken." In March 2002 King's College dedicated the Franklin-Wilkins building in her honor.

See also Crick, Francis; deoxyribonucleic acid (DNA); Pauling, Linus; Watson, James D.; Wilkins, Maurice H. F.; X-ray crystallography.

FURTHER READING

Franklin, R., and R. Gosling. "Evidence for 2-Chain Helix in Crystalline Structure of Sodium Deoxyribonucleate." *Nature* 172 (1953): 156–157.

———. "Molecular Configuration in Sodium Thymonucleate." *Nature* 171 (1953): 740–741.

Maddox, Brenda. *Rosalind Franklin: The Dark Lady of DNA*. New York: HarperCollins, 2002.

Frisch, Karl von (1886–1982) German *Ethologist*

Karl von Frisch is considered one of the founders of the comparative study of animal behavior. During his 60 years as a research scientist, he studied the senses, communication, and social organization of honeybees. He is most famous for demonstrating that bees have color vision and that they communicate information about the distance and location of food sources to their colony mates by genetically programmed characteristic dance movements. His research on the chemical and visual senses of bees stimulated the interest of other zoologists in ethology.

VISION AND SMELL IN HONEYBEES

Karl von Frisch was born on November 20, 1886, in Vienna, Austria, to Anton Ritter von Frisch, a university professor, and Marie Exner. He began university studies in medicine at the University of Vienna but switched to zoology. For his doctoral thesis he studied the color adaptation and light perception of minnows. After receiving his doctorate from the University of Vienna in 1910, he joined Richard von Hertwig at the University of Munich of the Federal Republic of Germany, where he obtained his teaching certificate in zoology and comparative anatomy. In Munich, Frisch began studying the behavior of bees, the major focus of his six-decade-long research career.

Honeybees are social insects that live in hives consisting of approximately 60,000 individuals. A single bee, called the queen bee, lays eggs. Drones are male bees that fertilize the eggs. The infertile female worker bees perform all the work to support the colony: feed larvae, build the honeycomb, and gather nectar and pollen for the colony to eat. Bees obtain food by visiting flowering plants, and in turn they carry pollen from one plant to another, assisting

the plants in reproduction. Many biologists believed that the bright colors of many flowers serve to attract insect pollinators. One of Frisch's first studies at the University of Munich was to examine whether or not bees had color sense. Biologists knew that bees could distinguish colors from the following experiment: Experimenters placed honey on a piece of blue cardboard and bees were allowed to feed off it. After a period of time, they removed the cardboard and replaced it with two new pieces, one red and one blue, neither of which contained food. The bees landed on the blue piece, indicating they could distinguish red from blue.

Frisch next wondered whether the bees perceived red and blue, or simply different shades of gray. To examine this, he placed a blue card on a table and surrounded it with cards of several shades of gray. All of the cards contained tiny watch glasses, but only the glass on the blue card contained sugar water. He allowed the bees to visit the cards, rearranging the placement of the blue card containing the food at frequent intervals. Then he replaced all the cards with clean cards and placed empty glass dishes on all the cards. The bees went right to the blue card, indicating they could perceive the color blue. Frisch obtained the same result when using orange, yellow, green, violet, or purple cards. The bees could not, however, distinguish red from black and had some trouble distinguishing blue from violet or purple when used together. They also confused yellow with orange and green, when those colors were tested together. Research performed by others later showed that bees have a sense of four different colors: yellow, blue-green, blue, and ultraviolet. Interestingly, hummingbirds and honey birds typically pollinate red flowers. These studies suggest that insect pollinators affect the adaptation of flower color.

In a related set of experiments, Frisch examined the effect of coloring hive entrances in houses where beekeepers kept many beehives. If a queen bee leaves the hive and returns to the wrong one, the residents will kill her, leading to the demise of her colony. Beekeepers often paint the entrances to prevent this from happening. Frisch tested whether or not the colored sheets surrounding the hive entrances were useful in orienting the bees to the correct hive. By painting the sheets different colors, moving them around, and observing the bees' response, he demonstrated that the colors do help bees distinguish their own hives from others nearby.

In the bees' natural environment, flower color clearly plays a role in the search for food, but flowers also exhibit a variety of shapes. Frisch examined this phenomenon by placing colored cards with cutout patterns over the entrance of boxes containing sugar water. After a short period of training the bees to enter a certain box by recognizing the shape at its entrance, he placed new cards with the cutout patterns in front of the boxes. The bees flew into the boxes with the familiar patterns, even though no sugar water was inside. Frisch found that the bees had trouble distinguishing some shapes from others (e.g., a triangle from a square) but were more successful at recognizing broken patterns such as the shape of the letter *X*. Following Frisch's example, other animal behaviorists performed more detailed analyses of bees' ability to distinguish certain shapes and patterns between the late 1920s and the 1950s.

CHEMICAL SENSES OF BEES

Frisch realized that the types of flowers pollinated by bees far outnumbered the different colors that bees could distinguish and was curious about which other features helped bees recognize different plants. Bees typically visited the same species of plant. In order to determine whether bees could distinguish the odors of particular flowers as humans can, Frisch set up experiments similar in method to his color experiments. He set up boxes on a table and inside one of them placed a dish of sugar water and either a fragrant flower or a few drops of an essential oil. Every so often he rearranged the position of the boxes on the table to ensure that only scent directed the bees to a particular box. After giving the bees sufficient time to associate the smell with the presence of the sugar water, Frisch replaced all the boxes with clean empty boxes. He placed a flower or oil in one of those boxes. The bees hovered around the entrance to all the boxes but only entered the box with the scent, indicating they could smell the odor that they learned to associate with food.

To see whether the bees could distinguish among numerous scents, he next arranged 24 boxes on the table. All the boxes contained different scents, but only one contained sugar water. After a training period, Frisch and his coworkers placed 24 clean, scented boxes on the table, but none of them contained food. They counted the number of times that bees entered each box during a five-minute period. Many more bees entered the box containing the scent that was associated with food during the training period. A significant number of bees entered three of 46 other boxes (they performed the experiment twice, for a total of 48 boxes, two containing the training scent). These three boxes contained oils that were made from fruits belonging to the same genus as the training scent, and the odor was similar to the training scent, as determined by the human nose.

From these experiments Frisch concluded that bees have a well-developed sense of smell. He found this intriguing, since the olfactory organs of insects and humans are so different. Today, biologists have

a better understanding of the cellular and molecular processes involved in olfaction; thus the results are not so surprising. To locate the sense organs in bees, Frisch cut off their antennae after training them in a particular odor. After the surgery, the bees could no longer distinguish the training scent. Cutting off the antennae had no effect on the bees' ability to distinguish colors, as expected. Thus, he concluded that the olfactory organs in bees are located on their antennae.

The sense of taste also depends on detection of chemicals by certain organs. Frisch researched different aspects of this sense. Bees can recognize different degrees of sweetness—different concentrations of sugar in a solution—and even display individual preferences for acceptable concentrations. The threshold of acceptance is the concentration at which a bee refuses to suck up a solution after tasting it. The threshold of perception is the lowest concentration that stimulates the sense of taste. When conditions are poor and the bee struggles to find food, the threshold of acceptance lowers considerably, approaching the threshold of perception.

Frisch tested bees' sensitivity to different concentrations of salt and to different types of sugars. He found that bees do not like salty substances and that they will consume a greater volume of a substance if it is sweeter than another less concentrated sugar solution. Other findings included the fact that bees tolerated bitter substances more than salty substances and that they could also distinguish sourness.

LANGUAGE OF BEES

During the course of his experiments examining the senses in bees, Frisch often found himself waiting for the bees to find his food source. Sometimes it took a few hours, other times it took a few days, but once a single bee found his supplied sugar water, hundreds would soon follow. This made him wonder how the other bees knew that the first bee found a food source and how the first bee communicated this information to the other bees.

The experimental setup required to study this phenomenon was quite complex. Because one cannot observe the events occurring between the honeycombs within a beehive, Frisch built an observation hive in order to learn how the bees shared information regarding a food source with the other members of their colony. The observation hive consisted of one large comb that could be observed through glass windows. He also devised a marking system so he could identify individual bees. He used different combinations of five colors of paint spotted onto different body parts of the bees to indicate the numbers 1–599. For example, bee 431 had a yellow spot on the abdomen to indicate 400, a blue spot on the front of the thorax to indicate 30, and a white spot on the hind part of the thorax to indicate the digit 1 in the ones place. Frisch set out a glass dish filled with sugar water near the observation hive and proceeded to wait and observe.

After finding the glass dish and sucking up sugar water, a bee flew back to the hive and transferred most of the sugar water to other bees. Then the worker bee performed a round dance, in which she remained in the same general spot and spun in circles to the right then to the left for up to 30 seconds or even longer. She then moved to another spot on the honeycomb and repeated her performance. While she was dancing, the other bees swarmed around her, then they exited the hive and found the food source. When they returned to the hive, they also performed the dance, and more bees found the food source.

To figure out whether and how the dancing conveyed information about direction, Frisch fed several bees from a dish exactly 32.8 feet (10 m) to the west, north, east, and south of the observation hive. Minutes later bees appeared at all the dishes regardless of direction. To see whether the bees could give more definite directions regarding the food source, he put out two large dishes of flowers, cyclamen and phlox. The cyclamen flowers contained a coating of sugar solution. After several bees fed on the cyclamen and returned to the hive, the new bees flew straight to the cyclamen and showed no interest in the phlox. Clearly the bees were giving more specific information than "Go look outside several meters away from the hive," and Frisch suspected scent played a role.

When the phlox contained the sugar solution, the bees sought out the phlox. When Frisch repeated the experiments but used flowers with different fragrances, he learned that after the bee returned to the hive, she carried the fragrance on her body, and other bees detected the particular fragrance. Not only does the upper body of the bee retain the scent for a long period, but she also regurgitates a bit of the nectar gathered from the bottom part of the flower. That nectar gives off the characteristic scent. When new foraging bees went out, they sought that specific scent to find the sugar water. Odorless flowers were untouched. When Frisch set glass dishes containing sugar water on cardboard sheets dabbed with peppermint oil, then bees from the hive would go out and seek sugar water near anything that smelled like peppermint. The new foragers learned the scent from the dancer. The dances were more vigorous when the sugar solution was more concentrated or when the quantity was abundant.

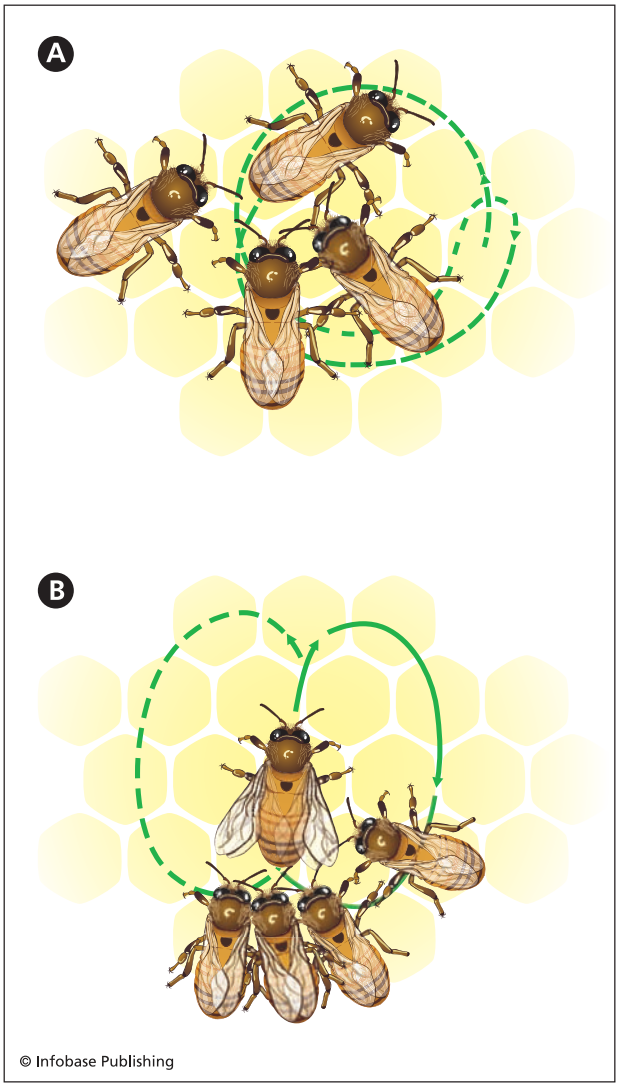
After performing these sorts of experiments for a few decades, Frisch hypothesized that the bees were communicating information about the distance in addition to the scent of the food source. He arranged

food sources at 32.8 feet (10 m) and 984 feet (300 m) from the hive. Glass dishes containing sugar water were placed on scented cards. In the first experiment, food was only available at the nearby food source. After bees visited the nearby food source, they returned to the hive and danced for the other worker bees. The new bees went out and fed mostly at the near source (174 bees), although a few (12 bees) visited the distant source. In the second experiment, food was available at the distant food source, but not the nearby one. After bees fed at the distant food source, they returned to the hive and danced, and new bees flew out to seek food from the distant source. Within a one-hour observation period, 61 bees visited the distant source, while only eight visited the nearby source. Thus, the bees somehow communicated information regarding distance during their dance.

The bees that fed from the nearby source performed the same round dance described earlier. Bees that fed from the distant source performed a different style of dance, one described as a tail-wagging or waggle dance. In this dance, the bee moves a short distance forward while moving its abdomen from side to side, then turns 360 degrees to the left, continues straight forward a bit, then turns 360 degrees to the right, and so on. The particular style of dance conveys information regarding the distance of the food source. Frisch found that the waggle dance changed to a round dance between 164 and 328 feet (50 and 100 m). A relationship also exists between the tempo of the dancing and the distance to the food source. The dance tempo (turns per unit of time) is faster when the food source is nearer.

The dance of the bees also conveys information about the direction of the food source, as demonstrated by the following experiment: Frisch fed bees from a scented card 656 feet (200 m) south of the hive, then placed similarly scented cards in all directions at a similar distance from the hive. After observing the dance by the first few bees, worker bees traveled to the food source south of the hive and to nearby cards that carried the same scent but without food on them. When they shifted the food source to another direction, such as east, the bees soon followed. Frisch and his coworkers noticed that when several bees returned from the same food source, they all "waggled" in the same direction between turns. They figured out that the direction of the food source was related to the direction of the short straight path during the waggle dance.

Amazingly, as the day progressed, the direction of the straight path during the waggle dance shifted slightly but continuously even when the location of the food source was kept constant, suggesting that



To communicate information about food sources to other workers, bees perform characteristic dances such as A) the round dance and B) the tail-wagging or waggle dance.

the Sun was involved. Even more surprising was the fact that the bees performed the dance inside a hive near complete darkness; the dancing bees relied on gravity to orient themselves and the observing bees recognized the angle of the dance movement with respect to gravity. The directions seemed to be as follows: a vertical movement during the dance indicated a location toward the Sun: a straight path that points downward indicates away from the Sun, a straight path of the waggle dance that heads 60 degrees to the left of vertical means fly 60 degrees to the left of the Sun, and so on.

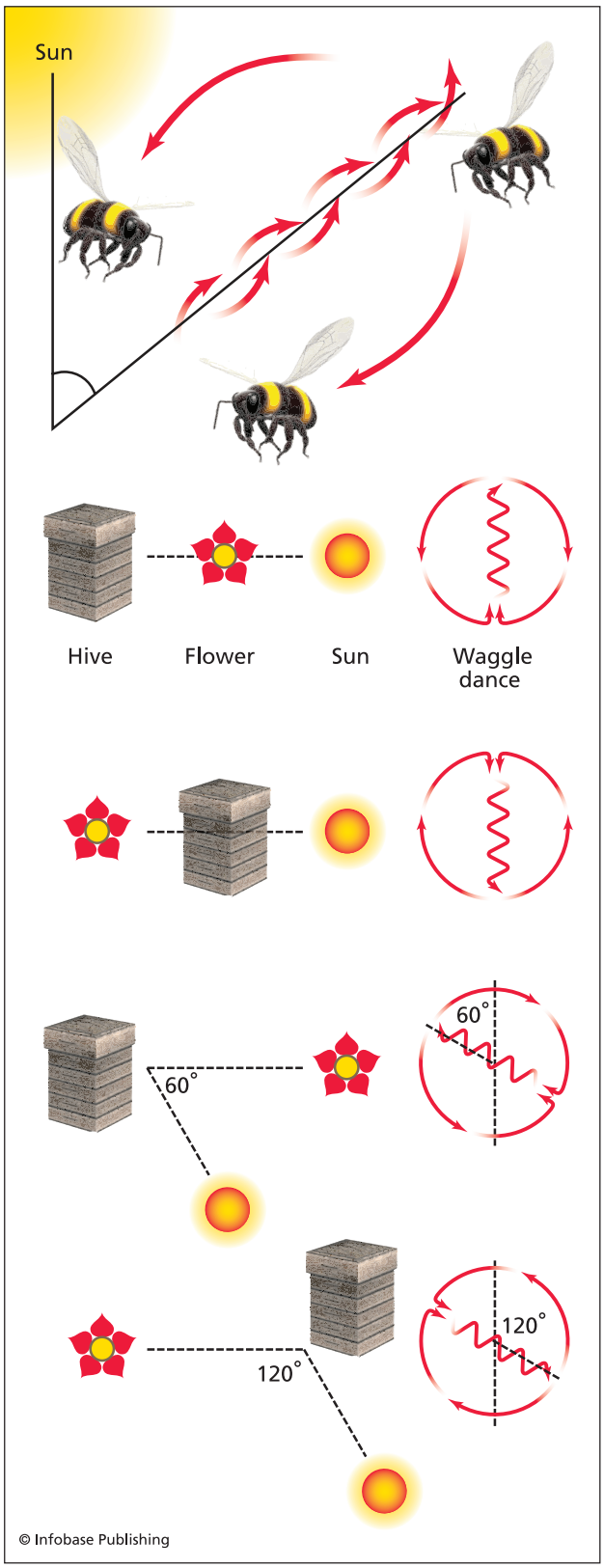
Even when the Sun was not out the bees were able to communicate the correct location efficiently. Frisch observed bees when the sky was overcast and

found that the bees could still perceive the position of the Sun when it was hidden behind the clouds. When Frisch placed a black plate that allowed the passage of ultraviolet light between the Sun and the beehive, the bees were still able to perform a dance that gave accurate location information. When Frisch obstructed the rays with a glass plate that absorbed ultraviolet radiation, the bees were disoriented. Thus the bees were able to detect the direction of the polarized (traveling in the same direction) ultraviolet light and interpret it to determine the position of the Sun.

This work on communication in bees earned Frisch the most coveted scientific award. In 1973 he received the Nobel Prize in physiology or medicine, shared with two other pioneering ethologists, Konrad Lorenz of Austria and Nikolaas Tinbergen of the United Kingdom, "for their discoveries concerning organization and elicitation of individual and social behavior patterns." Though many obviously considered Frisch's work outstanding, others seriously doubted his conclusions. Many scientists questioned that if the coded dance truly provided all of the specific information that Frisch suggested, then why did it take so long for the newly informed bees to reach the destination of the communicated food source? They proposed that the bees just traced the smell carried by the original dancing bee or that they simply followed the original bee back to the source. In May 2005 scientists published a paper in *Nature* supporting Frisch's conclusions. The group attached radar transponders to the bees and tracked them as they flew to food sources. They found that the bees flew immediately to the vicinity of the source, then flew around a bit in search of the exact location, accounting for the time lag. Their results support Frisch's original conclusions.

In addition to his work on communication in bees, Frisch studied pigments in fish skin, color changes in animals, hearing in fish, sensation of chemicals in fish and in insects, and vision in insects. He also authored numerous books, including a general biology textbook.

In 1921 Frisch briefly joined the faculty at the University of Rostock and served as the director of zoology. After only two years he took a similar position at the University of Breslau, and in 1925 he returned to Munich to assume Hertwig's former position. Frisch oversaw the building of the Zoological Institute at Munich, which was destroyed during World War II. While the institute was being rebuilt in 1946–50, he worked at Graz. After returning to the Zoological Institute, he remained there the rest of his career. He became a professor emeritus in 1958 but continued his studies. Frisch received the Balzan Foundation Award in 1963 and was a member of the



Bees indicated the direction of the food source by the angle at which they moved in a short, straight path during their waggle dance, using the Sun as a reference.

National Academy of Sciences and the Royal Society of London.

Karl von Frisch died on June 12, 1982, in Munich, Germany. Biologists and psychologists alike remember him for his pioneering work on the sensory capacities and behavioral patterns in bees and other animals. His most significant research demonstrated that bees perform genetically programmed dances to communicate information regarding the distance and direction of a source of nectar to other bees in the colony. This is one of the most complex communication methods to be demonstrated in invertebrates. Not only did Frisch help found the field of ethology, but his discoveries forced zoologists to reconsider the notion of intelligence and the concepts of language and communication.

See also ANIMAL BEHAVIOR; ETHOLOGY; LORENZ, KONRAD; TINBERGEN, NIKOLAAS; TURNER, CHARLES HENRY.

FURTHER READING

Frisch, Karl von. *Bees: Their Vision, Chemical Senses, and Language*. Rev. ed. Ithaca, N.Y.: Cornell University Press, 1971.

———. *A Biologist Remembers*. Oxford: Pergamon Press, 1967.

———. *The Dance Language and Orientation of Bees*. Translated by Leigh E. Chadwick. Cambridge, Mass.: Belknap Press of Harvard University Press, 1967.

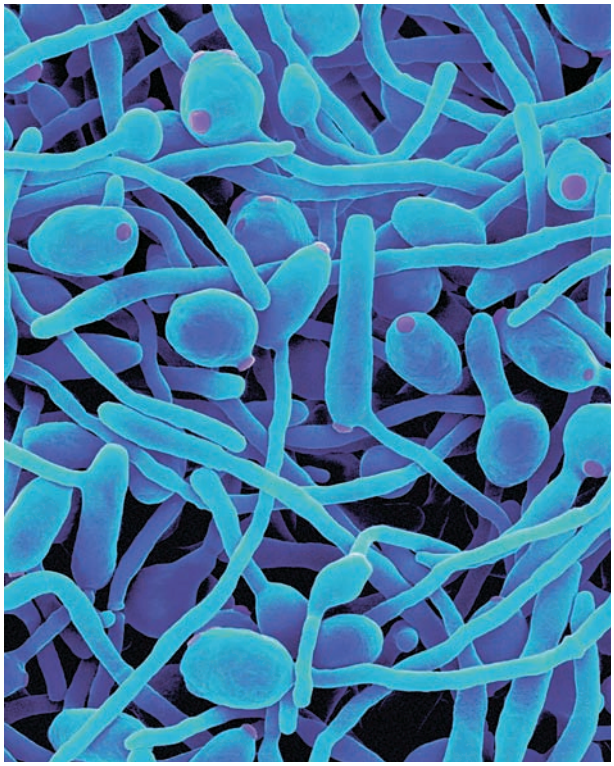
The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1973." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1973/index.html. Accessed January 25, 2008.

fungi Members of the eukaryotic kingdom Fungi are unique and diverse. Fungi include molds, mushrooms, yeasts, rusts, smuts, blights, morels, and truffles. Biologists once considered fungi as plants because they were immobile, appeared to have roots, and had cell walls. Plants, however, are photosynthetic, meaning they can harvest light as an energy source and use it to synthesize organic molecules. Fungi are chemoheterotrophs, meaning they cannot synthesize their own organic molecules from inorganic substances and therefore must obtain energy and nourishment from organic substances present in the environment. The bodies of fungi consist of long, slender filaments and are mostly multicellular, though one group, the yeasts, are unicellular. The polysaccharide chitin, the same material found in arthropod exoskeletons, composes fungal cell walls, compared to cellulose in plants. Unlike other eukaryotic organisms, fungi are haploid. They only exist as diploids during a brief phase of sexual reproduction.

Fungi cannot undergo photosynthesis; nor can they engulf food. Instead, they obtain their nutrition by secreting, into the environment, enzymes that digest organic matter. After the enzymes break down the organic matter into smaller components, the fungi absorb the organic molecules into their cells. Many fungi are saprophytic, meaning they obtain nourishment from decaying organic matter, dead organisms, or organic material from other organisms such as animal carcasses, leaf litter, and eliminated waste. Parasitic fungi absorb nutrients from living hosts, including both plants and animals, and can cause infectious diseases. Fungi can also grow on foods such as bread or fruit and even in substances such as house paint. Slightly acidic conditions (pH of about 5) that are unfavorable for most bacteria favor fungal growth. Fungi are also more resistant to low-moisture and high-salt environments.

Molds and fleshy fungi consist of filaments called hyphae, which range in size from microscopic to covering acres of land. Septate hyphae are composed of uninucleate (containing one nucleus) cells separated by septa, or walls, though openings between cells join the cytoplasms of adjacent cells. Coenocytic hyphae do not have septa; they consist of extended cells containing many nuclei. Hyphae grow by elongation at their tips, but if a piece of hyphae breaks, the new fragment can grow into another organism. Specialized types of hyphae carry out the functions of obtaining nutrition and reproduction. Vegetative hyphae must contact the surface on which they grow, so nutrients can be absorbed. When environmental conditions favor growth, the fungus spreads over the surface by branching out to form an intertwining network, or colony, called a mycelium. Reproductive hyphae produce reproductive spores and project outward into the air, so when the spores are released, the air can carry them away.

Yeasts are nonfilamentous, unicellular spherical or oval-shaped fungi. Budding yeasts, such as *Saccharomyces cerevisiae* (baker's yeast), can reproduce asexually by forming a small protrusion from the parent cell. After the parent nucleus divides, the bud receives one-half, continues growing, and eventually pinches off into a smaller but fully functional yeast cell. Structures called pseudohyphae form when the buds fail to separate completely and allow pathogenic yeasts such as *Candida albicans* to penetrate into tissues. Fission yeasts divide into two equally sized daughter cells. Yeasts are facultative anaerobes, meaning they can grow in the presence or absence of oxygen. When grown anaerobically (in the absence of oxygen), they undergo fermentation, a catabolic process that results in the production of ethanol and carbon dioxide. Yeasts are also easy to grow in the lab and are widely used in cell, molecular, and genetic research.



Yeastlike fungi such as *Candida albicans* have dimorphic life cycles and can grow in yeast or hyphal stages. *(© Dennis Kunkel Microscopy, Inc.)*

Some fungi are dimorphic—they can grow like molds with hyphae or like yeasts that bud. Temperature serves as the deciding factor. At 77°F (37°C) they resemble yeasts, but at 98.6°F (25°C) they resemble molds.

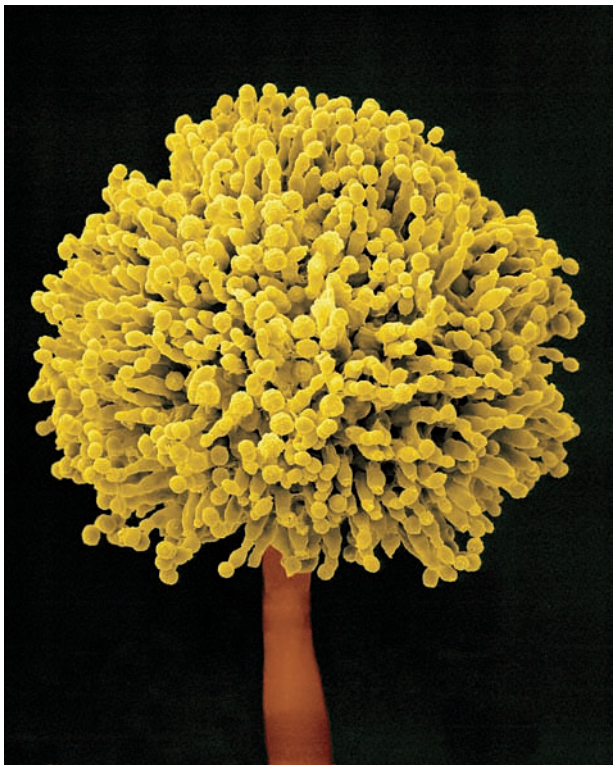
FUNGAL REPRODUCTION AND CLASSIFICATION

Fungi reproduce by forming and releasing spores from hyphal tips. Because fungal spores are very small, air currents easily transport them over long distances. Fungal spores differ from the more resistant bacterial endospores in that fungal spores are true reproductive structures. Each spore gives rise to a new organism; thus the production of fungal spores increases the number of organisms. Bacterial endospores allow cells to survive harsh or unfavorable conditions but do not increase the number of bacterial cells.

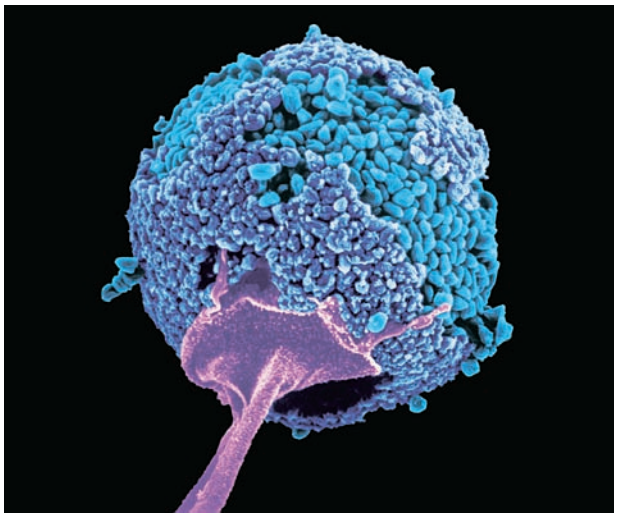
In asexual reproduction, the offspring are genetically identical to the parent. Filamentous fungi can reproduce asexually by fragmentation, when a separated piece of mycelium develops into an entire new colony. Asexual fungal spores form by mitosis and cell division. The two main subtypes of asexual fungal spores are conidiospores (also called conidia) and the less common sporangiospores. Conidiospores occur in chains at the end of aerial hyphae called conidiophores and are not enclosed by sacs. They develop by pinching off of the tip or by segmentation of hyphae. The several forms of conidiospores include arthrospores, chlamydospores, blastospores, phialospores, microconidia, macroconidia, and porospores. Sporangiospores develop by successive cleavages inside a sac called a sporangium at the end of aerial hyphae called sporangiophores. When the sporangium breaks open, the spores are released.

In sexual reproduction, two different mating types (a donor cell "+" and a recipient cell "-") of hyphae join together, fuse, and develop into a new organism that forms spores containing genetic information from both parents. The formation of sexual spores occurs in three main stages: plasmogamy, karyogamy, and meiosis. During plasmogamy the haploid nucleus from a "+" mating type invades the cytoplasm of a "-" mating type. The two haploid nuclei fuse in a process called karyogamy, resulting in a diploid nucleus. Meiosis occurs, forming nuclei of sexual spores.

Because sexual spores develop in a variety of diverse ways, one means of classifying fungi is by their mode of sexual reproduction. Three of the most common divisions are Zygomycota, Ascomycota, and Basidiomycota. Members of the phylum



The green mold *Aspergillus flavus* forms asexual spores called conidiospores at the end of aerial hyphae called conidiophores. *(© Dennis Kunkel Microscopy, Inc.)*



The common bread mold *Rhizopus stolonifer* forms asexual spores called sporangiospores that are enclosed in a sac at the end of an aerial hypha called a sporangiophore. *(© Dennis Kunkel Microscopy, Inc.)*

Zygomycota usually reproduce asexually, but when opposite mating types fuse, sexual spores called zygospores form. The zygospores are large, thick-walled spores that undergo meiosis and germination. The resulting mycelium gives rise to haploid sporangia that resemble the asexual sporangium, except that it contains nuclei with genetic information from both parents. Hyphae of zygomycetes usually have no cell walls. Black bread molds such as *Rhizopus stolonifer* belong to the phylum Zygomycota. Members of the phylum Ascomycota generally produce ascospores inside a tubelike sac called an ascus that forms when two mating types join to form a diploid nucleus. Subsequent meiosis and germination result in the formation of haploid ascospores that disperse when the ascus breaks open. Examples of ascomycetes include *Histoplasma* (the causative organism of Ohio River Valley fever, or histoplasmosis), truffles, and the yeasts. The phylum Basidiomycota includes the mushrooms, rusts, puffballs, and smuts. The fusion of hyphae of different mating types forms a fruiting body (such as a mushroom) that is common to members of this phylum. Club-shaped structures called basidia line the gills underneath the cap of the fruiting body. Fusion of haploid nuclei occurs within a basidium. Meiotic division produces haploid spores that germinate to form haploid hyphae.

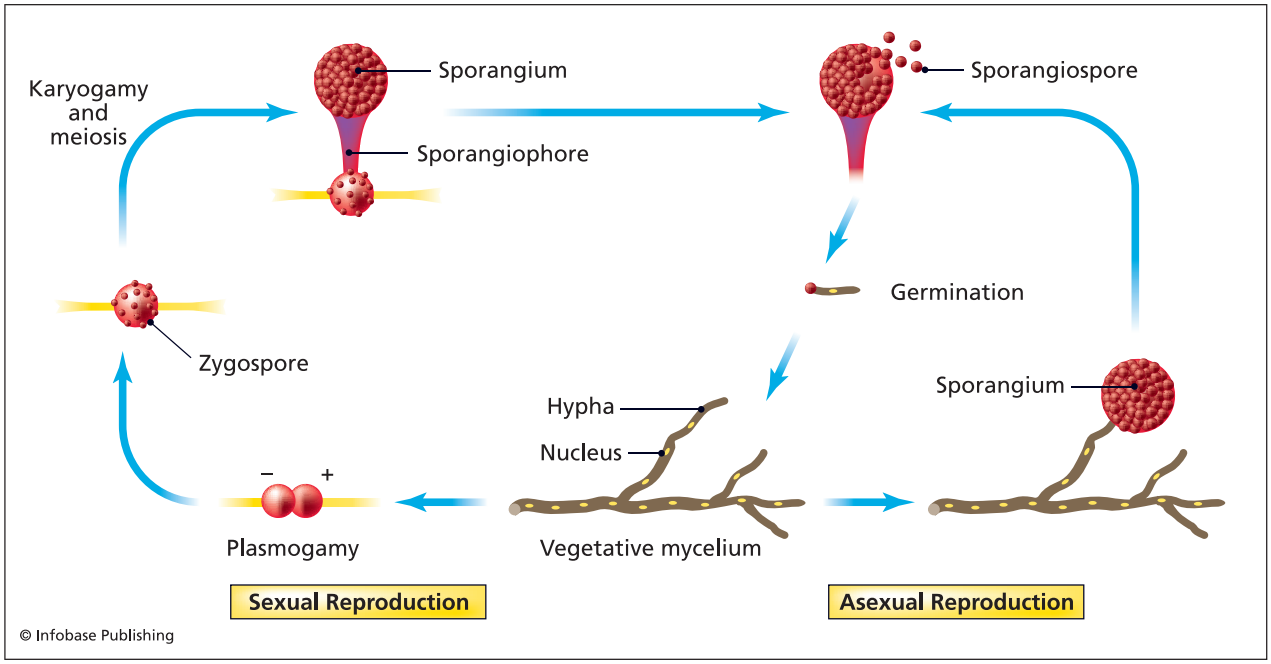
Two less well-known phylum are Deuteromycota and Chytridiomycota. Deuteromycota, commonly called the imperfect fungi, includes various fungi that are incapable of sexual reproduction or organisms for which the means of sexual reproduction is not yet known. The organisms that cause athlete's foot and that give Camembert and Roquefort cheeses their unique flavors belong to this phylum. Members of the phylum Chytridiomycota are usually unicellular and aquatic and produce flagellated, motile gametes and spores. Slime molds and water molds were once classified as fungi because they appeared to share similar life cycles and under certain conditions form structures resembling sporangia. Biologists now believe they are unrelated to fungi.

ECOLOGICAL IMPORTANCE

Fungi closely interact with other organisms in many different ways. A mycorrhiza is a mutualistic symbiotic association formed between a fungus and a plant. The fungus grows inside or wraps around the roots of the plant, and the mycelia branch out to increase the surface area through which absorption of water and nutrients such as phosphorus and minerals occurs. The fungus benefits from carbohydrates synthesized by the plant by photosynthesis. Plants with mycorrhizae can inhabit soil that is less fertile or live in environments that are drier than plants without them.

Lichens are symbiotic associations formed by a fungus with a photosynthetic organism such as a photosynthetic bacterium or an alga. The photosynthetic organism provides a food source for the fungus, while in return the fungus protects its partner from the environment, allowing it to live in habitats that would normally be too harsh, such as on the surface of a rock, in an arid desert, or on a tree trunk. The mycelia absorb moisture and carbon dioxide from the atmosphere for use in photosynthesis. Lichens play an important role in ecological succession, the replacement of one type of community by another at a single location over time, such as after a forest burns down. Lichens help prepare the previously barren terrain for other species by secreting an acid that breaks down rocks into soil and liberates nutrients. Over time, the organic material from dead lichens nourishes future inhabitants. Lichens also serve an important role monitoring the air for pollutants such as sulfur dioxide. Because they are sensitive to such manufactured pollutants, their growth is a good indicator of air quality.

Because most fungi are saprophytes and obtain their nutrition from decomposing organic material of other species, they play a crucial role in the food chain. Plants contain cell walls made of cellulose, a carbohydrate that animals are incapable of digesting. Fungi break down the decaying leaf litter on the forest floors, recycling the nutrients back into the environment in a form that other organisms can utilize. Without fungi, many nutrients would become depleted as they became incorporated into forms unusable to other organisms. Fungi are essen-



Fungi reproduce both asexually and sexually, as depicted in this generalized life cycle of a zygomycete. In asexual reproduction, spores form by mitosis and cell division. Sexual reproduction involves the fusion of two different mating types and the formation of spores by meiosis.

tial to maintaining conditions that support other life-forms.

IMPACT OF FUNGI

The ecological role played by fungi translates into important economic consequences. The maintenance of soil conditions that support many crops depends on relationships that fungi engage in with plants and other microorganisms. Some fungi inflict devastating damage to crops by causing diseases such as potato blight, black stem rust of wheat, covered smut of barley, powdery mildew, fruit rots, and Dutch elm disease. Other fungi are used in the biological control of costly plant diseases. For example, the yeast *Candia oleophila* protects fruits from harmful molds. The fungus *Trichoderma harzianum* not only protects fruits and vegetables from botrytis, a fungus that causes gray mold, but is also believed to enhance plant growth, degrade pesticides, and prevent the synthesis of toxins produced by other fungi. A mycorrhiza that grows on the roots of trees helps extract calcium from the mineral apatite in forest soils that are calcium poor because of the leaching effects of acid rain. The fungus *Entomophaga maimaiga* attacks the caterpillars of gypsy moths, insects that destroy entire forests by defoliation.

The production of many types of foods depends on fungi. Food and beverage manufacturers exploit the ability of yeasts to produce ethanol and carbon dioxide, two end products of fermentation. The formation of carbon dioxide gas causes bread dough to rise, and ethanol is the form of alcohol found in beer, wine, and liquor. Different fungi impart unique flavors and textures to cheeses, and edible fungi such as mushrooms are a food source themselves. Other fungi cause fruit to rot or bread and cheese to mold. Acidic conditions, as in jams and jellies and inside fruit, inhibit bacterial growth but encourage fungal growth. Fungi can also withstand high solute concentrations used to preserve many foods.

Antibiotics are chemicals produced by one organism that kill or inhibit the growth of another. Many antibiotics are produced by fungi or are derivatives of fungal products. The mold *Penicillium* produces penicillin, the first antibiotic discovered. Other fungi synthesize other commercially available chemical compounds. *Taxomyces andreanne,* a fungus that grows on the bark of Pacific yew trees, secretes taxol, an important anticancer drug. The immunosuppressant drug cyclosporine is derived from the fungus *Tolypocladium inflatum*. *Aspergillus niger* is the source for the flavoring ingredient citric acid and proteases, enzymes that digests proteins.

Less than 1 percent of fungal species are pathogenic to humans and animals. Some species of fungi that are pathogenic to humans cause conditions such as athlete's foot, ringworm, yeast infections, *Pneumocystis* pneumonia (commonly found in acquired immunodeficiency syndrome [AIDS] patients), and histoplasmosis. Diseases caused by fungi are called

mycoses and are usually chronic as a result of the slow-growing nature of fungi. Many mycoses are superficial or penetrate to just below the surface of the skin, but some, including histoplasmosis and coccidioidomycosis, are systemic and affect a number of organs.

See also EUKARYA; EUKARYOTIC CELLS; INFECTIOUS DISEASES; MICROBIOLOGY; REPRODUCTION; SLIME MOLDS.

FURTHER READING

Blackwell, Meredith, Rytas Vilgalys, Timothy Y. James, and John W. Taylor. "Fungi: Eumycota: Mushrooms, Sac Fungi, Yeast, Molds, Rusts, Smuts, etc." in *The Tree of Life Web Project*. Available online. URL: http://tolweb.org/Fungi. Content changed February 21, 2008.

Burnett, John H. *Fungal Populations and Species*. Oxford: Oxford University Press, 2003.

Cowan, Marjorie Kelly, and Kathleen Park Talaro. *Microbiology: A Systems Approach*. New York: McGraw Hill, 2006.

Gadd, G. M. *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press, 2006.

Money, Nicholas P. *Mr. Bloomfield's Orchard: The Mysterious World of Mushrooms, Molds, and Mycologists*. Oxford: Oxford University Press, 2002.

Walting, Roy. *Fungi*. Washington, D.C.: Smithsonian Books, in association with the Natural History Museum of London, 2003.

gene expression Gene expression comprises all the events that lead to the synthesis of a functional gene product from a gene on the deoxyribonucleic acid (DNA). Most genes encode for proteins, and the rest for ribonucleic acid (RNA) molecules that play a role in the synthesis of proteins. Genes, the basic units of inheritance, take the form of stretches of DNA on a chromosome. The sequence of the approximately 1,000 or more nucleotides that make up a gene determines the structure of the product it encodes. The central dogma of molecular biology outlines the flow of genetic information as follows:

$$\text{DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{protein}$$

During the process of transcription, an RNA molecule is made from DNA. If the gene encodes a protein, then the RNA is translated into a sequence of amino acids via the process of translation.

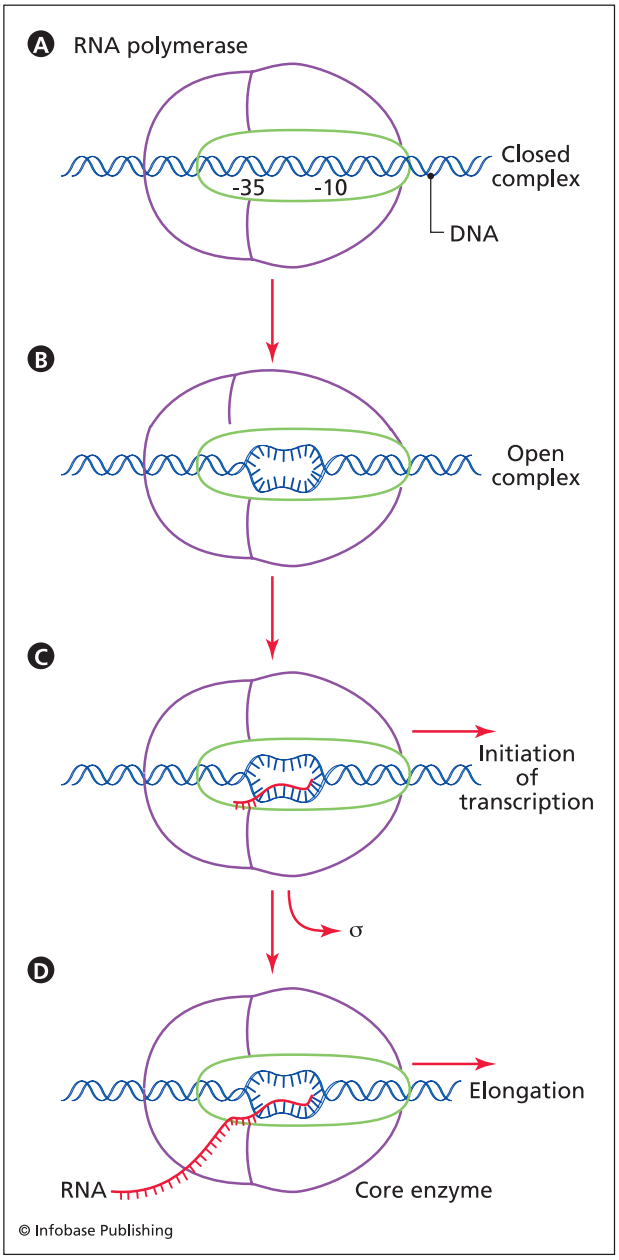
TRANSCRIPTION

The location of the DNA in a cell determines where the first step of gene expression, transcription, occurs. In prokaryotes the DNA exists in the nucleiod region of the cytoplasm, whereas in eukaryotes the DNA exists on chromosomes located in the nucleus. During transcription, basically, an enzyme called RNA polymerase scans along the DNA template and constructs a molecule of RNA based on the sequence of DNA it reads.

Transcription results in the synthesis of one of three different types of RNA, all of which participate in the synthesis of proteins from genes. Messenger RNA (mRNA) carries the information necessary to build a protein. Ribosomal RNA (rRNA) is a component of ribosomes, the sites of protein synthesis. Transfer RNA (tRNA) molecules carry the amino acids to the ribosomes during assembly of the nascent polypeptide.

Though DNA is double-stranded, only one strand within a gene encodes the information necessary to build a protein. The two strands of DNA are complementary, meaning the nitrogenous bases of the deoxyribonucleotides form specific base pairs held together by hydrogen bonds. Adenine always pairs with thymine and guanine always pairs with cytosine. RNA contains uracil instead of thymine and the sugar ribose rather than deoxyribose as part of the nucleotide subunits, but otherwise RNA can participate in complementary base pairing with a single strand of DNA. The major difference is that an adenine on the strand of DNA will pair with a uracil on the strand of RNA. Just as the two strands of DNA run antiparallel to one another (one strand runs 5'→3' and the other runs 3'→5'), a hybrid DNA-RNA molecule will also contain antiparallel strands.

A region called a promoter marks the beginning of a gene. Consisting of a segment approximately 60 base pairs long in prokaryotic organisms and more than 100 base pairs in eukaryotic organisms, the promoter signals to the RNA polymerase where to start transcription and plays an important role in the regulation of transcription. Specific regions of the RNA polymerase recognize and bind to the promoter region of a gene, and the DNA opens up. At the transcriptional start site, the RNA polymerase finds the first ribonucleotide from the pool in the cytoplasm of prokaryotes or the nucleus of eukaryotes. RNA polymerase scans the DNA template in the 3'→5' direction and because it builds an antiparallel complementary strand, it synthesizes RNA in the 5'→3'



Transcription begins when RNA polymerase recognizes the promoter, opens up the DNA, and begins adding ribonucleotides complementary to the DNA template. Elongation proceeds until RNA polymerase reaches a terminator sequence.

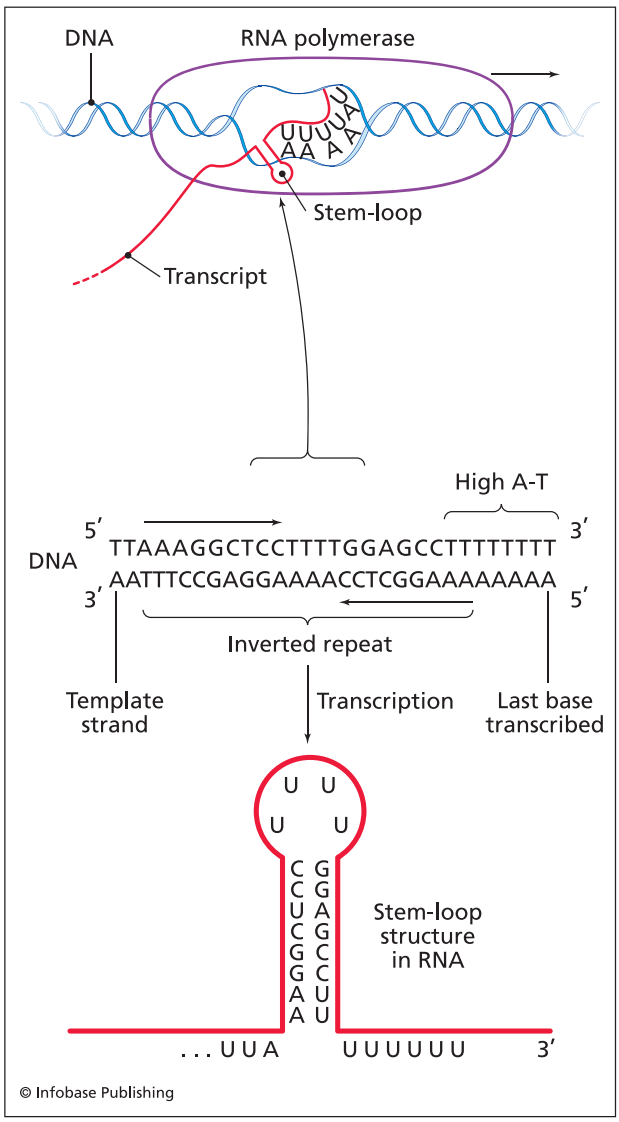
direction. As it scans the template, RNA polymerase adds ribonucleotides, following the rules of complementarity, pairing adenines with uracils (or with thymines on the DNA) and guanines with cytosines. The enzyme forms a covalent linkage between each incoming ribonucleotide and the nucleotide previously added to the new strand of RNA.

Transcription halts when the RNA polymerase reaches a terminator sequence on the DNA. Terminator sequences usually contain an inverted repeat, a specific sequence followed by four intervening deoxyribonucleotides, then the same specific sequence but in the reverse order on the complementary strand. The result is a segment in the newly transcribed RNA that can form a stem-loop structure by forming base pairs with itself. In some cases, a sequence of thymine-adenine base pairs follows the inverted repeat segment of the terminator. This results in a string of uracils on the transcribed RNA. In some prokaryotic genes and most eukaryotic genes, additional protein molecules assist in the process of termination. The hydrogen bonds between the DNA template and the new RNA transcript denature, the RNA molecule falls free, and the RNA polymerase becomes available to start transcribing another gene.

The next step depends on the type of RNA that the cell has synthesized. If the RNA is rRNA, the molecules combine with other molecules to assemble into ribosomes. Prokaryotic ribosomes contain three segments of rRNA: a 16S (1,540 bases) segment in the smaller 30S subunit and a 5S (120 bases) and a 23S (2,900 bases) segment in the larger 50S subunit. Typically, the smaller subunit also contains 21 polypeptides, and the larger subunit also contains 31 polypeptides. Eukaryotic ribosomes contain four segments of rRNA: an 18S molecule (1,900 bases) in the smaller 40S ribosomal subunit and a 5S (120 bases), 5.8S (160 bases), and 28S (4,800 bases) segment in the larger 60S ribosomal subunit. Except for the 5S segment, the rRNAs all belong to the same gene and are transcribed as one molecule. The smaller subunit also contains approximately 33 polypeptides, and the larger subunit also contains approximately 50 polypeptides. The exact number may differ among organisms.

If the newly transcribed RNA is tRNA, then the molecules must fold into a unique three-dimensional structure to become functional. The length of tRNA molecules ranges between 74 and 93 nucleotides, and at least 20 different types exist, one for each amino acid. The tRNAs carry amino acids to the ribosome during the synthesis of polypeptides, and the presence of a specific anticodon (discussed under the subheading Translation on page 367) ensures that the correct amino acid is added to the growing chain. All tRNAs have the same general shape, with three stem-loop structures formed by base pairing between ribonucleotides within the RNA molecule. After transcription of the tRNA, modifications occur, including the removal of extra sequences at the beginning and the end of the molecule and the chemical conversion of some of the nucleotides to unusual nucleotides.

If the RNA serves as a message encoding for the synthesis of a protein, as is most often the case, then in eukaryotic cells, several posttranscriptional modifi-



Transcription terminator regions in DNA consist of stem-loop structures formed from inverted repeat sequences.

cations must precede translation. A methyl-guanosine residue called a cap is added to the 5' end of primary transcripts. This cap aids in ribosomal recognition of the transcript before translation. The enzyme poly-A polymerase adds between 20 and 200 adenine residues to the 3' end of the transcript. The purpose of the polyadenylate tail is to stabilize the transcript and to aid in its transport from the nucleus, where transcription occurs, to the cytoplasm, where translation occurs. Another major posttranscriptional modification is splicing. Eukaryotic genes contain introns, intervening sequences that are not translated into proteins. The regions of the gene that are expressed in proteins are called exons. During splicing, the ends of the exons in the primary transcript are brought into close proximity. The intervening intron is excised, and the ends of the exons are joined. Numerous factors consisting of both polypeptides and RNA participate in the splicing process. The resulting mature mRNA contains only the coding region that is translated and a leader sequence that precedes the coding region of the mRNA and a trailer sequence, neither of which is translated.

TRANSLATION

During the process of translation, the protein synthesizing machinery translates the information embedded in the sequence of ribonucleotides of the mRNA into a protein. In prokaryotic cells, as soon as RNA polymerase has synthesized a long enough segment of mRNA, a ribosome can attach to the 5' end and begin translation. Then, as soon as that ribosome progresses a certain distance along the length of the mRNA, another ribosome can join and begin translation of a second polypeptide. The result is the formation of a polysome, a cluster of ribosomes simultaneously translating the same mRNA transcript. In eukaryotic cells, however, the processed mRNA must first move to the cytoplasm, where the ribosomes, tRNA molecules, and amino acids are located.

In order for translation to occur, the supply of "charged" tRNA molecules must be sufficient. Enzymes called aminoacyl-tRNA synthetases charge the tRNAs by attaching the appropriate amino acid to the ribose sugar at the 3' end of the tRNA molecule. A different tRNA synthetase specifically recognizes each of the 20 different amino acids and covalently links them to a tRNA molecule that has the corresponding anticodon based on the genetic code. An anticodon is the sequence of three consecutive nucleotides on one loop of a tRNA molecule that forms complementary base pairs with a codon on the mRNA. Each codon contains three ribonucleotides that specify one of the 20 amino acids. Because there are four different ribonucleotides, and they are read in sets of three, there are 64 (4^3) different codons. One of these is the start codon, AUG, and always encodes methionine. Three codons serve as stop signals for translation: UAG, UGA, and UAA. The remaining 60 triplet codons encode for the 19 other amino acids. Because there are many more possible codons than amino acids, several amino acids are represented by more than one codon, a characteristic termed redundancy. When the anticodon of the tRNA binds to the complementary codon on the mRNA, the ribonucleotide at the third position of the codon is often flexible. This reduced constraint at the third position is referred to as wobble. To illustrate these concepts, consider the codons CUU, CUC, CUA, and CUG. The fact that they all encode

Codons in mRNA

First base	Second base U	Second base C	Second base A	Second base G	Third base
U	UUU, UUC — Phenylalanine UUA, UUG — Leucine	UCU, UCC, UCA, UCG — Serine	UAU, UAC — Tyrosine UAA, UAG — Stop	UGU, UGC — Cysteine UGA — Stop UGG — Tryptophan	U C A G
C	CUU, CUC, CUA, CUG — Leucine	CCU, CCC, CCA, CCG — Proline	CAU, CAC — Histidine CAA, CAG — Glutamine	CGU, CGC, CGA, CGG — Arginine	U C A G
A	AUU, AUC, AUA — Isoleucine AUG — Start	ACU, ACC, ACA, ACG — Threonine	AAU, AAC — Asparagine AAA, AAG — Lysine	AGU, AGC — Serine AGA, AGG — Arginine	U C A G
G	GUU, GUC, GUA, GUG — Valine	GCU, GCC, GCA, GCG — Alanine	GAU, GAC — Aspartic acid GAA, GAG — Glutamic acid	GGU, GGC, GGA, GGG — Glycine	U C A G

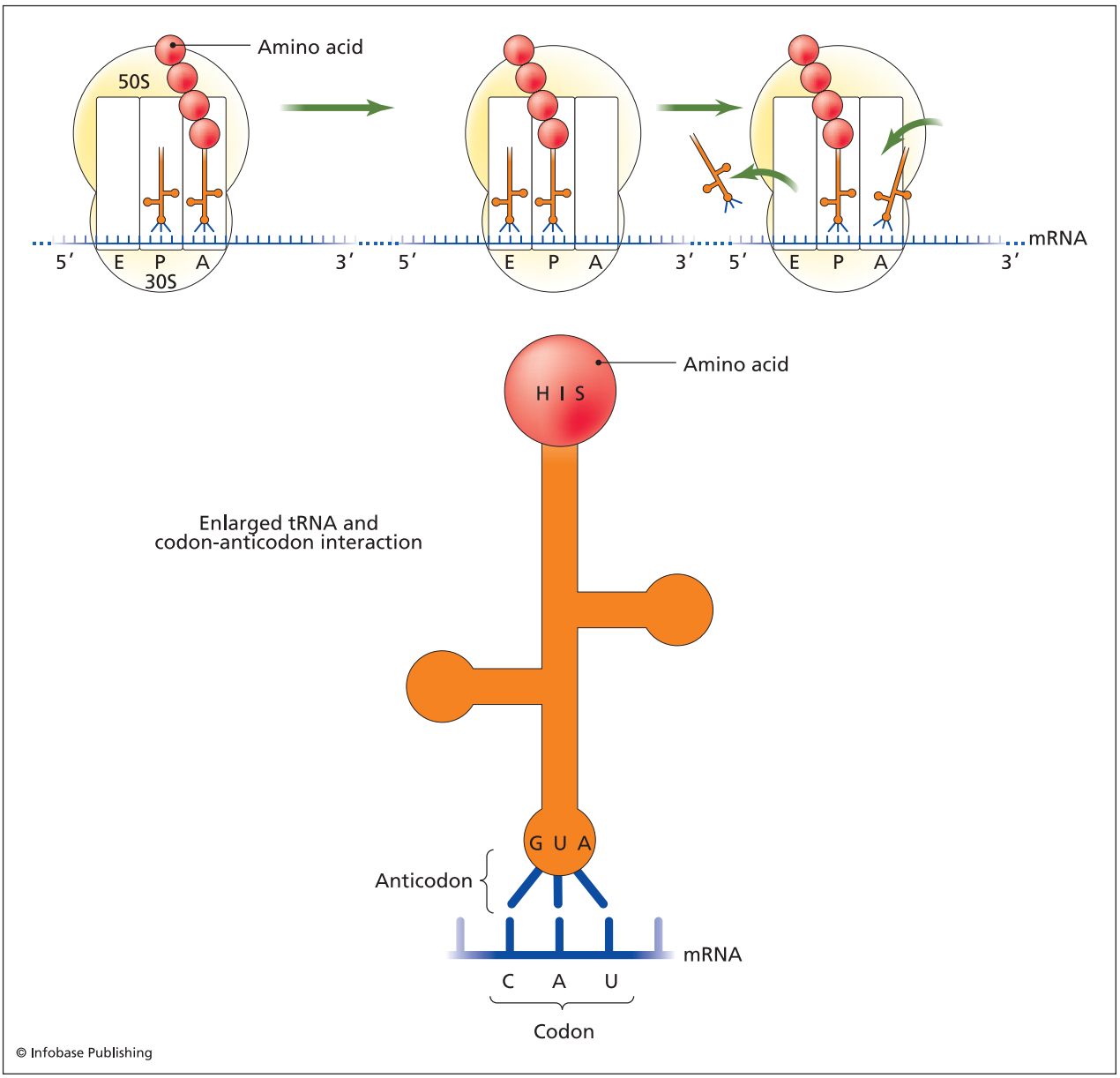
The genetic code specifies the amino acid represented by each triplet codon.

for the amino acid leucine demonstrates redundancy in the genetic code, and actually, UUA and UUG also encode leucine. The fact that the anticodon of the tRNA that carries leucine binds to any codon that has CU as its first two ribonucleotides no matter which ribonucleotide occurs in the third position demonstrates wobble.

Translation begins when the small subunit of a ribosome attaches to the 5' leader sequence of an mRNA transcript. The 16S rRNA of the 30S subunit in prokaryotes facilitates this interaction by forming temporary base pairs with a complementary sequence in the leader portion of the mRNA, the Shine-Dalgarno sequence, which also serves to position the ribosome at the translation start codon. In eukaryotes, the 5' cap helps the small subunit of the ribosome bind to the mRNA, and then it typically scans the RNA moving in the 3' direction until it reaches the first AUG, where the large subunit assembles to complete formation of the ribosome. An initiator tRNA charged with methionine, which in prokaryotes contains a formyl group attached to it, joins the complex, followed by the large ribosomal subunit. Formation of the initiation complex and ribosome assembly also involve the participation of several initiation factor proteins and require energy.

The ribosome contains three sites: the aminoacyl (A) site, the peptidyl (P) site, and the exit (E) site. The mRNA runs along the bottom of these sites. After the initiator tRNA carries in the first amino acid, methionine, the ribosome is positioned such that the codon that occurs immediately after the start codon is positioned under the A site, and the tRNA with the methioinine is in the P site. Elongation occurs when the tRNA that has an anticodon that pairs with the mRNA codon in the A site takes the next amino acid to the ribosome. Proteins called elongation factors assist in the correct positioning of the ribosome. If the correct tRNA is sitting in the A site, then a peptide bond forms between the amino acid in the P site and the amino acid in the A site. The enzymatic activity that performs this reaction is part of the 50S subunit of the ribosome and contains both protein and RNA components. After the peptide bond forms, the dipeptide is attached to the tRNA in the A site. Next, with the aid of another elongation factor, the mRNA moves relative to the ribosome such that the third codon is now positioned under the A site, the tRNA with the attached dipeptide is in the P site, and the initiator tRNA sits in the E site. The uncharged tRNAs fall from the E site into the cytoplasm, where they become recharged. Elongation proceeds with the next tRNA moving to the A site, a peptide bond forms between the amino acids attached to the tRNAs in the P and A sites, translocation of the mRNA with respect to the ribosomes, and so on.

The energy burning process of elongation continues until a stop codon sits in the A site of the ribosome. No tRNAs exist that have anticodons complementary to the stop codons. Proteins called release factors help free the nascent polypeptide from the final tRNA. The ribosome disassembles and the mRNA dissociates. The polypeptide adopts its three-dimensional conformation. Sometimes, before becoming functional, the polypeptide must combine with other polypeptides to form a complete protein.



During elongation of translation, tRNA molecules carry the correct amino acids to the growing chain, peptide bonds form, and the mRNA slides over one codon at a time for the addition of the next amino acid.

Modifications such as the addition of sugar moieties or phosphate groups may also be necessary before the protein is fully functional.

Molecular geneticists have encountered several exceptions to the typical processes involved in gene expression. RNA editing is one such exception, in which uracil residues are added to the mRNA or ribonucleotides are chemically modified after transcription but before translation. The discovery of reverse transcription in retroviruses challenged the dogma regarding the flow of genetic information. These viruses, including the human immunodeficiency virus and the Rous sarcoma virus, have RNA as their genetic material but synthesize a complementary DNA molecule using the RNA as a template during the viral replication process. Other viruses, RNA phages that infect bacteria, make an enzyme that enables the single-stranded RNA genome to self-replicate.

DIFFERENCES IN PROKARYOTIC AND EUKARYOTIC GENE EXPRESSION

As briefly mentioned in the preceding discussion, gene expression in prokaryotes and eukaryotes differs in several ways. First of all, the structure of the genes on the DNA varies. In prokaryotes, genes are often polycistronic, meaning they encode for several proteins to be translated from a single product

of transcription. In eukaryotes, one gene typically encodes one protein. Another major difference is that in prokaryotic cells, both transcription and translation occur in the cytoplasm and therefore can occur simultaneously. Ribosomes can attach to and begin translation of a molecule of mRNA that is still being transcribed by RNA polymerase. In eukaryotic cells, transcription occurs in the nucleus, where the DNA is housed, and translation occurs in the cytoplasm. Many more factors are involved in the initiation of transcription in eukaryotes than in prokaryotes, and eukaryotic genes often involve regions called enhancers far upstream of the coding region of the gene in addition to the promoter that play a role in regulation of the expression. Prokaryotes have a single RNA polymerase that transcribes the DNA template, whereas eukaryotes have three different RNA polymerases. RNA polymerase I transcribes the nucleolar organizer, the region around which the nucleolus forms and where ribosomal RNA is made. RNA polymerase II transcribes most genes that encode proteins. RNA polymerase III transcribes the 5S and the tRNA genes. After transcription, eukaryotic RNA must undergo several modifications, including the addition of a 5' cap, a poly A tail, and splicing, before it is transported to the cytoplasm. The first amino acid of a polypeptide in prokaryotes is chemically modified by the attachment of a formyl group to its ribose sugar. The ribosomal structure differs between prokaryotes and eukaryotes also, with slightly different components and overall sizes. Despite all of these differences, the general processes of transcription and translation are remarkably similar in prokaryotic and eukaryotic cells.

REGULATION OF GENE EXPRESSION

Cells utilize many strategies to achieve a balance between synthesizing sufficient quantities of gene products when needed and conserving energy. The mechanisms that cooperate to perform this function are collectively termed the regulation of gene expression. Some genes are constitutive, or are always expressed at relatively constant levels in active cells. These are often called housekeeping genes, and they are necessary to maintain the cell's normal activities. For example, rRNA genes are necessary for the construction of ribosomes, which are always needed by active cells to synthesize proteins. Other gene products are only necessary under specific conditions, during certain stages of development or differentiation, or only in specialized cell types.

Regulation of these gene products are made and of the quantity produced can occur at any step during expression. The DNA itself can be chemically altered or packaged into chromatin in a way that either allows or completely prevents the recognition or access of those genes. Transcription initiation is the most commonly regulated step of gene expression. Proteins called transcription factors can bind regions of gene promoters to up-regulate, or increase the expression of certain genes. In some cases, such as genes that are only expressed in specific tissues, certain factors beyond the basal factors are required to initiate transcription. Repressors down-regulate expression by binding to promoters and interfering with RNA polymerase binding or progression through a promoter. Activators are proteins that bind to enhancer regions far away from the promoter but can still interact with proteins bound at the promoter by looping out of the intervening DNA.

Though the mechanisms that regulate the expression of genes are numerous and complex, the end result is that cells express proteins when they need them. For example, steroid hormones such as testosterone, produced only at certain times in mammalian development, bind to tissue-specific protein receptors that are only present in target tissues or organs. Upon recognition of the hormone, the receptor then binds to the DNA and activates the expression of genes that result in the onset of sexual maturation. In another example, to prevent wasting energy, bacterial cells only synthesize the enzymes involved in certain metabolic pathways when the appropriate substrates are available. If the carbohydrate lactose is not present, then a repressor protein binds to the regulatory region of the operon that encodes the genes for lactose utilization and inhibits RNA polymerase from transcribing those genes. (Operons are sequences of adjacent genes that are all under the same regulatory control.) When lactose is present, it binds to the repressor protein and inhibits it from binding to the DNA and interfering with RNA polymerase. The genes are transcribed and translated into the proteins that enable the cell to utilize lactose as an energy source.

See also BIOMOLECULES; CHEMICAL BASIS OF LIFE; DEOXYRIBONUCLEIC ACID (DNA); DNA SEQUENCING; GENETIC DISORDERS; GENETICS; GENOMES.

FURTHER READING

Clark, David P. *Molecular Biology Made Simple and Fun.* 3rd ed. St. Louis: Cache River Press, 2005.

Lewin, Benjamin. *Essential Genes.* Upper Saddle River, N.J.: Prentice Hall, 2005.

Mattick, John S. "The Hidden Genetic Program of Complex Organisms." *Scientific American* 291 (2004): 60–67.

Ptashne, Mark. "Gene Regulation by Proteins Acting Nearby and at a Distance." *Nature* 322 (1986): 697–701.

Raineri, Deanna. *Introduction to Molecular Biology.* Malden, Mass.: Blackwell Science, 2001.

Tjian, Robert. "Molecular Machines That Control Genes." *Scientific American* 272 (1995): 54–61.
Watson, James D., Tania A. Baker, Stephen P. Bell, Alexander Gann, Michael Levine, and Richard Losick. *Molecular Biology of the Gene*. 6th ed. San Francisco: Benjamin Cummings, 2007.
Weaver, Robert F. *Molecular Biology*. 4th ed. New York: McGraw Hill, 2008.

gene therapy Diseases and disorders result from a variety of causes: pathogenic microorganisms cause infectious diseases; degenerative diseases result from either aging, excessive wear and tear, or continuous exposure to harmful substances; several intrinsic and extrinsic factors can lead to developmental disorders; and genetic disorders result from harmful mutations in one's hereditary material, or genome. Genes are the units of heredity that encode all of an organism's characteristics, from skin tone to the ability to tolerate dairy products. Deoxyribonucleic acid (DNA) is the biomolecule that physically carries the genes and consists of four different nucleotides (abbreviated *A, C, G,* and *T*). The specific order of these nucleotides along the length of a DNA molecule encodes the information necessary to build proteins, the molecules that carry out most cellular activities and ultimately determine the characteristics of an individual. Mutations are changes to the sequence of the nucleotides of DNA, and if they occur within a region that encodes a protein, they can affect the protein's structure and possibly destroy the ability of that protein to perform its function. Researchers have identified specific mutations responsible for many disorders including cystic fibrosis, muscular dystrophy, Huntington's disease, hemophilia, severe combined immunodeficiency (SCID), and some types of cancers. People who have genetic disorders such as these may obtain relief someday from gene therapy, as might people who have other chronic diseases that have a genetic component. Disorders that result from multiple mutations or from other extrinsic factors, such as Alzheimer's disease, diabetes, heart disease, and high blood pressure, are not good candidates for gene therapy.

Gene therapy is an experimental treatment that involves the insertion of normal genes into an individual to replace defective genes. Medical researchers are currently investigating gene therapy to treat certain genetic disorders, and a few clinical trials are under way. Unsolved difficulties currently prevent the widespread use of gene therapy, but the technique offers hope. Though researchers have launched hundreds of clinical trials around the world since the first one in 1990, the only gene therapy product currently in use other than in clinical trials is one approved in China for cancer. Safety concerns and ineffectiveness plague human trials, but researchers continue to develop new strategies and techniques.

In order to attempt treating a genetic disorder using gene therapy, the specific mutation that causes it must be identified and the normal gene must be cloned. The normal gene is packaged inside a vector that carries the gene into the targeted cells. Ex vivo methods introduce the DNA to host cells that have been removed from the body, such as blood cells or bone marrow cells. The cells are returned to the body intravenously after exposure to the DNA. In vivo methods involve the transfer of the vector or liposome to cells in the patient's body. Once inside the cells, the gene must be expressed, meaning the cells must produce the protein that the gene encodes. The goal is that once the cells receive a normal copy of the gene, they produce the protein and restore the function that was lacking in the individual with the disorder. Viruses are the common choice for vectors—specifically, viruses that have been genetically engineered so they cannot cause disease. Scientists knock out the genetic information that allows the virus to replicate itself once it has infected a human cell. Theoretically, once the virus transports the recombinant DNA into the human cell, its job is complete, and the virus cannot cause illness. A disabled adenovirus, one of the many types of viruses that cause the common cold, is the most frequently used virus vector. Adeno-associated viruses can insert their DNA directly into one of the human chromosomes rather than simply releasing it into the cytoplasm of a cell and hoping the cell incorporates it on its own. Retroviruses are also useful because they make double-stranded DNA that integrates itself into the host cell chromosomes, becoming a permanent part of the genome for those cells and their progeny. Another approach is to introduce naked DNA directly to the target cells, but this method is inefficient, requires a lot of DNA, and can only be used for certain tissues. A nonviral method for delivering DNA is to package it into tiny spheres surrounded by artificial lipids, called liposomes. The lipid exterior allows the spheres to penetrate the membrane of the host cell and release the DNA upon entry.

Sometimes the therapeutic gene being introduced is not a gene that is mutated in the individual, but rather a gene that encodes a protein that has the potential to overcome or reverse the effects of the individual's symptoms. This strategy may be useful in treating cancer or autoimmune disorders. More than 67 percent of gene therapy trials are for treating cancers. In addition to inserting normal copies of genes that are known to cause cancer when mutated, researchers are examining ways to improve

(continues on page 374)

GENE THERAPY AND CYSTIC FIBROSIS

by Caleb Hodson, Ph.D.
Howard Hughes Medical Institute, Yale University

In an ideal world the air people breathe everyday would be clean. In reality, each breath carries particles of dust as well as other potentially harmful substances such as bacteria into the lungs. To remain healthy, the body must remove or clear these foreign materials from the lungs where they can irritate the airways, reduce lung performance, or cause infection. People with the genetic disease cystic fibrosis, among other symptoms, are unable to clear their airways efficiently. Mutations in their DNA lead to abnormal secretions, which impair an important defense mechanism responsible for maintaining a sterile lung and respiratory environment. As a result, these patients suffer from reduced lung capacity and are more likely to acquire airway infections. Normally, removal of unwanted debris from the airways occurs through a defense process called mucociliary clearance. The two essential elements of mucociliary clearance are found within its name—mucus (*muco-*) and cilia (*-ciliary*).

Mucus, a mixture of water and protein, forms a protective coating over the cells of the respiratory tract. This layer of mucus traps foreign materials such as dust or bacteria before they have a chance to contact and damage the underlying cells. To produce mucus, specialized cells along the airways generate the protein component of mucus, called mucin, which the cells export onto the airway surface. Once at the surface, mucin absorbs water and becomes hydrated into the characteristic gellike consistency of mucus. If not enough water is present to hydrate the mucin properly, the resulting mucus becomes thick and sticky, similar to paste. Mucus must be the correct consistency in order for ciliary action to move it up and out of the airways successfully. Cilia are microscopic hairlike projections that extend out from the surface of airway cells. The millions of cilia present along the respiratory tract beat in a coordinated motion, providing the force necessary to propel mucus up and out of the airways. Watery mucus does not efficiently trap bacteria and particulates, and they remain inside the airways. If mucus is too viscous, then cilia are unable to move it out of the airways.

Individuals who have cystic fibrosis possess a mutation that reduces the amount of water available to hydrate mucin along the airway surface. As a result, the mucus of cystic fibrosis patients is too viscous and is inefficiently cleared from the airways. Over time, mucus accumulates in the lungs, forming large masses or cysts of thick and fibrous mucus, hence the name *cystic fibrosis.* As the mucus accumulates, so do bacteria and other foreign materials that often cause life-threatening infections. Despite treatment with antibiotics and other strategies, half of the people diagnosed with cystic fibrosis die before reaching their upper 30s. Daily life with cystic fibrosis is often difficult and requires strict adherence to medication schedules and participation in physical therapy exercises to increase lung performance.

While the treatment options for patients who have cystic fibrosis have improved over time, at best they provide temporary fixes to a permanent problem, mutations in a gene critical for mucus production. Because the DNA sequence of genes specifies the information needed to construct cellular proteins, changes or mutations within genes can result in changes within the corresponding proteins. Such changes sometimes alter the proper function of proteins as happens with the gene *CFTR.* Specific mutations in the *CFTR* gene sequence are responsible for cystic fibrosis. For example, the most common mutation in the *CFTR* gene that causes cystic fibrosis results in the deletion of a single amino acid from the CFTR protein. However, without this one amino acid, the CFTR protein has very little to no functional activity. Replacement of the defective *CFTR* gene with a normal copy could possibly reverse the ultimate cause of cystic fibrosis. This concept forms the basis for gene therapy and genetic therapeutics. Overcoming the harmful effects of mutated genes by transferring normal copies of the genes into cells holds great promise for the treatment of numerous genetically based diseases. In order for this strategy to work, several obstacles must be overcome. First, the mechanism for transferring the genes must efficiently target the affected cells. Second, the expression of the normal genes must be long-lasting or even persist for the rest of the patient's lifetime. Third, the transfer of genetic material must not cause unmanageable side effects such as inappropriate activation of the body's immune responses. While several clinical trials have been performed to test gene therapy treatments in cystic fibrosis patients, none showed a significant improvement linked to the therapy. One major reason for this lack of success is the incomplete understanding of how to balance each of these requirements for optimal gene therapies.

In order to introduce new DNA into cells, the DNA molecules must be contained within a delivery mechanism to prevent damage and degradation as the DNA is sent to the target cells. In addition, the surface of the target cells must be accessible to the delivery mechanism. The cells lining the airways directly contact the external environment, unlike cells in other organs such as the liver. This exposed location means targeting the affected airway cells in order to treat cystic fibrosis is relatively straightforward and could occur by directly applying gene therapies in an aerosolized form that can be inhaled. Although this characteristic is definitely an advantage over other tissues, there are still significant challenges to targeting airway cells. A substantial hindrance is the protective barrier of mucus within the airways that blocks contact between the DNA targeting mechanism and the cell surface.

Since DNA cannot be directly applied to cells because of damage and degradation, technologies have been developed to assist in the transfer of DNA into cells. The most promising approach for use in gene therapy

is to package DNA within virus particles, which are then used to infect the cells of interest. In the simplest terms, viruses are nucleic acid molecules enclosed in a coat of proteins that allow viruses to associate selectively with receptor proteins on the surface of host cells. Viruses attach to the plasma membrane of the target cells and the genetic material is transferred into the cells. Once inside, the viral genes encode the information needed to generate new virus particles that are ultimately released and go on to infect other cells. Through genetic engineering, one can create viruses that contain only a few essential genes plus normal copies of human genes affected in specific disorders such as cystic fibrosis. Additionally, viruses for gene therapy are unable to replicate inside human cells, therefore limiting the possibility of the virus's spreading throughout the body. Ideally, exposure of the airway cells to these engineered viruses should lead to the transfer of the normal gene copies into the airway cells. Subsequently, these genes will produce normal versions of the encoded proteins to compensate for the presence of mutated ones.

While sound in theory, the application of viral technology to gene therapy in the lungs is far from perfect. A well-studied type of virus for gene therapy, adeno-associated virus (AAV), provides high-efficiency transfer of genes to the targeted airway cells; however, its effects are short-lived and production of normal protein diminishes over time. Nonetheless, several clinical trials using AAV-based therapy have been performed. In a 2004 study, patients receiving virus containing the human *CFTR* gene sequence all showed efficient targeting of the gene to airway cells (Moss et al., 2004.) Furthermore, modest improvement in lung function occurred in patients receiving *CFTR*-containing virus compared to patients receiving placebo virus when tests of lung function were made 30 days after the treatment. Unfortunately when the patients were retested at two and three months after viral therapy, the investigators found no difference between patients in the test group and placebo control group patients. The virus can be administered multiple times; however, efficiency of gene transfer is reduced in subsequent applications, limiting its usefulness. Importantly, adeno-associated viruses do not seem to activate the immune system in significant ways, and that is a strong advantage over other types of viruses previously considered for gene therapy. While continuing research may provide solutions to the current shortcomings of AAV-based gene therapy, alternative strategies are also being investigated. Another class of viruses known as lentiviruses offers the advantage that genes transferred by this viral class can integrate into the existing genome. If genes integrate, they may be more stable and remain functional for longer periods. This distinction, in contrast to AAV technology, would alleviate the need for repeated administration of virus to the airways, which loses effectiveness over time.

Besides viral technology, several non-viral strategies show promise. DNA can be packaged within synthetic vesicles called liposomes made of compounds that mimic the biochemistry of the cellular plasma membrane. Fusion of liposomes with the plasma membrane introduces the contained gene sequence to the interior of the cell, where the gene has access to the normal cellular machinery. Current clinical trials show mixed results concerning activation of the immune system, and the efficiency with which cells uptake DNA appears to be lower than with viral techniques. Advances in the engineering of the chemical properties of liposomes should increase efficiency, making this approach more appealing for gene therapy. Finally, DNA may be transferred into cells by complexing a gene sequence with nanoparticles. These small molecular compounds are readily taken up by cells, and they rapidly deliver the transported genes to the cell nucleus. A 2004 study using this emerging technology demonstrated measurable CFTR function in patients treated with nanoparticles versus saline solution–treated controls (Konstan et al., 2004). The study showed no adverse side effects to the application; however, the improved CFTR function only persisted a few days, meaning lasting effects require repeated administration of the therapy. More recently, in 2007, a gene therapy strategy using nanoparticle technology successfully reduced the growth of cancerous tumors in laboratory animals. (Deng et al., 2007). Further development of nanoparticle applications is hoped to increase their usefulness as treatments for diseases such as cystic fibrosis. Although no gene therapy treatment currently balances all the requirements needed to cure cystic fibrosis, the lessons that have been learned, the continued research, and future technological developments will put society closer to effective gene therapies and truly novel ways to conquer genetic diseases.

FURTHER READING

Deng, Wu-Guo, Hiroyaki Kawashima, Guanglin Wu, Gitanjali Jayachandran, Kai Xu, John D. Minna, Jack Roth, and Lin Ji. "Synergistic Tumor Suppression by Coexpression of FUS1 and p53 Is Associated with Down-Regulation of Murine Double Minute-2 and Activation of the Apoptotic Protease-Activating Factor 1-Dependent Apoptotic Pathway in Human Non-Small Cell Lung Cancer Cells." *Cancer Research* 67 (2007): 709–717.

Konstan, Michael W., Pamela B. Davis, Jeffrey S. Wagener, Kathleen A. Hilliard, Robert C. Stern, Laura J. H. Milgram, Tomasz H. Kowalczyk, Susannah L. Hyatt, Tamara L. Fink, Christopher R. Gedeon, Sharon M. Oette, Jennifer Payne, Osman Muhammad, Assem G. Ziady, Robert C. Moen, and Mark J. Cooper. "Compacted DNA Nanoparticles Administered to the Nasal Mucosa of Cystic Fibrosis Subjects Are Safe and Demonstrate Partial to Complete Cystic Fibrosis Transmembrane Regulator Reconstitution." *Human Gene Therapy* 15, no. 12 (2004): 1,255–1,269.

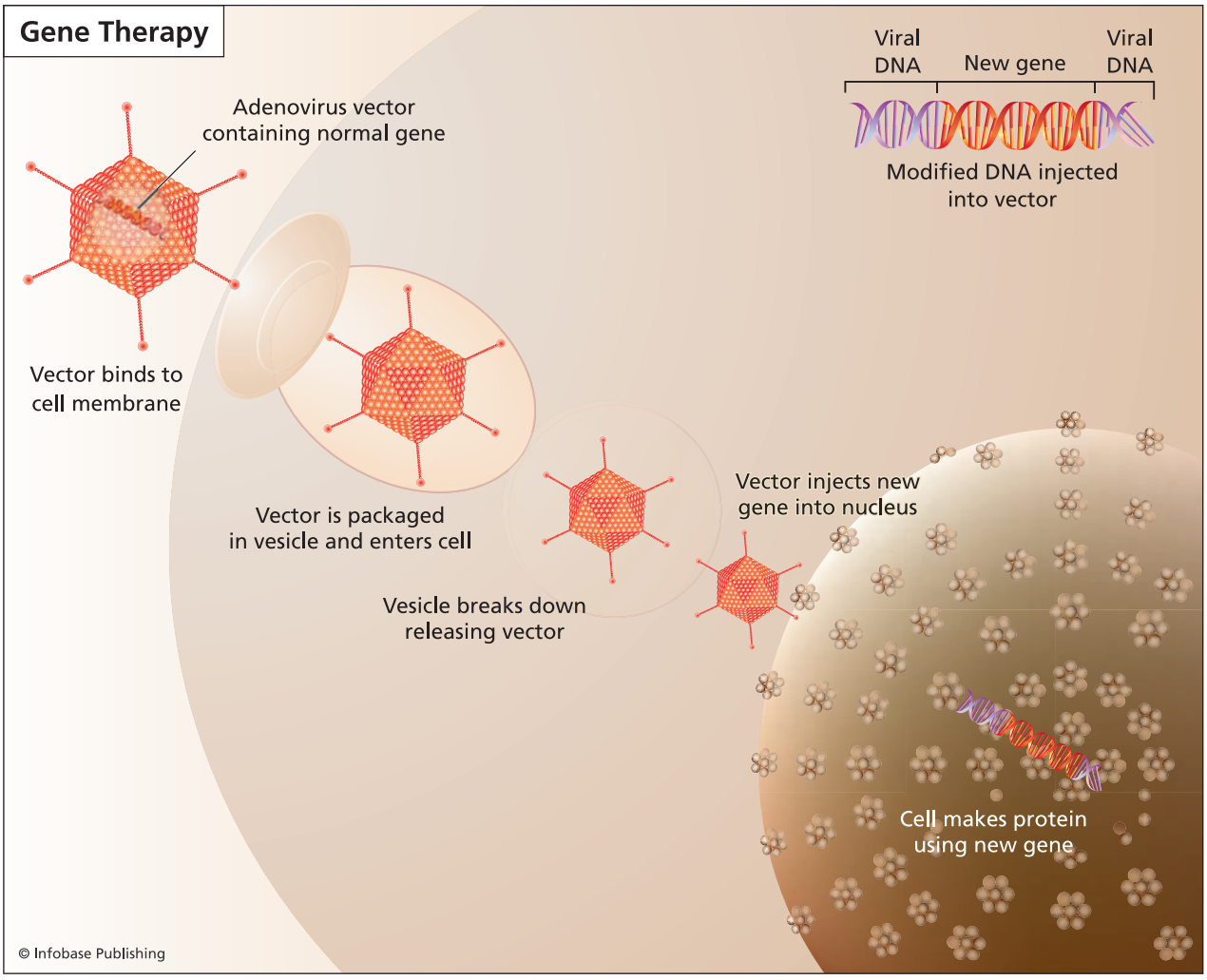
Moss, Richard B., David Rodman, L. Terry Spencer, Moira L. Aitken, Pamela L. Zeitlin, David Waltz, Carlos Milla, Alan S. Brody, John P. Clancy, Bonnie Ramsey, Nicole Hamblett, and Alison E. Heald. "Repeated Adeno-Associated Virus Serotype 2 Aerosol-Mediated Cystic Fibrosis Transmembrane Regulator Gene Transfer to the Lungs of Patients with Cystic Fibrosis: A Multicenter, Double-Blind, Placebo-Controlled Trial." *Chest* 125 (2004): 509–521. Available online. URL: http://chestjournal.org/cgi/content/full/125/2/509. Accessed August 3, 2008.

(continued from page 371)
the effectiveness of a patient's own immune system to attack cancer cells by introducing genes into white blood cells to help them recognize and attack tumor cells. The insertion of genes encoding for the production of certain cytokines, chemicals produced by the immune system that increase its efficiency, may also help. Two other ideas for treating cancer by gene therapy are inserting genes that cause the target cells to self-destruct or that prevent the growth of blood vessels to feed tumors, depriving them of nutrition. An alternative approach is the introduction of genes to a patient's blood-forming stem cells, with the aim of making the individual more resistant to the side effects of chemotherapy, allowing him or her to withstand traditional treatments better.

PROBLEMS AND PROGRESS

Major problems with current gene therapy protocols include inserting the normal genes into the targeted cells within the body and having them function properly after insertion. The body can naturally suppress their expression, preventing the cells from synthesizing functional protein, even if the gene is successfully inserted. In order for gene therapy to cure a condition, the therapeutic DNA must remain functional, and the target cells must be long-lived and stable. Currently, moderately successful therapies involve multiple rounds of treatment. Another problem is that the host's immune system often attacks the viral vectors. This is particularly troublesome when the host's immune system has previously encountered the virus used as a carrier, because the immune response is quicker and stronger upon the second and subsequent exposures. This characteristic hinders the effectiveness of multiple rounds of treatment.

According to the National Institutes of Health (NIH), hundreds of gene therapy trials have been registered, but the trials have had mixed results. Theoretically and in lab animal experiments, gene therapy



Viruses are often used as vectors to carry therapeutic genes into host cells during gene therapy.

looks promising, but when it is tested on humans, success is rare.

The first trial, launched by an NIH research physician named William Anderson in 1990, involved two girls who had inherited a form of SCID that left them unable to fight infections because of the lack of the enzyme adenosine deaminase (ADA). Researchers took some of the girls' cells, cultured them in vitro, inserted a normal copy of the ADA gene, then introduced their own genetically engineered white blood cells back into their bodies. Both girls were able to live normal lives afterward. Critics claim, however, that their apparent success is due to injections of a synthetic ADA enzyme that the patients started receiving shortly after the gene therapy treatments, not to restored immune function.

In one well-publicized case from 1999, a patient died of a reaction to gene therapy treatment for a potentially fatal metabolic disorder. The patient, 18-year-old Jesse Gelsinger, had a metabolic condition caused by the lack of an enzyme, ornithine transcarbamylase, that the liver uses to break down ammonia. The disorder led to frequent hospitalizations during childhood and required him to take numerous daily medications to maintain his health. After treatment, his immune system attacked the adenovirus vector designed to carry the normal gene to his liver cells. He suffered from multiple organ failure and died four days after receiving treatment. Investigations by the U.S. Food and Drug Administration (FDA) revealed questionable practices by the researchers at the University of Pennsylvania, where the trial was conducted.

In January 2003 the FDA placed a hold on all active gene therapy trials that used retroviral vectors to insert genes in blood stem cells after a second child who had been treated for X-linked severe combined immunodeficiency disease (X-SCID) in France developed an illness similar to leukemia. Initial results from the trial, which began in 1999, were promising, and all of the 10 treated children left the hospital with functional immune systems and seemed to be living normal lives, but in 2002 some of the children developed a condition resembling leukemia, in which some of the white blood cells started growing uncontrollably. After discussing appropriate safeguards for using retroviral vectors, in April 2003 the FDA lifted the ban on the use of such vectors in blood stem cells.

After learning that gene therapy researchers were making unsupported claims and not reporting unexpected adverse reactions of gene therapy trials, the public support and willingness to volunteer for trials diminished. In 2004 the NIH and the FDA, the governmental agency that oversees gene transfer research, launched a Genetic Modification Clinical Research Information System (GeMCRIS), a public database containing accurate information related to ongoing research and clinical trials of gene therapy. GeMCRIS helps the NIH and FDA, researchers, physicians, patients, and the general public to monitor progress, share successes, and address concerns related to safety of gene transfer research.

In 2003 researchers at the University of California at Los Angeles reported the successful delivery of genes across the blood-brain barrier, opening the door for gene therapy treatments for disorders such as Parkinson's or Alzheimer's disease. They used liposomes coated with a polymer called polyethylene glycol as the vector. Another avenue of active research involves RNA interference, a new potential type of gene therapy that uses double-stranded RNA to block the production of certain proteins. This form of gene therapy may be useful for disorders such as Huntington's disease, in which the symptoms result when the presence of a mutant protein rather than the simple lack of a protein's ability to function causes disease symptoms. Recent successes in gene therapy include the following:

- In 2005 the delivery of the *Atoh1* gene, which stimulates hair cell growth, into the cochlea of deaf guinea pigs using adenovirus triggered growth of the hair cells and restored 80 percent of the hearing to the animals.
- In 2006 two adults were successfully treated for a myeloid disease with gene therapy.
- Also in 2006 patients with advanced metastatic myeloma were treated with genetically engineered immune cells that were altered to target and attack cancer cells.
- In 2007 the delivery of two tumor suppressing genes via lipid-based nanoparticles reduced the number and size of lung cancer tumors in mice.
- Also in 2007, human gene therapy trials for a type of inherited childhood blindness commenced.

Though gene therapy has suffered many difficulties and setbacks, the potential benefits are worth continued research. Among the attraction is the promise of curing disease by attacking the underlying cause, rather than simply treating the signs and symptoms.

See also CLONING OF DNA; GENE EXPRESSION; GENETIC DISORDERS; POINT MUTATIONS; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Department of Health and

Human Services. "Cellular and Gene Therapy." Available online. URL: http://www.fda.gov/cber/gene.htm. Updated July 26, 2007.
Judson, Horace Freeland. "The Glimmering Promise of Gene Therapy." *Technology Review* 109, no. 5 (November 2006): 40–47.
Panno, Joseph. *Gene Therapy: Treating Disease by Repairing Genes*. New York: Facts On File, 2004.
Preuss, Meredith A., and David T. Curiel. "Gene Therapy: Science Fiction or Reality?" *Southern Medical Journal* 100, no. 1 (2007): 101–104.
U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program. "Human Genome Project Information: Gene Therapy." Available online. URL: http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml. Updated September 19, 2008.

genetic disorders The human genome contains more than 3 billion nucleotide base pairs, and each nucleated cell, except gametes, contains 46 total chromosomes. Abnormalities can occur at the molecular level or the chromosomal level, leading to genetic disorders. Some genetic disorders have been passed down numerous generations while others result from errors that occur during gamete formation. Genetic disorders can be classified into four main types: single-gene disorders, multifactorial disorders, chromosomal disorders, and mitochondrial disorders.

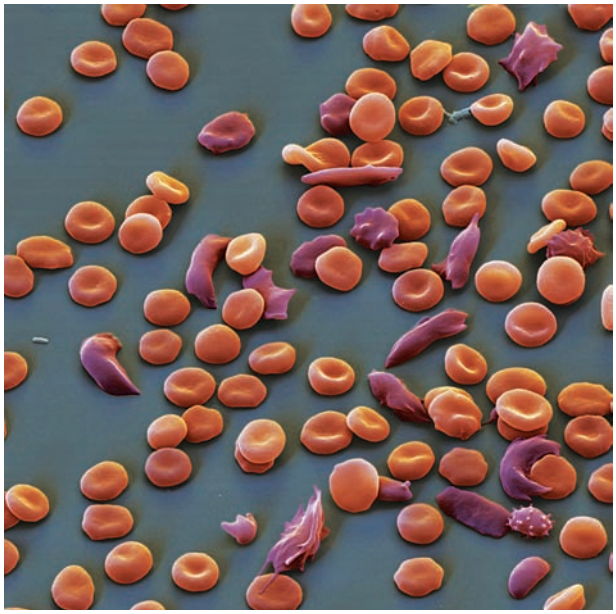
SINGLE-GENE DISORDERS

More than 6,000 genetic disorders characterized to date result from the absence of a functional protein product from one gene. A mutation at that locus might result in the production of abnormal protein that cannot perform its cellular function, the gene might be expressed at inappropriate levels (either too much product or too little product), or the gene might be missing altogether. Many single-gene disorders involve a mutant protein product resulting from an altered nucleotide sequence within the coding region of the gene, and therefore an altered amino acid sequence within the protein. These so-called monogenic disorders follow the simple Mendelian patterns of autosomal dominant, autosomal recessive, or X-linked inheritance. Autosomal disorders are encoded for by genes located on any of the 22 chromosomes other than the X or Y chromosome. Genes responsible for X-linked disorders are found on the X chromosome.

In autosomal dominant disorders, a person needs only to receive one mutant gene to inherit the disorder. If one parent has a mutant copy, 50 percent of the gametes from that parent will contain the mutant version of the gene. Thus, there is a 50 percent chance that any child born to the affected parent will have the same disorder. Huntington's disease follows an autosomal dominant pattern of inheritance and affects a person's ability to think, talk, and move by destroying parts of the brain. The responsible gene encodes the protein huntingtin, which helps direct the transport of secretory vesicles. The gene normally contains between 10 and 26 repeats of the triplet CAG. In people who have the disorder, the repeat occurs too many times, more than 40, and affects the protein's function. In the case of Huntington's disease, a person may reach middle age before exhibiting any symptoms and may have children before knowing he or she has a mutant gene. Other examples of autosomal dominant genetic disorders include neurofibromatosis, which causes the growth of noncancerous tumors called neurofibromas; achondroplastic dwarfism, which causes short stature; and polydactyly, which is characterized by the presence of extra digits.

Autosomal recessive conditions only manifest when a person inherits two mutant copies of the gene. If even one copy of the normal gene is present, the person is said to be a carrier and exhibits the normal phenotype (the observable characteristics and properties of an organism as determined by a individual's genes and their interaction with the environment). Someone who is a carrier has a 50 percent chance of contributing the mutant gene to offspring. When both parents are carriers, the probability that a child will receive two mutant alleles and express the mutant phenotype is 25 percent. The probability of the child's being a carrier but not having the disorder is 50 percent, and the probability that the child inherits two normal alleles is 25 percent. Sickle-cell disease, characterized by misshapen red blood cells, is an example of an autosomal recessive disorder. The protein hemoglobin carries oxygen throughout blood circulation within red blood cells, which are normally flexible. They must be able to travel through tiny capillaries that penetrate the body tissues. A single nucleotide replacement causes a different amino acid at the sixth position in one of the hemoglobin polypeptides. This causes a sickling of the red blood cells and hinders their ability to carry oxygen to vital organs efficiently. The red blood cells have a shorter life span; thus people who have sickle-cell disease suffer anemia, and when the blood cells become stuck in blood vessels, episodes of severe pain can occur. Other autosomal recessive conditions include cystic fibrosis, adenosine deaminase deficiency (ADA), phenylketonuria, and galactosemia.

X-linked disorders are encoded for by genes located on the X chromosomes. Normally, people with two X chromosomes are female, and people with one X chromosome and one Y chromosome are male.



A mutation in the hemoglobin protein causes sickle-cell disease, characterized by sickle-shaped red blood cells. *(Eye of Science/Photo Researchers, Inc.)*

The inheritance patterns of X-linked disorders, which are most often recessive, are unique since females have two copies of all the X-linked genes and males only have one. Because of this, X-linked disorders more commonly afflict males, who will have the disease even if they inherit only a single copy of a recessive mutant gene. Mothers contribute only X chromosome to their offspring, whereas fathers contribute either X or Y chromosomes equally. Fathers who are carriers of a mutant allele will pass the allele to half of their daughters but to none of their sons, since they contribute a Y chromosome to their sons. Mothers who are carriers will pass the mutant gene to half of their offspring, whether male or female. Because females will also receive an X chromosome from their father, they usually also get a normal copy of the gene. But sons only receive the one X, so 50 percent of males born to a carrier mother will inherit an X-linked disorder. One example is hemophilia A, a disease in which afflicted individuals have a reduced ability to clot blood because of the lack of a key factor involved in the clotting process. Because clotting is slow, even minor injuries are serious. Duchenne muscular dystrophy is an X-linked neuromuscular condition in which afflicted individuals have a mutant dystrophin gene and suffer progressive muscular weakness and a shortened life span.

MULTIFACTORIAL OR POLYGENIC DISORDERS

Multifactorial genetic disorders, also called polygenic disorders, result from numerous mutations and often also have an environmental component. Breast and ovarian cancer exemplify many aspects of multifactorial disorders. Between 5 and 10 percent of breast and ovarian cancers are believed to be inherited. Two genes associated with these cancers are BRCA1, located on chromosome 17, and BRCA2, located on chromosome 13. The BRCA gene products are involved in DNA and chromosomes repair, so individuals who have mutant versions accumulate mutations and damaged chromosomes, because cancer results from the accumulation of mutations that ultimately compromise the regulation of the cell cycle, leading to uncontrolled cell growth and division and potentially cancer. Other examples of complex multifactorial genetic disorders include heart disease, hypertension, Alzheimer's disease, diabetes, colon cancer, and obesity.

CHROMOSOMAL DISORDERS

In chromosomal disorders, pieces of chromosomes can be duplicated or missing; an incorrect number of genes can be encoded on the segment or whole chromosomes can be present in too many or too few copies as a result. Because many genes exist within a section of or on an entire chromosome, the effects are severe and often lead to spontaneous miscarriages early in a pregnancy. Some chromosomal disorders do result in live births, though they may reduce the life span of afflicted individuals. Aneuploidies are conditions in which the number of chromosomes is abnormal. Human aneuploidies include trisomy 21 (47, XX or XY, +21), trisomy 18 (47, XX or XY, +18), trisomy 13 (47, XX or XY, +13), Turner syndrome (45, X), Klinefelter syndrome (47, XXY), triple-X female (47, XXX), and XYY karyotype (47, XYY). The most common is trisomy 21, commonly known as Down syndrome, which occurs in about one in 700 live births and is characterized by moderate to severe mental retardation, slanted eyes, a broad short skull, and broad hands with short fingers. Aneuploid disorders result from chromosomal nondisjunction during meiosis, the failure of a pair of homologous chromosomes to separate during the first meiotic division. Maternal age is one factor that increases the risk of nondisjunction in gametes.

Other chromosomal abnormalities result when a segment of a chromosome is absent or duplicated. In Williams syndrome, a portion of chromosome number 7 is missing as a result of breakage during gamete formation. Among other genes, the deleted region includes the gene for the protein elastin, which is important in the formation of blood vessels; thus individuals who have Williams syndrome suffer from heart defects and circulatory problems. Other symptoms of this rare disorder include mental retardation and distinguishing facial features. Cri du chat (meaning "cry of the cat" in French) syndrome is a disorder

Individuals with Down syndrome have distinguishing facial morphologies including a round head with slanted eyes and a flat nose. *(PhotoCreate, 2007, used under license from Shutterstock, Inc.)*

resulting from a deletion in chromosome number 5. Children born with this disorder have a distinctive cry resulting from abnormal larynx development.

MITOCHONDRIAL DISORDERS

Mitochondrial disorders result from mutations in mitochondrial proteins. The mitochondria are organelles that carry out cellular respiration, the metabolic processes by which cells break down energy-rich organic molecules to extract energy from them. Mitochondria contain their own DNA, a closed, circular form resembling that found in prokaryotic organisms, but this DNA only encodes approximately 13 of the 1,000 proteins found in the mitochondria. Because the egg contributes most of the cytoplasmic contents, including organelles, to an embryo, mitochondrial genes are passed to children through the mother. Symptoms of mitochondrial diseases are similar whether the mutation is on a gene encoded for by the mitochondrial DNA or by nuclear DNA. The range and severity of symptoms vary tremendously as do the genetic causes, complicating diagnosis. Symptoms may include brain dysfunctions (such as seizures, mental retardation, and developmental delays), neurological problems, heart defects, hearing or vision deficits, short stature, diabetes, kidney disease, and metabolic problems.

See also CANCER, THE BIOLOGY OF; DEOXYRIBONUCLEIC ACID (DNA); GENE EXPRESSION; GENETICS; GENOMES; INHERITANCE; POINT MUTATIONS; REPRODUCTION.

FURTHER READING

Centers for Disease Control and Prevention. "Single Gene Disorders and Disability." Available online. URL: http://www.cdc.gov/ncbddd/single_gene/default.htm. Accessed January 21, 2008.

U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, and the Human Genome Program. "Genetic Disease Information—Pronto!" Available online. URL: http://www.ornl.gov/sci/techresources/Human_Genome/medicine/assist.shtml. Updated July 21, 2008.

genetic engineering Genetic engineering is the purposeful manipulation of genes or of an organism's genome. Using recombinant DNA technology, genetic engineers can cut up the deoxyribonucleic acid (DNA) from one organism, isolate genes, study them in vitro, modify them, replace them, or insert them into the genome of another organism. The result is often an organism with a different genotype, or genetic makeup, and sometimes a different phenotype, or observable characteristic. Genetic engineering has revolutionized agriculture, medicine, and biotechnology and significantly impacted other fields as well.

AGRICULTURAL APPLICATIONS

The purpose of agriculture is to produce crops, raise animals, or cultivate and prepare the products for market. For hundreds of years farmers have performed controlled breeding to alter the genetic makeup of plant and livestock populations. The careful selection and breeding of parents with desirable traits increase the probability that their offspring will exhibit the same characteristic or an increased degree of expression of that characteristic. This process of artificial selection and breeding is slow and does not guarantee results. In addition, because of independent assortment of alleles during meiosis and the random nature of fertilization, the individual offspring exhibit variable success. Another problem is that inbreeding, the mating of closely related individuals to preserve their desirable characteristics or to eliminate unfavorable characteristics, can lead

to decreased vigor or health of the individuals and populations. Genetic engineering gives farmers more control in the manipulation of an organism's genetic makeup and achieves results more rapidly than traditional breeding.

Genetically modified organisms (GMOs) are organisms whose genomes have been artificially altered by recombinant DNA technology. Most of the genes inserted into crop plants function to increase yield, improve nutritional value, or protect crops against adverse conditions such as drought, frost, salty soil, disease, and pests. Hardier plants that can withstand environmental stresses and healthier plants with higher yields will help meet the demand for staple crops such as rice while reducing the need for additional acreage and freshwater. Genetic engineering may also reduce the need for chemical pesticides, which can leach into groundwater and harm ecosystems. Hawaiian papaya growers credit genetic engineering with saving the industry from the insect-borne papaya ring spot virus in the late 1990s. The soil bacteria *Bacillus thuringiensis* naturally produces a protein toxin that kills numerous lepidopteran insect pests when ingested. Genetic engineers have inserted the gene that encodes this toxin into several crops, including corn, cotton, potatoes, and tomatoes, increasing the yields and reducing the need for chemical pesticides. *Agrobacterium* is a type of bacterium that naturally causes tumor formation in plants but is used as a tool for agricultural biotechnology because it can transfer DNA into plant cells via a plasmid called Ti (for tumor-inducing). For such purposes, the tumor causing genes are removed from the plasmid, and the genes that produce the desired trait are inserted. After reintroduction of the recombinant plasmid into *Agrobacterium,* the plants are purposely infected with the bacteria. When the process is successful, the plant genome takes up the DNA and expresses the desired trait.

One specific recent example of genetic engineering of crops involves trypsin modulating oostatic factor (TMOF), a hormone from mosquitoes that acts as a natural insecticide and has no effect on humans. Scientists have engineered a harmless strain of tobacco mosaic virus to contain this gene. Plants inoculated with this virus start producing TMOF, which prevents insects that ingest it from being able to digest food. As a result, the insects that eat the leaves of the virus-infected plant starve to death. Another potential benefit of genetically engineering crop plants is the elimination of common allergy-inducing substances in foods. For example, many young children and infants are allergic to soy, which is a common ingredient in many processed foods, including baby formula and cereals. Researchers are exploring means of silencing the gene products from soy that commonly trigger allergic reactions.

Crops can also be engineered to make them more nutritious. Vitamin A deficiency, which can lead to blindness, is common in poorer regions of Asia, but the insertion of genes for vitamin A production into rice crops may help to alleviate this problem. The insertion of genes for the production of healthy fatty acids into plants and animals is another potential beneficial application of genetic engineering to agriculture.

Agriculturists have also used genetic engineering to improve the quality of farmed animals. For example, aquaculturists genetically engineered salmon to produce greater than normal levels of growth hormones, so the fish grow large enough to sell faster. Scientists in the United States and Japan claim to have engineered cows so they are resistant to bovine spongiform encephalopathy (BSE). Also known as mad cow disease, BSE causes progressive, fatal nervous system degeneration in cattle, and the ingestion of contaminated meat products from cattle with BSE can lead to Creutzfeldt-Jacob disease, a similar neurodegenerative disease in humans.

Some people worry about the safety and possible negative environmental impact of genetically engineered foods. The U.S. Food and Drug Administration, the Environmental Protection Agency, and the U.S. Department of Agriculture work together to oversee the introduction of genetically modified plants into the environment and the sale of genetically modified foods, which have been declared to be as safe for consumption as their traditionally bred counterparts. The first genetically modified food entered the market in 1994—the Flavr Savr tomato, developed by the biotech company Calgene. The tomato contained a gene that inhibited the softening process that naturally occurs during ripening, so they could theoretically be vine-ripened for better flavor and then shipped without bruising and rotting. (In reality, the original Flavr Savr tomato was more difficult to ship than anticipated, thus was withdrawn from the market a few years later.) In the United States, soybeans, corn, and cotton are the major genetically engineered crops, and their products are found in substances such as cornstarch, soy protein, and canola oil. Estimates of the percentage of processed foods that contain these or other products from genetically engineered plants available in U.S. grocery stores range from 70 to 75 percent.

APPLICATIONS TO MEDICINE

Genetic engineering has already benefited the field of medicine in numerous ways, and the potential for future applications continues to grow. Because of genetic engineering, pharmaceutical companies can

produce many drugs more cheaply and in practically unlimited supplies. In 1982 the U.S. Food and Drug Administration approved the first drug produced as a result of genetic engineering—insulin, a hormone necessary for treating many diabetics. Previously, the hormone had to be extracted from the pancreas of slaughtered animals and thus was expensive and available in a limited supply, but now biotech companies manufacture large quantities using genetically altered microorganisms. Microorganisms can be quickly, easily, and cheaply grown in large quantities. Insertion of the gene for insulin production turns the microorganisms into insulin-producing factories. The hormone can then be isolated and purified from the bacterial cultures. Biotechnology companies now manufacture many other therapeutic agents by using genetically engineered microorganisms: human growth hormone for stimulating growth in children who do not adequately produce the hormone on their own, interferon for treating various viral diseases and cancers, the cytokine interleukin-2 for use in treating some cancers, blood clotting factors to treat hemophilia, tissue plasminogen activator to dissolve blood clots, erythropoietin to treat anemia, and tumor necrosis factor to treat tumors.

Pharmaceutical companies also use microorganisms to produce antibiotics, chemical substances that some microorganisms naturally produce to inhibit the growth of other specific microorganisms. Genetic engineering is employed to make recombinant vaccines to stimulate immunity against specific diseases. In contrast to whole-agent vaccines made from weakened or inactivated viruses, recombinant vaccines are made by recombinant DNA technology and only contain a few genes or proteins of the pathogen. Though they are not as effective as whole-agent vaccines, recombinant vaccines are generally safer.

Scientists have engineered some plants to make proteins called antibodies that help the immune system fight illnesses such as cancer and heart disease. Researchers have also inserted genes from viruses that cause measles, hepatitis B, Norwalk virus, cholera, and diarrhea into potatoes and bananas. The hope is that ingestion of these foods will stimulate people to develop a specific immune response against those viruses, just as if they had received a vaccine by injection.

Since scientists completed the sequencing of the human genome, the number of genes identified and associated with certain diseases has increased and will continue to do so. Once a mutation within a particular gene has been identified as the cause of a genetic disorder, researchers can explore treatment through gene therapy. Though medical researchers have not yet demonstrated the successful treatment of a genetic disorder through gene therapy, the potential exists and many clinical investigations are currently under way.

Xenotransplantation is the transplantation of a tissue or organ from one species to another species. Some tissues and organs from animals such as pigs can function effectively in humans, but the recipient's immune system often attacks the donated tissue or organ. Medical researchers are trying to use genetic engineering to create organisms that express molecules that will prevent the recipient's immune system from recognizing the transplanted tissue as being foreign, in hopes of preventing rejection.

In a recent example of genetic engineering with medical applications, scientists at the Roslin Institute in Edinburgh, Scotland, have modified chickens so they produce and secrete certain proteins in their egg whites. Specifically, these engineered chickens make proteins that are used to treat skin cancer and multiple sclerosis

OTHER BIOTECHNOLOGICAL APPLICATIONS

Broadly defined, *biotechnology* refers to any practical application of life science, and especially the application of technology to living organisms or the products of living organisms. Any use of genetic engineering thus falls under the umbrella of biotechnology. Other applications not discussed previously include the manipulation of organisms for the commercial production of various enzymes or chemicals.

The benefits of genetic engineering to basic research are enormous. Cloned genes, meaning genes that have been isolated for further use or study, are typically inserted into plasmids or viral vectors, which are maintained in bacterial strains. They allow molecular biologists to examine the gene structure, to determine its sequence, and to study how it is regulated. The researchers might use the altered bacteria to synthesize the protein for analysis of its structure and function or to observe the effects of various mutations. Some microorganisms naturally digest chemicals, such as spilled oil, that are considered pollutants. Bacteria may be genetically engineered to enhance their ability to degrade such chemicals for bioremediation purposes. Genetic engineering may also aid in the recovery of threatened or endangered species by inserting genes that make them heartier or more resistant to disease.

In January 2008 Craig Venter announced that a team of scientists at the J. Craig Venter Institute in Rockville, Maryland, had successfully created an entire bacterial genome from chemicals in a test tube. They have not yet been able to insert the genome into a living organism, where it would serve as the hereditary information. In 2002 scientists had created and assembled an active poliovirus in vitro, but viruses are not considered living organisms. This achieve-

ment of manufacturing an entire bacterial genome puts genetic engineers one step closer to creating synthetic forms of life, a pursuit known as synthetic biology. Applications of synthetic biology include designing organisms to perform specific tasks, such as bioremediation of a certain pollutant, and helping biologists attain a better understanding of the minimal requirements for life.

See also AGRICULTURE; BIOINFORMATICS; BIOTECHNOLOGY; CLONING OF DNA; DEOXYRIBONUCLEIC ACID (DNA); DNA SEQUENCING; MOLECULAR BIOLOGY; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

Grace, Eric S. *Biotechnology Unzipped: Promises and Realities*. Rev. 2nd ed. Washington, D.C.: Joseph Henry Press, 2006.

Spangenburg, Ray, and Diane Kit Moser. *Genetic Engineering*. New York: Benchmark Books, 2004.

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. "Biotechnology." Available online. URL: http://www.cfsan.fda.gov/~lrd/biotechm.html. Updated November 4, 2008.

Wexler, Barbara. *Genetics and Genetic Engineering*. Farmington Hills, Mich.: Thomson Gale, 2006.

genetics Genetics is the branch of the life sciences concerned with heredity, the transmission of characteristics down generations, and variation, the structural or functional divergence of characteristics among organisms. The smallest unit of heredity is the gene, a segment of deoxyribonucleic acid (DNA) consisting of a specific sequence of nucleotide subunits that encodes information for building biomolecules such as proteins. The types of proteins that a cell expresses determine the functions a cell can perform. Since cells are the basic unit of life, an organism's genes ultimately determine many of the characteristics an organism will possess, including traits inherent to that species, though environment also plays a significant role. Genetic information passes from a parent cell to daughter cells during cellular reproduction and from parents to offspring via DNA, which exists in cells in the form of chromosomes. Genetic variation among individuals results from differences in the alleles, or forms of a gene, which are simply different sequences of nucleotides within a gene. These variations among organisms can lead to adaptations upon which natural selection acts to drive evolution.

HISTORY OF GENETICS

For thousands of years, humans have recognized and exploited concepts of genetics. People observed that offspring often resembled their parents and extended this phenomenon to efforts growing crops and domesticating animals. Society established plant and animal breeding as tools for increasing the quantity and quality of food production long before the term *genetics* was coined. In 1665 the English scientist Robert Hooke discovered and described cells in his book *Micrographia,* but more than 200 years passed before scientists recognized their role in inheritance or variation within or between species. In 1833 the British botanist Robert Brown discovered cell nuclei, spots within plant cells. Today biologists know that nuclei contain and protect DNA, the genetic information. In the late 1930s, the German botanist Hugo von Mohl described mitosis, the duplication of cellular nuclei, and Theodor Schwann and Matthias Schleiden articulated the cell theory, stating that cells are the basic unit of living organisms. In 1858 Rudolf Virchow articulated that cells arise from preexisting cells.

During the 1860s, an Austrian monk named Gregor Mendel, now considered the father of classical genetics, performed numerous studies on inheritance in pea plants. His work led to the description of dominance, the law of segregation, and the law of independent assortment. Though these concepts form the basis for understanding transmission genetics, his work was largely unnoticed until 1900. During this time, Walther Flemming in Germany found a structure that bound to basic dyes and named it chromatin. Flemming also observed threadlike strands splitting and separating during mitosis. These strands were later called chromosomes, and now Flemming is considered the father of cytogenetics. The German biologist Oskar Hertwig first described meiosis in 1876, the Belgian zoologist Edouard Van Beneden described the actions of chromosomes during meiosis in *Ascaris* (a type of roundworm) eggs in 1883, and the German biologist August Weismann noted that two cell divisions were necessary to create haploid cells that would fuse during fertilization. Between 1887 and 1890, Theodor Boveri published findings that supported that chromosomes are individual entities that persist throughout the cell cycle and that sperm and egg contribute equal numbers of chromosomes during fertilization. Thus by 1900 the events of mitosis and meiosis, even with respect to chromosomes, had been delineated, but because nobody was aware of Mendel's work, the connection between these cellular events and inheritance was not made.

In 1900 three biologists (Hugo de Vries, Carl Correns, and Erich von Tschermak) independently rediscovered Mendel's work from 1865. This led to a flurry of activity repeating Mendel's experiments and widespread recognition of their significance. In 1902 the American biologist Walter Sutton figured out that the behavior of chromosomes during meiosis

mimicked Mendel's factors of heredity, and thus genes were located on chromosomes. The American geneticist Thomas Hunt Morgan observed crossing over (a process resulting in genetic exchange between chromosomes) during meiosis in fruit flies in 1911 and obtained experimental evidence for phenomena including sex linkage, genetic distance, and nondisjunction. This work clearly established the chromosomal theory of inheritance.

Researchers at the Rockefeller Institute, Oswald Avery, Colin MacLeod, and Maclyn McCarty, provided proof that the DNA component of chromosomes was the molecular carrier for genetic information in 1944, ushering in the era of molecular genetics. James Watson and Francis Crick revealed the structure of DNA in 1953, paving the way to determine how the molecule transmits genetic information between generations and how it encodes for the synthesis of proteins. With the discovery of restriction enzymes and the use of cloning vectors such as plasmids, recombinant DNA technology has taught biologists much about how genes confer characteristics on an organism and the effects of different types of mutations. Through genetic engineering, researchers have cloned genes and introduced them into other species, resulting in the large-scale production of biochemicals, transgenic animals, and genetically modified foods. Most recently, the field of genomics has grown explosively, culminating in the mapping of numerous genomes that will further advance the applications of biological and medical research.

SUBDISCIPLINES OF GENETICS

The broad field of genetics can be divided into a few major subdisciplines, each encompassing several fields of specialty: classical genetics, molecular genetics, and evolutionary genetics. Classical genetics includes topics concerned with the transmission of traits from one generation to the next as described by the chromosomal theory of inheritance. Genes exist as physical entities on chromosomes and segregate with the chromosomes during the first division of meiosis. In sexual reproduction, gametes (eggs and sperm) each contain one-half of the normal number of chromosomes (they are said to be haploid), so when they unite during fertilization, the normal number is restored, and two copies of each type of chromosome exist (a condition called diploid). The members of a pair of chromosomes, called homologues, contain all of the same genes, but they can contain different alleles, or forms of those genes. In order to create haploid cells from diploid cells during gamete formation, the homologous chromosomes pair up, and an event called crossing over occurs; it can result in recombination, the exchange of pieces or sections of a chromosome with its homologue. In the end, the haploid eggs contain random combinations of the chromosomes from the mother, and the sperm cells contain random combinations of chromosomes from the father. The unique combination of chromosomes an individual receives from his or her parents determines the biological gender and the allele combinations (the genotype) that the offspring will possess. The collective effect of all the specific alleles an individual inherits and the manner in which the gene products interact will affect that individual's characteristics, or phenotype. Classical geneticists study the processes by which all of these events occur.

Cytogenetics is a subfield of classical genetics. Cytogeneticists study genetics at the cellular level, in other words, chromosomes. They study the processes involved in chromosomal duplication and division during mitosis and meiosis, both when the events occur normally and when problems, which may lead to aberrations in chromosomal structure or number, arise. Many abnormalities of chromosomal number, such as too many or too few, do not result in viable offspring. Abnormalities in the number of sex chromosomes are viable more often than abnormalities in autosomal chromosomes (the ones not involved in sex determination). In humans, the sex chromosomes are called the X and the Y chromosomes. Genes located on these are said to be sex-linked and follow unique, gender-specific patterns of inheritance.

Classical genetics also encompasses gene mapping studies. The farther apart two genes are along the length of a chromosome, the more likely it is that recombination will occur between them. Geneticists can use the frequency with which two genes are inherited together to determine the genetic distance between them, which in turn can be used to map the position of various genes linked on the same chromosome.

Another major subdiscipline of genetics is molecular genetics, which focuses on genetics at the molecular level. Molecular geneticists study the structure and function of nucleic acids and the molecular events involved in synthesizing new DNA, called DNA replication, and in the expression of genes, a process involving multiple steps and resulting in the synthesis of new, functional proteins. The first step in the expression of a protein is transcription, the synthesis of a messenger ribonucleic acid (mRNA). As the first step in the process of making proteins, this step is highly regulated. In eukaryotic cells, the mRNA is processed and modified before transportation to the cytoplasm. Next, ribosomes assemble a polymer of amino acids based on the sequence of ribonucleotides in the mRNA transcript. This new molecule, called a polypeptide, undergoes folding and sometimes additional modifications or joins with additional polypep-

tide chains to become a functional protein. Changes in the DNA sequence, called mutations, may occur spontaneously or as a result of the action of chemical agents or radiation exposure. Cells have mechanisms for repairing molecular mutations to the DNA but are not always 100 percent effective. If not repaired, the mutations may affect the sequence of the final protein and therefore its function. All aspects relating to the regulation and synthesis of functional proteins are of interest to molecular geneticists.

The major subdiscipline of evolutionary genetics encompasses the subfields of quantitative and population genetics, which involve the examination of frequencies of certain alleles in a population and ways they change over time. Changes to allele frequencies within a population can occur over time as a result of biological processes including mutation, migration, and natural selection in addition to circumstances such as population size and randomness of mating. In stable populations, allele frequencies may reach equilibrium, a situation allowing one to make predictions concerning the genotypic and phenotypic frequencies of alleles and traits. Evolutionary geneticists also study the rate of evolutionary change and can use this information to establish the degree of relatedness among different species and to draw conclusions about the history of biological evolution.

APPLICATIONS OF GENETICS

Genetic research complements the research performed by other kinds of biologists. Geneticists observe organisms that have mutations and look for the effects of the improperly functioning genes. This approach allows for the determination of the function of the gene product as well as its importance and relationship to other genes—for example, is the protein crucial for a specific anatomical or physiological characteristic, is the mutation dominant or recessive, or does the gene interact with other genes? This information helps provide a more complete picture of how organisms develop, reproduce, and perform other life functions. The advent of genetic engineering and recombinant DNA technology has allowed researchers to examine these questions more directly. They can isolate specific genes and control the conditions under which the gene product and its action are studied.

Genetic research impacts the field of medicine tremendously. Using knowledge of cytogenetics and molecular genetics, physicians can diagnose genetic diseases caused by chromosomal abnormalities and molecular mutations. Genetic counselors educate couples and assist them in understanding their risks of conceiving a child who has a genetic disorder on the basis of family medical histories or gene testing. Research related to the cell cycle and its regulation has led to a better understanding of diseases such as cancer, which results from the accumulation of many mutations to DNA. With the human genome mapped, medical researchers will be able to relate many more specific genes and mutations to certain medical disorders or conditions. Genetic research in other organisms has also improved the ability of physicians to treat human disease. For example, knowing the mechanisms by which bacteria develop resistance to certain antibiotics and the relative frequencies of the genes in different types of bacteria that confer resistance helps a physician decide which medications to prescribe.

Agriculture and horticulture have also benefited immensely from an understanding of genetics. Controlled breeding programs have been used for centuries to improve yields, such as more wool from sheep, greater quantities of milk from dairy cows, or more grain per acre of wheat. The tools of molecular genetics allow agriculturists to use molecular markers such as restriction fragment length polymorphisms to follow the transmission of different traits without having to wait a season for a plant to grow and mature to the point where the characteristic can be directly observed, saving time and increasing efficiency. They can also use recombinant DNA techniques to transfer genes that make a plant more resistant to harsh environmental conditions or to pests, increasing vigor and yields.

Forensic analyses often rely on genetic techniques to investigate crimes. For example, detectives can collect blood or other bodily fluids from a crime scene as evidence that a suspect was present because every person has a unique DNA fingerprint, left behind in samples containing nucleated cells. These same fingerprints can be used to determine paternity or the degree of relatedness of individuals.

The most important benefit of genetic research may be attaining a greater understanding of life itself. Many cell and molecular characteristics and events are shared by all organisms, and biologists can use the degree of similarity to construct phylogenies. All living things use DNA to carry genetic information from one generation to the next. The steps involved in protein synthesis (transcription of DNA to make RNA, followed by translation using the genetic code to build a polypeptide) are very similar even across kingdoms. Vertebrate animals, plants, bacteria, fungi, protists, archaeans, and even viruses share a practically universal genetic code, meaning the same nucleotide codons encode for the same amino acids. The field of genetics has granted scientists insight into life itself and the evolutionary processes that led to the development of the species present in the world today.

See also AVERY, OSWALD; CELL BIOLOGY; CHROMOSOMES; CLONING OF DNA; CRICK, FRANCIS;

DEOXYRIBONUCLEIC ACID (DNA); DNA FINGERPRINTING; DNA SEQUENCING; FRANKLIN, ROSALIND; GENE EXPRESSION; GENE THERAPY; GENETIC DISORDERS; GENETIC ENGINEERING; GENOMES; HOOKE, ROBERT; INHERITANCE; MACLEOD, COLIN MUNRO; MCCARTY, MACLYN; MCCLINTOCK, BARBARA; MENDEL, GREGOR; MOLECULAR BIOLOGY; MORGAN, THOMAS HUNT; POLYMERASE CHAIN REACTION; RECOMBINANT DNA TECHNOLOGY; VARIATION, GENETIC VARIATION; VIRCHOW, RUDOLF; WATSON, JAMES D.; WILKINS, MAURICE H. F.

FURTHER READING

Carlson, Elof Axel. *Mendel's Legacy: The Origin of Classical Genetics.* Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

genomes A genome is the complete DNA content of an organism. Characterization of an organism's genome includes a description of the number of chromosomes in addition to the size of the genome expressed as number of kilobase (kb) pairs (one Kilobase equals 1,000 base pairs) in a haploid genome. Eukaryotic organisms generally have between two and 10 times as many genes as prokaryotic organisms, and their genomes are often thousands of times larger. The amount of deoxyribonucleic acid (DNA) in a cell, however, is not necessarily related to the evolutionary complexity of the organism.

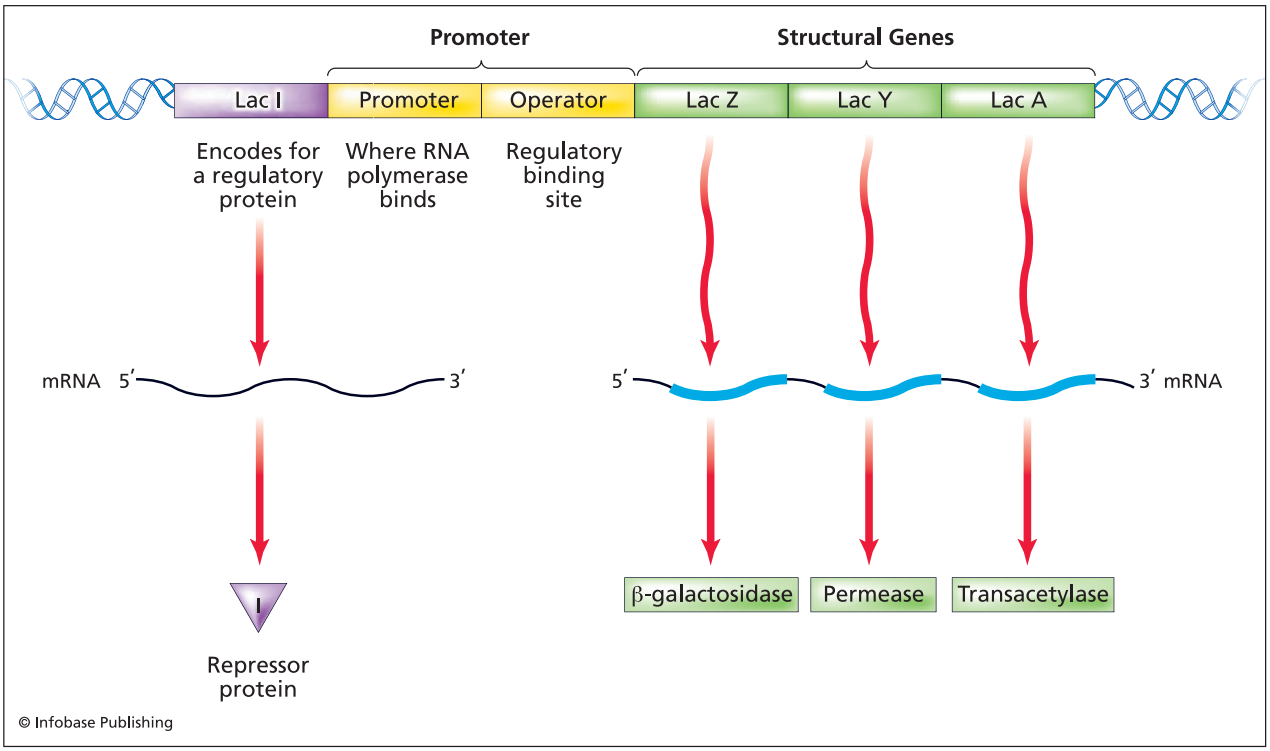
Genomics refers to the discipline of life science concerned with the characterization of genomes and the methods used to achieve that goal. Scientists are actively working toward obtaining complete genome sequences for many different species. According to Genomes OnLine Database (GOLD), as of January 2008, almost 700 completely sequenced genomes had been published, including numerous viruses, bacteria, and archaeans as well as higher organisms including the yeast *Saccharomyces cerevisiae,* the nematode *Caenorhabditis elegans,* the fruit fly *Drosophila melanogaster,* the mouse *Mus musculis,* and humans. This information will be useful for numerous applications such as identifying genes that cause disorders, examining evolutionary relationships among organisms, determining the function of proteins, and achieving a better understanding of life at the molecular level.

PROKARYOTIC GENOME ORGANIZATION

The majority of DNA in a prokaryotic genome exists in one closed, circular molecule of DNA and includes between 1,000 and 10,000 genes. The size ranges between 580 kb and 1.0×10^4 kb, with most prokaryotic genomes averaging $4–5 \times 10^3$ kb, and most single genes averaging 1,000–2,000 base pairs (bp). The widely studied bacterium *Escherichia coli* has 4.5×10^3 kb. Obligate intracellular parasites have characteristically small genomes since they rely on host organisms to fulfill many metabolic needs and therefore do not need to encode or express the genes necessary for those functions. Exceptions to the norm do exist. The genome of *Bacillus megaterium* is very large, 3×10^4 kb. The bacterium responsible for Lyme disease, *Borrelia burgdorferi,* has linear chromosomes. The genome of the organism that causes cholera, *Vibrio cholerae,* consists of two circular chromosomes, and the archaean *Methanococcus jannaschii* has three. Archaean chromosomes exhibit a range in chromosome size from about 500 kb to 5.8×10^3 kb, similar to that of bacteria. Compared to eukaryotic genomes, prokaryotic genomes are characterized by very little junk DNA, the term for DNA that does not encode for anything. The gene density in prokaryotes is high—approaching one gene per kilobase of DNA in *E. coli.* Introns, noncoding sequences found within the coding region of a gene, are rare in bacteria and only found in transfer RNA genes in archaeans. Topoisomerases, enzymes that wind up and twist the DNA, package the prokaryotic chromosome, which attaches to the inner cell membrane. Few proteins remain associated with the chromosome, a condition described as naked.

Many bacteria also have plasmids, small extrachromosomal pieces of DNA that can carry additional genes. A few linear plasmids also exist, and these have specialized ends to protect the DNA from degradation. Plasmids replicate independently of the bacterial chromosome and can be present in multiple copies, ranging from one to hundreds of copies per cell. Copy number is a property of the type of plasmid, and genes encoded by the plasmid control the number of copies present in a cell. The size of plasmids averages between 2,000 and 100,000 base pairs, and they often encode for genes that confer antibiotic resistance. The range of potential host bacteria varies among plasmids: some have narrow host ranges, and some can exist and replicate in many different bacterial types. Bacteria can transfer plasmids from one cell to another through bacterial conjugation, a property that the plasmid being transferred encodes. Plasmid DNA can also be introduced into bacteria cells by transformation, a process in which naked molecules of DNA are taken up by competent recipient cells.

Prokaryotic chromosomes contain operons, organized units of several genes that are transcribed as one molecule of messenger ribonucleic acid (mRNA). An operon typically includes regulatory protein binding sites and a few structural genes. The structural genes are usually functionally related; for example, they may all encode proteins necessary for one par-



Bacterial genes are often organized into operons, sets of structural genes whose mRNA is synthesized as one molecule. The *lac* operon contains genes necessary for the utilization of lactose.

ticular metabolic pathway to proceed. By transcribing several of the genes onto a single mRNA, they are regulated as a unit by a single regulatory element. In bacteria, the lactose (lac) operon encodes for proteins necessary for the metabolism of lactose and includes the gene that encodes its own regulatory protein, the *lac* repressor.

EUKARYOTIC GENOME ORGANIZATION

Eukaryotic genomes vary in size by up to four orders of magnitude but typically contain between several million and several billion base pairs in a haploid genome. Examples of genome size in a few well-researched eukaryotic organisms are humans, 3.0×10^6 kb; *Drosophila melanogaster* (fruit fly), 1.7×10^5 kb; maize (corn) 2.0×10^6 kb; *Arabidopsis* (a small flowering plant) 7.0×10^4 kb; lily, 1.0×10^8 kb. The wide range in size is due to varied numbers of introns and repetitive sequences. The chromosomes of eukaryotic organisms are linear, occur in pairs, and contain thousands of genes each. The number of chromosomes varies among species and ranges from two to hundreds in polyploid organisms. Humans have 46 chromosomes, fruit flies have eight, and maize has 20. A karyotype reveals the structure of a genome at the chromosomal level, with the chromosomes paired with their homologues and arranged according to shape, size, and centromere position. Within a species, particular genes always reside at the same gene locus, the position on a specific chromosome where a gene resides. With the exception of the roundworm *Caenorhabditis elegans,* genes are not organized into operons as in prokaryotes. Each mRNA transcript encodes for a single protein.

The cells of eukaryotic organisms contain mitochondria, organelles that function in cellular respiration. Plant and algal cells also contain chloroplasts that function in photosynthesis. Both mitochondria and chloroplasts possess their own DNA, believed to be derived from a prokaryotic endosymbiont. According to the endosymbiotic theory, eukaryotic mitochondria and chloroplasts evolved from smaller prokaryotic organisms that lived in an endosymbiotic relationship with a larger prokaryotic cell. The smaller organisms performed specialized tasks, cellular respiration in the case of mitochondria and photosynthesis in the case of chloroplasts, while living inside the larger cell. Eventually both cell types lost their ability to live independently. The DNA inside mitochondria and chloroplasts resembles a prokaryotic genome, being closed, circular, and naked. Many of the genes on the DNA from these organelles share sequence similarities with prokaryotic organisms, lending further support to the endosymbiotic theory. These genes encode for proteins that perform critical

cellular functions, thus must not be omitted from discussions about eukaryotic genomes.

Though eukaryotic genomes are millions or billions of base pairs long, the majority of the DNA is noncoding, meaning it does not encode for the synthesis of a protein or RNA molecule. Humans, for example, have approximately 30,000 genes, with one gene averaging 27 kb in length, though the average-sized mRNA transcript is only 2,000 nucleotides long. Less than 2 percent of the human genome encodes instructions for the synthesis of proteins. In comparison, between 5 and 10 percent of the *Drosophila* genome encodes for proteins, and 85–90 percent of bacterial genomes are coding. Compared to that in prokaryotes, the overall gene density in eukaryotes is low, but eukaryotic genes are organized into clusters on the chromosomes.

REPETITIVE DNA IN EUKARYOTIC GENOMES

The sequence organization of eukaryotic genomes is very complex but can be generally divided into three main classes. The single-copy, functional genes encode for proteins necessary to carry out cellular functions. Spacer DNA, as the name implies, are regions of DNA that are not transcribed and do not have any characteristics of the known types of repetitive elements, described later. The third type of DNA is repetitive DNA—DNA that is present in multiple copies in the genome. Some repetitive DNA sequences are functional, and others have no known function.

Satellite DNA, which consists of highly repetitive DNA sequences, makes up about 5 percent of the human genome and 10 percent of the mouse genome. Density gradient centrifugation of eukaryotic DNA results in two bands, one main band and a second, smaller and less dense band. The lighter band results from satellite DNA, which contains a higher percentage of adenine-thymine base pairs than the rest of the DNA. The separation of the two bands on the gradient represents the difference in nucleotide composition. Prokaryotic genomes do not exhibit this phenomenon. The sequences found in the satellite band are present in heterochromatic regions surrounding the centromeres of chromosomes and in telomeres, the ends of chromosomes. These inactive regions are packed more tightly than regions of the chromosomes that contain actively transcribed genes; thus they appear darker when stained and viewed under a light microscope. Though the role of highly repetitive sequences is not known, the heterochromatic region near the centromeres binds the kinetochore, the structure to which spindle fibers attach in order to separate homologous chromosomes during mitosis and chromatids during meiosis. The sequence that composes the centromere regions has been defined for several species and contains numerous repeats extending up to 1 million base pairs. In *Drosophila* the repeated sequence is 10 bp long, and in humans it is 170 bp. Telomeres also contain highly repetitive DNA. Telomeric DNA sequences contain short tandem repeats (six nucleotides long in humans) that provide stability to the chromosome. Other telomeric-associated sequences are found near and within the telomere. Their function is unknown.

Another type of repetitive DNA for which there is no known function is middle repetitive DNA, including tandemly repeated sequences and interspersed sequences. Variable number tandem repeats (VNTRs) are 15–100 bp long and repeat up to 1,000 times at locations throughout the genome, forming minisatellites. The human genome has about 30,000 minisatellite DNA loci. Because the number and the size of the repeats vary among individuals, minisatellite DNA is used in the technique of DNA fingerprinting. Microsatellites are also dispersed throughout the genome; they consist of shorter tandem repeats than minisatellites. For example, dinucleotides contain just two nucleotides, and they repeat between five and 50 times. The human genome has approximately 200,000 microsatellite loci.

Transposons are repetitive DNA sequences that can replicate and insert themselves into other locations within the genome. Short interspersed elements (SINEs) are less than 500 bp long and can have as many as 500,000 copies in a genome. One SINE family in humans, called the *Alu* family, makes up more than 5 percent of the genome. Long interspersed elements (LINEs) are similar to SINEs but are longer, between 1,000 and 5,000 kb, and occur in multiples of 20,000 to 40,000 in humans. LINEs are referred to as retrotransposons, because they use the enzyme reverse transcriptase to propagate themselves by first making a strand of DNA using a piece of RNA as the template, a strategy used by retroviruses.

Some middle repetitive DNA is functional, for example, genes that are present in multiple copies to increase their level of expression. Ribosomal RNA (rRNA) is one example of a multiple copy gene that occurs as tandem repeats in the nuclear organizer, the region of the chromosome set that is physically associated with the nucleolus, a dark-staining, spherical region located inside the nucleus. In contrast to the organization of the rRNA genes, some proteins or families of proteins are encoded by copies of genes that are dispersed throughout the genome. For example, actins are encoded by between five and 30 gene copies, keratins by more than 20, and the variable region of immunoglobulins (antibodies) is encoded for by 500 different genes. When members of multiple copy gene families become nonfunctional, they are called pseudogenes. These can also result

from duplicated genes that have undergone destructive mutations. The presence and location and the sequence of pseudogenes in a species reveal information about evolutionary relationships.

GENOMICS AND PROTEOMICS

Genomics is the branch of biotechnology concerned with the genetic mapping and DNA sequencing of the genes and genomes of organisms, identifying the genes, developing organized databases of this information, and applying this knowledge. *Bioinformatics* refers to the development of hardware and software and the use of computers to acquire, store, organize, and analyze biological information such as genetic data. Many databases and software tools are freely available online to assist genetic researchers at public and private institutions. Several specialized fields within genomics explore different aspects of genome structure and function. Functional genomics focuses on how the genome determines the characteristics of individuals and species and how the DNA and proteins encoded by the DNA interact with each other within and among organisms and their environment. *Transcriptomics* refers to the large-scale study of mRNAs and the regulation of their synthesis.

A proteome is the entire set of proteins expressed in a cell, tissue, or organism, and proteomics is the study of their expression, regulation, structure, modification, cellular location, function, and interactions and the organization and application of this information using bioinformatics. One tool for exploring protein function is knockout studies, in which genes encoding certain proteins are inactivated in a living organism so the effect can be studied. While knockout studies are limited to model organisms, such as yeast, *Drosophila,* or mice, bioinformatics facilitates the comparison of sequences between humans and these model organisms. Thus information obtained in other species benefits human proteomics as well. Comparative genomics involves close examinations of the DNA sequences and patterns between genes and species. The aim of structural genomics is to construct three-dimensional models of proteins, which will complement functional studies. Traditional biochemistry techniques to separate and characterize proteins such as chromatography and gel electrophoresis are still useful, but the emergence of bioinformatics has greatly advanced proteomics. During the 21st century, the applications of the knowledge gained from genomics studies will not only improve human health care, but provide much deeper insight into many aspects of living systems ranging from how species evolve to how to sustain livable conditions within the Earth's biosphere.

See also CHROMOSOMES; DEOXYRIBONUCLEIC ACID (DNA); GENE EXPRESSION.

FURTHER READING

Campbell, A. Malcolm, and Laurie J. Heyer. *Discovering Genomics, Proteomics, and Bioinformatics.* 2nd ed. San Francisco: Benjamin Cummings, 2006.

Gerstein, Mark, and Deyou Zheng. "The Real Life of Pseudogenes." *Scientific American* 295, no. 2 (August 2006): 48–55.

Jeffreys, A. J., V. Wilson, and S. L. Thein. "Hypervariable Minisatellite Regions in Human DNA." *Nature* 314 (1985): 66–73.

Korenberg, J. R., and M. C. Rykowski. "Human Genome Organization: *Alu,* LINEs, and the Molecular Organization of Metaphase Chromosome Bands." *Cell* 53 (1988): 391–400.

Richards, Julia E., and R. Scott Hawley. *The Human Genome: A User's Guide.* 2nd ed. Burlington, Mass.: Elsevier Academic Press, 2005.

Ridley, Matt. *Genome: The Autobiography of a Species in 23 Chapters.* New York: HarperCollins, 2000.

U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program. "Facts about Genome Sequencing." Available online. URL: http://www.ornl.gov/sci/techresources/Human_Genome/faq/seqfacts.shtml. Updated September 19, 2008.

Geoffroy Saint-Hilaire, Étienne (1772–1844) French *Naturalist* The French naturalist Étienne Geoffroy Saint-Hilaire was a skilled comparative anatomist who put forth the principle of unity of composition, claiming that all animals shared an archetypical structural design. His anatomical research was highly regarded, but Geoffroy Saint-Hilaire's greatest influence was on the development of the study of pre-Darwinian evolution.

Étienne Geoffroy was born on April 15, 1772, in Étampes, near Paris. His father, Jean-Gérard Geoffroy, was a procurator at the tribunal and later became a judge. Étienne had 13 older siblings. He received the surname *Saint-Hilaire* as a child and later added it to his family name. At age 15 he began preparations to devote his life to the church, but the onset of the French Revolution in 1789 disrupted his plans. At his father's recommendation, he pursued the study of law. After receiving a law degree in 1790, he followed his own interests and studied medicine and science at the Collège du Cardinal Lemoine. During the revolutionary turmoil in 1792 Geoffroy attempted to free an imprisoned priest. Because of his efforts, in 1793, when Geoffroy was only 21, one of the priest's friends arranged for him to be appointed professor of vertebrate zoology at the Jardin des Plantes (the royal botanical gardens), which became the Muséum National d'Histoire Naturelle (National Museum of Natural History) in June 1793. There

he became friends with Jean-Baptiste Lamarck, who was the museum's professor of invertebrates.

After a brief correspondence, Geoffroy invited the up-and-coming comparative anatomist Georges Cuvier to Paris, and they collaborated on many projects. Shortly after his arrival in 1795, Cuvier was appointed an assistant professor of animal anatomy at the museum. Together the two men discussed the notion that all animals were derived from a single type of animal, an idea that Cuvier later rejected. In 1796 Geoffroy formally proposed this principle of unity in his *Mémoire sur les rapports naturels des* Makis lémur *L.* (Memoir on the natural relations of the *Makis lemur* L.) He said, "It seems that nature is confined within certain limits and has formed living beings with only one single plan, essentially the same in principle, but that she has produced variation in a thousand ways in all her accessory parts." He initially applied this principle to mammals but then later extended it to vertebrates and eventually to invertebrates as well.

From 1798 to 1801 Geoffroy joined Napoleon's scientific staff in Egypt. He visited archaeological sites and collected mummified animals and humans for scientific research. His analysis of these materials was reported in *Description de l'Égypte par la commission des Sciences* (1808–24). One of his conclusions was that animals from 3,000 years ago were similar to living forms. Scientists who believed in the immutability of species used this finding to defend their viewpoint, though geologically speaking, 3,000 years is very short.

Geoffroy became a member of the French Academy of Sciences in 1807, and in 1809 he joined the zoology faculty at the University of Paris.

During the early 19th century, biologists held divided opinions on the nature of anatomical differences in animals. Formalists believed that form determined function and that all vertebrates developed from a single archetype and thus shared the same underlying organization, from which functions were derived. In contrast, the so-called functionalists thought that form developed as the manifestation of a particular function: in other words, form followed function. The controversy boiled down to whether organization or activity was more important. Cuvier was a functionalist who believed that God had created all organisms with the structures necessary for their survival in their particular habitats and that the form of each species was permanently fixed. Geoffroy was a formalist, as were Georges-Louis Leclerc, comte de Buffon, and Lamarck. He believed that species transformed over time. In Geoffroy's books, the two-volume *Philosophie Anatomique* (Anatomical philosophy, 1818–22) and *Histoire naturelle des mammifères* (Natural history of mammals, 1819), he described three laws that summarized his philosophy of anatomy. The law of connections stated that similar structures retained the same pattern of connections to other structures. The law of permanence said that new organs are not created, but structures are simply modified from preexisting structures. The law of balance required that when one organ developed, another degenerated or became reduced.

Geoffroy claimed that all vertebrate animals shared the same underlying anatomical organization with only slight modifications to accessory parts. He cited vestigial organs, body parts that are degenerate or imperfectly developed in comparison with ancestral forms or related species, and embryonic stages of development as proof that organization dictated function. Because he believed that all of an organism's habits, activities, and functions were the result of the animal's structure, he focused on the overall body plan or major anatomical features of different organisms. Varied structures in different animals were considered similar if they had the same pattern of organization. The modern term for this concept, *homology,* illustrates Geoffroy's and the formalists' viewpoint. Homologous structures of two different organisms are derived from the same structure in a common ancestor. For example, an arm of a human, a front leg of a dog, and a wing of a bat are all homologous. Though the structures appear different in these three animals, they are all organized in a similar manner, with bones in the same positions relative to other bones. Geoffroy outlined a set of rules for determining whether two varied structures were homologous.

In *On the Origin of Species* (1859), the British naturalist Charles Darwin used the concept of homologous structures to illustrate and identify evolutionary relationships. Modern evolutionary biologists do the same. Unfortunately, Geoffroy carried his examples of related connections too far and ended up incorrectly describing some homologous relationships that biologists have since dismissed as false. For example, he suggested the carapace of insects corresponded to the vertebrae.

A colleague named Robert Edmund Grant studied marine invertebrates in Edinburgh, Scotland, in the late 1820s. While examining mollusks, Grant found they had a pancreas and wrote to Geoffroy. This led Geoffroy to extend his unity principle to invertebrates. In 1829 a formalist paper was published. The authors, Laurencet (first name unknown) and Pierre Stanislas Mayranx, made structural comparisons between cephalopods (squids, cuttlefish, and octopuses) and vertebrates and proposed an account for a transition from cephalopods to fish. Cuvier thought this was ridiculous and tried to interfere with the Academy of Science's review of the paper. This led to a series of debates that took place in 1830

between Cuvier and Geoffroy regarding the unity of composition principle. Cuvier took advantage of this opportunity to point out several inaccurate descriptions of uniformities in structure. He declared that similarities in structure resulted from the need to perform similar functions; they were not due to common descent. Geoffroy defended his plan of uniform structure among animals, claiming that philosophically only a single animal existed. In *Principles de philosophie zoologique* (Principles of zoological philosophy), published in 1830, Geoffroy attempted to explain his debate with Cuvier and their conflicting views on the form of species.

Today, aspects of both formalism and functionalism permeate the fields of anatomy and physiology; thus the controversy was never resolved, but rather, the two perspectives were merged or synthesized. An organism's current form restricts or limits the possible modifications that may alter its function over time, but at the same time, the conditions of a particular environment strongly influence the functions or activities an organism exhibits. Similar environmental conditions can lead to the emergence of analogous structures that are not homologous. For example, the wings of a bat and the wings of a mosquito perform similar functions, but they are not derived from a single structure in a common ancestor, and their structural strategies are dissimilar.

Geoffroy is not considered an evolutionary biologist, but rather an accomplished comparative anatomist. He did, however, make comments later in his career that suggested he believed that the environment shaped the evolution of animals, a kind of precursor to Darwin's proposed theory of natural selection. Another claim made by Geoffroy, that morphological changes occurred in quick bursts, rather than slowly, foreshadowed the theory of punctuated equilibrium proposed by the American paleontologists Stephen Jay Gould and Niles Eldredge in 1972. Punctuated equilibrium theory states that evolution occurs in sudden, rapid spurts followed by long periods of no substantial change, and today punctuated equilibrium is considered an important process in large-scale evolutionary change.

Geoffroy became blind in 1840 and later suffered an attack that left him paralyzed. He resigned his chair at the museum in 1841, and his son, Isidore Geoffroy Saint-Hilaire, succeeded him. His health failed, and Étienne Geoffroy Saint-Hilaire died in Paris on June 19, 1844. His prominence and influence on 19th-century science and on other 19th-century scientists, including Charles Darwin, have engraved a permanent place for him in the history of life science. Even his adversaries such as Cuvier could only praise Geoffroy's talents for description and classification.

See also ANATOMY; BUFFON, GEORGES-LOUIS LECLERC, COMTE DE; CUVIER, GEORGES, BARON; DARWIN, CHARLES; EVOLUTION, THEORY OF; GOULD, STEPHEN JAY; LAMARCK, JEAN-BAPTISTE.

FURTHER READING

American Philosophical Society. "Étienne Geoffroy Saint Hilaire Collection (1811–1840)." Available online. URL: http://www.amphilsoc.org/library/mole/g/geoffroy.htm. Accessed January 25, 2008.

Guyader, Hervé le. *Geoffroy Saint-Hilaire: A Visionary Naturalist*. Translated by Marjorie Grene. Chicago: University of Chicago Press, 2004.

Travis, John. "The Ghost of Geoffroy Saint-Hilaire." *Science News* 148, no. 14 (September 30, 1995): 216–217.

germ theory of disease The germ theory of disease states that microorganisms cause infectious diseases. Specific microorganisms cause specific diseases and can be spread directly from person to person or indirectly through shared contact with other objects. The germ theory of disease is one of the most important historical contributions of science to the welfare of society. Its acceptance opened the door to many other developments that advanced medicine: hygienic practices, aseptic techniques during surgery, development of vaccines and antibiotics. Knowledge and understanding of the causes of infectious diseases have saved millions of lives over the past 120 years.

Until the end of the 19th century, physicians were ignorant as to the cause of illness and disease. From the time of Hippocrates (460–370 B.C.E.), classical medicine taught that the body contained four humors (fluids) that must be kept in balance in order for a person to be healthy. The relative amounts of the four humors—black bile, yellow bile, phlegm, and blood—were also associated with one's personality. Different illnesses resulted from an imbalance in the levels of the fluids, a condition that could result from variations in diet or activity. For example, an excess of yellow bile caused illnesses associated with vomiting, which was considered the body's mechanism for reducing the levels of yellow bile to normal. Physicians attempted to restore the balance of these fluids by administering substances that induced vomiting and diarrhea or by forcing starvation or blood loss through the use of blood-sucking leeches or even intentional cutting of a vessel, procedures now considered primitive and barbaric as well as ineffective. Another hindrance to the advancement of medicine was society's belief that disease resulted from supernatural causes or divine powers. People believed that those who practiced witchcraft could cast a spell to make someone sick, that demons could cause illness

by inhabiting or possessing individuals, and that God punished sinful behavior by causing disorders or diseases.

Numerous people contributed to the development of the germ theory of disease. A Dutch draper, Antoni van Leeuwenhoek, first discovered microorganisms in pond water and in 1674 and subsequently found them to be ubiquitous. Many people, scientists included, believed that these simple life-forms arose spontaneously from nonliving matter. This belief, called spontaneous generation, originated thousands of years before and offered an explanation as to why maggots appeared on rotting meat and rats appeared in garbage piles.

Until the 1840s, puerperal fever, also called childbed fever, killed so many women that many preferred to give birth on the streets rather than in a hospital out of fear of contracting this disease. Ignorant of the cause of this condition, physicians routinely delivered babies without first washing their hands, often after performing autopsies on women who had died of the condition. The Hungarian physician Ignaz Semmelweis demonstrated that hand washing significantly reduced the incidence of puerperal fever, a practice that reduced the mortality rate at his hospital from 12.24 to 2.38 percent.

In 1859 the French chemist Louis Pasteur performed the last of a series of experiments that debunked the theory of spontaneous generation. He showed that microorganisms are present in the air, carried by airborne dust particles, but do not arise from the air. Afterward, scientists accepted microorganisms as independent life-forms that reproduced to perpetuate their species. Pasteur also showed that microorganisms caused fermentation and putrefaction, and that microorganisms could be killed by heating or treatment with harsh chemicals.

An English physician named Joseph Lister was aware of both Semmelweis's and Pasteur's research. Lister made a connection between their work and his studies on gangrenous wounds, wounds of soft tissue that died and decayed, believed by most to be caused by the exposure of the tissues to chemicals in the air. Lister believed that microorganisms caused the tissues to rot and applied the knowledge shared by Semmelweis and Pasteur to eliminate the microorganisms from wounded tissues in hopes of decreasing gangrene after surgery. In 1867 Lister published his findings that spraying a solution of carbolic acid onto surgical instruments and onto wounds prior to and during surgery markedly decreased the incidence of gangrene. Today Lister is considered the father of antiseptic surgery.

Also during the late 1860s, Pasteur examined the causes of diseases in silkworms and demonstrated an association between particular microorganisms and specific diseases. He soon turned his efforts toward demonstrating a relationship between microorganisms and diseases in humans and large animals. He developed the first vaccines against cholera, anthrax, and rabies. Vaccines are injections of microorganisms or parts of microorganisms that prevent future infection of a disease by stimulating specific immunity.

The German physician Robert Koch studied many of the same diseases as Pasteur including anthrax and cholera. They each benefited from knowledge of the other's experiments, and competition between the two hastened the advances that led to the establishment of the germ theory of disease. In the 1870s, Koch outlined a set of procedures for ascribing a particular microorganism to a specific disease. First, one must isolate the suspected microorganism from a disease victim. Second, one must grow a pure culture of the isolated organism in the lab. Third, injection of the microorganism into a healthy individual must cause the same specific disease as that in the individual from whom the organism was first isolated. Last, the same organism must be isolated from the injected individual. Meeting these conditions, referred to as Koch's postulates, demonstrates that the isolated organism is the etiological agent for the particular disease. Koch performed experiments that helped to identify the causative organism for anthrax to be *Bacillus anthracis,* a relationship experimentally proved by Pasteur. Koch also determined the causative organism for tuberculosis to be *Mycobacterium tuberculosis* and for cholera to be *Vibrio cholerae*.

History credits both Pasteur and Koch for major contributions to the development and acceptance of the germ theory of disease. Since then, improvements in sanitation, public health mandates, the development of vaccines, the discovery and use of antibiotics, and other improvements in health care and medicine have ended the era in which infectious diseases caused the majority of all deaths. As a result, the average life expectancy has increased from slightly less than 40 years for someone born in 1850 to almost 80 years for someone born today.

See also ANTIMICROBIAL DRUGS; HOST DEFENSES; KOCH, ROBERT; LEEUWENHOEK, ANTONI VAN; MICROBIOLOGY; PASTEUR, LOUIS; SPONTANEOUS GENERATION; VACCINES.

FURTHER READING

Adler, Robert E. *Medical Firsts: From Hippocrates to the Human Genome.* Hoboken, N.J.: John Wiley & Sons, 2004.

Waller, John. *The Discovery of the Germ: Twenty Years That Transformed the Way We Think about Disease.* New York: Columbia University Press, 2002.

Golgi, Camillo (1843–1926) Italian *Cell Biologist*

Camillo Golgi was the first Italian to receive the Nobel Prize in physiology or medicine (1906). He made a true scientific breakthrough when he developed a method for staining neurons. His work made possible the elucidation of the innermost anatomy of the nervous system, which eventually led to a basic understanding of its function.

TRAINING AND CAREER

Camillo Golgi was born on July 7, 1843, at Corteno (now called Corteno Golgi), in the province of Brescia, in northwestern Italy. His father, Alessandro, was a physician, and Camillo also decided to study medicine. He graduated in medicine in 1865 from the University of Pavia with a thesis on hereditary factors in mental illness. After obtaining his medical degree, he worked for seven years at the Hospital of St. Matteo in Pavia, where he studied the cause of mental diseases. Meanwhile, he worked at the Institute of General Pathology with the experimental pathologist and histologist Giulio Bizzozero, who taught him histological techniques. Golgi never practiced medicine but devoted his life to medical research instead. He left Pavia briefly in 1872–76 to serve as chief physician at the Hospital for the Chronically Ill at Abbiategrasso, where he started investigating the nervous system using a microscope set up on the kitchen table in his small living quarters. He returned to Pavia and in 1876 was appointed professor of histology; then in 1881 he succeeded Bizzozero as the chair of general pathology at the University of Pavia, and he remained there for the rest of his career. Many distinguished scientists spent time in Golgi's laboratory at the Institute of General Pathology. Golgi served the university as dean of the faculty of medicine and also as rector of the University of Pavia for several years. He became a senator of the kingdom of Italy in 1900. Later in his life, during World War I, he assumed responsibility for a military hospital in Pavia, and he created a center for the study and treatment of peripheral nervous lesions and for rehabilitation of the wounded. He retired in 1918 but remained as professor emeritus.

THE BLACK REACTION

Golgi's earliest scientific article, suggesting organic lesions in the neural centers caused psychiatric disorders, was published in 1869. Determined to find facts to support his own claims, he initiated histological studies of the nervous system; however, the methods at the time were inadequate for examining the complex nature of nervous tissue. The axons and dendrites of neurons are too thin to be seen using common staining methods. While at Abbiategrasso, he experimented with different staining techniques until in 1872 he finally had success at developing a procedure that left darkened outlines in some neurons. He published a brief note with the translated title "On the structure of grey matter," in the *Gazzetta Medica Italiana* in 1873. This contained the first mention of the black reaction, the fundamental discovery in neural anatomy that now bears his name and that gave Golgi Nobel fame. Golgi described the microanatomy of a nerve cell body including its cellular processes in much better detail than previously possible. A few years later he published the first sketches of neural structures in olfactory bulbs as seen by the Golgi staining method. In 1885 he published a monograph that contained the detailed illustrations of the microanatomy of the central nervous organs. Between 1877 and 1880 he was extremely prolific and published many more observations made possible by using his staining technique.

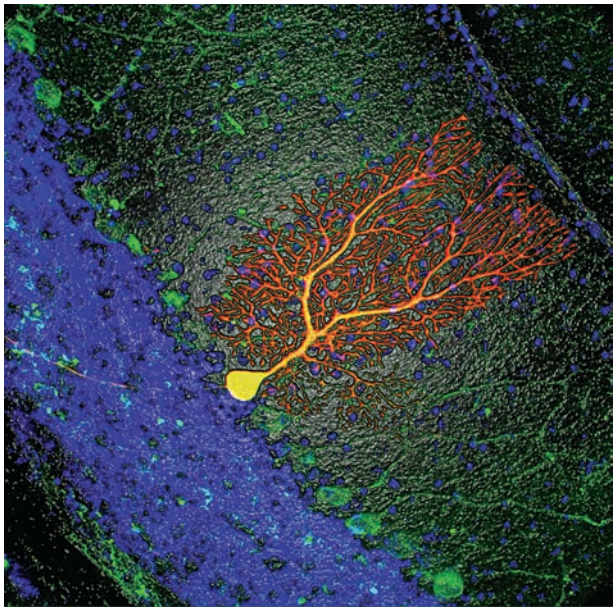
Histologists today still use Golgi staining or Golgi impregnation. A series of numerous attempts and adjustments led to the following procedure: After hardening, or fixing, a piece of neural tissue with potassium dichromate, he immersed it with silver nitrate. The salt silver nitrate formed, crystallized, and filled in the neuron along its entire length. For an unknown reason, only between 1 and 5 percent of cells become impregnated and stain black using this method. This turns out to be beneficial since nervous tissue is complex and contains many overlapping and intertwined fibers and cells. A comparison demonstrating its utility would be observing a shadow painting of a distant densely populated forest. If every trunk, branch, and leaf were painted black, the entire canvas would appear black. But if the structures of only one or two trees were outlined in black and the rest all remained colorless, the observer would be able to distinguish the detail and structure of the individual trees within the forest. Golgi later developed modifications of his basic silver staining technique by altering the length of time the tissue was immersed in the dichromate solution in order to emphasize the neurons, the glia, the cell process, or all at once.

One major ramification of Golgi's staining method was that it revealed morphological features hidden by previously used histological techniques. For example, in 1837 the Czech anatomist Jan Evangelista Purkinje described unique cells in the cerebellum, the area of the brain that maintains the body's position in space and subconsciously coordinates motor activity. He was only able to visualize the bodies of these cells, now called Purkinje cells, but Golgi staining later revealed that these cells contained more extensive branching than any other type of neuron. They contain intricate, oak-tree-like branching that is integral to their function of carrying all outputted

cerebellar information and is necessary for coordination of motor activities. Until the application of Golgi's technique, the branching had been invisible—only the bottommost part of the trunk had been observable. Being able to see the organization of the neuronal extensions of the Purkinje cells allowed neurobiologists to trace the projections and draw conclusions about their function.

The Golgi staining method also impregnated glial cells (also called neuroglia), allowing Golgi to describe their structure. These cells provide support and nutrition to the neurons. Golgi differentiated between two neuron types, Golgi type I and Golgi type II. The former possess long axons that extend long distances from the cell body, whereas axons of the latter cells project locally into the vicinity of the cell body to form local circuits or interneurons.

In 1838–39 Matthias Schleiden and Theodor Schwann had proposed the cell theory, purporting that tissues were composed of cells, but anatomists had not yet applied this concept to nervous tissue. This was mainly due to the fact that its structure and organization were still so mysterious. Until the late 1890s, two competing hypotheses described the structure of nervous tissue: the reticular theory and the neuron theory. According to the reticular theory, nervous tissue consisted of a large syncytial network made of many physically connected fibers. This diffuse arrangement would enable nervous impulses to flow continuously through the system. The neuronal hypothesis proposed that the nervous system was composed of numerous individual cells called neurons. Golgi preferred the reticular theory. Understanding how a nervous impulse could travel between individual separate cells, as the neuron theory demanded, was more difficult to imagine, yet the cellular detail disclosed by Golgi's staining method supported this school of thought. Biologists have since elucidated the method by which nervous impulses travel across synapses, the spaces between neurons. The presynaptic cell secretes chemicals called neurotransmitters that carry the information across the junction to the next cell, where they bind to receptors and initiate an impulse in the postsynaptic cell.



Golgi staining revealed the treelike branching pattern of axons in specialized nerve cells of the cerebellum, called Purkinje cells. *(David Becker/Photo Researchers, Inc.)*

After he had been considered every year since its inception in 1901, on the basis of the sharply perceptive observations of neurons that biologists were able to make using Golgi's staining method, Golgi was awarded the Nobel Prize in physiology or medicine in 1906 for his work on the structure of the nervous system. The Nobel Prize was shared with the Spanish anatomist Santiago Ramón y Cajal, one of the main supporters of the neuron theory, the correct view that the nervous system consists of anatomically and functionally distinct cells.

During the Nobel ceremonies, Golgi delivered a distasteful lecture that cost him the respect of others thereafter. In the first sentence of his lecture, Golgi admitted that he had always been opposed to the neuron theory (for which his corecipient Cajal had gathered much supportive evidence) and then stated that "this doctrine is generally recognized to be going out of favour." From that point on, he made incorrect, combative, and narcissistic statements until nearly all of the audience members and his colleagues were insulted. Those who were there reported that Golgi seemed oblivious to the hostile nature of his comments. After Golgi's lecture and in sharp contrast, Cajal spoke and humbly acknowledged the contributions of others (including Golgi) and described the brilliant research that supported the neuron theory. Scientific observations backed Cajal's claims, and soon after, the neuron theory became widely accepted.

INTERNAL RETICULAR APPARATUS

During the course of his cellular examinations, Golgi observed an intracellular structure that he called an internal reticular apparatus. He published his discovery of this structure in 1898, but for half a century many scientists doubted its existence, believing its appearance was an artifact from the staining procedure. In the 1950s, electron microscopy confirmed its existence, and today cell biologists recognize the importance of this cytoplasmic structure in eukaryotic cellular physiology. Referred to as the Golgi apparatus, the Golgi complex, or simply the Golgi,

this structure is responsible for modifying, sorting, packaging, and routing substances made in the cell. Portions of the Golgi pinch off to form membrane-bound vesicles that deliver membrane proteins and other components to the cell membrane by fusing with it. Substances produced by secretory cells are delivered to the cell's exterior by a similar mechanism. The Golgi apparatus also packages potentially destructive substances, such as lysosomal enzymes.

OTHER DISCOVERIES

In 1878 Golgi discovered nerve endings embedded among the fibers of a tendon that perceive changes in muscle tension, or stretch. These nerve endings are a type of proprioceptor and are called Golgi's tendon organs. Proprioceptors are specialized sensory organs that help individuals know the position of their body parts in space by providing information about joint angles and muscle tension and length.

Golgi's early research included discrimination of the different species of parasites that cause malaria, a disease characterized by periodic attacks of chills and fever. Between 1882 and 1892 he examined the life cycle of the causative agent, *Plasmodium,* and he developed a method for photographing blood cell preparations to identify different stages. When a female *Anopheles* mosquito bites a human, she releases *Plasmodium* sporozoites into the bloodstream with her saliva. The sporozoites quickly travel to the liver, and over five to seven days they multiply to form thousands of merozoites. The liver cells burst open, and the merozoites invade red blood cells, where they multiply again. After two days the newly infected red blood cells burst open, and the released merozoites invade more red blood cells. Golgi correlated the recurrent chills and fever with the release of the parasites from the blood cells. Some of the merozoites develop into gametocytes within the blood cells, and a mosquito may take these up during a blood meal. The life cycle continues in the mosquito, and if the mosquito bites another human, that person may become infected. Golgi's observations made it possible to diagnose the correct form of malaria afflicting a patient and to treat the disease more effectively.

Golgi's magnum opus was *Opera omnia,* consisting of four volumes that contained most of his publications. He published three in 1903, and his coworkers edited and published the fourth after his death.

In 1877 Golgi had married the former Donna Lina Aletti, Bizzozero's niece. The couple adopted his niece, Carolina. Golgi died on January 21, 1926, in Pavia.

The method of silver staining invented by Camillo Golgi enabled physiologists to study the anatomy of the nervous system at the cellular level and to observe subcellular structures clearly. Golgi's efforts transformed the mass of tangled fibers that made up the central nervous system into a structured network of neurons that exhibited complex anatomies. Investigators gained the ability to dissect individual components of nerve fibers from tissue specimens. Only after becoming able to define the structures could scientists efficiently explore the cellular mechanisms responsible for the functions of the nervous system. His work significantly impacted the debate over the neuron versus reticular theory of nervous tissue structure by providing evidence to confirm the neuron theory, which, ironically, Golgi did not support. In addition to the staining method and cellular structure that bear his name, the Historical Museum at the University of Pavia dedicated a hall to Golgi. In 1994 his portrait appeared on an Italian postage stamp.

See also EUKARYOTIC CELLS; INFECTIOUS DISEASES; NERVOUS SYSTEM.

FURTHER READING

Golgi, Camillo. "Sur la structure des cellules nerveuses." *Archives Italiennes de Biologie* 30 (1898): 60–71.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1906." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1906/index.html. Accessed January 25, 2008.

Rapport, Richard. *Nerve Endings: The Discovery of the Synapse.* New York: W. W. Norton, 2005.

Gould, Stephen Jay (1941–2002) American *Evolutionary Biologist, Paleontologist*

Trained in paleontology, Stephen Jay Gould was the 20th century's most prominent interpreter of evolutionary thought. In 1972 his fellow American paleontologist Niles Eldredge and Gould proposed punctuated equilibrium as a modification to the model of natural selection originally expounded by Charles Darwin. Though at first controversial, punctuated equilibrium is now considered an important process in large-scale evolutionary change. Gould was a prolific, award-winning author, particularly concerning the origins and diversity of life, who wrote for the general public as well as for a scientific audience.

AN EARLY INTEREST IN PALEONTOLOGY AND EVOLUTION

Stephen Jay Gould was born on September 10, 1941, in New York, New York. Steve was the older of two sons born to Eleanor Rosenberg Gould and Leonard Gould, a court stenographer who enjoyed natural history. At the age of five, Steve resolved to become a paleontologist after seeing the *Tyrannosaurus rex* exhibit at the American Museum of Natural History

Stephen Jay Gould was a prominent 20th-century evolutionary biologist and prolific author. *(Time & Life Pictures/Getty Images)*

in Manhattan. When he was 11 years old, he read *The Meaning of Evolution* (1949), written by the curator of the Department of Geology and Paleontology at the American Museum of Natural History, George Gaylord Simpson, who helped establish the modern synthesis of Darwin's theory of evolution by natural selection. Though he only minimally understood what he read, he was fascinated by it. The high school did not provide adequate instruction on evolution, so Steve began reading Darwin's work independently. He would later unite his two interests of paleontology and evolution.

Steve spent the summer after high school at the University of Colorado and then enrolled at Antioch College in Yellow Springs, Ohio. The intellectual and creative genius of the evolutionary biologist Charles Darwin impressed Gould, though he would later challenge his description of the progression of evolution. He completed his bachelor's degree with a double major in geology and philosophy in 1963. At Columbia University, Gould pursued his doctorate in evolutionary biology and paleontology by researching fossil land snails in Bermuda. To trace the evolutionary history of the snails, he searched strata representing millions of years but found basically no changes. A fellow graduate student and future collaborator, Niles Eldredge, observed a similar phenomenon in trilobites.

In 1965 Gould married an artist named Deborah Lee, with whom he had two sons, and accepted a position as an assistant professor of geology at Antioch College in 1966. The following year he completed his doctorate in paleontology and became an assistant professor of geology and assistant curator of invertebrate paleontology for the Museum of Comparative Zoology at Harvard University, where he continued researching the evolution of snails. He remained at Harvard his entire life, becoming an associate professor in 1971, and a full professor only two years afterward. In 1982 he was named the Alexander Agassiz Professor of Zoology.

PROPOSES THE THEORY OF PUNCTUATED EQUILIBRIA WITH NILES ELDREDGE

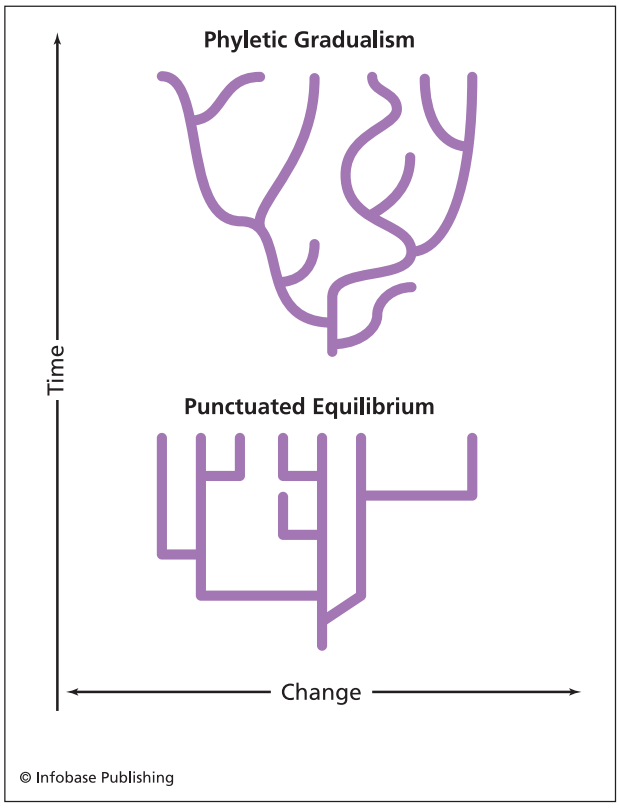
As an undergraduate student of geology, Gould dared to question the constancy inherent in uniformitarianism, the principle that asserts the Earth's physical features result from geological processes that have operated steadily and in the same manner since its formation 4.5 billion years ago. Why assume rates were constant and unchanging? As a student, he wrote a paper titled "Hume and Uniformitarianism" examining the assumption of constancy of natural laws in order to reach scientific conclusions about the past. He published a revised version, "Is Uniformitarianism Necessary?" in the *American Journal of Science* in 1965. Gould continued thinking about uniformitarianism and the other extreme, catastrophism, the belief that Earth's geological formations, such as mountains and lakes, resulted from tremendous catastrophes, such as floods or earthquakes. Years later, he described the concepts of time and direction in geology in the technical book *Time's Arrow, Time's Cycle* (1987).

Gould supported evolutionary theory, but without convincing evidence for slow, gradual transitions between species, he questioned the widely accepted manner by which it occurred. Phyletic gradualism, rooted in Darwinism but slightly modified, maintained that speciation occurred from the slow and steady transformation of entire populations over a large geographic range, but data indicated that evolution occurred in sudden, rapid spurts followed by longer periods with no substantial changes. Eldredge and Gould published "Punctuated Equilibria: An Alternative to Phyletic Gradualism" in *Models in Paleobiology* in 1972. This paper attempted to explain the tempo and pattern of evolution by fossil evidence and helped revitalize paleontology by generating a vast amount of literature in response. Although the German-born evolutionary biologist Ernst Mayr had initially proposed the basis of the concept, Eldredge and Gould's more thorough and extended presentation of the model that they named is considered the foundation of the punctuated equilibrium school of thought. Their paper also contained a warning to scientists about the danger of seeing only what an accepted theory dictated and suggested that paleontological research and comprehension of Earth's true history had been hindered by the assumption of phyletic gradualism.

Gould was disappointed in Darwin for attributing the absence of nongradualistic fossil evidence to

imperfections in the fossil record. He also noticed that some variations did not provide adaptive improvements and therefore could not be explained by Darwinian logic. Eldredge and Gould proposed that the abrupt appearance and stasis of species in the stratigraphical record were consistent with allopatric speciation, speciation occurring in small geographically separated subpopulations. Within a large population, new genetic variations became lost within an even larger mix of forms for a specific characteristic, but if an individual that carried the genetic variation were isolated from the rest of the population so that gene flow was reduced, then that variation had a greater chance of becoming established in a separated subpopulation, resulting in lineage splitting. One would expect transitional fossils to be rare if speciation occurred abruptly in small peripheral populations.

Eldredge and Gould believed that the breaks in the fossil record accurately depicted the past. New species did not evolve within the same geographic area, and entire populations did not gradually transform into new species. In contrast, they proposed that speciation was a rapid event that occurred in a small isolated population, followed by a long period of stasis, with no change, a model called punctuated equilibrium (PE).



In phyletic gradualism, slight variations accumulate over long periods, whereas in punctuated equilibrium, speciation occurs rapidly and is followed by long periods of stasis.

Reactions to PE varied. Some paleontologists felt the need to defend gradualism and flaunted the few well-documented examples. One classic example in paleontology is the slow, progressive increase in the number of whorls in the Liassic oyster *Gryphaea*. Biologists were surprised at the abundant evidence demonstrating that species remain virtually unchanged for millions of years, even in the face of rapid geological or climatological change. Organisms appeared to migrate rather than adapt to sudden climactic shifts. Critics of PE, those who are known as Darwin fundamentalists, labeled it "evolution by jerks." Gould wittingly responded by calling gradualism "evolution by creeps."

Though the title of the original paper introducing PE called it an alternative to Darwin's gradualism, Gould later explained that the two methods did not operate exclusively. Examinations of the overall fossil fauna overwhelmingly supported PE, whereas support for gradualism usually was found by investigating a specific lineage. PE explained why intermediate fossils connecting related species were absent, although transitional fossils between major lineages did exist.

Gould published several follow-up papers expanding the theory of PE. While some criticized his ideas, saying he made PE out to be more important than it actually was or periods of stasis were simply a weak attempt to explain missing links in the fossil record, others recognized his work as brilliant. The Paleontological Society awarded him the Schuchert Award in 1975 for excellence in paleontological research by a scientist less than 40 years of age.

PROLIFIC AUTHOR

In addition to more than 1,000 scientific papers, Gould authored at least two dozen books, interestingly, all using a manual typewriter. From 1974 to 2001, Gould published a series of 300 consecutive monthly essays for a column titled "This View of Life" in the magazine *Natural History*. His enlightening discourses ranged in scope over topics of science, philosophy, history, art, and literature and were collected and republished in 10 volumes under intriguing titles such as *Hen's Teeth and Horse's Toes* (1983) and *I Have Landed* (2002), explaining complex topics such as evolution and other natural phenomena without oversimplifying them. *The Panda's Thumb* (1980), a book that described a wrist bone that pandas use to help them strip bark from bamboo shoots and allowed them to switch from eating meat to eating plants, received the 1981 American Book Award in science. The following year Gould won the National Book Critics Circle Award

for his book that attacked the misuse of standardized intelligence tests to discriminate against certain races and religions, *The Mismeasure of a Man* (1981). His *Wonderful Life: The Burgess Shale and the Nature of History* (1989) won the Rhône-Poulenc Prize, a literary award for the best nonfiction science book written for the general reader. The best-seller described a British Columbia limestone quarry that formed 530 million years ago and holds a variety of unusual and complex fossil remains. Gould used this geological structure as an illustration of the characteristic randomness of evolution. He believed that evolution did not purposefully strive toward perfection and encouraged people to wonder "what if" biological history had proceeded down a different path.

Because of his recognized expertise on evolution and his ability to communicate scientific concepts lucidly, Gould served as a witness in an Arkansas state trial challenging the teaching in public schools of so-called creation science alongside evolution. Gould demonstrated that intelligent design, the belief that a higher being created the Earth, had no scientific basis and that, in fact, scientific evidence discredited many biblical stories that creationists interpreted as literal truths. The court ruled in favor of eliminating creationist teaching on the basis that it was religion and did not meet the criteria to qualify as science.

WRITES MAJOR TREATISE ON EVOLUTIONARY THEORY

Gould's last contribution to scientific libraries was a mammoth 1,433-page treatise, *The Structure of Evolutionary Theory,* written over two decades and published in 2002. In the book, he reviewed the undeniable facts of Darwinian evolution: more offspring are produced than can survive given competition for resources within a population, variations occur within individuals, and the variations are passed on to the next generation. Natural selection provided the mechanism by which variants that were better adapted to a particular environment achieved better reproductive success and passed on the favorable characteristics to their offspring. A master of analogy, Gould likened the frame of Darwin's evolutionary theory to a piece of coral with three major limbs branching from a central trunk. The trunk represented the core of Darwinian logic, the theory of natural selection, and the three branches represented a tripod of agency, efficacy, and scope. The central branch represented the agency by which natural selection worked—the claim that natural selection worked on organisms, not genes or clades or any other level in the hierarchical organization of life. The second branch represented the efficacy of natural selection—that natural selection alone was the mechanism for adaptive evolutionary change. The third branch symbolized the scope of natural selection, the extrapolation that small microevolutionary variations such as those that transformed wolves into dogs explained all taxonomic diversity given the immensity of geological time. Gould explained that severing the central trunk or disproving natural selection as an evolutionary force would destroy the theory (as it would kill the organism). Severing close to the branch points of the three major limbs would significantly compromise the theory, but excision and regrafting other parts would maintain its essential nature.

Gould proceeded to expand, add, and redefine aspects of classical evolution to restructure the symbolic coral, allowing growth of stronger branches to occur. The accumulation of new and different types of data over the 30 years since Eldredge and Gould first proposed PE allowed Gould to revise the structure of evolutionary theory by regrafting upon the original foundation. The recognition of species as Darwinian individuals capable of participating in natural selection led to a generalization of the hierarchical theory and the expansion of the agency branch by permitting selection to act on multiple levels in the hierarchy of life: genes, cells, organisms, demes, species, and clades. Gould cut back the efficacy branch a little, maintaining that creativity was necessary to build "evolutionary novelties" but allowing for a variety of additional mechanisms to guide evolutionary pathways by imposing some constraints (such as structural or developmental). For example, as the diameter of a single-celled organism increases, the ratio of its surface area to its volume exponentially decreases. Physical forces limit the maximal size of a cell since the surface area of the membrane of a very large cell could not support the required amount of material exchange with the environment. Gould did not feel microevolutionary processes were sufficient to explain the extent of diversity of life despite the vastness of geological time, so he modified the scope branch to include the role of broader-scale operations in the establishment of new species. The discovery of a catastrophic mass extinction occurring 65 million years ago supported this modification to evolutionary theory. Scientists found an unusually high level of iridium, an element detected in meteors, comets, and the Earth's mantle, within a layer of sediment deposited around that time, suggesting that an extraterrestrial impact and the resulting environmental disturbances caused the extinction of 85 percent of all species at the end of the Cretaceous period.

Gould exhibited an extraordinary breadth of knowledge, and people often asked his opinions on divisive issues. Though frequently questioned about the possibility of life on other planets, he never responded with a simple yes or no but took care to

explain that given the variety of earthly life-forms and the vastness of the universe, it seemed improbable that only Earth provided conditions permissive of the origin and support of life. Gould staunchly opposed biological determinism, the assumption that biology determines individual differences, making them unchangeable. Believing that science could never be detached completely from a personal dimension because scientists are human, Gould often spoke about the cultural embeddedness of science, the fact that society influences scientific endeavors.

In 1981 the MacArthur Foundation awarded Gould a fellowship, nicknamed the "genius grant," awarded to U.S. residents or citizens who show exceptional merit and promise for continued and enhanced creative work. *Discover* magazine named Gould Scientist of the Year for 1981 for developing the theory of punctuated equilibrium. In 2000 the U.S. Library of Congress named him one of 83 living legends who have "advanced and embodied the quintessentially American ideal of individual creativity, conviction, dedication, and exuberance." Gould also received the Medal of Excellence from Columbia University in 1983, the Silver Medal from the Zoological Society of London in 1984, the Gold Medal for Service to Zoology from the Linnean Society of London in 1992, and the Distinguished Scientist Award from the Center for the Study of Evolution and the Origin of Life at the University of California Los Angeles in 1997. He helped found *Paleobiology,* a journal that publishes articles focusing on processes and patterns in biological paleontology. He also received 44 honorary doctoral degrees and belonged to numerous scientific organizations including the National Academy of Sciences, Royal Society of Edinburgh, Paleontological Society, American Society of Naturalists, and American Association for the Advancement of Science, for which he served as president in 2000. In 1996 Gould became the Vincent Astor Visiting Research Professor of Biology at New York University and divided his time between New York and Cambridge.

In 1982 Gould was diagnosed with mesothelioma, a type of abdominal cancer. Though the median survival time was eight months, he lived two more decades and died of an unrelated lung cancer on May 20, 2002. His is survived by his second wife, Rhonda Roland Shearer, and his two sons, Jesse and Ethan, from his first marriage.

Gould was fluent in several languages and could read sources in their original languages. He was also a New York Yankees fanatic and a gifted baritone who sang with the Boston Cecilia Society. Though the public recognized him as a famous paleontologist, he described himself as a historian at heart and never limited his studies to a particular field. Wherever answers could be found, Gould ventured there to find them. His intellect surpassed the ordinary, and associates described him as both brilliant and arrogant. Gould's research and writings on punctuated equilibrium strongly influenced ideas about macroevolution and loosened the restrictions of classical evolutionary theory. He demonstrated that stasis is an important phenomenon worthy of examination and that punctuation is an interesting model of change. Alongside discussions on Darwin and his contributions to modern evolutionary theory, modern textbooks of Earth science, paleontology, biology, and evolution all include descriptions of punctuated equilibrium. Time will reveal how Gould's more recent ideas concerning the restructuring of evolutionary theory will influence future lessons on the history of life.

See also DARWIN, CHARLES; EVOLUTIONARY BIOLOGY; EVOLUTION, THEORY OF.

FURTHER READING

Chavez, Miguel. "The Unofficial Stephen Jay Gould Archive." Available online. URL: http://www.stephenjaygould.org. Accessed January 21, 2008.

Eldredge, Niles, and Stephen Jay Gould. "Punctuated Equilibria: An Alternative to Phyletic Gradualism." In T. J. M. Schopf, ed., *Models in Paleobiology.* San Francisco: Freeman, Cooper, and Company, 1972.

Gould, Stephen Jay. *The Structure of Evolutionary Theory.* Cambridge, Mass.: Belknap Press, 2002.

The Official Stephen Jay Gould Archive. Available online. URL: http://www.sjgarchive.org/. Accessed January 21, 2008.

Gray, Asa (1810–1888) American *Botanist*

Asa Gray was an American botanist who updated the classification and naming of the North American flora from the outdated Linnaean system to a more natural system based on biological similarity. He was one of the first paid professional botanists, *the* botanist at Harvard University for three decades, and the taxonomic authority on plants for the 19th century. Known for his staunch support of Charles Darwin, Gray helped prepare American scientists to accept the idea that species were mutable. His series of botany textbooks served as the standard and his *Manual of Botany of the Northern United States* survived well into the 20th century. When he retired in 1873, Harvard University hired four botanists to replace him.

CHILDHOOD AND TRAINING

Asa Gray was born on November 18, 1810, in Sauquoit, New York, to Moses and Roxana Howard Gray. When Asa was still an infant the family moved to Paris Furnace, where Moses set up a successful

tannery, allowing him to purchase a farm back in Sauquoit in 1823. Asa was introduced to Greek and Latin at Clinton Grammar School between the years of 1823 and 1825. A student of the nearby Hamilton College ate at the house where Asa lived during the school year, and he introduced Asa to the college's Phoenix Society librarian, who let Asa borrow novels, which he devoured. Asa entered Fairfield Academy in 1825 and started attending lectures at the College of Physicians and Surgeons of the Western District of the State of New York in Fairfield, in 1826. He received a doctor of medicine degree in 1831, at the age of 20.

Dr. James Hadley, the chemistry and materia medica teacher at Fairfield, had introduced Gray to botany and stirred in him an interest in science. Gray began collecting floral specimens. On a trip to New York City to purchase medical books with a letter of introduction in hand from Hadley, Gray visited the home of the foremost American botanist, Dr. John Torrey. Unfortunately, Torrey was out of town, but upon his return he admired the plant specimens that Gray left for him. Gray abandoned practicing medicine after only one year to devote himself to the study of plants. To earn money, Gray taught science part-time and worked as a librarian. He increased his scientific collection by sending dried specimens from America to overseas locations and receiving numerous samples in return. In 1833 he began assisting Torrey in studying the vegetation of the northern region of the United States and in reorganizing his herbarium by the natural method. He even moved in with the Torrey family for a while. For a few years he continued his involvement in other endeavors such as teaching at Hamilton College. Frustrated with the lack of suitable textbooks, he decided to write one based on the natural system of classification, with the goal of providing accurate information for the beginner as well as a useful reference for botanists. He published *Elements of Botany* in 1836, presenting botany as a balanced science. The well-reviewed book increased Gray's reputation and his communication with other natural historians. He later published revised and updated editions named *Botanical Textbook* (1850, 1853) and then *Introduction to Structural and Systematic Botany* (1858). Believing the available print resources sacrificed content and accuracy, Gray also published *First Lessons in Botany and Vegetable Physiology* (1857), a less technical version of his college textbook written for high school students, and *How Plants Grow: A Simple Introduction to Botany* (1858) for schoolchildren. The publisher's reports and sales of these books indicate his attempt at simplifying science while maintaining integrity was successful.

A CAREER BOTANIST

Gray also became a full collaborator with Torrey in researching and writing the two-volume *Flora of North America* (1838–43). Having established himself as a botanist, he was invited to join the U.S. Exploring Expedition (also known as the Wilkes Expedition because the commander was Charles Wilkes), a scientific naval expedition to explore the West Coast of North America, Oceania, and Australia. Two years later, after repeated delays and unending bickering between the scientists and the politicians involved, Gray opted to resign in favor of another opportunity; thus he never sailed with the survey team. The state of Michigan had joined the Union and the enthusiastic young new governor, Stevens T. Mason, planned to found a new university. In 1838 the board of regents of the University of Michigan appointed Gray the first permanent professor of the university. Because the institution only existed on paper at this point, Gray spent the first year of his professorship in Europe collecting books for the library, examining herbaria, and getting to know other botanists.

After his return to the United States, the university was not in immediate need of his services since still no students were enrolled, so he continued working on *Flora of North America* with Torrey while providing advice to the administration from afar. The voyage proved to his advantage for this task as he had many new notes and observations from his experiences and interactions with the scholars of Europe. Their major goal was to complete a survey and classification of the plants of North America. The continent lagged far behind Europe in cataloguing the indigenous flora. For almost a century, natural historians had been following the classification system proposed by the Swedish botanist Carl Linnaeus, which was based on the easily observable but superficial characteristics of number of male and female parts of a flower. European botanists had already switched to a more modern system for classifying plants (called the natural system), influenced by the work of the French botanist Antoine Laurent de Jussieu and and the Swiss botanist Augustin-Pyramus de Candolle. Gray and Torrey attempted to restructure the groups of North American flora on the basis of biological characteristics rather than arbitrarily selected flower parts. Collectors aware of their efforts sent specimens from all over North America and overseas. Gray and Torrey diligently worked, cataloguing specimens, recording observations, and writing summary reports of the samples from the numerous explorations.

The position at the University of Michigan never materialized because of the state's poor financial situation. After being on leave without salary for two

years, he formally resigned in 1942 when Harvard University offered Gray the Fisher Professorship of Natural History, a position that he accepted with the condition that he could focus on instruction in botany and the restoration and maintenance of the botanical gardens. At the time, botany was still considered an interest rather than a profession. Typical training consisted of finding a mentor willing to share his knowledge, and botanists struggled financially to support their botanical activities. Gray's arrangement at Harvard made him one of the first paid professional botanists. He remained at Harvard for the rest of his career, teaching introductory botany and a few upper-level courses while enjoying the ability to devote himself to his botanical researches.

Though both Gray and Torrey persisted in their efforts of reporting on specimens sent to them from expeditions, their formal collaborative efforts ended with Gray's move to Boston. The shadow of the incomplete *Flora* hung over Gray, but he set to work collecting seeds and roots to renovate the Harvard Botanic Garden. By the time he passed away, the Botanic Garden had become a hub of botanical science in the United States.

One of Gray's most successful endeavors was the *Manual of Botany of the Northern United States* (first edition published in 1848), which covered a more limited geographical range than the never-finished *Flora of Northern America*. The volume included all the flowering plants as well as some lower plants. The eighth edition, now called *Gray's Manual of Botany,* is still popular for identifying plants in the northeastern United States.

As America's leading botanist, Gray lent his expertise to many others, spreading himself too thin and not having sufficient time to devote to projects that he considered worthy. For the government he analyzed and reported on samples collected during the Pacific Railroad Surveys (1855–57). He corresponded with numerous individual collectors who obtained samples for him from all over the United States, but in return, he had to engage in the time-consuming activities of maintaining the correspondence, advising them, and sending them supplies. By 1948 Wilkes, the commander of the U.S. Exploring Expedition for which Gray worked for two years in the 1830s, practically begged Gray to help organize, analyze, and report on the botanical specimens that the so-called botanist who sailed on the expedition had collected. Many samples had been lost and others were damaged. No American botanist had expertise in tropical plants or other unique plants collected from the vast areas covered by the expedition, but everyone agreed Gray was the best choice for the job. Knowing this, Gray requested that the government support his traveling to Europe, where he could work in a well-equipped herbarium with access to scientific publications to aid him in his analysis. Wilkes agreed, and Gray spent one year overseas working in Sir William Hooker's herbarium, visiting the Royal Botanic Garden at Kew, and seeking expertise from other prominent botanists. Work continued back in Boston for several years. Other teams worked on special groups such as mosses, ferns, algae, and fungi (which are not plants but were included in this project). Petty disagreements over details of format and style plagued the project. Of the 100 official copies of Gray's first volume on flowering plants, 21 burned in a warehouse fire in Philadelphia.

SUPPORTS DARWIN

The British naturalist and evolutionist Charles Darwin began corresponding with Gray in 1855. Both were interested in the geographical distribution of plant species, a field called biogeography. By corresponding with amateur botanists around the country, Gray was able to define the ranges of many species. In 1857 Darwin shared his developing theory concerning the origin of species by natural selection with Gray, the third colleague to share the privilege. (Charles Lyell and Joseph Hooker were the other two.) One of Gray's most influential works was built upon Darwin's ideas. In response to a letter from Darwin, in which he questioned Gray about the geographical distribution of alpine plants in the United States, Gray performed a statistical analysis of the plants described in his *Manual* and published a paper in the *American Journal of Science* in 1856 on the distribution of plants, "Statistics of the Flora of the Northern United States." Three years later, he followed with a paper, "Diagnostic Characters of New Species of Phaenogamous Plants, Collected in Japan by Charles Wright, Botanist of the U.S. North Pacific Exploring Expedition," in the *Memoirs of the American Academy of Arts and Sciences,* proposing an explanation for why plant species in eastern North America resembled species from Japan more than species from western North America. He suggested that they evolved from a common ancestor in the Bering Strait region that migrated southward during glaciation and diverged into two lineages, one in North America and one in Asia. This work provided solid botanical evidence supporting Darwin's notion that the environment played a key role in the evolution of species, spelled out in his famous book, *On the Origin of Species,* published in 1859. As a result of religious and social conventions at the time, society accepted the idea of evolution in plants more readily than in animals or humans. Gray's work opened the minds of American biologists to the notion of evolution in all living organisms. The correspondence between the two men also served as

evidence of Darwin's priority in formulating the theory of evolution by natural selection, as the British naturalist Alfred Russel Wallace independently drew the same conclusion as Darwin around the same time. An outline of Darwin's theory was presented at the Linnaean Society meeting in July 1958.

One of Gray's adversaries was the Swiss-born naturalist Louis Agassiz, a colleague from Harvard whom Gray had hosted during a visit in 1846. Agassiz had been invited to the Lowell Institute, an educational foundation in Boston, to give a series of lectures titled "Plan of Creation in the Animal Kingdom." Agassiz believed that species were divinely created and distinct to each geological age. Gray, who was Presbyterian, also believed in creation but disagreed with Agassiz's point that Caucasians were created separately from African Americans and Malays, especially since this was the sort of argument favored by slavery proponents, who claimed the Caucasian race was superior. This point of contention served as a wedge that grew deeper after Agassiz joined the faculty at the Lawrence Scientific School of Harvard University in 1848. Gray grew to believe Agassiz did not follow scientifically sound logic when drawing conclusions and disliked Agassiz's showman style during lectures. Darwin's upcoming publishing of his fleshed out theory of evolution by natural selection and speciation by descent with modification gave Gray a platform on which he could publicly challenge Agassiz. A series of debates ensued, beginning with one at the Cambridge Scientific Club in December 1858, followed by several at the American Academy meetings. Gray outlined his conclusions about the relationship of North American and Asian flora, and Agassiz politely countered, saying his conclusions were drawn from the animal world. The discussions continued for several months, with comments made concerning the effects of climate on the distribution of plants, fossil evidence of flora, and the continuity of species. When pressed for written records of their arguments, Agassiz did not produce them.

After *On the Origin of Species* was published, Gray openly supported its main contention and collaborated with the Scottish geologist Charles Lyell to develop strategies for responding to religious objections to evolution. *Atlantic Monthly* published several essays written by Gray purporting that natural selection did not conflict with Judeo-Christian beliefs. Gray also published numerous anonymous articles attacking religious opponents and attempting to reconcile Darwin's ideas and the belief in God by proposing that God was responsible for evolutionary development; the book *Darwiniana* (1876) contained many of these essays and articles. Thus Gray's reputation and efforts prepared American scientists for accepting Darwinian evolution. Darwin and Gray's friendship persisted, and in 1877 Darwin dedicated a new book about structural differences between plants of the same species to Gray.

IMPACT

During his tenure at Harvard, Gray published numerous botany textbooks for all levels that were popular not only in academics but also with the general public. He trained and assisted countless collectors and amateur hobbyists to obtain and identify botanical specimens, which they in turn shared with him, expanding the database from which he could draw conclusions. He retired from teaching in 1873 but continued to live in the residence of the botanical garden. He spent his later years expanding *Flora* into *Synoptical Flora of North America* (1878). Illness forced him to give up his botanical studies in November 1887. When Gray passed away on January 30, 1888, in Cambridge, Massachusetts, he was survived by his wife, the former Jane Lathrop Loring, whom he had married in 1848.

Gray served as president of the American Academy of Arts and Sciences from 1863 to 1873 and president of the American Association for the Advancement of Science in 1872. He was also a regent of the Smithsonian Institution in 1874–88 and became a foreign member of the Royal Society of London in 1873. The herbarium that Gray established when he went to Harvard in 1842 grew into a world-class center of botanical research, and in 1864 he gave it to Harvard, in conjunction with his book collections. The Gray Herbarium, which specializes in vascular plants, held 1,939,914 specimens as of 2007. The small brick building that Harvard built around his small library has grown into the Library of the Gray Herbarium, holding more than 63,000 volumes. In 1984 the American Society of Plant Taxonomists established the Asa Gray Award to honor botanists who have made significant contributions to plant systematics.

As the undisputed leader in the field of plant taxonomy for the 19th century, Gray made contributions to plant classification and botanical education both within and outside the walls of Harvard University that also made him one of the most influential scientists. Gray left his mark in classrooms, libraries, herbaria, and botanical gardens around the world. He influenced schoolchildren, amateur plant collectors, and scientists alike. He helped turn botany into an established scientific profession by insisting on the use of scientific reasoning for classifying plant species, by authoring numerous textbooks used to train amateur and professional botanists, and by demonstrating that plants, and animals, evolved in the manner proposed by Darwin. Gray also established a world-class center of botanical study at the Harvard Botanical Garden and Gray Herbarium.

See also BIOLOGICAL CLASSIFICATION; BOTANY; DARWIN, CHARLES; HOOKER, SIR JOSEPH DALTON.

FURTHER READING

Dupree, A. Hunter. *Asa Gray: American Botanist, Friend of Darwin.* (Johns Hopkins Paperbacks edition) Baltimore: Johns Hopkins University Press, 1988.

Rodgers, Denny. *American Botany, 1873–1892; Decades of Transition.* Princeton, N.J.: Princeton University Press, 1944.

Sargent, Charles S., ed. *Scientific Papers of Asa Gray.* 2 vols. Boston: Houghton, Mifflin, 1889.

Griffith, Frederick (1879–1941) British *Microbiologist* Frederick Griffith discovered the transformation principle in 1928 while trying to develop a vaccine to prevent pneumonia. This experiment was crucial in revealing deoxyribonucleic acid (DNA) as the genetic material.

Frederick Griffith was probably born in 1879, though sources also state 1877 and 1881, in Hale, in Cheshire, England. After graduating from the University of Liverpool in 1901, he held many positions as a microbiologist before accepting one as a medical officer in the pathology laboratory of the Ministry of Health in London. At the time, pneumonia was a leading cause of death, and much of his scientific effort involved determining the specific strains of bacteria in sputum specimens from patients diagnosed with pneumonia. In his free time, he studied the differences among the numerous strains he accumulated. He observed that many samples contained four or five different pathogenic strains of bacteria. Thinking it was unlikely for one person to be infected simultaneously with so many different types of pneumococcal bacteria, Griffith hypothesized that one strain could convert into another. Bacteriologists already knew that, over time, a cultured pathogenic pneumococcal strain lost its ability to produce a capsule, a thick extracellular polysaccharide layer that gave the colonies a smooth appearance and contributed to their virulence. If cultured in the presence of antisera that contained antibodies against capsular components, the virulent smooth strain (termed S) changed into the harmless, nonencapsulated rough strain (termed R). Occasionally, the R form reverted to the S form. Griffith wondered which structural remnant allowed this reversion to occur.

To examine this process, he heated a culture of S type pneumococci to kill them and then he injected masses of the dead S cells in combination with a small inoculum of living R cells into mice, within a few days all of the mice were dead; however, mice that were injected with only living R cells survived. After spending considerable time and effort definitively proving that all of the heated S organisms were dead, Griffith published his results, "The Significance of Pneumococcal Types" in the *Journal of Hygiene* in 1928.

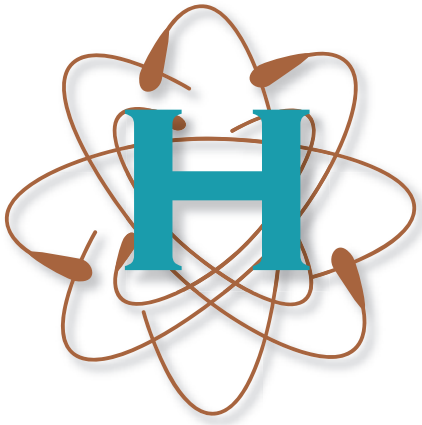
Though Griffith was primarily interested in the implications of his results for the epidemiology and treatment of pneumonia, across the Atlantic Ocean, Oswald Avery and his coworkers were interested in determining which substance from the S strain transformed the R strain into the encapsulated, virulent form. After confirming Griffith's results and duplicating them in vitro, Avery, Colin MacLeod, and Maclyn McCarty identified DNA as the molecular carrier of genetic information.

Frederick Griffith died during a World War II air raid in London in 1941 before he could realize the significance of his contributions to the field of genetics.

See also AVERY, OSWALD; DEOXYRIBONUCLEIC ACID (DNA); MACLEOD, COLIN MUNRO; MCCARTY, MACLYN.

FURTHER READING

Griffith, Frederick. "The Significance of Pneumococcal Types." *Journal of Hygiene* 27 (1928): 113–159.



Haeckel, Ernst (1834–1919) German *Zoologist* Ernst Haeckel was a 19th-century zoologist who is most famous for his support of Darwin and for his investigations of the relationship between the stages of development of an organism and its evolutionary history. Though many of his ideas were wrong, his lectures and publications stimulated debate and progress in understanding evolutionary theory and the history of life. Haeckel was also the first to formulate and define ecology, phylum, and phylogeny.

PERSONAL LIFE AND CAREER

Ernst Heinrich Phillip August Haeckel was born on February 16, 1834, in Potsdam, Germany, to Carl, a government official, and Charlotte (née Sethe). Ernst graduated from the public Domgymnasium in Merseburg, in 1852. As a schoolboy he collected plants in his free time and became adept at rapid identification. Following his father's wishes, Ernst pursued medicine as a course of study, realizing it offered him the greatest opportunity for scientific study. Knowing his intent was not to practice medicine, Haeckel focused on learning subjects such as embryology and comparative anatomy. One of his mentors was Johannes Müller, an expert in comparative anatomy, physiology, and ichthyology (the study of fishes). Haeckel studied at Berlin, Würzburg, and Vienna, and received a medical degree from Berlin in 1857, having submitted a dissertation on crawfish. He passed the state medical examination in 1858.

In 1862 Haeckel married his cousin, Anna Sethe. When she died two years later, he married Agnes Huschke, and they had three children together: Walter (1868), Elizabeth (1871), and Emma (1873). Haeckel obtained an academic appointment as a privatdozent (lecturer) at the medical school of the University of Jena in 1861, and he became a professor extraordinary (similar to an associate professor) in comparative anatomy and director of the Zoological Institute in 1862. A chair in zoology was established for him in 1865, and he held the chair until he retired in 1909.

ZOOLOGICAL RESEARCH

Haeckel joined a zoological expedition to the Mediterranean from 1859 to 1860. In the harbor of Messina, he launched his career as a zoologist. Having dipped his net into the water, he pulled out what local fishermen called strings of sea-eggs, but what Haeckel identified as radiolarians, planktonic marine protozoans that have gelatinous bodies, threadlike pseudopods, and intricate skeletons made of siliceous spicules. The trip resulted in his identification of 144 new species of radiolarians. In 1862 he published *Die Radiolarien* (The radiolarians), a monograph consisting of his research from this expedition, including 35 remarkable etchings. *Die Radiolarien* established Haeckel as a professional zoologist. The volume also expressed Haeckel's support of Darwin's evolutionary theory.

In 1859 the British naturalist Charles Darwin published one of the most influential books in science, *On the Origin of Species by Means of Natural Selection, or The Preservation of Favoured Races in the Struggle for Life*. The book caused considerable controversy for its author's claim, at a time when most people believed that a supernatural God created organisms in their current forms, that species are mutable and that all organisms descended from a few primitive life-forms. In summary, Darwin suggested that variation exists within a population, and this

variation caused some individuals to have increased success at survival and reproduction. Natural selection acts by competition to adapt the inhabitants to a particular environment; thus, new species emerge by the divergence of a common ancestor through the accumulation of slight, favorable variations over long periods of time. When the German translation came out in 1860, Haeckel recognized the work as revolutionary and became focused on research related to evolutionary theory. He also delivered numerous lectures about Darwin's theory of descent with modification to the public and to other scientists. Although some would later summarize Haeckel's work as simply popularizing Darwinism in Germany, Haeckel contributed much original thought to expand Darwin's theory in formulating his own synthesis.

Haeckel researched the morphology, systematics, and embryology of marine invertebrates, including radiolarians, medusae, siphonophores, sponges, annelids (segmented worms), and echinoderms. He sought not only to find evidence in support of Darwin's theory but to explore it further. Darwin laid the foundation, but Haeckel took on the reconstruction of zoology on the basis of the newly proposed theory by seeking connections among species.

GENERAL MORPHOLOGY

From the time of his reading *On the Origin of Species,* Haeckel sought to relate the anatomy and morphology of organisms to their evolutionary history. In 1866 Haeckel published the two-volume work *Generelle Morphologie der Organismen* (General morphology of organisms). In the 18th century the Swedish botanist Carl Linnaeus divided living organisms into two kingdoms, Plantae and Animalia. Haeckel proposed a third borderline kingdom, Protista. Each kingdom contained several groups that he called phyla, which comprised all the extinct and extant organisms that arose from a common ancestor. Haeckel used this concept of a clade to create genealogical or phylogenetic trees, the first of which appeared in *Generelle Morphologie.* The phylogenetic trees represented a natural system of classification. Within Protista, Haeckel included monerans, which he described as living particles of plasm that contained no nucleus and did not seem to have reached the level of a true cell. Biologists later distinguished between prokaryotic and eukaryotic cells; the term moneran is no longer common, but refers to prokaryotic organisms. Haeckel imagined that life on Earth originated by the spontaneous formation of complex organic molecules that assembled into a "moner," or a mass of plasma. From a moneran, or a group of them, all other life-forms emerged. Monerans therefore represented the lowest life-forms. Haeckel began his discussion of organic morphology here, with monerans, and then progressed to true cells, organs made up of numerous cells, then to whole individuals, and beyond that, to social communities. Thus he carried the concept of evolutionary progression into the realm of sociology, something for which he was later criticized.

Another major concept on which Haeckel expounded in *Generelle Morphologie* was what he called the fundamental biogenetic law. Sometimes this law is summarized by the statement "ontogeny recapitulates phylogeny," and 19th-century biologists used it as evidence in support of organic evolution. Ontogeny is the development or course of development of an organism. Phylogeny refers to the evolutionary history of a species. The phrase "ontogeny recapitulates phylogeny" means that the stages of development of an organism reflect the series of steps in the evolutionary history of that species. All multicellular organisms develop from a single cell that undergoes a series of mitotic cleavages or divisions to form a ball of cells. This stage, called a morula, is followed by the formation of a blastula, a sphere of cells with a hollow center. Gastrulation occurs next and results in the formation of the primary germ cell layers that develop into specialized tissue and organs. Eventually body parts and structures unique to the species form. The concept of recapitulation was based on the fact that the embryological stages of multicellular organisms appear to resemble one another. It should be noted that the 19th-century Estonian embryologist Karl von Baer, not Haeckel, was the first to observe and comment on the resemblance of the embryological stages between species.

Haeckel's biogenetic law consists of the following three main points:

- Law of correspondence: each embryonic stage corresponded to an adult stage of a more primitive life-form. For example, the fertilized egg or zygote of sexually reproducing multicellular organisms corresponded to the adult stage of protists.
- Law of terminal addition: each new species resulted from the addition of another step or stage of development. This would result in a linear rather than a branched phylogeny, a major departure from Darwinian evolution.
- Law of truncation: each preceding stage in development could be shortened in order to decrease gestation time and also because all developmental stages were not observed in all animals.

At the earliest stages of development, nearly all organisms undergo the same events. As development progresses to later stages, fewer types of organisms share the same stages or features. Haeckel believed

that each new process or distinguishing stage of development represented the divergence of a common shared ancestor into separate lineages, each defining a new clade. Thus the course of development repeats each stage or phase in the phylogeny of an organism. Haeckel saw, as an advantage to this law, the fact that embryological data was more abundant than paleontological data, and was therefore useful in establishing the emergence of species. He incorporated ontological data into the construction of tree diagrams that depicted evolutionary relationships and histories of different organisms.

While the proposed relationship between ontogeny and phylogeny stimulated much thought and research on evolutionary histories, the basis of the main idea as support for evolution is no longer considered valid, as much of it was based on faulty evidence. For example, Haeckel thought the gill slit (grooves that appear below the head) stage of human embryos corresponded to the adult stage of fish, a lower vertebrate in comparison to humans. However, in humans, these gill slits do not actually open into the larynx, whereas they do in fish. Moreover, in fish they play a role in breathing or respiration, whereas in humans, they develop into the lower jaw. Thus, anatomical and physiological evidence has eliminated the notion that the presence of gill slits recapitulated a stage in the shared phylogeny of humans and fish. Another example of superficial support for the biogenetic law is the resemblance between animal embryos and the larval stage of insects. Many anatomists and zoologists criticized Haeckel's drawings of embryonic stages, calling them forgeries and claiming they were incorrect, or at least overemphasized some similarities and de-emphasized others in order to prove his point.

Though the basis of *Generelle Morphologie* was scientific, Haeckel included some discussion of philosophy and sociopolitics. Haeckel was a monist, meaning he believed that there is only one kind of ultimate substance; only relative differences distinguished organic and inorganic substances, or physical and metaphysical, and physical laws explained biological diversity and biological processes such as growth and reproduction. Man was simply a higher vertebrate, and therefore subject to the same physical laws and methods of analysis as any other animal. Carrying monism even further, Haeckel sought unity in all realms of the world, including economics, politics, and ethics. Haeckel felt that evolutionary theory should not be limited to the organic sciences, but could be applied to the social sciences, political science, and also ethics. For example, anthropologists adopted Haeckel's concept of evolution as advancement by viewing primitive cultures and races as unfit or "lower." The Nazis later applied this unscientific rationale to defend their stance that "lower" or "inferior" races should be exterminated.

OTHER ZOOLOGICAL RESEARCH

After publishing *Generelle Morphologie,* Haeckel's perspectives and methods essentially remained the same, though he continued to describe and outline the phylogenies of different animal species (up to 4,000) over the course of his career. The translated titles of some of his other monographs include *Siphonophora* (1869), *Monera* (1870), and *Calcaerous Sponges* (1872). The latter work provides an example of his use of the fundamental biogenetic law to classify organisms. He divided sponges into three types, all of which, he said, descended from a common ancestral life-form. This work was also important because it presented the foundation of Haeckel's gastraea theory, which stated that the cup-shaped gastrula stage of development suggested the existence of an ancestral form that he called a gastraea, common to all metazoans.

Though Haeckel believed that Darwinian evolution explained all biological phenomena, he did not wholeheartedly embrace natural selection as the mechanism by which evolution occurred. His beliefs more closely resembled those of Jean-Baptiste Lamarck, who propounded that inheritance of characters resulted from environmental effects. He described two forms of heredity: conservative heredity and progressive heredity. He suggested that the nucleus controlled conservative heredity, which was responsible for the inheritance of heritable characters. The cytoplasm directed progressive heredity, the inheritance of characters acquired as adaptations to the environment. The two types of heredity interacted to cause change in species. In 1876 Haeckel published *Die Perigenesis der Plastidule* (The genesis of things around the protoplasmic molecules), a book that explained heredity as a result of molecular motions within the cytoplasm transmitted from the mother to the daughter cells. Motions of molecules within the cytoplasm that occurred in one cell as a result of environmental conditions caused adaptations that were passed on to the next generation of cells.

From 1870 to 1872, C. Wyville Thomason led a scientific expedition aboard the HMS *Challenger,* during which thousands of unknown species were collected. After the expedition returned, Haeckel helped analyze, identify, and report on many of the marine invertebrate specimens. From these investigations he published *Deep-Sea Medusae* (1881), *Radiolaria* (1887), *Siphonophora* (1888), and *Deep-Sea Keratosa* (1889).

Recognizing the importance of environmental conditions in shaping the form of living organisms,

Haeckel developed two new concepts in life science. He defined ecology as the comprehensive science of the relationships of organisms to the environment and chorology as the entire science of the spatial (geographical and topographical) distribution of organisms over the Earth's surface. Both of these concepts persist in biology. Ecology has become a major broad, interdisciplinary branch of biology. Chorology is more commonly called biogeography today, though the recognized importance of the external environment in the distribution of life-forms remains the same.

Systematische Phylogenie (1894–96) contained a summary of Haeckel's work undertaken in the years after publication of *Generelle Morphologie* in 1866. In no small part stimulated by his observations and ideas, researchers made further advances in studies in phylogeny. Unfortunately, this book was not as well received as he had hoped. His later works focused on monism, which Haeckel described as a necessary consequence of evolution. *Die Welträthsel,* published in 1899, presented Haeckel's view on anthropology, psychology, cosmology, and theology. This work was more popular than any of his scientific publications.

Haeckel belonged to more than 90 academic societies and organizations, including the Leopoldine Academy, the Bavarian Academy of Sciences at Munich, the Imperial Academy of Sciences at Vienna, the American Philosophical Society, the Royal Swedish Academy of Sciences at Stockholm, and the Royal Society of Edinburgh. In 1901 Haeckel received the Bressa Prize of the Royal Academy of Sciences at Turin.

Haeckel died in Jena in 1919. His major works were not widely read by his colleagues, but his popular articles were and his lectures about evolutionary theory were well attended, making him as famous as Charles Darwin in his time. Though many of the scientific ideas Haeckel supported later turned out to be false, he stimulated much thought leading to progress in the life sciences. He is credited with creating the kingdom Protista, which he described as containing organisms intermediate between plants and animals, and he was the first to utilize tree diagrams to represent genealogical relationships. Words that Haeckel coined, including ecology, phylogeny, and phylum, and the concepts they represent helped shape evolutionary biology and ecology.

See also Darwin, Charles; evolution, theory of; Lamarck, Jean-Baptiste.

FURTHER READING

Bölsche, Wilhelm. *Haeckel: His Life and Work*. London: T. F. Unwin, 1906.

Breidbach, Olaf. *Visions of Nature: The Art and Science of Ernst Haeckel*. Munich: Prestel, 2006.

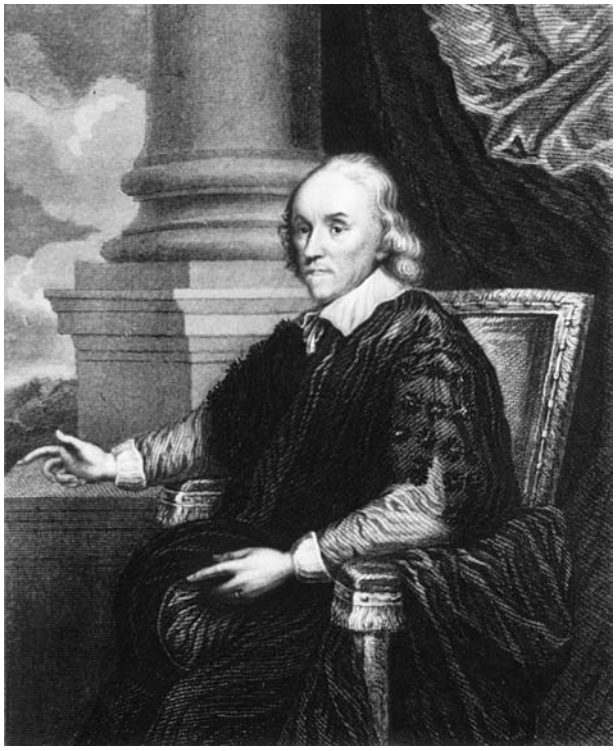
Harvey, William (1578–1657) English *Physician, Anatomist, Physiologist* William Harvey was a 17th-century physician who definitively disproved long-held theories when he discovered that blood circulates throughout the body, pumped by the muscular heart. Because of his pioneering methods in studying the human body and how it functions, Harvey is considered the father of modern physiology.

BECOMES A PHYSICIAN

William Harvey was born on April 1, 1578, in the small coastal town of Folkestone, England. He was the eldest of seven sons. Thomas Harvey and his second wife Joan Halke also had two daughters. Harvey senior was a farmer and a landowner and later in life became a successful businessman. As a child helping out on his father's farm, William became interested in how the body was built and functioned. He reportedly dissected small animals in his mother's kitchen. When William was 10 years old, he entered the King's School in Canterbury. He received a scholarship to study arts and medicine at Gonville and Caius College at Cambridge University and obtained his bachelor's degree in 1597.

In 1602 Harvey received his doctorate of medicine from the University of Padua in Italy, the most reputable medical school at the time. Most of the anatomy Harvey learned at Padua stemmed from the teachings of Aristotle and Galen. The second-century Greek physician Galen was extremely prolific on the topic of anatomy, yet he based his writings on animal observations. Human dissections were considered sacrilegious mutilations and therefore prohibited in his day. Because Galen's doctrines were consistent with Christian theology, after the faith became predominant his works and reputation were well-protected for 1,500 years. Most physicians still relied on his teachings, though a few dared to begin questioning some of his assertions.

After obtaining his medical degree, Harvey set up a private practice in London. He obtained his full licensure to practice medicine, and he began to acquire many famous patients. In 1604 he married Elizabeth Browne, the daughter of one of King James I's many physicians. They had a happy marriage but never had any children. Harvey was elected to membership in the Royal College of Physicians in 1607, and, two years later, he was appointed physician to St. Bartholomew's Hospital, one of two hospitals that treated poor patients. This position required him to visit the hospital about once each week, examine some of the patients, and direct the staff of three surgeons, one apothecary, and 13 nurses. From 1615 to 1656, he lectured on anatomy as the Lumleian lecturer of surgery for the Royal College. In 1618 he replaced his father-in-law as



William Harvey was an English physician who discovered that the blood circulates throughout the body, pumped by the heart. *(National Library of Medicine)*

one of the king's physicians, a position he continued to hold when King Charles I assumed the throne in 1625. Harvey became a close companion of King Charles and was loyal to him during and after the English civil war.

DISCOVERS BLOOD CIRCULATES

In order to appreciate the contribution that Harvey made to modern anatomy, one must first understand what was then accepted as true concerning the circulatory system. The ancient Greek philosopher Aristotle had proposed that the liver made the blood, which was carried through the body in the veins and was cooled by the brain. He also believed that the heart was the body's main organ and was responsible for emotions and intelligence. Galen expanded these ideas into the belief that the body was organized into three separate physiological systems. The main organ of the first system was the liver, which made the dark blood from food cooked in the stomach. Veins then carried these "natural spirits" around the body, and different body parts absorbed blood as needed. The main organ of the second system was the heart, the so-called seat of the soul that served as an innate source of heat for the body. The heart was also responsible for preparing the life-giving "vital spirits" by mixing the blood with air from the lungs, a process that also acted to cool the heart. After being mixed with air, the bright red blood then would be carried throughout the body via the arteries. Lastly, the brain served as a source of "animal spirits" that were responsible for sensation and motion and were distributed throughout the body through hollow nerve tubes. Galen also noted that blood moved from the right side of the heart to the left and he suggested it moved through tiny pores or openings in the septum, the wall that separates the two sides of the heart. The blood was thought to move through the body by a force contained with the spirits themselves. Because these ideas were sanctioned by the Roman Catholic Church, Galen's ideas went unchallenged for centuries.

The Flemish anatomist and physician Andreas Vesalius was the first to question Galen publicly. He noticed that Galen's observations and anatomical descriptions about humans must have been made from observations of monkeys and apes. A member of the faculty at the University of Padua in the early 16th century, Vesalius bravely and innovatively used human dissections as part of his anatomy lectures. Based on his own observations, he proposed that Galen never even dissected a human and proceeded to publish the most accurate textbook on human anatomy to that date. In 1559 one of Vesalius's students, Realdo Columbo, wrote *On Anatomy,* which stated that blood did not move through pores within the septum, but rather from the right side of the heart to the left via the lungs. Resistant to new ideas, the medical faculty at Padua continued to follow Galenic tradition. Girolamo Fabrici, more commonly known as Fabricius, was one of Harvey's teachers. In 1603 he discovered that veins contained structures that looked like little flaps. He called these valves and suggested their purpose was to control the amount of blood sent to certain body parts. They prevented blood from collecting in the lower body parts. Fabrici was devoted to Galen, however, and did not extend his observations further.

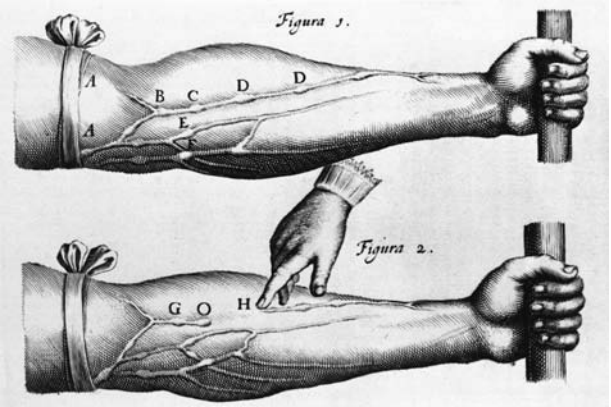
This was where the doctrine stood in the early 17th century, when Harvey grew troubled by the apparent conflict between what he was taught to believe and what he observed on his own. From his Lumleian lecture notes, scholars learned that Harvey began formulating his opinions on the heart and blood before 1616. Only after he published a 68-page booklet titled *On the Motion of the Heart and Blood in Animals* in 1628 did the world finally, though still reluctantly, begin to consider the Galenic doctrines more critically. Within a few decades, physicians widely accepted Harvey's discoveries.

The overwhelming conclusion that revolutionized thought concerning the heart and blood was that blood circulated through the body. That is, the same blood passed through the veins, heart, and arteries

over and over, hence the current nomenclature, the "circulatory" system. Ignoring such nonsense as spirits and mysterious forces, Harvey determined by observation that the heart was simply a muscle, a mechanical pump that alternately contracted and relaxed with each beat. When the heart contracts, its chambers shrink, expelling blood into the arteries and making them pulse or temporarily bulge. This action sends the blood traveling throughout the body. The veins carry blood in a single direction back to the heart. The action of valves, the structures in the veins discovered by Fabricius, prevents the reverse flow. Similar valves are found between the atria and the ventricles in the heart. These valves prevent blood from returning to the atria after moving to the ventricles and into the arteries.

Today, the pathway of blood circulation is well known. Blood enters the right atrium of the heart through the vena cava, then passes through a valve into the right ventricle. Blood travels from the right ventricle to the lungs via the pulmonary artery. Oxygenated blood returns to the heart through the pulmonary vein at the left atrium and moves through another valve into the left ventricle, the strongest chamber of the heart. The left ventricle pumps the blood into the aorta, which carries the freshly oxygenated blood to all the body parts. Within the body tissues, the arteries empty into capillaries that connect to veins and eventually dump their now deoxygenated blood into the vena cava.

Harvey had performed many dissections and vivisections (dissections performed on living animals), and he presented several types of evidence in defense of his revolutionary statements. He crudely measured the amount of blood the heart pumps with each contraction. Using this estimate, he calculated that in a single hour the heart would pump a quantity of blood three times the weight of an average man. Thus, it was difficult to fathom the liver producing this much blood and the body parts absorbing as much. The same blood must flow continuously throughout the arteries and veins. He described several experiments that confirmed his conclusions. For example, he tied off the vena cava of a living snake. The heart became pale and empty of blood. When the aorta was tied off, the heart filled with blood that could not escape. If he tied a cloth tightly around a man's upper arm, the deep arterial blood flow was blocked, the arm became pale, and a bulge appeared on the heart side of the cloth. If the cloth was loosened, arterial flow brought blood down the arm, but the blood could not return through the more superficial veins, and as a result the arm turned purplish and bulged. He also detailed the anatomical evidence based on the structure and orientation of the valves within the veins and within the heart. He was able to insert probes through the little doorways only in one direction, the same direction that lead to the heart. If he probed through the valves in the other direction, they were functionally destroyed.



This artwork from *On the Motion of the Heart and Blood in Animals* (1628) shows that valves in veins of the forearm prevent blood from flowing back toward the hand on the way to the heart. *(National Library of Medicine)*

Harvey did not expound on the purpose of blood circulation. Today physiologists know that the circulatory system is responsible for transporting all materials into and out of body tissues. Oxygen necessary for metabolism is picked up from the lungs by pulmonary circulation and sent to all parts of the body. At the body tissues, veins collect the waste products of metabolism, such as carbon dioxide, and carry them away from the body tissues. Blood also carries sugars and amino acids for anabolic purposes to all parts of the body as well as hormones that allow communication between body parts. Harvey was unaware of capillaries, the tiny vessels that connect arteries and veins within the tissues. These structures were discovered by the Italian physician Marcello Malpighi with the assistance of a microscope.

ROYAL TROUBLES

Believing in the divine right of kings to rule, King Charles abolished Parliament in 1629. In 1642 civil war broke out between King Charles's supporters and Parliament's supporters. Harvey's research papers were all destroyed when the living quarters of the king's palace were ransacked. By 1639 Harvey had been promoted to chief physician to the king. In return for Harvey's loyalty, the king supported his research by providing him with deer from the royal parks, which Harvey used for dissections. When Harvey followed the king to Oxford, as a favor, Harvey was given the position of warden for Merton College at Oxford University. In 1646 the king surrendered to Parliament and was beheaded in 1649.

In 1649 Harvey responded publicly for the first time to criticism—raised especially by French doctor Jean Riolan—of his *On the Motion* treatise. Harvey not only responded, but he provided additional evidence supporting his original claims. Around this time, the criticism began to die down as his work was accepted by a younger generation of physicians.

While Harvey's discoveries regarding blood circulation immeasurably advanced the field of physiology and rendered him famous, he was also interested in animal reproduction. While at Merton, a professor allowed Harvey access to his hens, and Harvey spent much time there examining the eggs. In 1651 he published *On the Reproduction of Animals*. While this treatise was not nearly as controversial as his publication *On the Motion*, it earned Harvey recognition for presenting the most accurate and well-documented day-to-day development of the chick.

Harvey pondered questions about the generation of life and, specifically, about what each parent contributed to an embryo. There had been many suggestions through the years. One idea, preformation, suggested that an entire miniature organism was already present within each egg—at birth, each female has eggs containing entire beings already inside them, and those eggs also contain even smaller entire beings already inside them, and so on. The church endorsed this belief. Other physicians believed that semen mixed with menstrual blood to form an embryo. In his text, Harvey stated that all animals, including mammals, arise from eggs, a doctrine called the primacy of the egg. His beliefs that led to this finding were actually incorrect; however, his publication did shift the focus of reproduction to eggs, which eventually led to an appreciation of the roles of both the egg and sperm in reproduction of animals.

William Harvey remained an active member of the Royal College of Physicians over the years. When his duties as a royal physician became too time-consuming to actively participate in the business of the college, he donated money to build a library, pay a librarian, and fund an annual lecture. He was offered the position of president in 1654, but he declined due to advanced age. He suffered a fatal stroke at Roehampton, London, in 1657 and was buried in the family vault at Hempstead Church in Essex.

Harvey's discovery of blood circulation marked a tremendous jump forward for the field of medicine. His method of making careful observations and drawing conclusions based only on findings from research constituted a revolutionary new approach that paved the way for modern physiology. Before Harvey, physicians relied heavily on the texts of ancient philosophers in order to understand the workings of the human body. The writings were based on conjecture and feeble analysis, and they delayed the advancement of medical knowledge for centuries. Harvey demonstrated the importance of discovery by direct observation and exploration rather than reading alone. He was a quiet, conservative man, and it took great courage for him to speak against the millennium-old Galenic tradition, but his meticulous methodology gave him the confidence to question the recognized authority. Because he taught others to accept only that which could be observed, thus removing a long-standing obstacle to further progress, Harvey is considered the father of modern physiology.

See also ANATOMY; CIRCULATORY SYSTEM; PHYSIOLOGY.

FURTHER READING

Bylebyl, Jerome J. "Harvey, William." In *Dictionary of Scientific Biography*. Vol. 6, edited by Charles Coulston Gillispie, 150–162. New York: Scribner, 1970–1976.

French, Roger. *William Harvey's Natural Philosophy*. Cambridge and New York: Cambridge University Press, 1994.

Spangenburg, Ray, and Diane Kit Moser. *The Birth of Science: Ancient Times to 1699*. New York: Facts On File, 2004.

Hershey, Alfred (1908–1997) American *Microbiologist* Alfred Hershey is famous for his demonstration that deoxyribonucleic acid was the carrier of genetic information in bacteriophage. He shared the Nobel Prize in 1969 with Max Delbrück and Salvador Luria "for their discoveries concerning the replication mechanism and the genetic structure of viruses."

EARLY CAREER

Alfred Day Hershey was born on December 4, 1908, in Owosso, Michigan. He earned his bachelor of science degree in bacteriology in 1930 and his doctorate in chemistry in 1934 from Michigan State College (now Michigan State University). As a graduate student, he studied the bacteria *Brucella*, which causes brucellosis, or undulant fever, a disease characterized by flulike symptoms. He joined the department of bacteriology at the Washington University School of Medicine in St. Louis as a research assistant. In 1936 he became an instructor, then in 1938, an assistant professor, and finally, in 1942, an associate professor. His research first focused on bacterial growth and metabolism, then moved into viral replication, specifically, that of bacteriophages, viruses that infect bacteria, and the interactions between bacteriophage and antibodies. More familiar viruses cause diseases such as chicken pox, the common cold, polio, and measles. In the 1930s and 1940s scientists were try-

ing to discover how viruses replicated themselves in hopes of better understanding viral diseases and how to treat them.

In the 1940s Hershey made some important discoveries regarding viral genetics. He demonstrated that bacteriophages, also called phages, could rapidly and spontaneously mutate and that when more than one phage strain was grown simultaneously in the same host cells, the strains could exchange genetic information in a process termed recombination. He then proceeded to study phage structure and the biochemistry of phage development. He often collaborated with Max Delbrück and Salvador Luria, the two other phage geneticists who shared Hershey's genius when it came to phage. Together the trio came to be known as the trinity of the "Phage Church," and they are considered the founders of the field of phage genetics.

DEMONSTRATES DNA IS CARRIER OF GENETIC INFORMATION

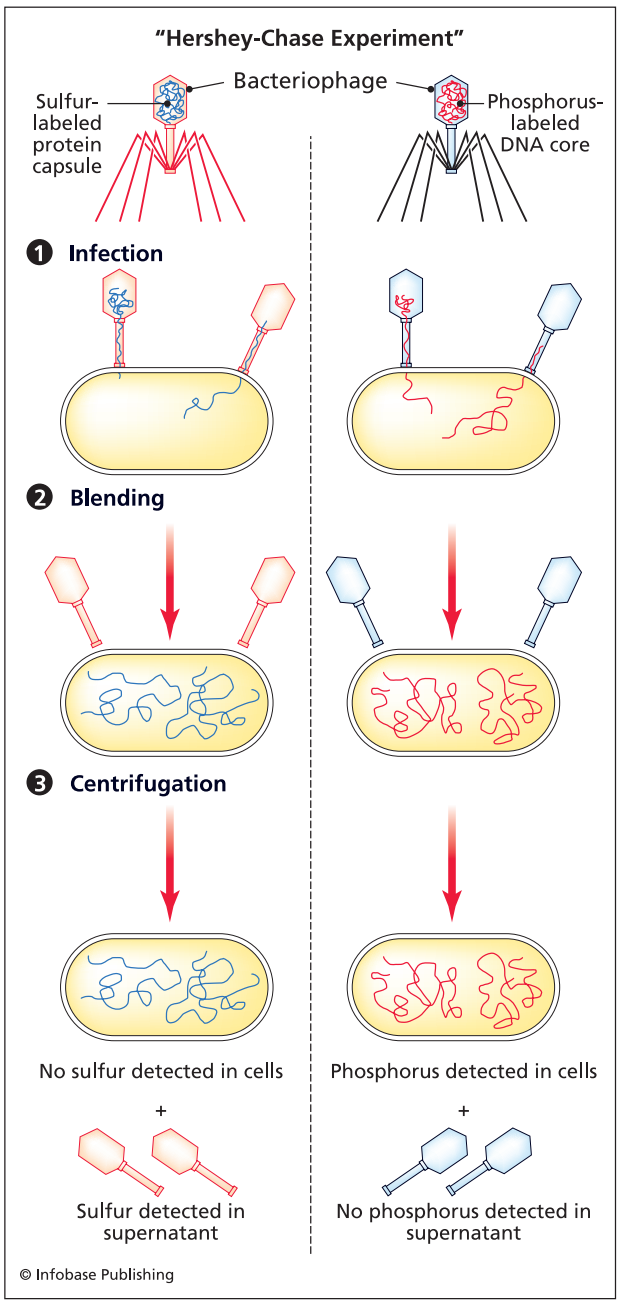
In 1950 Hershey moved to New York, where he worked as a staff scientist in the department of genetics at the Carnegie Institute of Washington at the Cold Spring Harbor Laboratory. Earlier, in 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty, researchers from the Rockefeller Institute, had demonstrated that deoxyribonucleic acid (DNA) was the molecular carrier of genetic information in pneumococcal bacteria. Avery and his colleagues could transform a nonvirulent bacterial strain into a virulent strain by the addition of purified DNA. Chemically isolated DNA transmitted the property of having a capsule from one strain to another. Though the experiment was beautifully orchestrated and controlled, many scientists failed to recognize or accept DNA as the carrier of genes until Hershey performed an experiment to demonstrate this in 1952.

With his lab assistant, Martha Chase, Hershey first labeled two different cultures of T2 bacteriophage with either radioactive sulfur (^{35}S) or radioactive phosphorus (^{32}P). Sulfur is found in proteins but not nucleic acids, and phosphorus is found in nucleic acids but not proteins. The radioactive tracers allowed Hershey and Chase to follow the location of the proteins and nucleic acids during their experiment. They allowed the radioactive phage particles to infect two separate *Escherichia coli* cultures and then put the cultures in a high-speed blender to displace any viral particles that were attached to bacterial cells. After centrifuging the blended mixtures, the pellet at the bottom of the centrifuge tubes contained the bacterial cells and their contents, while the supernatant contained the viral particles. Examination of the pellets and supernatants from the two cultures revealed that radioactive protein was found mostly in the supernatant, while much of the radioactive nucleic acid had entered the bacterial cells. Furthermore, the DNA inside those cells was still able to direct the synthesis of new phage progeny. Hershey and Chase concluded that the role of the proteins was to encapsulate and protect the DNA, to function in attachment to bacterial cells, and to inject the DNA into the host cells while it remained on the outside. These results also showed that the phage nucleic acid, not protein, carried the molecular instructions for programming growth and development of the next generation of viral particles. Hershey named Chase as a coauthor of their landmark publication, "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage," that appeared in the *Journal of General Physiology* in 1952.

The following year, James D. Watson and Francis Crick discovered the double helical structure of DNA. The existence of specific base-pairings between the two strands revealed a simple mechanism for self-replication. DNA had joined proteins in the realm of interesting biomolecules.

Hershey continued studying bacteriophage DNA. A lack of understanding about the structure of the phage genome was interfering with further genetic analyses. He developed unique methods for examining phage chromosome structure and found that some phage DNA is not double-stranded and some is not linear. Viruses display all possible combinations of double and single-strandedness, having either ribonucleic acid (RNA) or DNA as their genetic material. Developing new methods brought Hershey great satisfaction and his lab contributed to the development of several methods, including measures to handle DNA without denaturing it, methods for breaking DNA into smaller pieces in a controlled manner, means for fractionating DNA, fixed angle cesium gradient centrifugation, and ways to measure the molecular weight of DNA.

Hershey became director of the Genetics Research Unit (formerly the department of genetics) of Cold Spring Harbor in 1962 and retired in 1974. The National Academy of Sciences elected Hershey to membership in 1958 and gave him their Kimber Genetics award in 1965. He was elected to the American Academy of Arts and Sciences in 1959. He received the prestigious Albert Lasker Award for Basic Medical Research from the American Public Health Association for his role in discovering the fundamental role of nucleic acid in viral reproduction and heredity in 1958. In 1969 Hershey shared the Nobel Prize in physiology or medicine with his colleagues Max Delbrück from the California Institute of Technology and Salvador Luria from the Massachusetts Institute of Technology. Hershey's



The famous blender experiment performed by Alfred Hershey and Martha Chase in 1952 convinced the scientific community that DNA was the material that composed genes.

colleagues described him as original, logical, industrious, modest, and economical in words. His contributions to the understanding of the chemical basis for heredity have earned him mention in most textbooks on genetics and microbiology.

Alfred Hershey married a research assistant named Harriet Davidson in 1945. They had one child, a son named Peter. Hershey died at his home in Syosset, New York, on May 22, 1997.

See also AVERY, OSWALD; CHASE, MARTHA; CRICK, FRANCIS; DEOXYRIBONUCLEIC ACID (DNA); MACLEOD, COLIN MUNRO; MCCARTY, MACLYN; RADIOACTIVITY; WATSON, JAMES D.

FURTHER READING

Hershey, A. D., and Martha Chase. "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage." *Journal of General Physiology* 36 (1952): 39–56.

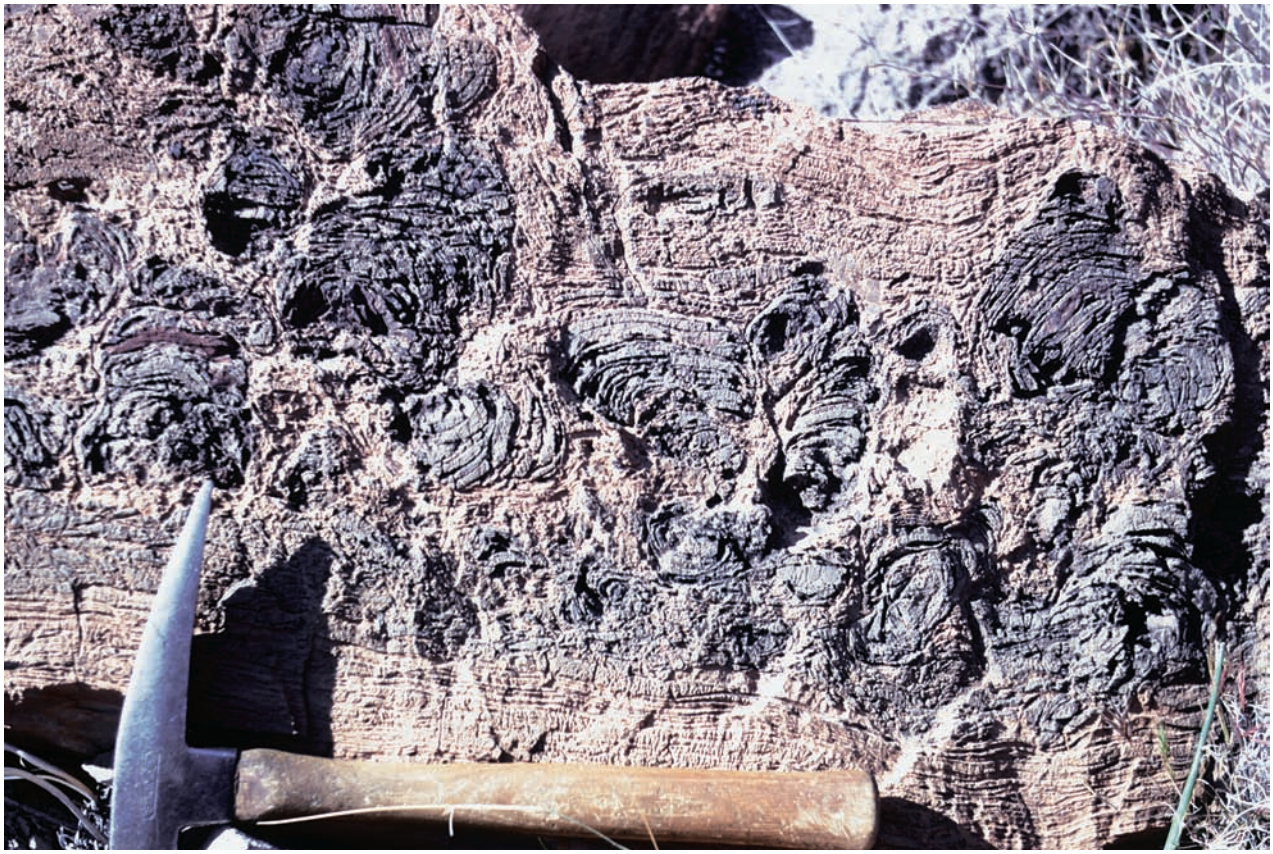
The Nobel Foundation. The Nobel Prize in Physiology or Medicine 1969. Available online. URL: http://nobelprize.org/medicine/laureates/1969. Accessed on January 14, 2008.

Stahl, Franklin W. "Alfred Day Hershey (December 4, 1908–May 22, 1997)." In *Biographical Memoirs. National Academy of Sciences*. Vol. 80. Washington, D.C.: National Academy Press, 2001.

history of life Microfossil evidence indicates that life first appeared on the planet Earth more than 3.6 billion years ago. The first life-forms were prokaryotic (unicellular organisms that have no distinct nucleus) and faced conditions much different from those present today. As they grew in numbers, diversified, and adapted to the changing physical conditions on the young planet, the organisms influenced the environment. Biologists must rely on fossil evidence to learn about past life-forms but they can infer evolutionary relationships by studying existing life. While all living organisms likely diverged from a universal common ancestor, the variety of life with respect to size, morphology, food sources, preferred habitats, modes of reproduction, and other anatomical and physiological features is remarkable. Major milestones in the history of events leading to the diversity of life in existence today include the first appearance of life, the advent of oxygenic photosynthesis and aerobic respiration, the development of eukaryotic cells, the rise of multicellularity, and the colonization of land by plants and later by fungi and animals. Along the way, at least five major mass extinctions occurred, each one wiping out a significant percentage of all life and making way for divergent evolution to fill the emptied niches.

FIRST APPEARANCE OF LIFE

Life first appeared during Precambrian time, the time from the earliest era of geological history until Cambrian time, including the Hadean (4.5 to 3.8 billion years ago), the Archaean eon (3.8 to 2.5 billion years ago), and the Proterozoic eon (2.5 billion to 543 million years ago). The universal ancestor was certainly anaerobic, as no oxygen was present in the atmosphere, and prokaryotic. The oldest known fossils



The layers of mineralized mats in fossilized stromatolites such as these provide evidence of prokaryotic life-forms that existed billion of years ago. *(U.S. Geological Survey)*

are of prokaryotic organisms from western Australia and South Africa and date to approximately 3.6 billion years. The ancient forms of these bacteria grew in filaments that formed mats, over which layers of calcium carbonate and other minerals precipitated and became trapped. As new layers formed, rock-like structures called stromatolites took shape. The stromatolites fossilized, and today they display not only the layers formed by the growth of bacterial communities, but, preserved fossils of prokaryotic cells are also occasionally observed. Reefs of these stromatolites still exist in warm, salty areas such as Shark Bay, Australia.

The first cells would have required a source of energy and had to obtain the nutrient carbon, the basis of organic molecules. Life-forms present on Earth today fulfill these needs by a variety of mechanisms. Autotrophs make their own organic substances by fixing carbon, meaning they incorporate inorganic carbon from the environment into organic molecules such as carbohydrates. Heterotrophs obtain their carbon in the form of premade organic molecules, thus they are ultimately dependent on autotrophs for food; they either eat the autotrophs, eat organic matter produced by the autotrophs, or eat other organisms that have eaten the autotrophs. With respect to an energy source, radiant energy from the Sun serves as the energy source for phototrophs, and chemotrophs obtain their energy from oxidizing reduced inorganic (chemolithotrophs) or organic (chemoorganotrophs) molecules. The conditions on the early Earth would have been toxic to many of today's life-forms. The atmosphere lacked any oxygen, but contained the gases methane, ammonia, and hydrogen, and the waters were hot and salty. (Over time, radiant energy would have broken up the molecules of gas in the atmosphere and led to the formation of carbon dioxide and nitrogen.) Organisms that can withstand harsh environments are called extremophiles, and most belong to the domain Archaea. Modern organisms that resemble what biologists believe the ancient universal common ancestor was like can be found living in extreme conditions such as in hot springs, the hypersaline Dead Sea, and surrounding hydrothermal vents in the deep ocean floor. Research on modern archaeans provides insight as to how ancient cells may have obtained energy and nutrients necessary to sustain life.

Biologists cannot know for sure how the first cells obtained their nutrition and energy, but the

earliest cells must have been able to utilize the nutrients and energy sources that were available to them. Though the oldest fossils (stromatolites) are of photosynthetic organisms, the first life-forms may have been heterotrophs and obtained their carbon from available organic compounds. At first the organic compounds must have been made by chemical rather than biological processes. Heat and the reducing conditions caused by high concentrations of hydrogen and the absence of oxygen could have allowed for their formation. Though some scientists have questioned whether hydrogen levels were sufficient to create the highly reducing conditions necessary for the abiotic synthesis and accumulation of organic molecules, recent studies suggest that hydrogen gas was a sufficient component in the early atmosphere. Clay or other mineral deposits in shallow waters could also have aided in the formation and accumulation of these abiotically formed organic molecules that could then have served as both a carbon and an energy source. As the populations expanded, the ancient cells could have fed on waste products of others and on dead cells.

Another possibility is that life began with chemoautotrophy. If metabolic systems developed before self-replicating genes, protobionts may have begun as simple autocatalytic metabolic systems that formed on the surfaces of precipitates of sulfides of iron and other metals. These systems may have produced small organic molecules by fixing carbon monoxide (CO) and later carbon dioxide (CO_2), using energy obtained by the oxidation of FeS to FeS_2.

Whether life began with chemoautotrophy or heterotrophy, all three domains of life—Archaea, Bacteria, and Eukarya—have electron transport systems, series of membrane-bound proteins that transfer electrons obtained from energy rich molecules to electron acceptors, and in the process, generate chemical energy in the form of ATP (adenosine triphosphate), which is readily used by the cell. Because these electron transport systems are common to all domains, they likely were present in the universal common ancestor. In the universal ancestor, CO_2 probably served as the major source of carbon to synthesize organic molecules, and hydrogen gas (H_2) and reduced sulfur compounds such as hydrogen sulfide (H_2S) probably served as the electron donors—the energy source—and as they accumulated, CO_2 and oxidized sulfur compounds as the final electron acceptors.

EMERGENCE OF PHOTOSYNTHESIS AND AEROBIC RESPIRATION

Scientists do not know for certain whether chemoautotrophy or photoautotrophy developed first, but by about 3.4 billion years ago, life-forms had developed the ability to harness light energy from the Sun and use it to synthesize organic molecules such as simple carbohydrates. Early photosynthetic organisms used light energy to make glucose from CO_2 and H_2S and released elemental sulfur as a waste product. Today species of purple and green bacteria still utilize this process, called anoxygenic photosynthesis.

As hydrogen gas escaped from the atmosphere and H_2S levels decreased, the ability to utilize water (H_2O) as an electron donor emerged. When water gives up its electrons, the protons from the hydrogen atoms follow, creating oxygen (O_2) as a by-product; this process is called oxygenic photosynthesis and was carried out by ancient cyanobacteria. The oxygen released into the ocean oxidized the free iron dissolved in the water, forming iron oxides that precipitated and created banded iron formations. These layers of hematite and other iron oxides date between 2.5 and 1.8 billion years ago, suggesting that oxygenic photosynthesis emerged about 2.5 billion years ago. Other geological evidence indicates that weathering due to oxidation did not occur until approximately 2.4 billion years ago, only after oxygen began to accumulate as a result of oxygenic photosynthesis.

The increasing concentrations of oxygen in the oceans and in the atmosphere would have killed off many life-forms. Oxygen is a good electron acceptor, but because of this trait, it can cause damage to biomolecules such as proteins and nucleic acids. (The prebiotic synthesis of organic molecules was possible only because the atmosphere was void of oxygen.) Some organisms found anaerobic environments in which they lived, others acquired mechanisms for preventing oxidative damage from oxygen. Several lineages not only developed a tolerance for oxygen, but also exploited its very characteristic that made it

This banded iron ore from the Ord Ranges of Western Australia formed 2.4 to 2.8 billion years ago. *(François Gohier/Photo Researchers, Inc.)*

toxic by evolving means for using molecular oxygen as the final electron acceptor in electron transport systems. Because the tendency of oxygen to accept electrons from other molecules is so great, aerobic cellular respiration is more efficient than anaerobic respiration.

APPEARANCE OF EUKARYOTIC ORGANISMS

Phylogenies based on molecular analyses indicate that the common universal ancestor diverged into two lineages, one that evolved into Bacteria, and the other that subsequently split into two lines, Archaea and Eukarya. These events preceded the date of the oldest fossil stromatolites. One must keep in mind, however, that though the appearance of the cell line that developed into eukaryotes may have appeared more than 3.5 billion years ago, the first eukaryotic cells did not appear until much later. For at least 1.4 billion years, the only living organisms were prokaryotic. Prokaryotic cells consist of a cell membrane (also called plasma membrane) that encloses the cytoplasm and all of its contents, including a nucleoid region where the chromosome resides. The advent of eukaryotic cells was significant in the evolutionary history of life, because all complex cells and multicellular organisms are eukaryotic. Scientists do not know exactly when the first eukaryotic cells appeared; fossils suggest this event may have occurred between 2.1 and 1.6 billion years ago, but the fossil record is ambiguous, and the earliest eukaryotic cells may have been too small to leave fossil evidence.

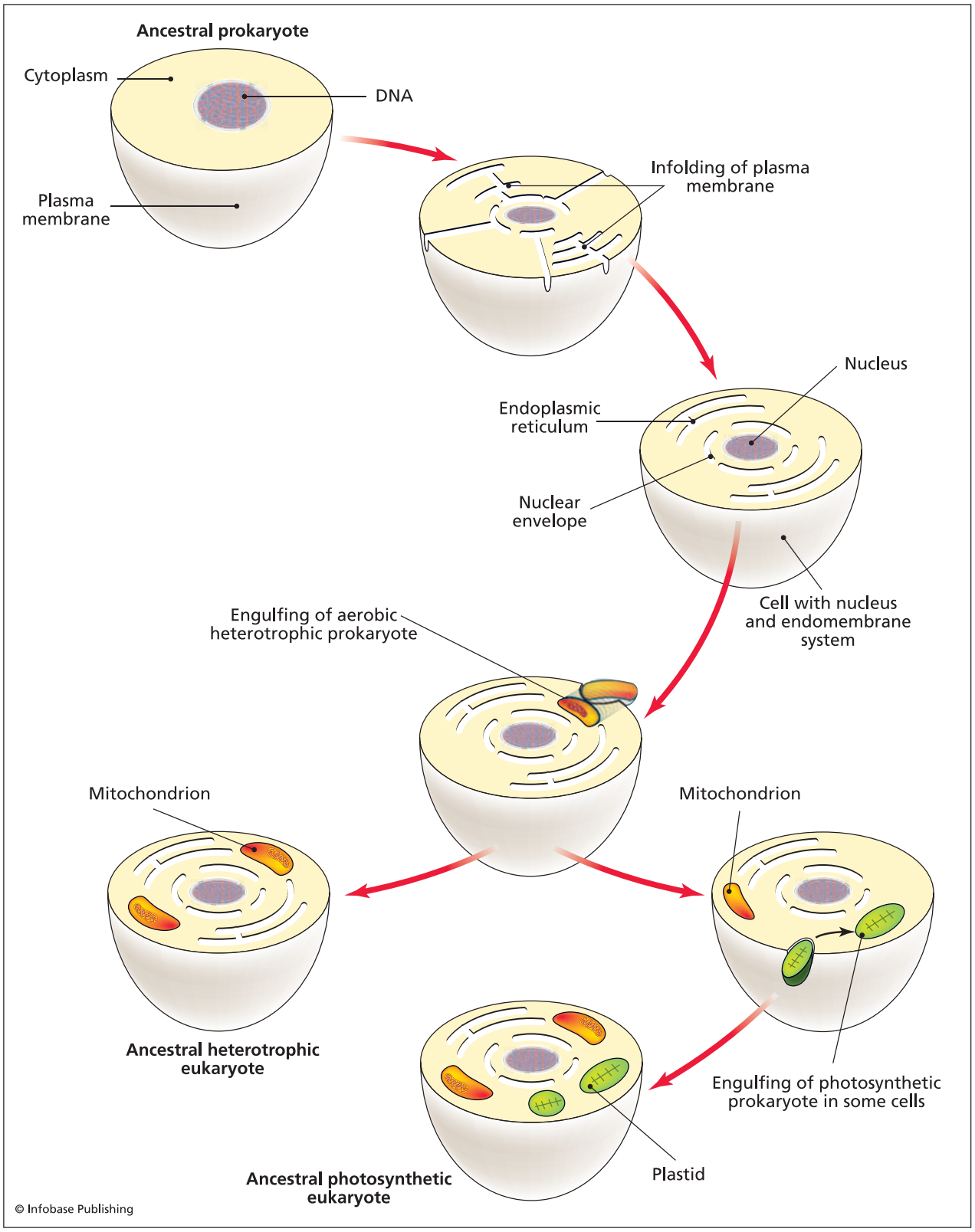
Eukaryotic cells have a cell membrane, like prokaryotic cells, but they also have a nucleus, other membrane-bound organelles, and a cytoskeleton. The nucleus contains the DNA and is surrounded by a double membrane that originated by infolding of the plasma membrane around the prokaryotic nucleoid. The evolutionary advantages offered by compartmentalization of the DNA are twofold. First, the hereditary information is better protected against damage and mutation. Other molecules and chemicals must gain entry not only to the cell, but also pass through the nuclear envelope in order to access (and potentially damage) the DNA. Second, during gene expression, translation of the messenger RNA cannot proceed until after transcription of the genes and splicing of the introns (noncoding segments within the messenger RNA transcript) from the RNA is complete. Additional infoldings could lead to the formation of an endomembrane system, the basis for other cellular organelles such as the endoplasmic reticulum and the Golgi apparatus. Protein and lipid synthesis occur on and in the endoplasmic reticulum, and the Golgi apparatus is responsible for protein modification, sorting, and packaging. Vesicles formed by pinching off sections of the endoplasmic reticulum and Golgi apparatus store and transport molecules to different intracellular locations and to the cell membrane if they are to be secreted.

The American biologist Lynn Margulis popularized endosymbiosis, the accepted theory for the origin of mitochondria and plastids, two eukaryotic organelles that played a significant role in the history of life. Drawing on ideas from 19th- and 20th-century biologists, she was the first to provide microbiological evidence for the endosymbiotic theory. Though rejected at first, today mainstream scientists embrace the endosymbiotic theory, for which convincing supportive cell and molecular evidence has accumulated over the past four decades. Mitochondria carry out aerobic respiration and synthesize ATP. Plastids are cytoplasmic photosynthetic organelles such as chloroplasts that capture energy from sunlight and use it to drive the synthesis of organic molecules. Mitochondria and chloroplasts share many similar characteristics: they both have their own DNA—a closed, circular chromosome as is found in prokaryotic cells; they both are bound by a double membrane; they both contain their own ribosomes, which are similar in structure to prokaryotic ribosomes; many proteins important to the organelle's particular function are synthesized within the organelle; and many of their metabolic activities are carried out by proteins embedded within the inner membranes of the organelle, which contain many invaginations and specialized folded structures that increase the area that carries out those metabolic activities.

Primitive heterotrophic eukaryotes had to hunt for food. With the development of a cytoskeleton, they would have had the ability to change the shape and structure of their cells, enabling them to engulf other smaller cells for intracellular digestion. The endosymbiotic theory purports that if a larger heterotrophic cell engulfed a smaller prokaryotic cell capable of aerobic respiration but did not digest it, a symbiotic relationship could have developed. The larger cell would provide protection and nutrients for the endosymbiont (an organism living inside another organism), while the endosymbiont would make ATP for the larger cell. Both organisms would benefit from such a relationship and eventually come to depend on one another, each losing the independent ability to carry out the function performed by the other. Thus, mitochondria likely originated from endosymbiosis. Chloroplasts evolved in a similar manner, except that the endosymbiont was a smaller photosynthetic prokaryotic cell. The endosymbiont would have utilized sunlight to synthesize sugars for the larger cell, which over time would have lost the ability to move around and hunt food on its own. All eukaryotic cells have mitochondria, or the cells from

which they are derived have mitochondria, but only plant and algal cells have chloroplasts. This suggests that mitochondria evolved first.

Much evidence supports the endosymbiotic theory. The DNA within the mitochondria and chloroplasts resembles prokaryotic DNA both in form (as



The endosymbiotic theory purports that mitochondria and chloroplasts in eukaryotic cells originated from smaller prokaryotic cells living inside larger cells.

a singular, closed, circular chromosome) and in the similarity of sequences for the genes it encodes. The ribosomes contained within the mitochondria and chloroplasts resemble prokaryotic ribosomes. They consist of a 30S small subunit and a 50S larger subunit, whereas typically eukaryotic ribosomes consist of 40S and 60S subunits. The composition of the inner membrane of the mitochondria and chloroplast differs from the composition of the cell membrane and other membranes in eukaryotic cells, suggesting it is of different origin. The organelles replicate in a manner that resembles binary fission, the mechanism by which prokaryotic organisms reproduce.

MULTICELLULARITY

The next milestone in the evolution of life is multicellularity, the appearance of organisms consisting of more than one cell, with a division of functions among specialized groups of cells. Molecular analysis of gene sequences suggests that the common ancestor to metazoans (multicellular animals) existed about 1.5 billion years ago, and the oldest fossils of multicellular organisms are of primitive algae that date to 1.2 billion years ago. The existence of colonies of eukaryotic organisms that lived in close association probably preceded the development of true multicellular organisms. *Volvox* are an example of green algae that live in colonies in which the cells have specialized functions and are dependent on one another. Such dependence could lead to the emergence of organisms made of many types of specialized cells. In multicellular organisms after division, the cells remain in close contact, and adhesions between the plasma membranes and extracellular matrixes hold them together.

With refined specialization for the functions necessary to sustain life, such as respiration, detection of sensory input, digestion of food substances, and reproduction, tissues soon developed, then organs and organ systems. This higher level of complexity opened the door to many new anatomical and physiological adaptations. Multicellularity evolved within many different lineages, leading to tremendous diversity. The fossil record reveals the first soft-bodied invertebrate animals such as sponges, cnidarians, and worms, during the Vendian Period, 650 to 543 million years ago. This led into the Cambrian explosion, the period in Earth's history when most of the major groups of complex animals first appear in the fossil record. The diversity of marine life included worms, as evidenced by their burrows; brachiopods, animals that resemble but are not related to clams; echinoderms, members of the phylum that includes starfish and sea urchins (but Cambrian echinoderms looked very different); trilobites, extinct segmented arthropods characteristic of this period; gastropods, or snails; early cephalopods,

Trilobites are arthropods that were common during the Paleozoic era, beginning with the Cambrian period. This fossil was found in Devonian rock. *(Russell Shively, 2007, used under license from Shutterstock, Inc.)*

ancestors to modern octopuses and squids; the first vertebrates, jawless fishes similar to modern hagfishes and lampreys; predatory anomalocarids, the largest and now extinct of the Cambrian animals; and some animals resembling sponges. The Burgess Shale in the Rocky Mountains of British Columbia has many well-preserved Cambrian fossils of soft-bodied creatures, which are rare since soft-bodies do not mineralize readily. The first shelled protozoans also appeared during the Cambrian, and marine plants and algae were abundant.

Whereas the Cambrian brought an explosion in the number of types of animals, the Ordovician (490 to 443 million years ago) brought diversification. Following an extinction event at the end of the Cambrian, life-forms diverged to fill the empty niches. During the Ordovician, numerous diverse invertebrates inhabited the surface of the ocean floor. Coral reefs appeared, creating ecosystems dominated by red and

This fossil worm from the Burgess Shale area lived 500 million years ago. *(Alan Sirulnikoff/Photo Researchers, Inc.)*

MAJOR EVENTS IN THE HISTORY OF LIFE

Time	Significant Biological Events
4.55 billion years ago	Earth formed
3.9–3.8 billion years ago	crust solidified, oceans form
3.6 billion years ago	oldest evidence of life
3.4 billion years ago	anoxygenic photosynthesis
2.5 billion years ago	oxygenic photosynthesis
2.2 billion years ago	aerobic respiration
2.1–1.6 billion years ago	first eukaryotes appeared
1.5–1.2 billion years ago	multicellular organisms appeared
~600 million years ago	oldest invertebrate animal fossils
600–580 million years ago	oldest known bilaterians
500–450 million years ago	first vertebrates, fish, appear
480–460 million years ago (could be as early as 700 million years ago)	plants colonize land
400–360 million years ago	insects
370–355 million years ago	amphibians
340–300 million years ago	reptiles
248 million years ago	largest known mass extinction
230–200 million years ago	first dinosaurs and mammals
195–150 million years ago	birds
141–100 million years ago	first flowering plants
65 million years ago	mass extinction
2.4 million years ago	ice age
<100,000 years ago	beginning of modern humans

green algae. Though vertebrates first appeared during the Cambrian, more complete fossil evidence of bony vertebrates comes from the Ordovician. Another mass extinction occurred, perhaps caused by a drop in temperatures, as evidence of glaciation in the polar regions of the landmasses exists. This led to another round of diversification of the surviving groups.

COLONIZATION OF LAND

Because the terrestrial environment was harsh, the first terrestrial life was probably an association between photosynthetic cyanobacteria or algae and fungi. These symbioses, called lichens, would have contributed to more habitable conditions for plants by breaking down soil surfaces, physically penetrating it, and chemically attacking it. Cyanobacteria, which existed as free-living cells as well as in lichens, have the ability to fix nitrogen, which would have contributed to the organic nitrogen available for use by other organisms. Even today, fungi often form symbiotic associations with the roots of many plants; the fungus helps the plant absorb more water and minerals, and in return the fungus receives organic nutrients from the plant. Adaptations such as a waxy coating on leaves to prevent water loss helped green algae evolve into terrestrial plants. Microfossils of plant spores and fungi from about 480 to 460 million years ago suggested that plants colonized land during the Ordovician. Molecular analysis pushed this backward in time to about 600 million years, and, until recently, biologists believed this major evolutionary milestone occurred close to this time frame. In 2001, however, a team of researchers led by S. Blair Hedges from Pennsylvania State University published the paper "Molecular Evidence for the Early Colonization of Land by Fungi and Plants," an extensive molecular analysis of proteins from aquatic and terrestrial fungi, concluding they diverged between 1.5 billion and 966 million years ago, in contrast to the previously accepted 660 to 370 million years ago—a significant difference. Their analysis of land plants led to the suggestion that plants first colonized land 700 million years ago, during the Precambrian. They proposed that the spores were from more complex plants, rather than the earliest terrestrial plants.

The fossil record clearly demonstrates the existence of complex vascular plants in the Silurian (443 to 417 million years ago). This supports the belief that plants emerged before the Ordovician. In addition, fungal fossils that resemble modern forms are also present. Once plants colonized the land, they added organic nutrients to the soil, making it more fertile, and changed the Earth's ecosystems. In turn, the plants diversified. True roots and leaves developed, and trees formed the first forests.

During the mild temperatures with alternating floods and droughts that characterized the Devonian (417 to 354 million years ago), ferns, horsetails, and seed plants evolved. Meanwhile, marine life continued to diversify, leading to the formation of the ammonoids, one of the most diverse groups of extinct animals. The Devonian also brought the first animals onto land. Fossils of spiders, mites,

scorpians, millipedes, and, later, centipedes and primitive winged insects have all been identified from Devonian rock. Aquatic vertebrates also diversified; one of the most significant events was the evolution from lobe-finned fish to tetrapods—four-limbed vertebrates—by the end of the Devonian. By 355 million years ago, the first vertebrates had wandered onto land. Amphibians, which probably fed on the terrestrial arthropods, still depended on water for reproduction, but in the Carboniferous (354 to 290 million years ago) the development of the amniote egg allowed vertebrates to move farther onto land by enclosing a moist environment within an egg. The common amniote ancestor developed into modern reptiles, birds, and mammals. The types of vegetation present during the Carboniferous indicate that the climate was tropical, and new types of insects appeared, including cockroaches, grasshoppers, and leafhoppers. Seedless vascular plants thrived and formed the first forests of the Carboniferous, greatly contributing to the reduction of CO_2 in the atmosphere and to the formation of coal over the millions of years that followed. Eventually the seed plants became more dominant due to adaptations that gave them an evolutionary advantage. The outer coating of seeds is tough, offering protection against harsh environments. Seeds also allowed plants to disperse more widely than spores of mosses and ferns, and they can remain dormant for months or years until conditions are optimal for germination.

During the Permian (290 to 248 million years ago), many of the insects that emerged during the Carboniferous became extinct, as did many amphibians. The interior land of the continents became drier, and gymnosperms—plants that produce naked seeds—became more prominent.

The end of the Permian brought the end of the Paleozoic era, and the largest known mass extinction in the history of life on Earth. The first period of the Mesozoic era, the Triassic (248 to 206 million years ago), ushered in the age of the dinosaurs, a group that really diversified during the Jurassic (206 to 144 million years ago), and became extinct following the Cretaceous (144 to 65 million years ago). Pangaea—a single, global continent—existed during the Triassic and significantly affected global climate and ocean circulation patterns. Pterosaurs, flying reptiles, and the first birds appeared (from the diapsid lineage, which also includes snakes, lizards, and crocodiles) during the Jurassic, as did small mammals from the synapsid amniote lineage. During this time, ichthyosaurs (fishlike dinosaurs), giant crocodiles, cephalopods, modern-looking sharks, and ammonites all shared the oceans, which contained abundant phytoplankton such as dinoflagellates and single-celled algae with calcareous plates.

During the Cretaceous, angiosperms, or flowering plants first appeared, and they eventually took over due to the evolutionary advantage brought by fruits and flowers. Fossils indicate the first ants and butterflies also appeared. Another mass extinction occurred at the Cretaecous/Tertiary boundary, perhaps one of the best known due to the eradication of the dinosaurs at this time. The impact of a large asteroid may have disrupted the environment and initiated the extinction episode. At the same time, Pangaea was breaking up; thus afterward, adaptive radiation filled the continents with different characteristic species of plants and animals, types that are familiar today.

The Tertiary (65 to 1.8 million years ago) and the Quaternary (1.8 million years ago to the present) of the Cenozoic era are sometimes referred to as the age of mammals. After the nonavian dinosaurs became extinct, mammals became—and they remain—the largest land animals. Though they coexisted with the dinosaurs during the Mesozoic era, they were mostly nocturnal and fed on insects until the dinosaurs became extinct. With regard to numbers and diversity, insects, flowering plants, birds, and fish also dominate the Cenozoic.

See also Archaea; Bacteria (Eubacteria); Eukarya; eukaryotic cells; evolution, theory of; origin of life; prokaryotic cells.

FURTHER READING

Bottjer, David J. "The Early Evolution of Animals." *Scientific American* 293 (2005): 42–47.

Butterfield, Nicholas J. "Macroevolution and Macroecology through Deep Time." *Paleontology* 50 (2007): 41–55.

Heckman, Daniel S., David H. Geiser, Brooke R. Eidell, Rebecca L. Stauffer, Natalie L. Kardos, and S. Blair Hedges. "Molecular Evidence for the Early Colonization of Land by Fungi and Plants." *Science* 293 (2001): 1,129–1,133.

Knoll, Andrew H. *Life on a Young Planet: The First Three Billion Years of Evolution on Earth*. Princeton, N.J.: Princeton University Press, 2003.

Sagan, Lynn. "On the Origin of Mitosing Cells." *Journal of Theoretical Biology* 14, no. 3 (1967): 255–274.

Tomitani, Akiko, Andrew H. Knoll, Colleen M. Cavanaugh, and Terufumi Ohno. "The Evolutionary Diversification of Cyanobacteria: Molecular-Phylogenetic and Paleontological Perspectives." *Proceedings of the National Academy of Sciences of the United States of America* 103 (2006): 5,442–5,447.

University of California Museum of Paleontology. History of Life through Time. Available online. URL: http://www.ucmp.berkeley.edu/exhibits/historyoflife.php. Accessed on January 21, 2008.

Hodgkin, Dorothy (1910–1994) British *Biophysicist* Dorothy Hodgkin was a bold scientist who pushed to expand the capabilities of the methodology available for structural analysis of biomolecules, taking on molecules that others claimed were too difficult to decipher. Understanding that structure determines function, Hodgkin helped establish a new approach to studying biomolecules while solving the molecular structures of cholesterol, penicillin, vitamin B_{12}, and insulin. Spanning seven decades, her pioneering research influenced the field of X-ray crystallography as well as chemistry and biochemistry.

INTRODUCTION TO CRYSTALS

Dorothy Mary Crowfoot was born in Cairo, Egypt, on May 12, 1910. At the time, her father, John Winter Crowfoot, worked as an administrator for the Egyptian Education Service, and in 1916 he became assistant director of education in the Sudan. Her mother, Grace Mary Hood Crowfoot, though never formally educated beyond finishing school, was an amateur expert in botany and became proficient in early weaving techniques and ancient textiles. Dorothy's family traveled extensively until World War I broke out, after which Dorothy and two of her three younger sisters settled in Worthing, England, where a nanny and their paternal grandmother cared for them until Dorothy was eight. After having seen her parents only once during the past four years, the family settled close to relatives in the village of Geldeston, near Beccles, in northeastern Suffolk.

Dorothy was introduced to chemistry when she was 10 years old and attended a local Parents National Educational Union class. Her chemistry booklet included experiments on growing copper sulfate and alum crystals that she found so captivating, she repeated them at home in a makeshift attic laboratory. Crystals are solids composed of atoms that are arranged in a regular, repeating pattern. At the Sir John Leman School in Beccles, which she attended from 1921 to 1928, Dorothy and a friend convinced the teachers to let them take the chemistry class, though it was supposed to be open only to boys. Ironically, the teacher was female. Dorothy performed well enough to choose chemistry as her major in college. During this time, she also volunteered for various peace campaigns and began her lifelong association with the Labour Party, a democratic socialist party in Great Britain.

Crowfoot took six months off from school during 1922–23 to visit her parents, who had moved to the Sudan, where she visited the Wellcome Laboratory in Khartoum, met the director, soil chemist A. F. Joseph, and learned to pan for gold. While practicing this new technique in her parents' backyard, she discovered shiny black chunks of a mineral that she analyzed and identified as ilmenite, iron titanium oxide. Her enthusiasm impressed Joseph, who gave her a professional kit for surveying and identifying minerals.

Upon graduation, Dorothy realized that she needed to learn Latin in addition to more mathematics and science if she wanted to study chemistry and crystallography in college. Extensive tutoring helped her prepare for the Oxford University entrance examination, which she passed. She then traveled to Jerusalem, where her father now was director of the British School of Archeology, and she assisted her parents in excavating Byzantine churches by recording patterns of the mosaic floors. She enjoyed the work and considered majoring in archaeology instead of chemistry.

At Somerville College for women at Oxford University, Dorothy developed a close relationship with Margery Fry, the principal of Somerville, and she became fascinated by the relatively new field of X-ray crystallography. This technique is used by scientists to gain information about the atomic structure of molecules. Because atoms are much smaller than the wavelengths of visible light, they cannot diffract the light rays or be seen, even with microscopes. The wavelengths of X-rays are much smaller, and thus can be used to "see" atoms and molecules. The substance to be x-rayed must first be crystallized, a procedure in which the molecules become highly ordered, with regular spacing occurring between atoms in the arranged molecules. When bombarded with X-rays, the crystallized molecules diffract, or bend, the waves, which pass through the created spaces as through a grating, forming a unique pattern of blurry circles on photographic film. Examination and interpretation of the resulting patterns of spots requires a good understanding of chemistry, mathematics, and physics. Dorothy first learned about crystallography as a teenager, when her mother gave her two books based on William H. Bragg's Royal Institution Christmas lectures for children, *Concerning the Nature of Things* (1925), and *Old Trades and New Knowledge* (1926). Bragg received the Nobel Prize in physics in 1915, shared with his son, William Lawrence Bragg, for their services in the analysis of crystal structure by means of X-rays. For her fourth-year research project, Dorothy synthesized and crystallized thallium dialkyl halides, then used X-ray diffraction to analyze the structures.

After Crowfoot graduated from Somerville College in 1932, her friend Joseph helped her obtain a position in the laboratory of John Desmond Bernal at Cambridge University, where she began graduate studies in X-ray crystallography. Bernal, a pioneer in the use of X-ray crystallography to study biological molecules, had been researching metals, but he

started studying sterols around the time Crowfoot entered his laboratory. Scientists from all over the world sent Bernal crystals for analysis, and, as his assistant, Crowfoot had access to plenty of material for practicing her new skills. Though her dissertation research concerned the crystallographic investigation of steroid crystals, she also studied minerals, metals, other organic and inorganic molecules, proteins, and viruses. Between 1933 and 1936 Bernal listed her as coauthor of 12 scientific papers.

In 1934 Crowfoot visited a specialist to inquire about tenderness and inflammation in the joints of her hands. The doctor diagnosed rheumatoid arthritis, a painful condition caused by the body's attack on its own tissues and one that worsened throughout her life, which ultimately deformed and crippled her. The depressing news of her disease did not dampen her professional drive—when she returned to the lab that afternoon, she found that Bernal had successfully photographed a protein crystal of pepsin. Having figured out the trick of keeping the crystal wet, he became the first to obtain a good X-ray photograph of a protein. Crowfoot soon immersed herself in deciphering the photographs of pepsin, a digestive enzyme that hydrolyzes peptide bonds between amino acids of proteins in the stomach. According to the memoir written by Guy Dodson in the *Biographical Memoirs of Fellows of the Royal Society of London,* this was "the beginning of protein crystallography, and it was one of the most important scientific episodes in Dorothy's life."

Though she received financial assistance from her aunt and through a research grant from Cambridge, money was tight and in 1933 Crowfoot accepted a research fellowship to be held for one year at Cambridge and the second at Oxford. She reluctantly returned to Somerville College at Oxford in 1934, not wanting to leave the intellectually stimulating environment in the lab at Cambridge, but she had to consider her future, and this position at Oxford could lead to a permanent job. She continued her doctoral studies on sterols and earned her Ph.D. in 1937. She remained at Oxford for the duration of her career.

In December 1937, she married Thomas Hodgkin, a researcher and African political historian who was Fry's cousin. Like Bernal, Hodgkin was a member of the Communist Party. During the early years of their marriage, he organized adult education classes, and then later he became the director of the Institute of African Studies at the University of Ghana. During the years 1938–46, the Hodgkins had three children, all of whom grew up to become respected professionals in their own fields. During her third pregnancy, Dorothy Hodgkin became the first woman at Oxford to receive paid maternity leave. She was a devoted, loving, patient mother who never complained about the demands of having children in a two-career family. She exhibited the same likeable qualities to her colleagues and to the young scientists she mentored.

CHOLESTEROL

At Oxford, Hodgkin continued the research she began in Bernal's lab on sterols, a group of mostly unsaturated alcohols, including cholesterol, found in plant and animal tissues. The laboratory facilities available for her use at Oxford were much more primitive than those at Cambridge. In order to study a crystal with her polarizing microscope, she had to climb a rickety ladder to a gallery located just under the only window in her basement lab that allowed sufficient light for viewing. Though space and equipment were limited and hardly adequate, she obtained a series of grants and other financial support from the Rockefeller and Nuffield Foundations, and she did not let the pitiable working conditions affect the quality of her work.

Though Hodgkin studied over 100 steroids, she focused on cholesterol, a greasy molecule produced in the liver of animals. Cholesterol lends structural rigidity to cell membranes and acts as a biochemical precursor for manufacturing vitamin D and for the production of steroid hormones involved in the development and function of the reproductive system and in maintaining physiological homeostasis. The role of cholesterol in the development of heart disease has led to increased interest in this complex biomolecule.

The chemistry of cholesterol was understood, and the basic sterol formula was worked out, but nobody knew how the atoms of carbon, hydrogen, and oxygen were arranged in three dimensions to form the functional molecule, and most thought it was too complicated for X-ray analysis. Liking a challenge, she methodically attacked and deciphered the molecular structure of cholesteryl iodide and published her findings in the *Proceedings of the Royal Society* in 1945 in an article, "The Crystal Structure of Cholesteryl Iodide." Cholesterol was the most complex organic structure solved to date and her work constituted the first three-dimensional study of a biochemically important molecule.

PENICILLIN

During World War II, Bernal started conducting war research and donated his crystallographic equipment to Hodgkin. As the molecules she studied became increasingly complex, the mathematical calculations required to solve crystal structures also multiplied. She gained access to an early type of computer, a Hollerith punch card machine, to assist her with calculations. Hodgkin's graduate student, Barbara Rog-

ers Low, wrote the first three-dimensional computer program and punched data into cards that then were inserted into the computer.

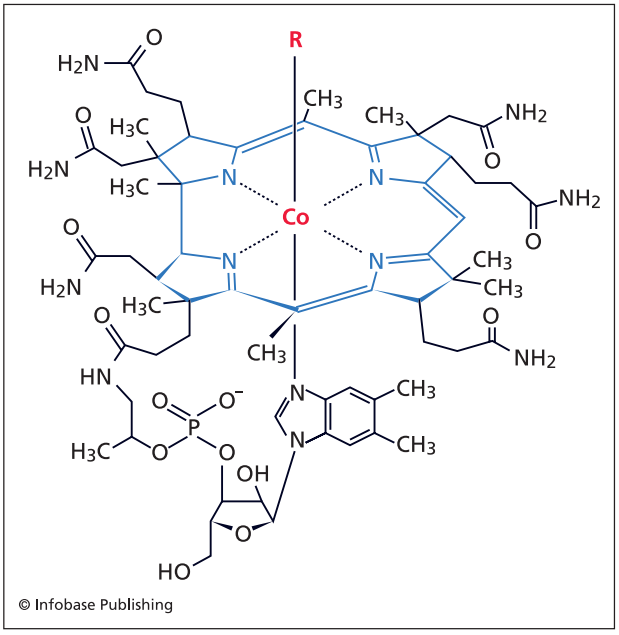
Hodgkin had switched her research focus to penicillin, a newly discovered, highly effective antibiotic made by the mold *Penicillium notatum*. The effectiveness of penicillin in treating bacterial infections that caused diseases such as pneumonia, syphilis, and gas gangrene in wounds led to a demand difficult to meet using early production methods. Pharmaceutical companies were very interested in knowing the structure of the drug naturally produced by the mold *Penicillium* in hopes of manufacturing it in bulk. Different forms of the molecule crystallized in different ways, complicating its analysis. In 1945, after analyzing hundreds of spots on X-ray diffraction photographs, Hodgkin announced the arrangement of the atoms in penicillin. Her research proved that penicillin contained a unique structure called a beta-lactam ring that was joined to a five-sided thiazolidine ring, an unusual attachment. Though solving the structure did not lead to its synthetic production, it did allow for the eventual manufacture of semi-synthetic penicillins when bacteria developed resistance to natural penicillin.

In 1946 Hodgkin helped form the International Union of Crystallography (IUCr) to encourage and facilitate the exchange of scientific information between all nations, including communist countries. The following year, Britain's foremost scientific organization, the Royal Society of London, elected her the third woman fellow. Oxford made her a university lecturer and demonstrator and increased her salary in 1946 but did not promote her to a university reader (a position similar to a full professor in the United States) until 1956 and did not give her a modern chemical crystallography laboratory until a few years after that.

VITAMIN B_{12}

The size of Hodgkin's research group grew with her fame, but she always tried to limit the number of workers to 10 to keep the working group manageable. After her discovery of the structure of penicillin, the drug company Glaxo sought her help in solving the structure of vitamin B_{12}, which had been discovered in 1926 but was not isolated and purified until 1948. Vitamin B_{12} was necessary for the body to manufacture red blood cells that carry oxygen to the body's tissues via the circulatory system. Without adequate quantities, people died from pernicious anemia, a potentially fatal disease caused by the absence of a glycoprotein called intrinsic factor that assists in the absorption of vitamin B_{12} in the intestines. The chronic disease can be successfully treated with intramuscular injections of vitamin B_{12}. Pharmaceutical companies wanted to manufacture the vitamin but needed to know its structure, which was too complex for chemical analysis and the standard methods of degradation and synthesis. When Glaxo brought Hodgkin some crystals of vitamin B_{12} in 1948, she knew solving its structure would require more than the four years it took to solve the structure of penicillin, as vitamin B_{12} was much larger. The fact that no one had ever solved a structure so complicated did not deter her from consenting to the challenge. She was anxious to demonstrate the power of X-ray analysis.

Her initial studies suggested the structure of vitamin B_{12} was similar to porphyrin, a flat ring made of four smaller rings called pyrroles, found in hemoglobin. Others who lacked Hodgkin's intuition and imagination did not recognize the indications for pyrrole rings. She took over 2,500 X-ray photographs of vitamin B_{12} ($C_{63}H_{88}N_{14}O_{14}PCo$) over a six-year period. For analysis, Hodgkin sent her data by telegram and airmail to the University of California at Los Angeles, where crystallographer Kenneth Trueblood and his colleagues ran the findings on a very sophisticated computer. The National Bureau of Standards Western Automatic Computer was housed on UCLA's campus, and staffers had programmed it to perform crystallographic calculations 100 times more rapidly than standard methods. Anxious to



Hodgkin solved structures of biomolecules that other scientists thought were too difficult, such as vitamin B_{12} shown here, the most complicated biomolecule ever analyzed by X-ray diffraction analysis at the time. The corrin ring, pictured in blue, was new to organic chemists.

test their newly developed software, Trueblood had offered Hodgkin computational assistance at no cost. The structure of vitamin B_{12}, elucidated in the 1955 *Nature* article "The Crystal Structure of the Hexacarboxylic Acid Derived from B_{12} and the Molecular Structure of the Vitamin," contained a structure new to organic chemists, called a corrin ring, that is structurally similar to porphyrin. Biochemists discovered a completely synthetic method for producing this vitamin in 1979.

In 1960 the Royal Society of London awarded Hodgkin the first Wolfson Research Professorship, a position she held until she retired from Oxford in 1976. For her determinations by X-ray techniques of the structures of important biochemical substances, Hodgkin received the Nobel Prize in chemistry in 1964, becoming the first British female recipient. In 1965 Queen Elizabeth II awarded her Britain's Order of Merit, the highest honor any British citizen can receive.

INSULIN

Internationally recognized for her work, Hodgkin resumed doing what she loved—research. Long ago, even before she received her doctorate, the Nobel Prize–winning chemist (1947) Sir Robert Robinson gave Hodgkin a crystalline sample of insulin. A hormone made by the pancreas, insulin stimulates cells to absorb sugar from the bloodstream into the cell. Without insulin, the body's cells starve regardless of the amount of food ingested. Diabetics that are insulin-deficient depend on administration of the hormone by shots or pumps. The protein insulin consisted of 777 atoms, her most complex molecular puzzle yet. She filtered, grew, and regrew insulin crystals, collecting data from wet and dry samples. In 1939 she published "X-Ray Measurements on Wet Insulin Crystals," in *Nature*. Other molecular mysteries interrupted her 35-year-long research project on insulin, yet she always kept it in her thoughts.

She prepared derivatives from several different heavy atoms, a technically difficult task, but one that paid off. Her lab group analyzed 70,000 X-ray spots and chugged through the onerous calculations. She graciously allowed one of her postdoctoral students, Thomas Blundell, to announce the results on the structure of insulin at an IUCr meeting at the State University of New York at Stony Brook in 1969. Two years later, Hodgkin's lab group refined their model to a resolution of 1.9 angstroms, but she still thought it needed improvement. In 1988 she published her last scientific paper, "The Structure of 2Zn Pig Insulin Crystals at 1.5 Angstroms Resolution," in the *Philosophical Transactions of the Royal Society of London*. Her research led to understanding of insulin's behavior in solution, its chemical reactivity, and its folding properties. By expanding the capabilities of X-ray crystallography, she demonstrated to organic chemists the potential utility and superiority of the technique over the tedious tasks of chemical analyses and degradations.

OTHER HONORS

The Royal Society of London gave Hodgkin the Royal Medal in 1957 for her beautiful, complex analysis of vitamin B_{12} and their Copley Medal in 1976. She became a foreign member of the Royal Netherlands Academy of Sciences (1956) and the American Academy of Arts and Sciences (1958). From 1976 to 1988 she served as president of the Pugwash Conferences on Science and World Affairs, whose purpose is to bring together scholars from around the globe who are concerned with world affairs in order to find ways to reduce the danger of armed conflict. She was elected chancellor of Bristol University in 1970 and worked much harder than the ceremonial title required for 18 years. Though she refrained from ever discussing politics in the laboratory in order to protect her students, her associations with communists caused conflicts as the cold war worsened, and her beliefs prevented her from keeping too quiet. In the late 1980s, Hodgkin wrote one of her former research students, Margaret Thatcher, the only prime minister of Great Britain who had earned a science degree, offering advice on how to improve relations with the Soviet Union. Though she had visited several times previously, Hodgkin was refused a visa to visit the United States for an important protein structure meeting in 1953, and she had to fight to regain permission to enter the country. During her lifetime, she traveled to many different countries for the purpose of exchanging scientific knowledge.

Hodgkin's husband passed away from emphysema in 1982. She suffered from rheumatoid arthritis for most of her life and officially retired in 1977, but it has been reported lightheartedly that she never realized that she had retired. In her later years, her debilitating disease, in combination with a broken pelvis, confined her to a wheelchair, but her condition did not prevent her from traveling internationally to scientific and peace conferences—she attended the IUCr meeting in Beijing in 1993. She died at her home in Ilmington at the age of 84 on July 29, 1994.

Hodgkin's colleagues and former students remember her extraordinary gentleness and natural authority, as well as her great memory and keen intuition. Structural biologists and physical chemists revered Hodgkin for her amazing ability to see molecular constructs in enigmatic X-ray diffraction patterns and her confidence in tackling seemingly impossible challenges. Her popular fame, however, lies in her intuitive choices of biomolecules for research. Not

only were they chemically interesting, but they were also medically important. Her solution of the molecular structures for cholesterol, penicillin, vitamin B_{12}, and insulin demonstrated that X-ray analysis was the best method for determining three-dimensional structures, especially when the classic approaches did not work. Today Hodgkin's achievements may seem trivial to students who have been trained in crystallography using very advanced, modern computers, but at the time she undertook her studies, she stood alone in her willingness to take risks for the advancement of X-ray crystallography.

See also ANTIMICROBIAL DRUGS; BIOMOLECULES; DIABETES; NUTRITION; ORGANIC CHEMISTRY, ITS RELEVANCE TO LIFE SCIENCE; X-RAY CRYSTALLOGRAPHY.

FURTHER READING

Dodson, Guy. "Dorothy Mary Crowfoot Hodgkin, O.M. 12 May 1910–29 July 1994." In *Biographical Memoirs of Fellows of the Royal Society of London.* Vol. 48, 170–219. London: The Royal Society, 2002.

Ferry, Georgina. *Dorothy Hodgkin: A Life.* Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2000.

McGrayne, Sharon Bertsch. *Nobel Prize Women in Science.* Washington, D.C.: Joseph Henry Press, 1998.

The Nobel Foundation. The Nobel Prize in Chemistry 1964. Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1964/. Accessed on January 25, 2008.

homeostasis

Homeostasis is the maintenance of a stable internal environment despite alterations in the external environment. Animals have adapted to live in a variety of habitats, an observable fact demonstrated by their diverse body forms. Even within a particular habitat, however, the conditions of the external environment change; for example, the temperature varies, or the availability of water increases or decreases. Internal conditions also fluctuate, but the body cells of an organism function optimally within a specific range of temperatures, blood sugar levels, osmoregulation, water balance, calcium levels, pH, and other conditions. Organisms have a variety of homeostatic mechanisms to maintain constant internal conditions.

THERMOREGULATION

Animals exhibit two major strategies for maintaining their body temperatures within a tolerable range: ectothermy and endothermy. Ectotherms gain most of their body heat from the external environment and generally tolerate greater fluctuations in internal temperatures, whereas endotherms generate their own heat by metabolic processes and maintain relatively stable internal temperatures. Most invertebrates, including fishes, amphibians, and reptiles, are ectotherms and largely depend on behavioral adaptations such as seeking sunshine, shade, or water or huddling close to other individuals. Birds and mammals are endotherms. Endothermy requires more energy, thus endotherms must consume more food than ectotherms, but endothermy allows animals to live in many different habitats and to sustain intense activity for longer periods of time than ectotherms. For example, reptiles that depend on radiant energy from the sun to warm themselves could not survive the freezing winter temperatures common to many of the Earth's biomes. The normal range for body temperature in humans is 96–99.9°F (35.5–37.7°C), but as endotherms, humans have mechanisms that enable them to maintain a stable body temperature in both cold and hot environments. The hypothalamus receives information from temperature sensitive receptors in the skin and, in response, sends out signals that regulate the body's temperature by balancing the amount of heat generated or gained with the amount of heat lost.

Heat exchange occurs via conduction, convection, radiation, and evaporation. The heat, or thermal motion, moves from hotter to colder objects. Conduction is the direct transfer of heat between objects. Convection occurs when either air or a fluid flows across a surface of an object, picks up heat from the object, and carries it away. The emission of energy in the form of electromagnetic waves—radiation—allows heat transfer through a medium such as air, when objects are not in direct contact with one another. During the process of evaporation, heat energy converts a liquid to the gaseous phase, enhancing heat loss from the surface of a hot object.

Physiological adaptations that aid in thermoregulation include the degree and type of insulation and the arrangement of circulatory vessels. Insulation is an adaptation in animals that live in colder temperatures. The innermost layer of skin contains adipose tissue composed mostly of fat-storing cells. Thick layers of adipose tissue significantly reduce the amount of heat exchanged between an animal and its external environment. Fur and feathers also inhibit heat exchange to a degree dependent on how much air the fur or feathers trap. In cold weather, raising the fur or feathers will increase the amount of trapped air, thereby reducing the amount of heat lost to the environment. Countercurrent heat exchange plays an important role in thermoregulation of birds and marine mammals. This mechanism traps heat in the core of the body to reduce heat loss in the extremities. Arteries leading to the extremities are positioned near veins that return from the extremities. The blood in the veins is cooler than the blood in the arteries due to

heat loss at the surface of body parts exposed to cold water or snow and ice. As the chilled venous blood travels back toward the body core, the warmer arterial blood moving in the opposite direction transfers heat to it, minimizing the heat lost.

Vasoconstriction, vasodilation, sweating, shivering, and nonshivering thermogenesis are physiological responses to unfavorable temperatures. Heat loss to the surrounding air occurs across the surface of the skin, thus one mechanism for regulating internal temperature is to adjust the blood flow to vessels near the body surface. When heat needs to be conserved, the hypothalamus sends signals that cause the constriction of arterioles that lead to the skin's surface, a process called vasoconstriction. In contrast, when the body temperature increases, vasodilation increases the diameter of the surface blood vessels, allowing more blood to flow through and radiate heat to the environment. The blood returns to the body core cooler than before. Sweating is another physiological mechanism for reducing the body temperature. Humans have between two and three million sweat glands in their skin that produce and secrete sweat. Heat loss occurs by evaporation of the moisture on the skin's surface. The behavioral response of bathing accomplishes the same effect. Some animals have a high concentration of blood vessels on the floor of their mouth, and panting has an evaporative cooling effect.

Metabolic activity generates heat in endotherms. Muscular contraction increases metabolism, thus actions such as shivering or other movement replaces heat lost to the environment. Nonshivering thermogenesis (NST) is another metabolic adaptation for thermoregulation. Mammals have a special type of tissue called brown fat, located in the neck and shoulders, that produces heat by a mechanism known as mitochondrial uncoupling. Mitochondria are the organelles responsible for cellular respiration. Cells oxidize organic compounds such as glucose for energy and temporarily store the removed electrons on carriers such as nicotinamide adenine dinucleoide (NADH) and flavin adenine dinucleotide ($FADH_2$). During aerobic respiration, the electron transport system transfers energy from the electrons of NADH and $FADH_2$ to adenosine triphosphate (ATP), the molecule that serves as the currency of energy in the cell. The electron transport system exists on the inner membrane of mitochondria. In NST, the cell releases the energy as heat instead of using it to make ATP molecules. Human infants have more brown fat than white fat, which functions primarily in fat storage.

BLOOD SUGAR

Glucose, a simple sugar molecule, is the primary fuel source for cells. When glucose levels exceed the body's needs, muscle and liver cells store the excess in the form of glycogen, a polymer made of numerous glucose subunits. If even more glucose is present than the glycogen stores can hold, the body converts the excess to fat. Two types of specialized cells within the islets of Langerhans in the pancreas produce two hormones that play a major role in the regulation of glucose levels in blood circulation. The beta cells of the islets of Langerhans secrete the hormone insulin when the blood glucose levels are high, such as after a meal. Insulin enhances the uptake of glucose by body cells and slows down glycogen breakdown in the liver. As a result, the levels of glucose circulating in the blood decrease. If glucose levels get too low, the alpha cells of the islets of Langerhans secrete the hormone glucagon, which has the opposite effect. Glucagon stimulates the breakdown of glycogen in the liver and the release of glucose into the blood, increasing circulating levels. The antagonistic effects of insulin and glucagon maintain glucose levels within a small range. Diabetes mellitus results when insulin is deficient or no longer has an effect on body cells and the blood glucose levels remain elevated.

OSMOREGULATION AND WATER BALANCE

Animal cells are bathed in extracellular fluids, the composition of which is crucial to many physiological functions. Because some organisms live in freshwater, some in marine habitats, and some on land, different mechanisms help control the amount of water and solutes in body fluids, a process called osmoregulation. Differences in osmolarity, the total solute concentration expressed in moles of solute per liter of solution, direct osmosis across biological membranes. Water diffuses from an area of low solute concentration to an area with a higher relative solute concentration. The addition of water to a solution decreases its osmolarity. Because osmolarity and water balance are so closely related, the homeostatic mechanisms that compensate for fluctuations in either are integrated.

Animals have no cell walls to provide structural support, so those that live in freshwater habitats must have mechanisms to rid themselves of excess water that enters their bodies by osmosis, or their cells are in danger of lysing. One important adaptation of freshwater animals is to maintain a lower total solute concentration than marine animals. Diminishing the difference in solute concentrations between the external and internal conditions reduces the amount of energy required for osmoregulation. Many freshwater animals also excrete very dilute urine and transport salts against concentration gradients across membranes in the gills.

Animals that live in salt water must have adaptations that allow them to either bring in more water

or to balance the osmolarity of their cells with that of their environment to prevent water loss or dehydration. Though the total internal solute concentration usually equals that of the surroundings, the composition of the solutes differs. The intake of large quantities of salt water and the ingestion of salty foods also helps balance osmolarity. Gills of marine animals usually transport excess salts out and the kidneys produce very concentrated urine. Sharks and other cartilaginous fishes have structures called rectal glands that rid the body of certain types of salts or eliminate them with feces.

Terrestrial organisms must have efficient means to conserve water and avoid desiccation. Specialized body coverings such as shells, exoskeletons, and skin cells help prevent water loss. Utilization of water produced as a by-product of metabolism and the intake of water are important strategies. Land mammals produce very concentrated urine, and some animals such as birds, insects, and reptiles excrete nitrogenous wastes as uric acid, which is insoluble in water, and therefore can be excreted as a semisolid paste.

While anatomical and physiological adaptations allow organisms to live in a variety of environments, all animals must also have a means of monitoring their internal osmolarity and making adjustments to it in order to maintain appropriate solute concentrations for their physiological needs. The excretory system responds to fluctuations in internal osmolarity to maintain optimal conditions for cells of body tissues to function properly. Manipulation of solute concentrations by active transport, a process that costs energy, allows an organism to control the direction and rate of osmosis across cellular membranes in order to maintain a stable internal environment.

In mammals, the hypothalamus monitors osmolarity of the blood using special osmoreceptors. When the osmolarity of the blood gets too high, the hypothalamus signals the posterior pituitary to release antidiuretic hormone (ADH), also known as vasopressin. The distal tubules and the collecting ducts of the kidney respond to the presence of ADH by increasing their permeability to water. As a result, the kidney reabsorbs more water from the urine, concentrating it and preventing osmolarity of the blood from increasing any further. The only way to restore the lost water, however, is by the intake of more fluids. The hypothalamus triggers feelings of thirst, and drinking fluids replace the lost fluids and dilute the solute concentration of the blood. When the blood osmolarity returns to normal, the hypothalamus ceases to stimulate the release of ADH by the posterior pituitary, a form of negative feedback. In the absence of any ADH, water cannot diffuse out of the collecting duct. The degree of permeability of the collecting duct to water depends on the quantity of ADH present. Another hormone that plays a role in osmoregulation is aldosterone, a steroid hormone secreted by the adrenal cortex when osmolarity increases. Aldosterone increases the reabsorption of sodium (Na^+) by the distal tubules and collecting ducts of nephrons. If ADH is also present, water reabsorption will ensue.

Aldosterone also functions as part of the renin-angiotensin-aldosterone system (RAAS), a mechanism for maintaining blood volume and blood pressure. The juxtaglomerular apparatus (JGA), a structure located near the afferent arteriole that supplies blood to the glomerulus, also helps regulate water balance by responding to changes in blood pressure. Fluid loss due to persistent diarrhea, vomiting, or excessive blood loss leads to a drop in blood pressure that stimulates the JGA to secrete renin. This enzyme initiates a series of events that results in the conversion of the plasma protein angiotensinogen to angiotensin II, which stimulates the secretion of aldosterone by the adrenal gland, resulting in increased Na^+ reabsorption. Angiotensin II also induces ADH secretion to conserve blood volume, stimulates thirst to increase fluid levels, and constricts arterioles, which increases blood pressure even if the volume remains constant. The end result of both the RAAS and ADH is an increase in water levels and therefore blood volume, but the two mechanisms respond to different signals. ADH responds to dehydration, or water loss evidenced by an increase in osmolarity, for example, sweating a lot or not taking in enough fluids. RAAS responds to a decrease in blood volume despite the osmolarity of the blood, such as when someone loses large amounts of blood or has excessive diarrhea, situations in which both fluids and salts are lost.

Atrial natriuretic peptide (ANP) is a peptide hormone made by certain heart cells in response to an increased blood volume. Also called atriopeptin, ANP works to rid the body of excess Na^+ and fluids, though the mechanism is not yet clearly understood. A better understanding of ANP could lead to new treatments for high blood pressure.

CALCIUM LEVELS

More than 99 percent of the body's calcium is found in the matrix of bone tissue, but free calcium ions (Ca^{2+}) are important to normal cell function. The concentration of Ca^{2+} in the extracellular fluids is kept much higher than cytoplasmic levels, and the cell uses the potential energy stored in this gradient to perform work, such as the stimulation of muscular contraction, the release of neurotransmitters from a neuron into a chemical synapse, and the transmission of molecular signals across cellular membranes during signal transduction. Ca^{2+} also plays a role in

the coagulation of blood and acts as a cement for tight junctions between cells. Because Ca^{2+} has so many crucial functions, the body closely monitors and regulates the concentration of Ca^{2+} circulating in the plasma of the blood.

Bone tissue stores Ca^{2+} and releases it when circulating levels are low. Two different types of cells found in bone tissue regulate calcium storage and release. Osteoclasts secrete acid and enzymes that dissolve bone tissue and release free Ca^{2+}, and osteoblasts produce bone, a process that removes excess Ca^{2+} from circulation. The four parathyroid glands, found just behind the thyroid gland in the neck, produce hormones that affect Ca^{2+} storage and release. In response to dropping Ca^{2+} levels, the parathyroids secrete parathyroid hormone, which stimulates the activity of osteoclasts, and they dissolve some of the calcified matrix of bone tissue. Parathyroid hormone also causes the kidneys to reabsorb more Ca^{2+} from the filtrate during urine production and stimulates the conversion of vitamin D to an active form. Vitamin D acts on the small intestine, causing the absorption of more Ca^{2+} during digestion, as well as reinforcing the action of parathyroid hormone on the kidneys and bone tissue. The effects of calcitonin, a hormone produced by the thyroid gland, oppose those of parathyroid hormone. When the concentration of Ca^{2+} in the plasma is higher than necessary, calcitonin signals the kidneys to decrease the amount of Ca^{2+} that is reabsorbed, lowering the circulating Ca^{2+} levels.

See also ANATOMY; ANIMAL FORM; DIABETES; DIGESTIVE SYSTEM; ENDOCRINE SYSTEM; EXCRETORY SYSTEM; MUSCULOSKELETAL SYSTEM; NERVOUS SYSTEM; NUTRITION; PHYSIOLOGY.

FURTHER READING

Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

Hooke, Robert (1635–1703) English *Experimental Scientist* Robert Hooke was one of the most industrious and insightful men of the 17th century. He was a brilliant engineer, inventor, scientist, architect, and surveyor, but he had a temper that caused Sir Isaac Newton to remove Hooke's name from the permanent records of the Royal Society. Hooke's accomplishments include *Micrographia,* an extraordinary volume that contained intricate sketches of biological specimens as well as observations of other natural phenomena. Historians of science have heralded *Micrographia* as second in influence only behind Sir Isaac Newton's *Principia*. Hooke also coined the term *cells* while observing cork under high magnification, devised an equation that described elasticity (now called Hooke's law), and participated in the rebuilding of London after the Great Fire of 1666.

HOOKE'S EARLY CAREER

Robert Hooke was born on July 18, 1635, at Freshwater, on the Isle of Wight, off the southern coast of England. His father, the Reverend John Hooke, mostly schooled Robert at home. Robert was a sickly child who spent much time playing with mechanical toys and even constructed a ship model with working firing guns. When his father died in 1648, Robert moved to London, where Sir Peter Lely, the leading portrait painter of the age, took him in as an apprentice. The smell of paint bothered Robert, so at age 13, he entered Westminster School, where he is said to have mastered all six of Euclid's *Elements* in a week, learned several languages, and invented more than 30 ways of flying. Hooke went to Christ Church, Oxford, in 1653. Due to financial difficulties, he had to serve as a chorister and as a servant to a wealthier student. At Oxford he met and befriended Christopher Wren, who became a renowned architect. Though Hooke never earned his bachelor's degree, he was nominated for a master of arts degree from Oxford in 1663. For a while he served as an assistant to the English anatomist Thomas Willis, who recommended him to Robert Boyle. In 1655 Hooke became an assistant to Boyle, one of England's premier physical chemists.

Hooke's mechanical talents were immensely useful to Boyle. One aspect of Boyle's research involved vacuums, which can be created with the use of an air pump. When the leading pumping engineer failed to produce a machine sufficient for Boyle's studies, Hooke stepped in and built one in 1658 or 1659. While still imperfect, the pump allowed Boyle to generate a vacuum after a few minutes of pumping. Using the pump, Hooke and Boyle examined burning coal, charcoal, and candles under low and high pressure. They concluded that fire was a chemical event rather than an element, a concept that dated back to the ancient Greek philosopher Aristotle. In all, the two performed 43 experiments with the air pump, the results of which were published in *New Experiments Physico-Mechanical, Touching the Spring of the Air and Its Effects* in 1660. Though Hooke's exact role is unclear, in 1661, with Hooke's assistance, Boyle performed the experiments that led to his formulation of what is now called Boyle's law, stating that for a

given mass at a constant temperature, the pressure times the volume is a constant.

$$p \times V = \text{constant}$$

This relationship between pressure and volume of a gas was Hooke's first foray into studies of elasticity, a subject that held his interest for many years. He later used the pump to explore the expansion and contraction of gases at different temperatures.

Hooke was also interested in combustion and performed experiments that led to his conclusion that air consisted of an inert portion and a combustible portion. Physiologists at the time were examining the properties of air in relation to respiration and circulation. Hooke bravely sealed himself inside a chamber and monitored the effects on his own body as the air pressure in the chamber was lowered by pumping air out. He experienced pain and deafness in his ears, a symptom of high altitude sickness. Later in his career, Hooke showed that the function of respiration was to bring fresh air into the lungs, not to cool the blood, as one prevailing hypothesis suggested.

In 1661 Hooke published a pamphlet on capillary action, the tendency of one substance to draw another substance into it. When he stood a narrow glass tube in a bowl of water, the water within the tube rose up to a level higher than the surface level of the water in the bowl. Impressed with this, and having several members remember him from Oxford, in 1662 the Royal Society of London named Hooke curator of experiments. His responsibility was to set up and demonstrate several experiments at the weekly meetings, but his intellectual contribution was recognized, and the following year he was elected a fellow. He is credited with providing the enthusiastic impetus needed to sustain the Royal Society through its early years. The Royal Society is now one of the world's premier scientific organizations. Hooke's position as curator initially did not come with a stipend. In 1664 merchant and financier Sir John Cutler founded a lectureship to provide Hooke with a salary. With an academic appointment, Hooke gained the privilege of lodging in the chambers at Gresham College. The following year, Hooke became a professor of geometry, and he remained in London until his death.

SPRINGS, CLOCKS, AND ELASTICITY

The pendulum clock invented by the Dutch mathematician and astronomer Christiaan Huygens in 1656 was the most accurate timekeeper of the 17th century, but the fact that the pendulum had to hang from a frame made it unsuitable for a clock aboard a ship at sea or for a pocketwatch. In the late 1650s, while still working as Boyle's assistant, Hooke began exploring springs and elasticity and discovered that when the spring was stretched and released, its oscillations were isochronous, in other words, they occurred at regular intervals. Around 1660 Hooke realized that spring tension and release could be used to create rotations at regular intervals to keep time in a portable watch. Though Boyle and others attempted to get Hooke to patent his spring-regulated watch, Hooke did not agree to the terms. A letter written in 1664 to King Charles II by Hooke establishes that in 1661 he had devised a mechanism that used springs to regulate watches, including the use of balance wheels to prevent shaking from interfering with the clock's functioning. Since the details regarding Hooke's spring-driven balance wheel are not available, whether or not he conceived of a spiral spring is unknown.

In 1675 Huygens communicated to the Royal Society his new invention of a coiled spiral spring that could be used in watches. Hooke accused the secretary, Henry Oldenburg, of sharing Hooke's ideas from his similar invention from a decade prior and called him a "transcriber of intelligence," a comment the Royal Society later compelled him to publicly withdraw. Hooke claimed priority and rushed to put together a working model, a requirement to obtain a patent in those days. Huygens and Hooke fought over priority for years, but in the end, the king did not grant a patent to either of them. Hooke published *Helioscopes* in 1676, describing his design of a spring-regulated watch. Watchmakers utilized the principles of this design until the 20th century, when electronic chips were invented.

In *A Description of Helioscopes* (1675), which was based on Cutlerian lectures Hooke delivered in January 1675, Hooke announced a discovery in the form of an anagram code, as was fashionable at the time. He claimed to have explained the theory of elasticity or springiness and defined a way to compute the velocity of bodies moved by them. The anagram was "*ceiiinosssttuu*" which two years later he deciphered in *De potentia restitutiva* (Of springs)—"*ut tension sic vis*." Translated, this means the power of a spring is proportional to its extension, a concept now known as Hooke's law. Tension resulted from suspending weight to a coiled spring, causing it to extend; thus, tension can be viewed as the force. Hooke's law can be interpreted to state that the force exerted by a spring is proportional to the distance it is extended (or compressed for that matter). In addition to elasticity, *De potentia restitutiva* also revealed Hooke's appreciation of vibrations and offered a demonstration of the isochronal nature of springs.

After Oldenburg died in 1677, Hooke assumed the position of secretary of the Royal Society, a position he held until 1682.

THE GREAT FIRE

The Great Fire of London began on September 2, 1666, and the conflagration completely destroyed the medieval sections of the city within five days. The disaster consumed 13,200 houses, 87 churches, and 50 livery halls, but only six people are known to have died. The fire put an end to the bubonic plague, which had killed 17,440 of the city's population of 93,000 the year before, by killing all the rats that spread the disease. The fire started when a baker to King Charles II, Thomas Farynor, forgot to put out his cooking fire, and by the early morning hours his house was burning down. Houses in London stood close to one another, and many consisted of timber and pitch, which ignited easily, causing the fire to spread beyond control rapidly. Hooke submitted a plan that involved laying out the city on a grid. Though his model for rebuilding the city was never implemented, it impressed the city council, who appointed Hooke, along with Wren and a few others, to posts as city surveyors. For 10 years, Hooke worked tirelessly to reestablish property lines and to supervise the rebuilding. Hooke's architectural talent was apparent in his design of the Bethlehem Hospital or Bedlam, Montagu House in Bloomsbury, the Royal College of Physicians, Merchant's Taylors' Hall and Ragley Hall in Warwickshire, and Willen Church in Buckinghamshire. Together Hooke and Wren created the Monument to the Great Fire, which was built in 1677 at the site of Farynor's house. At 202 feet high (61.6 m), the Monument is the tallest isolated stone column in the world.

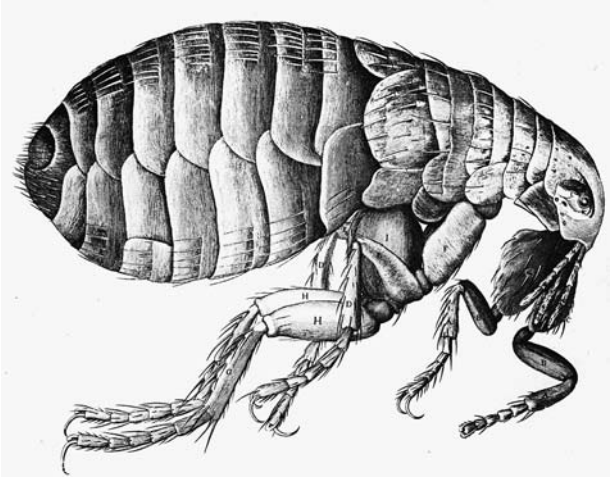
MICROGRAPHIA

Hooke's major contribution to life science was his profoundly influential book *Micrographia* (1665), the first publication devoted to microscopic observations. *Micrographia* provided a wealth of data for the scientific community, who had never before considered that the microscopic universe was as vast as the telescopic universe, leaving much to be studied. Many observations recorded in the book served as stimuli for further research by other scientists. The microscope had been around since the early 1600s, but few had used it to make scientific discoveries. A clever inventor, Hooke invented a practical compound microscope that had better resolution than most others at the time. The book contained comprehensive descriptions and 58 enlarged, detailed engravings of his observations using the microscope. Today visual depictions and illustrations are commonplace in scientific literature, but this was not so in the 17th century.

The book contained Hooke's observations from examination of common everyday objects, such as the point of a needle and silk, in addition to life-forms and their body parts, including fleas, bee stingers, the compound eye of flies, other insects, bryozoans (a type of marine invertebrate), sponges, moss, wild oats, and bird feathers. The accompanying engravings were graphic and almost unbelievable in their detail. For example, the seemingly smooth fine point of a needle looked rough. Insects, to which many had not given much thought, suddenly seemed monstrous under the magnification of Hooke's microscopes. The expensive *Micrographia* was a best seller, and the book inspired and influenced other scientists. Samuel Pepys, a naval administrator who became famous for his private diaries that detailed his personal accounts of major historical events such as the Great Plague and the Great Fire, admitted to staying up until 2:00 A.M. reading what he called "the most ingenious book that I ever read in my life." Some scientists ridiculed Hooke for spending his time examining what they felt were insignificant details of unimportant objects such as fleas. The influence of *Micrographia* spread to the general public as well, and a theatrical performance whose lead character was based on Hooke mocked his interest in trifles such as "mites of cheese." A demonstration of the ignorance of those who undermined the book's importance and the ingenious insight of Hooke was his observation that cork (from tree bark) consisted of numerous boxlike packed pores, whose structure he likened to honeycomb. The pores reminded him of cells in a monastery, thus he named them "cells." Though Hooke did not understand their significance (nobody did), his description of cells in *Micrographia* is the first recorded mention of these fundamental units of all living organisms. The tissue he was examining was not living, and what he actually saw was the outline of the cells left by the material forming the cell walls of the plant. Hooke also noted that he observed similar structures in other plants.

In addition to illustrations of microscopic biological specimens, *Micrographia* contained astronomical observations made with magnifying lenses, such as craters on the Moon and descriptions of the Pleiades star cluster. The nature of colors was addressed through his investigations involving thin plates of mica, soap bubbles, and layers of air between glass plates. This led to a discussion of Hooke's proposed theory of light as a wave (a transverse vibrational motion transmitted through a medium). The book also included his description of heat as a property of a body that arises from the vibration of its parts, his previously published description of capillary action, as well as a discussion on combustion and Hooke's earlier research on air performed in Boyle's laboratory.

In 1673 a Dutch draper named Antoni van Leeuwenhoek began a 50-year correspondence with the



This detailed drawing of a flea created by Robert Hooke and published in *Micrographia* (1665) demonstrated the power of microscopy in the study of biological specimens. *(HIP/Art Resource, NY)*

Royal Society during which he shared his meticulous descriptions of microscopic specimens such as fungal spores, a louse, and bee parts. Though he had never received any formal education or training in science, Leeuwenhoek was a skilled lens grinder and made simple microscopes of higher quality than any other known at the time. In 1676 Leeuwenhoek wrote of his remarkable discovery of "animalcules" swimming in rain water. He had also found these microscopic "beasties" in pond water, melted snow, and well water. Their shapes and sizes varied, though Leeuwenhoek commented that up to 1,000 might fit on the head of a pin. The claim of microscopic life was dubious and many scientists tried unsuccessfully to repeat Leeuwenhoek's experiments. As the reputed expert on microscopy and curator of experiments at the Royal Society, Hooke also attempted to reproduce Leeuwenhoek's observations. In 1678 Hooke published his own observations of motile, microscopic life, substantiating Leeuwenhoek's claims.

CONTRIBUTIONS TO ASTRONOMY AND PHYSICS

At Oxford, Hooke studied astronomy with Seth Ward, an English mathematician and astronomer who researched planetary motion. Many of Hooke's later investigations related to astronomy, the field in which he made his most extensive contributions. In 1664 he discovered a fifth star in the Orion trapezium. He was among the first to observe the surface of Jupiter, and he explained the scintillation of stars. He also created several drawings of Mars that were useful to astronomers two centuries later and made observations of the comet that illuminated the skies of the Northern Hemisphere during the winter of 1664–65.

When Sir Isaac Newton published a paper on color and light in 1676 (he had already published one on colors in the *Philosophical Transactions* of the Royal Society in 1672), Hooke commented that the content of Newton's paper simply promoted the ideas he had expressed in *Micrographia,* but that he did not have time to develop further. After some bickering, Newton finally acknowledged that Hooke's ideas contributed to the development of his own, although he insisted that the main work was original. The timing of this quarrel during the same year as Hooke's dispute with Huygens for the timepiece spring-balance mechanism led some to disregard Hooke's claims as manifestations of arrogance and perhaps jealousy.

Hooke continued to deliver the Cutlerian lectures in addition to serving as city surveyor. His lecture topics included discussions of some of his earlier research on combustion and optics. A volume titled *Lectiones Cutlerianae,* published in 1679, contained a collection of his lectures. Among other topics, this treatise contained a formal proposal of Hooke's law of elasticity and his likening of a vibrating spring to a pendulum. He also described what is recognized as the basis for his claim on discovering the law of universal gravitation. He had previously explored the concept of gravitation as it related to heavenly bodies in *An Attempt to Prove the Motion of the Earth* (1674). As early as 1666, during a Royal Society meeting, Hooke read a paper correctly explaining planetary motion. Hooke drew three conclusions from his research:

- All celestial bodies have a center of gravity. He argued that the fact that the Moon and other planets were spherical suggested that they had gravitational centers, just as astronomers believed Earth did.
- Bodies put in motion will remain in straight-line motion until acted upon by other forces. Gravity, he purported, also explained the altered pathway of comets as they traveled past heavenly bodies such as the Sun and Earth.
- Gravitational attraction is stronger when the bodies are closer to one another (though he could not expound on the exact relationship).

While Hooke's insight was ingenious, he did not excel at mathematical demonstration or in-depth analysis. In 1679 he reported in a letter to Newton his conclusion that gravitational attraction varied as the inverse of the square of the distance to the Sun (the inverse square law), but he did not offer any evidence that led him to this conclusion nor did he offer any mathematical expression of the concept.

When Newton published his findings on this topic in 1686 in his masterpiece *Philosophiae Naturalis Principia Mathematica* (Mathematical principles of natural philosophy), often referred to simply as *Principia,* Hooke claimed Newton stole his idea. Newton had been working on the mathematical proof explaining the elliptical orbits of the planets for two decades, but there is no evidence that Newton had thought to explain the orbits in this manner before Hooke's letter to him in 1679. Hooke had a flash of insight but he proved incapable of performing the required mathematical demonstration to convince the scientific community. However, Newton later acknowledged that Hooke's insight had stimulated his own work that led to his demonstration that the elliptical orbits of the planets involved an inverse square force.

Newton became so annoyed with Hooke that he avoided publishing his work. He refused to publish *Opticks,* a treatise on light (a topic explored by Hooke in *Micrographia*), until 1704, one year after Hooke died.

OTHER CONTRIBUTIONS

Fossils and geology also interested Hooke. In the 17th century, the origin of fossils was unknown. While some believed fossils were preserved lifeforms, one other hypothesis explaining their origin purported that a plastic force shaped their formation deep within the Earth. This explanation was popular because many fossils did not resemble the extant lifeforms, and the concept of extinction was not widely accepted. In order to shed light on the nature of fossils, Hooke examined fossils under the microscope and divided them into two general categories: those resembling living organisms and those that were inorganic in nature. He compared the differences between fossilized wood and living wood and between fossil shells and living mollusk shells. After combining his observations with that of differences between rotting wood and petrified wood, he suggested that wood fossilized by the deposition of minerals into the wood by what he called petrifying water. He also concluded that the shells he studied formed from the action of "petrifying water" on formerly living mollusks. Because of this, Hooke recognized and proposed that they be used as documentation of the history of life. This notion contradicted the widely accepted biblical account of creation, which did not allow for the mutability of species, but Hooke stood firm in claiming that the changes documented by the fossil record should be used to outline the succession of life-forms.

Hooke did not limit his geological explorations to the origin of fossils. In *Lectures and Discourse of Earthquakes,* read before the Royal Society in 1668 and published in 1705, after his death, he suggested that places once submerged were now inland and that former mountaintops were now submerged. Upheavals from earthquakes displaced Earth's surface.

Though Hooke never married, his niece Grace served as his housekeeper and his companion. After she died in 1687, Hooke's own spirit began to decline, and within a decade his health began to deteriorate. He suffered symptoms, including blindness and swelling of the legs, that may have been due to diabetes. Hooke died in London on March 3, 1703. He was buried at St. Helens's Bishopgate. Sir Isaac Newton assumed the presidency of the Royal Society and, soon after, he had all records of Hooke expunged. No portrait remains of this brilliant polymath.

Some science historians think it unfair that such a brilliant man has not received the historical recognition he deserves. Although he excelled in many pursuits, his attentions were widely spread, and he did not carry his pursuits through to completion. His contributions always seemed to be made at the fringes of a major discovery and so they never received priority. His arrogance, stubborn personality, and tendency to offend may have also played a role in limiting his historical stature. Nevertheless, his multifarious contributions advanced the fields of biology, physics, astronomy, mathematics, and technology. On January 31, 2007, the lord mayor of London dedicated a memorial tablet to Robert Hooke at the foot of the Monument to the Great Fire of 1666.

See also Leeuwenhoek, Antoni van.

FURTHER READING

Bennett, Jim, Michael Copper, Michael Hunter, and Lisa Jardine. *London's Leonardo: The Life and Work of Robert Hooke.* New York: Oxford University Press, 2003.

Hooke, Robert. *Micrographia: Or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses, With Oberservations and Inquiries Thereupon.* London: Jo. Martyn and Ja. Allestry, printers to the Royal Society, 1665.

Inwood, Stephen. *The Forgotten Genius: The Biography of Robert Hooke 1635–1703.* San Francisco: MacAdam/Cage, 2003.

Jardine, Lisa. *The Curious Life of Robert Hooke: The Man Who Measured London.* New York: HarperCollins, 2004.

Robert Hooke Web site. Available online. URL: http://www.roberthooke.org.uk/. Accessed on January 25, 2008.

Hooker, Sir Joseph Dalton (1817–1911)

English *Botanist* Sir Joseph Hooker was a famous 19th-century botanist, renowned for his taxonomic skills and for transforming the Royal Botanic

Gardens at Kew into a highly respected botanical research center. His work on the geographical distribution of plants is considered pioneering.

Joseph Dalton Hooker, the son of Sir William Jackson Hooker and his wife Maria, was born on June 30, 1817, at Halesworth, Suffolk. He attended high school in Glasgow, Scotland, and entered the University of Glasgow, where his father had accepted the regius professorship of botany in 1820. In 1837 Hooker published his first scientific paper, in which he described three newly identified Indian mosses. In 1839 Joseph Hooker obtained his medical degree, but he preferred botanical research.

HMS *EREBUS* AND EVOLUTION

His father's friend, Sir James Clark Ross, enlisted Hooker to serve formally as assistant surgeon, though, in reality, as a naturalist, aboard the HMS *Erebus* on an expedition to Antarctica. The ship left England on September 30, 1839. Many of the geographic areas they visited were previously unexplored; thus, Hooker had the opportunity to break much new ground. He made sketches, recorded observations, and collected dried plant specimens. When the expedition returned in 1843, Hooker published a six-volume work collectively titled *The Botany of the Antarctic Voyage of H.M. Discovery Ships* 'Erebus' *and* 'Terror.' In two volumes he described the flora of the Antarctic islands, in another two New Zealand flora, and in a further two Tasmania flora. This series described a total of 5,340 species and contained 528 colored lithographs created by Walter Hood Fitch, the renowned botanical artist who made the plates for almost all the Kew publications. The volumes were important historical works. In *Flora Antarctica* (1844–47), Hooker disputed the theory of multiple origins, which stated that the same species arose in several areas. Instead, Hooker proposed that a species originated in one location and then became distributed geographically. In particular, he noted the resemblance between plants in the subantarctic islands, South America, New Zealand, and Australia. He suggested that a large circular tract of land that once connected these regions had disappeared, leaving species spread out on distant continents. Later, in an 1866 paper titled "Insular Floras" that he read to the British Association at Nottingham, he said that he did not like the "sinking imaginary continent" idea and believed transoceanic migration was more likely. *Flora of New Zealand* (1853–55) and *Flora of Tasmania* (1855–60) are important because they demonstrate the current scientific thoughts immediately before the publication of *On the Origin of Species* (1859), one of the most influential works of all time, written by the British naturalist Charles Darwin.

Darwin's major work resulted from decades of analyzing and synthesizing data collected during his own five-year expedition aboard the HMS *Beagle* from 1831 to 1836, during which he made observations that convinced him of the mutability of species. Darwin began a correspondence with Hooker upon Hooker's return to England. Darwin sought Hooker's assistance in classifying the plants he had collected from the Galápagos. Hooker read his paper *On the Vegetation of the Galápagos Archipelago* to the Linnean Society of London in 1846, but the work was not published until 1851. Like Darwin, Hooker found much evidence supporting the evolution of species during his trip. Darwin wrote Hooker a letter in January 1844 claiming to have solved the mechanism by which species evolved. Hooker was the first person with whom Darwin shared his theory of natural selection. This would prove important later in establishing priority for the theory of evolution by means of natural selection, when Alfred Russel Wallace proposed a practically identical theory in 1858 in a letter he wrote to Darwin. Together, Hooker and Scottish geologist Charles Lyell persuaded Darwin and Wallace to present their research on evolutionary theory jointly to the Linnean Society of London.

Hooker later admitted that though Darwin shared with him all of his latest ideas and views concerning species mutability and evolution, he did not adopt them himself until 1858, after he had collected sufficient evidence from his botanical taxonomic work and studies on the geographical distribution of plants. No real evidence of Darwin's influence appeared in Hooker's writings until 1860, in the introduction to *Flora Tasmaniae*. From that point on he staunchly defended Darwin. The introductory essay of this book is also well-known for Hooker's clear sketch of a hypothesis for the geographical distribution of plants.

KEW AND INDIA

Sir William Jackson Hooker became director of the Royal Botanic Gardens, Kew, in 1841. The younger Hooker moved in with his parents at Kew after his Antarctic voyage. The resources at Kew were invaluable for analyzing his botanical data. In 1846 he accepted a position for the Geological Survey as a paleobotanist, a scientist who studies fossil plants. He wrote several papers in this capacity.

Hooker left with a commission in the Royal Navy in November 1847 to go to India. He traveled for almost four years, making observations, collecting botanical specimens, and surveying the geography of northeastern India. Most of his efforts focused on the Himalayan state of Sikkim and eastern Nepal, areas that were practically unexplored. In 1849 the Sikkim authorities arrested and held him for several weeks.

From Hooker's work, the India Trigonometrical Survey published a map of the mountain terrain. While in India, Hooker wrote a book titled *Rhododendrons of Sikkim-Himalaya* (1849–51). He sent several dried specimens and sketches back to England to Fitch, who created the illustrations for the work. Another major contribution from this expedition was the introduction of several new *Rhododendron* species to England. Hooker also wrote *Flora Indica* (1855) with Thomas Thomson, a colleague from his school days. This evolved into the *Flora of British India* (1872–97), a 6,000-page, seven-volume work that required the assistance of several other botanists and described about 17,000 species. *Flora of British India* remains a valuable resource concerning seed-bearing plants in India. Hooker had been knighted in 1877, and, in 1897, this work earned him the title of grand commander. Another book written by Hooker from his Indian expedition, *Himalayan Journals* (1854), provided an account of his adventures written for a general audience. He dedicated this book to Darwin.

In 1855 Hooker became assistant director at the Royal Botanical Gardens. In 1865 William Hooker died, and Joseph succeeded him as director. The older Hooker had done much to improve the state of the gardens, and the younger Hooker carried on his father's work. One task he accomplished was adding his father's herbarium and library to the Royal Botanic Gardens collections. Indian plants, a mycological herbarium, mosses, and lichens soon followed, requiring an addition to the building in 1877. Kew progressed into a world famous botanical research center. The library became one of the most important botanical reference sources in the world, and today the gardens hold one in eight of all known plant species. Hooker retired as director in 1885.

With the botanist George Bentham, Hooker took on the enormous task of attempting to develop a more modern and uniform system for classifying plants. They began work in 1857, but soon, because Hooker's other duties took up too much of his time, his contribution totaled less than half of the work. The compilation of their efforts, *Genera plantarum* (1862–83), contained detailed descriptions of all the known seed-bearing plants, their many different names, and their geographical distribution.

In 1870 Hooker published *Student's Flora of the British Isles,* with the goal of not only describing the plants, but also including information regarding their variation and geographical distribution. Hooker continued his research, going on expeditions, and writing about the geographical distribution of plants. In 1877 he joined his American colleague Asa Gray and others in the Rocky Mountains of Utah and Colorado, where he surveyed the botany of those states. Hooker submitted a report to the U.S. Geological and Geographical Survey of the Territories, published in 1881. His main conclusion was that glaciation exterminated the Miocene flora in western North America, but the flora survived in eastern North America and in eastern Asia. His last literary work was a biography of his own father.

In 1851 Hooker married Frances Harriet Henslow, with whom he had six children. She died in 1874, and, in 1876, Hooker married Hyacinth Symonds Jardine, with whom he had two sons.

Hooker received due recognition for his brilliance in botanical research and was awarded many honors. The Royal Society elected him to membership in 1847, and he served as president from 1873 to 1878. The society awarded him a royal medal in 1854, and he received the Copley Medal in 1887 and the Darwin Medal in 1892. The Swedish Academy of Sciences presented him with the Linnean medal for serving as "the most illustrious living exponent of botanical science" during the bicentennial celebration of Carl Linnaeus's birth. He received numerous honorary degrees, including from the universities of Oxford, Cambridge, Dublin, Edinburgh, and Glasgow. Queen Victoria appointed Hooker companion (C.B.) of the Order of the Star of India in 1869, knight commander in 1877, and knight grand commander in 1897. In 1907 he was presented with the Order of Merit for his distinguished service to science. Sir Joseph Dalton Hooker died on December 10, 1911, in Sunningdale, Berkshire.

Hooker was the greatest expert on the geographical distribution of plants in the 19th century; one of his most enduring contributions to botany was his work as the director of the Royal Botanical Gardens at Kew. He is also remembered as one of Darwin's greatest confidants.

See also Darwin, Charles; Gray, Asa.

FURTHER READING

Desmond, Ray. *Sir Joseph Dalton Hooker: Traveller and Plant Collector*. Woodbridge, England: Antique Collectors' Club with Royal Botanic Gardens, Kew, 1999.

Endersby, Jim. *Imperial Nature: Joseph Hooker and the Practices of Victorian Sciences*. Chicago: University of Chicago Press, 2008.

———. Joseph Dalton Hooker Web site. Available online. URL: http://www.jdhooker.org.uk. Last updated on November 14, 2008.

host defenses A living organism must defend itself against potentially pathogenic foreign invaders and harmful molecules. Vertebrates have a highly developed immune system composed of three lines of defense. The first two lines of defense include

nonspecific physical and chemical barriers that do not distinguish between invaders. The third line of defense, the immune system proper, specifically attacks microorganisms, toxins, and other foreign substances that penetrate the nonspecific physical and chemical barriers.

NONSPECIFIC DEFENSES

Animals are constantly in contact with microorganisms in their surrounding environment—in the air and water, indoors as well as outdoors. While the overwhelming majority of these microbes are harmless, the body must have mechanisms for dealing with invaders that are potentially pathogenic and have the ability to cause disease. The first line of defense involves nonspecific, innate, anatomical barriers and secreted chemicals that attempt to prevent microbes from gaining entry into the body. The skin is a mechanical barrier that covers the body. Composed of the fibrous proteins keratin, the skin is difficult for microorganisms to penetrate. In addition to acting as a physical barrier, secretions from the sweat and oil glands inhibit microbes from colonizing on the skin. The high salt concentration of sweat and the acidic nature of sebum secreted from the sebaceous glands inhibit the growth of many microbes. In addition, most microbes require more moisture than the skin provides.

Mucous membranes line the portals of entry to the body's interior. Like skin, these membranes physically block microbes from entering into the tissues, but they also secrete mucous, a thick, sticky substance that traps microbes so they can be carried out of the body. Mucous contains lysozyme, an enzyme that punches fatal holes in the cell walls of microbes. Cilia that line the upper respiratory tract beat in unison to move mucous that might contain pathogens up toward the pharynx, where it can be coughed up or swallowed. Microbes can also be removed by the mechanical washing action of tears, saliva, and urine. Microorganisms that make it as far as the stomach must face the acidic environment created by the secretion of hydrochloric acid in the gastric juices. Mechanical processes such as peristalsis, defecation, and vomiting also act to move potentially dangerous microorganisms out of the body.

Once microbes have entered the body tissues, several components of the immune system interact to prevent the invaders from colonizing and causing an infection: the bloodstream, the lymphatic system, the reticuloendothelial system (RES), and the extracellular fluids. In addition to erthryocytes (red blood cells), the blood contains leukocytes (white blood cells) that have immune system functions. Leukocytes can be divided into two general categories based on their appearance when specially stained and viewed under the microscope: granulocytes and agranulocytes. Granulocytes include the neutrophils, basophils, and eosinophils and have a grainy appearance due to vesicles containing digestive enzymes and other chemicals. The main function of neutrophils, which make up about 60 to 70 percent of all white blood cells, is phagocytosis, the ingestion of large, particulate substances by a cell. Basophils constitute less than 0.5 percent of leukocytes and secrete histamine, an important mediator of the inflammatory response. The function of basophils is very similar to that of mast cells, except that mast cells are distributed in the tissues and do not circulate. Approximately 1.5 percent of leukocytes are eosinophils whose main job is to attack larger parasitic invaders such as helminths by secreting destructive enzymes. The agranulocytes include monocytes and lymphocytes. Making up approximately 5 percent of all leukocytes, monocytes circulate only for a few hours before migrating into the body's tissues where they develop into macrophages, the largest and most effective phagocytic cells. Macrophages also process foreign antigens and present them to lymphocytes and secrete chemicals that mediate other immune functions. Some monocytes differentiate into dendritic cells, cells that are found in lymphatic tissues and the RES and that trap pathogens and present them to lymphocytes, the primary white blood cells involved in specific immunity. Natural killer cells resemble lymphocytes and attack cells that are infected with viruses or that might be cancerous.

The lymphatic system is a key component of the second and third lines of defenses. Consisting of vessels, lymphoid tissues (such as the spleen, adenoids, tonsils, Peyer's patches, and lymph nodes), and lymph, the lymphatic system is responsible for collecting interstitial fluids in lymphatic capillaries and returning the fluids and proteins that have escaped from cells and tissues to blood circulation. After entering the lymphatic vessels, the fluid, now called lymph, circulates through the system where it passes through structures called lymph nodes, located throughout the body. The lymph nodes contain large quantities of macrophages and lymphocytes that attack foreign invaders present in the lymph.

The RES is a network made up of connective tissue that supports and interconnects tissues and organs. Macrophages embedded in the RES attack invaders as they enter tissues. The tissues are bathed in extracellular fluid that flows from the bloodstream through capillaries and enters tissues bringing nutrients, hormones, chemicals, and other substances with it. Substances needed by the body cells diffuse from the extracellular fluid into the cells, and excess fluid is picked up by lymphatic vessels and eventually returned to blood circulation.

Organisms that penetrate the first line of host defenses must contend with a second line that includes internal mechanisms such as the inflammatory response, phagocytosis, and other actions performed by antimicrobial proteins. The nonspecific processes that make up the second line of defense are carried out by specialized blood cells and other components of the blood and lymph.

The inflammatory response involves a coordinated set of reactions following damage to a tissue that can be the result of an injury, infection, or other distress. The goals of the inflammatory response are to bring immune components to the affected site, to begin removal of harmful substances or dead cells, to initiate tissue repair, and to immobilize and destroy microorganisms. Inflammation is characterized by the dilation of blood vessels in the vicinity and increased permeability of the nearby capillaries. Bringing more blood to the site causes symptoms including redness, warmth, swelling from more fluid accumulating in the extracellular tissues of the area, and pain from pressure on the neighboring nerve endings. Increasing the blood flow and permeability of capillaries allows more immune system components to access the affected area, so as to prevent infection and heal the damaged tissue, but chronic inflammation itself can be damaging.

Fever often accompanies inflammation and may be the first sign that an infection is present. Certain leukocytes produce cytokines that raise the body's thermostat, as do toxins produced by some microbes. While an extremely high fever or a prolonged fever can be dangerous, fever is also beneficial in fighting infections. An increase in body temperature inhibits the growth of some temperature-sensitive microorganisms, increases the rate of antibody synthesis, speeds up phagocytosis, stimulates the activity of lymphocytes, increases the activity of interferon, and interferes with iron availability, which retards bacterial growth.

Phagocytosis is the ingestion of large particles or cells by endocytosis. Several types of white blood cells are capable of phagocytosis, including neutrophils and monocytes that develop into macrophages. Phagocytes seek out microorganisms, dead host cells, and other particulate matter, often aided by a gradient of chemical mediators secreted by injured tissue cells. They come into contact with the particle or cell and form a phagosome by extending pseudopods out and around the object to form an intracellular vesicle. Fusion of the phagosome, which encloses the ingested particulate matter, and granules containing digestive enzymes results in a phagolysosome. If a microorganism was ingested, it is killed and then digested. The phagocyte releases smaller bits of indigestible debris by exocytosis.

Interferons are a family of proteins that aid in inhibiting viral replication and in fighting cancer. Host cells that are infected with a virus start producing and secreting interferon, which then binds to neighboring cells and stimulates them to synthesize proteins that either degrade viral RNA or prevent viral protein synthesis. This limits the spread of infection. Interferons function nonspecifically; thus, they can inhibit viruses other than the virus that initially triggers their release. Other interferons stimulate lymphocyte development, enhance phagocytosis by macrophages, or assist in the inflammatory response.

The complement system consists of more than 26 proteins that also nonspecifically attack bacteria and some viruses. Complement can be activated by any of three separate pathways; the classical pathway, the lectin pathway, or the alternative pathway. All lead to the same result, the formation of a membrane attack complex that causes cells to lyse and die. Classical activation is the most efficient and involves antibodies, a component of specific immunity. The lectin pathway is stimulated by the binding of host lectins (proteins that bind carbohydrates) to mannan, a carbohydrate present in many fungal cell walls. The alternative pathway is the slowest, but can be activated by the binding of complement proteins to bacteria, enveloped viruses, fungi, or other parasites. In general, complement activation results in initiation of a series of biochemical reactions that end in the assembly of circular-shaped protein complexes that effectively punch holes through the cell wall of a microorganism. This allows the cellular contents to leak out, and the cell dies. In the case of a virus, the membrane attack complex perforates the envelope, leading to viral inactivation.

SPECIFIC IMMUNITY

While facing the second line of defense, microorganisms and other foreign substances that penetrate through the first line of defense will also stimulate a specific immune response. Considered the third line of defense, specific immunity is acquired rather than innate and is stimulated by specific antigens. The specific immune response in vertebrates is led by two types of lymphocytes, B and T cells, and leads to memory, so if the host encounters the same antigen in the future, the immune system can mount a much more rapid response.

An antigen, also called an immunogen, is any substance that provokes an immune response. Proteins and polypeptides, lipoproteins from cell membranes, glycoproteins, nucleoproteins, and certain polysaccharides can all act as antigens. Usually the antigen is a foreign macromolecule that does not belong in the organism and whose presence could cause harm

to the host. In addition to molecules belonging to microorganisms such as a protein expressed on the surface of a bacterial cell or a glycoprotein spike on a viral capsid, antigens also include toxins or molecules found on the surface of cancer cells or transplanted tissue cells. B and T lymphocytes circulate in the bloodstream and lymphatic system waiting to come into contact with an antigen. Recognition occurs through interaction of the specific antigen with a protein receptor on the surface of the lymphocyte. Following recognition, the B and T lymphocytes work in concert to inactivate or destroy the particle or foreign invader associated with that antigen. The branch of the specific immune response involving B cells is called the humoral branch, or humoral immunity, whereas the other branch involving T cells is referred to as cell-mediated immunity.

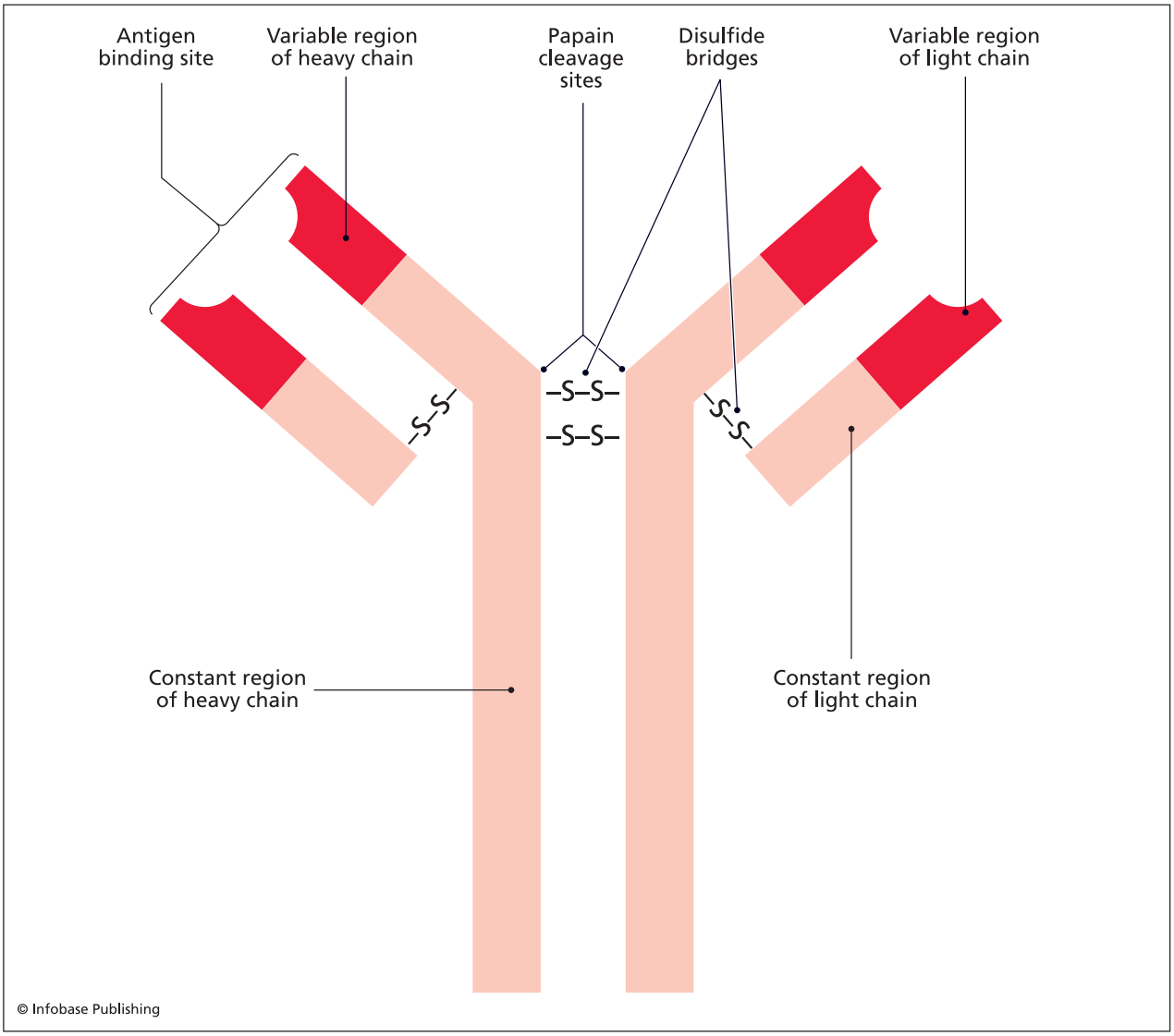
Hematopoiesis, the formation of blood cells including lymphocytes, begins in the bone marrow or fetal liver. Both B and T lymphocytes arise from the same stem cell type, but they differentiate and mature either in the bone marrow (B cells) or in the thymus (T cells). During maturation they undergo clonal selection, a process by which they develop specificity for a particular antigen. Though human DNA only has about 500 different genes that encode for the lymphocyte receptors, extensive genetic rearrangements and recombinations result in the theoretical potential to create between 10^{14} and 10^{18} different clonal types, all with the capability of recognizing a unique antigen. During development, lymphocytes that have randomly acquired the specificity for self molecules, molecules that are a normal part of the host tissues, are marked for deletion and subsequently destroyed. The ability to recognize self versus nonself is termed tolerance. Autoimmune disorders result when someone does not have or has lost immunotolerance and their immune system starts attacking and destroying their own tissues and organs.

After maturation, lymphocytes migrate to lymphoid tissue where they await activation by encountering a specific antigen. B cells display large glycoprotein molecules called immunoglobulins on their cell surface. The recognition and binding of an antibody, also called an immunoglobulin (Ig), on a B cell to a specific antigen activates that B cell and triggers an immune response. This leads to an increase in the cell's metabolism, growth, proliferation, and differentiation. The activated cell divides mitotically, producing daughter cells that share the same antigenic specificity. Some differentiate into memory B cells that can persist for many years and react with the same antigen in the case of future exposure. Other cells differentiate into plasma cells whose main job is to synthesize antibodies, which they can do at an amazing rate of 2,000 per second.

Antibodies are large protein molecules that specifically recognize a single, unique antigen. B cells can produce five different classes of antibodies, all with the same specificity. The main circulating antibody is IgG, which is constructed from two identical polypeptides called heavy chains and two identical polypeptides called light chains. The chains are connected by disulfide linkages to form a Y-shaped structure. Each chain consists of a constant region that is unique for the class of immunoglobulin (for example, IgG) and a variable region that makes up the antigen binding site. IgGs are the most abundant circulating immunoglobulin and contain two antigen binding sites. IgM molecules are pentamers that can bind up to 10 antigens at the same time, and they are the first immunoglobulin to be synthesized the first time a host encounters a particular antigen. IgA molecules are dimers and are the main antibody secreted into body fluids such as mucous, saliva, and breastmilk. IgD is the form of immunoglobulin that attaches to the surface of B cells and acts as the receptor for antigens. IgE is the least common immunoglobulin, and it plays a role in the inflammatory response and in fighting infections by parasitic worms. IgE also is responsible for mediating allergic responses.

After antibodies are synthesized and secreted by B cells, they perform several different functions to aid in the destruction of the foreign invader. One main function is to fix complement, that is, to activate it by the classical pathway. This is an example of nonspecific and specific defenses working together. The antibody-antigen interaction is specific, but the same complement proteins form membrane attack complexes on any microorganism. Another function of antibodies is neutralization. When the antibody is bound to the antigen, the antigen cannot inflict further damage to the host. For example, if the antigen is a neurotoxin that binds to nerve cells and interferes with the normal transmission of neural impulses, when the antibody binds to the neurotoxin, the toxin cannot bind to the nerve cells. Another example would be neutralization of a virus. If the glycoprotein spike by which a virus attaches to a host cell is occupied by an antibody, then the virus cannot attach to nor penetrate the host cell. Antibodies can also opsonize, or coat a bacterial cell with antibodies to facilitate its recognition and engulfment by phagocytosis. The ability of antibodies to bind more than one antigen at a time allows them to crosslink cells or particles into large clumps or aggregates. This process is called agglutination, and it renders the microorganisms immobile and enhances their phagocytosis.

While the humoral branch is working to fight infection through the action of B cells, the cell-mediated branch is also functioning. Antigen presenting



Antibodies consist of two heavy chains and two light chains that combine to form two antigen binding sites.

cells (APCs) such as macrophages or dendritic cells express a class of molecules called the major histocompatibility complex (MHC) on their cell surfaces. Also called human leukocyte antigens (HLA), the glycoproteins control immune responses and also play a role in the rejection of transplanted tissues. After processing an antigen, the APC places it on its cell surface near an MHC marker. In order to be stimulated, a T cell must make cell-to-cell contact with the APC and recognize the antigen in conjunction with an MHC protein. Depending on the type of T cell that binds the antigen-MHC complex, the cell will differentiate into memory T cells, cytotoxic T (T_C) cells, or helper T (T_H) cells. T_C cells directly attack cells that express the specific antigen for which they are specific by secreting chemicals called perforins that punch holes in cell membranes and granzymes that enter through the holes and destroy proteins inside the target cell. T_C cells destroy virally infected host cells, cancer cells, or transplanted tissue cells in this manner. T_H cells can be divided into two categories: T_H1 and T_H2. T_H1 cells secrete cytokines that activate the cell-mediated immune pathway, and T_H2 cells secrete cytokines that stimulate proliferation of B cells.

Though the humoral and the cell-mediated branches of specific immunity play different roles in fighting infections, they work together to keep a host healthy. Antibodies are most effective against extracellular antigens, while cell-mediated responses are more effective against intracellular pathogens, but both contribute to the elimination of the pathogen. In the case of a viral infection, the cell-mediated branch can attack infected host cells to prevent the spread and further replication of the virus. Meanwhile, antibodies can bind and neutralize viral par-

ticles that are in the extracellular fluids to prevent attachment of the viral particles to healthy host cells and to opsonize them for phagocytosis.

In addition to specificity, memory is a hallmark of specific immunity. The memory cells of the humoral and the cell-mediated branches respond more quickly to subsequent exposures with a specific antigen. The primary response that follows the first exposure to a specific antigen involves a latent period, during which the antigen collects in lymphoid tissue and B cells become activated. As the number of plasma cells increases, the concentration of antibodies in the blood rises. IgM is produced first, followed by IgG. The next time the host encounters the same antigen, the memory cells act immediately, and IgG is the predominant class of antibody produced. The more vigorous secondary response, also called an anamnestic response, is characterized by an increased rate of antibody synthesis and length of antibody persistence.

Specific immunity is acquired naturally from exposure to an infectious agent in the course of normal life experience. Immunity is considered active when someone develops an immune response after coming into direct contact with a microorganism, or passive as happens during breastfeeding or when immunoglobulins cross the placenta. Artificial specific immunity occurs as a consequence of medical intervention. For example, vaccines, or immunizations, stimulate an artificial active immune response, and the administration of preformed immunoglobulins made by another person is considered artificial passive immunity.

See also ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS); ANATOMY; CIRCULATORY SYSTEM; IMMUNE SYSTEM DISORDERS; INFECTIOUS DISEASES; MICROBIOLOGY; PHYSIOLOGY; VACCINES.

FURTHER READING

Bowers, William, Abdul Ghaffar, and Gene Mayer. "Immunology." In *Microbiology and Immunology Online*. Edited by Richard Hunt. Department of Microbiology and Immunology, University of South Carolina School of Medicine. Available online. URL: http://pathmicro.med.sc.edu/book/immunol-sta.htm. Accessed on January 14, 2008.

Cruse, Julius M., and Robert E. Lewis. *Atlas of Immunology*. 2nd ed. Boca Raton, Fla.: CRC Press, 2004.

The Nobel Foundation. The Immune System—Overview. Available online. URL: http://nobelprize.org/educational_games/medicine/immunity/immune-overview.html. Accessed on January 14, 2008.

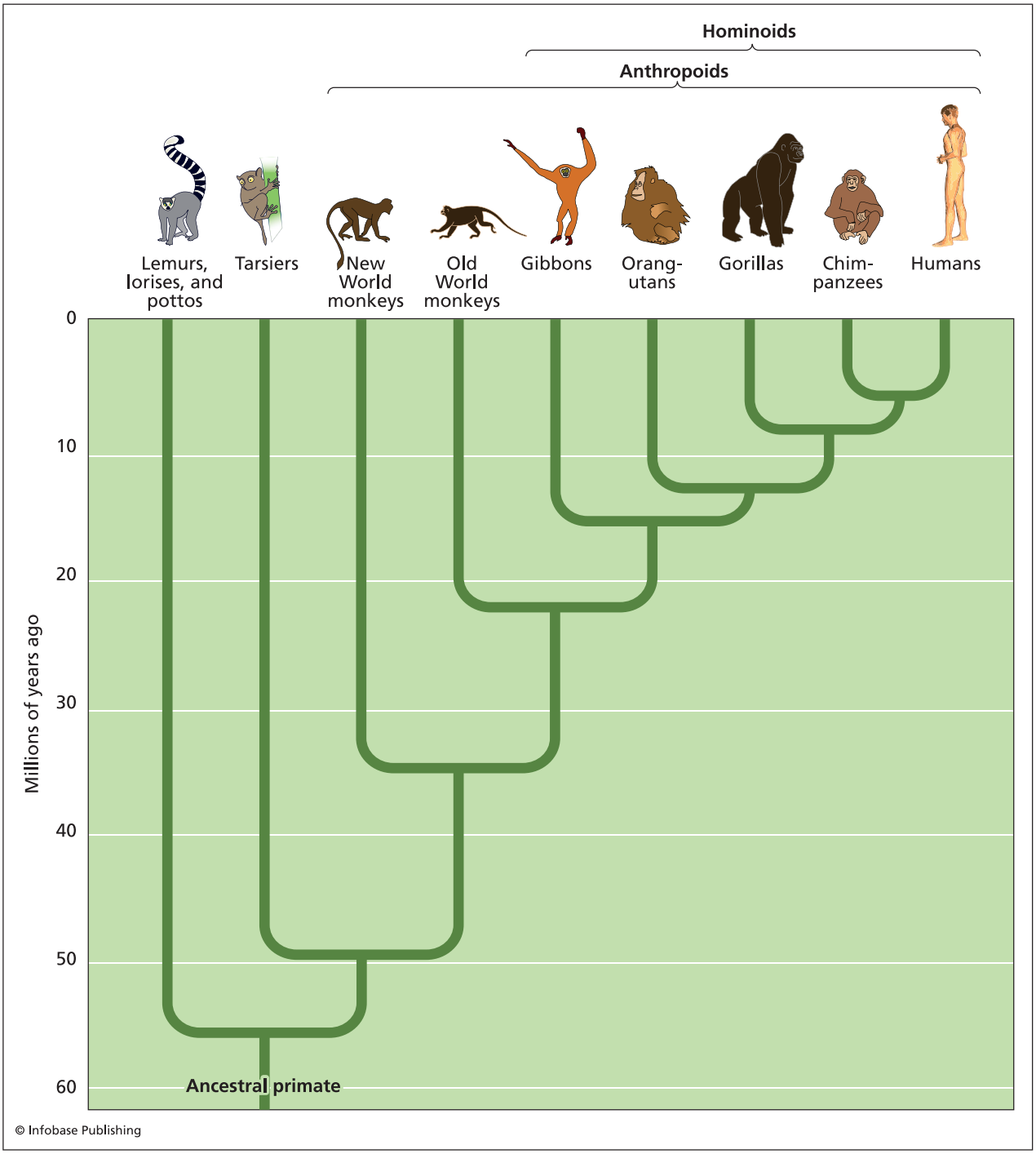
Roitt, Ivan M., Seamus J. Martin, Peter J. Delves, and Dennis Burton. *Roitt's Essential Immunology*. 11th ed. Malden, Mass.: Blackwell Science, 2006.

Wise, Darla J., and Gordon R. Carter. *Immunology: A Comprehensive Review*. Ames: Iowa State University Press, 2002.

human evolution In 1863 the British biologist Thomas Huxley described many similarities between humans and apes in his book *Evidence As to Man's Place in Nature*. Then in 1871 Charles Darwin published *The Descent of Man and Selection in Relation to Sex,* another seminal text that purported human beings shared a common ancestor with apes. Society took offense to the idea, calling the notion preposterous, and religious organizations battled against the implications, mainly that humans did not share a physical form with God, nor were they more special than the lowliest worm or any other living organism. Even today, many groups that have accepted the concept of evolution struggle with the fact that man is simply a primate. Like apes, monkeys, lemurs, and tarsiers, humans are mammals with appendages for grasping on all four limbs, fingernails in place of claws, enlarged cerebral hemispheres, eyes that point forward, and binocular vision with developed depth perception.

The scientific name for humans is *Homo sapiens,* belonging to the domain Eukarya, kingdom Animalia, phylum Chordata, class Mammalia, order Primates, and family Hominidae. Two suborders of primates include Strepsirrhini and Haplorrhini. Streptsirrhini includes the mostly arboreal, wet-nosed lemurs, aye-aye, lorises, pottos, and bush babies. Haplorrhini consists of the dry-nosed tarsiers, marmosets, New and Old World monkeys, gibbons, gorillas, chimpanzees, orangutans, and humans. The family Hominidae has four living genera and five species: *Gorilla,* which contains one species; *Pan,* which contains two species of chimpanzees; *Pongo,* which contains one species of orangutan; and *Homo,* which contains one species, *sapiens*.

Ancestors of the first primates were small mammals that had large eyes, sharp teeth, and ate insects. The earliest known undoubted primate fossils are 55 million years old. The development of hands and feet that could grasp allowed these earliest primates to hang on branches, cling to their mothers, and seize food. Binocular vision from forward-facing eyes improved depth perception, a marked advantage for leaping between branches or trees, where the earliest primates lived. Their ability to capture food efficiently and to escape predators quickly, in combination with their agility, evolved along with larger brains. Primates are also characterized by larger cerebral hemispheres and short jaws, and they exhibit complex social behavior and the ability to care for their young. All primates have five digits on the terminal portion of all four limbs, but only anthropoids have fully opposable thumbs, meaning they can touch the tip of each of the four fingers on one hand with the inner tip of the thumb on the same hand. (Humans have even more dexterity



Fossil evidence shows the first primates lived about 55 million years ago. The anthropoid lineage diverged about 50 million years ago, and hominoids between 20 and 25 million years ago.

than monkeys and apes due to modifications at the base of their thumbs.) The anthropoids can be further subdivided into two groups: Platyrrhini, which includes the New World monkeys, and Catarrhini, including the Old World monkeys, apes, and humans. From a common ancestral primate, the lemurs, lorises, and pottos diverged between 50 and 60 million years ago, and then the anthropoids diverged from what became the tarsiers shortly after that. Approximately 36 million years ago, many primate species became diurnal, meaning they were active and fed during the daytime, in contrast to their ancestors, which were mostly nocturnal, active at night.

Living anthropoids include the New World and Old World monkeys and the hominoids. The New

World monkeys are all tree-dwellers, many have prehensile tails that can be used for grasping and broadly spaced nostrils that open outward toward the sides. Old World monkeys live in trees and on the ground, do not have prehensile tails, and their nostrils point downward. The hominoids diverged from the Old World monkeys approximately 20 to 25 million years ago. Also called apes, the hominoids include gibbons, orangutans, gorillas, chimpanzees, and humans, all of which have no external tail. Except for humans, all dwell in trees at least part-time. The apes have flexible limbs, broad chests, mobile hips and ankles, and display more intelligence than other mammals.

As a species, humans, or *Homo sapiens*—the only primate not fully covered by hair—are only about 160,000 years old and are more closely related to chimpanzees than other hominoids. The genomes of chimpanzees and humans are approximately 99 percent identical. The differences between the extant species of *Homo sapiens* and chimpanzees began emerging long before the current species did. Fossil evidence, mostly from eastern and southern Africa, give proof of more than 20 extinct hominoids, more closely related to humans than chimpanzees. These extinct species are called hominids, and the oldest lived 6 to 7 million years ago. Paleoanthropologists, scientists who study human origins, can gain information about the species by considering the biogeography of fossil finds and examining the skeletal remains; for example, they can compare the position of the hole at the base of the skull where the spinal cord joins it, the structure of certain teeth, and the flatness of the face to distinguish species. Different features evolve at different rates. One shared characteristic of hominids is the ability to walk upright on two legs. *Homo sapiens* also have larger brains than other primates. Thus, bipedalism and brain size are two features paleoanthropologists consider when studying the evolution of humans.

New World monkeys, such as the long-haired spider monkey shown here, have flared nostrils and prehensile tails that they can use to hang from trees. *(Art Wolfe/Photo Researchers, Inc.)*

HOMINID EVOLUTION

One of two misconceptions about human origins is that humans evolved from chimpanzees. Humans and chimpanzees share a common evolutionary ancestor, whose descendents diverged and evolved alongside one another. The other major misconception is that humans represent the pinnacle of a series of hominid ancestors. At times, several hominids coexisted and coevolved; all the lineages, except one, became extinct. It should also be noted that traditionally, the term *hominid* has meant protohumans and humans, but its meaning is evolving to encompass other apes such as gorillas and orangutans. The term *hominin* now refers to the traditional human subset.

Many hominids that lived between 4 and 2 million years ago were already bipedal and had humanlike hands and teeth. These species are called australopiths and are members of the genus *Australopithecus*. Compared to other modern apes, australopiths had several distinguishing features: their spinal columns were S-shaped rather than C-shaped, the spinal column exited the skull at the base rather than the rear, the legs were longer than the arms, only the legs were used for walking, the pelvis was more bowl-shaped to hold the body's weight over its center, and the femurs angled inward to help carry the body's weight when walking. The australopiths ranged from 40 to 100 pounds (18 to 45 kg) and from 3.5 to 5 feet (1.1 to 1.5 m) tall, with brains about 24 to 34 cubic inches (about 400 to 550 cm^3) in volume. In comparison, modern humans have brains of approximately 82 cubic inches (1,350 cm^3). The most famous australopith is Lucy, a 3.24 million-year-old female skeleton discovered in Ethiopia in 1974 by the American Donald Johanson, species name *Australopithecus afarensis*. Because the skeleton was 40 percent complete, they were able to learn a lot from it. They determined that Lucy

Old World monkeys, such as the mandrill shown here, do not have prehensile tails, and their nostrils point downward. *(Millard H. Sharp/Photo Researchers, Inc.)*

Chimpanzees are more closely related to human beings than to other primates, such as gorillas. *(AP Images)*

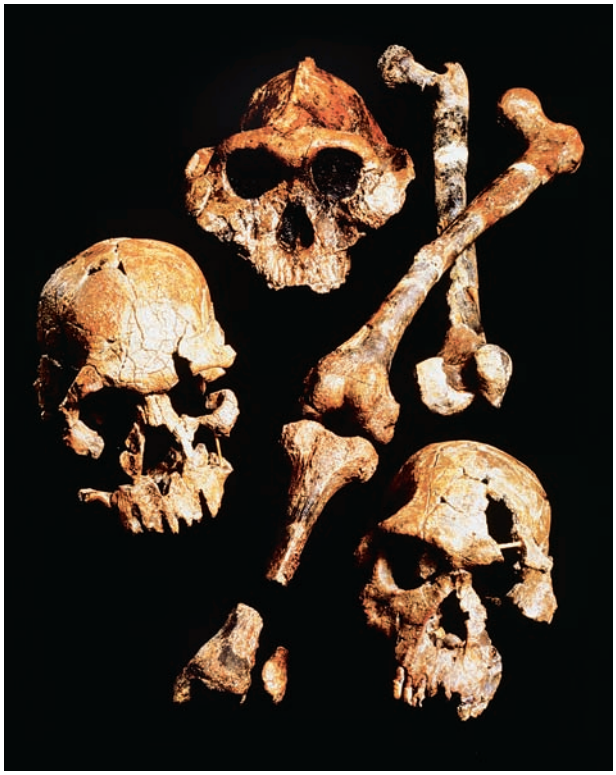
was approximately 3.28 feet (1 m) tall, had a head similar in size to a chimpanzee, a brain of approximately 30 cubic inches (500 cm^3), long arms, and a long lower jaw, but she was bipedal. In 1924 the Australian-born South African anthropologist Raymond Dart had discovered another *Australopithecus* species, *africanus,* in South Africa. The fossil skull he found had a flat face and an even row of teeth instead of long canine teeth. The opening of the skull to the spinal column pointed downward, suggesting this species stood upright. *Australopithecus afarensis* was probably ancestral to *Australopithecus africanus,* which had a slightly larger brain and was slightly taller.

More recently, in 2001 in Chad, a team led by the French paleontologist Michel Brunet discovered a nearly complete hominid cranium with an estimated age of 6 to 7 million years. The species, *Sahelanthropus tchadensis,* had small canine teeth, a short face, and prominent brow ridges, all hominid characteristics, but the brain was as small as a chimpanzee's.

A few hypotheses explaining the development of bipedalism exist. One proposes that adaptation began to favor moving over open ground as the climate became drier, forests shrank, and more savannas existed. Another suggests that selection for early hominids that could reach fruit hanging from trees played a role. Standing upright might have evolved as a means of defense. Whatever the explanation, walking erect, or bipedalism, is a major distinguishing characteristic of *Homo sapiens*. By about 1.9 million years ago, hominids began walking longer distances on two legs. As man became bipedal, he lost the ability to outrun predators and was forced to cooperate with other members of his species, in a tribe, to survive.

In addition to bipedalism, tool use is another human characteristic. Other hominids also use tools, such as sticks to stir up insect nests or rocks to break open certain foods, but obviously, none show as sophisticated an ability as humans in designing and using tools, an ability that developed in conjunction with increased brain size. The oldest evidence of hominid tool use—believed to be 2.5 million years old—consists of scratch marks on animal bones from using a stone or rock to cut the flesh off bone.

The human genus *Homo,* which probably diverged from *Australopithecus,* originated between 2.4 and 1.6 million years ago in South and East Africa. The British archaeologist Louis Leakey discovered the first remains of *Homo habilis,* the first fully erect known hominid, in the early 1960s. *Homo habilis* possessed brains larger than australopiths, approximately 39 cubic inches (640 cm^3) their jaws were shorter, and tools have been found near their remains, hence the species name *habilis,* which



These 1.5-million-year-old hominid fossils, found by Richard Leakey in Kenya, show that *Homo habilis* (center skull), *Australopithecus africanus* (bottom skull), and *Australopithecus robustus boisei* (top skull) coexisted. *(John Reader/Photo Researchers, Inc.)*

means handy. They averaged 4.2 feet (1.27 m) tall and weighed about 100 pounds (45 kg). Brains from *Homo ergaster,* which appeared from 1.9 to 1.6 million years ago, were even larger, and their legs and hips better suited for walking. The teeth were smaller, and they used more tools. Also, the difference in sizes between male and females was reduced, indicating more paired social interactions than in *Homo habilis*. Some *Homo ergaster* fossils were once thought to be members of the *Homo erectus* species, which lived between 1.9 million years ago and 50,000 years ago. Some scientists consider *Homo ergaster* an early subspecies of *Homo erectus,* which was larger, approximately 5 to 5.5 feet (1.5–1.68 m) tall and had a brain size of about 60 cubic inches (about 1,000 cm^3), intermediate in size between *Homo habilis* and present-day humans, and which had large brow ridges and smaller teeth than *Homo habilis*. Anthropologists believe *Homo erectus* lived in tribes of about 20 to 50 individuals, hunted, used fire, and built a variety of tools. They migrated from Africa to Asia and Europe.

In existence from 350,000 to 30,000 years ago, *Homo neanderthalensis,* commonly known as Neanderthals because their fossils were first discovered in 1856 in the Neander Valley in Germany, were once believed to be the link between *Homo erectus* and *Homo sapiens,* the only living species of the *Homo* genus. Their brains were as large as present-day humans. Now scientists believe Neanderthals descended from *Homo heidelbergensis,* which first appeared 600,000 years ago. Analysis of deoxyribonucleic acid (DNA) extracted from Neanderthal fossil remains showed that present-day humans do not share a common ancestor with Neanderthals from Europe or the near East. So what lineage gave rise to *Homo sapiens*? DNA and fossil evidence suggests that humans originated in Africa, probably from *Homo heidelbergensis,* which could have descended from either *Homo erectus or Homo ergaster*. The oldest known *Homo sapiens* specimen was found in Ethiopia and is believed to be 160,000 years old. All living human beings are believed to have originated from a central African lineage, as evidenced by molecular studies. In 2004 Peter Brown from the University of New England in New South Wales, Australia, and Thomas Sutika from the Indonesian Centre for Archaeology, together with other colleagues, discovered in Indonesia a fossilized skeleton of a new species that coexisted with *Homo sapiens, Homo floresiensis,* estimated to be 18,000 years old. *Homo floresiensis* was much shorter and had a smaller brain than humans, but structural similarities show it also descended from *Homo erectus*.

See also Darwin, Charles; evolutionary biology; evolution, theory of.

FURTHER READING

Crow, Tim J., ed. *The Speciation of Modern* Homo sapiens. Oxford: Oxford University Press, 2002.

Darwin, Charles. *The Descent of Man, and Selection in Relation to Sex*. With an introduction by John Tyler Bonner and Robert M. May. Princeton, N.J.: Princeton University Press, 1981.

Scientific American Special Edition. Becoming Human: Evolution and the Rise of Intelligence 16, no. 2 (June 2006).

Scientific American Special Edition. New Look at Human Evolution 13, no. 2 (July 2003).

Human Genome Project The Human Genome Project was an international effort that commenced in 1990, coordinated by the U.S. Department of Energy (DOE) and the National Institutes of Health (NIH). The goals were to identify all of the genes in the human genome, determine the complete sequence of the 3 billion nucleotide base pairs, store the information in databases, improve tools for analyzing the data, transfer the technologies to the private sector,

and address the related ethical, legal, and social issues. DOE also supports the Microbial Genome Program, whose goals overlap and complement those of the Human Genome Project. Synergies in technological development and knowledge gained from data obtained in each program benefit the other and has accelerated progress in both. The planned timeline for the Human Genome Project was 15 years, but due to advances in technology the project was successfully completed in 2003.

BACKGROUND

A genome consists of all the deoxyribonucleic acid (DNA) in an organism. DNA is the molecular carrier of genes, the functional units of heredity that determine the characteristics of an organism specific to that individual and to that species. The genes contain the information necessary for the organism to grow, metabolize, reproduce, and carry out other necessary functions of life. Individuals of the same species have all of the same genes, but they may have different forms of the genes. DNA consists of four nucleotides, abbreviated A, C, G, and T, depending on the base each contains. The sequence of these nucleotides within a gene determines the composition and order of the amino acids used to build the protein encoded by that gene. In addition to coding genes, genomes contain noncoding sequences, some of which have known functions and some that have no known function. The DNA is organized into chromosomes. Most prokaryotic organisms have only one single, closed circular chromosome, but the number of chromosomes in eukaryotic organisms varies between species. The number of chromosomes, the number of genes, the total number of nucleotide base pairs, and the sequence of the nucleotides are all characteristics used to describe the genome of an organism. The human genome consists of 24 different chromosomes—two sex chromosomes, called the X and the Y chromosomes, and 22 other chromosomes called autosomes. The normal chromosomal makeup of an individual consists of 22 pairs of autosomes, with one of each pair from the mother and one of each pair from the father, and two sex chromosomes, for a total of 46 chromosomes. Females have two X chromosomes, and males have one X and one Y chromosome.

METHODS

Sequencing the human genome involved numerous complicated and technologically challenging steps, but the emergence of bioinformatics, that is, the use of computers to acquire, store, organize, and analyze biological information, greatly facilitated the process. The main method used was whole-genome shotgun sequencing, developed by J. Craig Venter of the Institute for Genome Research and first utilized in 1994 by Venter and microbiologist Hamilton O. Smith to sequence *Haemophilus influenzae*, a bacterium that causes ear and respiratory tract infections. The 24 different human chromosomes first had to be broken into smaller pieces ranging in size from two to 40 kilobases. These fragments were subcloned into plasmids maintained in bacteria. The sequencing step itself was performed by using the cloned fragments as substrates in biochemical reactions that generate a set of fragments that differ in length by a single nucleotide. Gel electrophoresis separates the fragments based on differences in size, and fluorescent dyes indicate the nature of the last nucleotide base of each fragment synthesized during the sequencing reactions. The order of the colored bands (yellow, blue, green, and red) on the finished gel represents the sequence of the nucleotides in the chromosomal fragment. The size limit for electrophoretic separation is about 500 to 700 bases long. An automated sequencer can read the sequence from the colors. Computers then compile the sequenced fragments by finding overlapping regions and assembling them to form one linear sequence. The 24 chromosomes ranged in length from 50 to 25 million nucleotides each, thus this was a tedious process. Researchers completed sequencing and compiling a draft of the entire human genome in June 2000, and a high-quality reference sequence in 2003. In May 2006 the last of even more detailed sequences was completed.

The next step, still in progress, is to examine the 3 billion nucleotides in order to identify all the genes that encode proteins. Computers search the genome for open reading frames, those that could potentially encode proteins based on the presence of specific sequences known to be necessary for the initiation and termination of translation, the synthesis of polypeptides from mRNA transcripts, to occur. While effective for bacterial genomes, this step is complicated in eukaryotic genomes by the presence of noncoding introns interspersed within the coding regions of genes. Newer software programs utilize additional strategies to increase the effectiveness of this process. After a gene is located, the next step is to determine the function of that gene. Traditional methods such as mutational analysis and linkage mapping complements newer useful methods that rely on bioinformatics technology to locate similar genes with known functions, or regions with similar patterns (motifs) that have known common functions, such as binding to DNA or signaling secretion.

OUTCOME

The completed Human Genome Project has revealed many facts.

- The complete human genome contains slightly more than 3 billion nucleotides.
- The average gene consists of 3,000 bases.
- The largest gene, which encodes the protein dystrophin, is 2.4 million bases long (longer than many prokaryotic genomes).
- Scientists have found 1.4 million locations where single-nucleotide differences exist; translated, this means that 99.9 percent of all the bases are identical between individuals.
- Chromosome 1 contains more genes (2,968) than any other.
- The Y chromosome contains the fewest (231).
- One unexpected finding from the Human Genome Project is that the human genome only contains between 20,000 and 25,000 genes, encoded by less than 2 percent of the genome. (Scientists originally estimated humans had between 80,000 and 140,000 genes.) The function is unknown for about half of the identified genes.
- Genes occur in clusters on the chromosomes, with extended lengths of noncoding regions in between.
- The overwhelming majority of the human genome is noncoding; repetitive sequences that do not encode anything make up about 50 percent of the genome.
- Some of the noncoding regions perform useful functions such as stabilizing the chromosomes or regulating the expression of the structural genes, but the function of most of the noncoding DNA, if there is one, is not known.

Knowledge gained from the Human Genome Project will benefit many disciplines and have many applications. The potential individual differences in sequence at the 1.4 million locations throughout the genome will help pinpoint genes associated with specific diseases and genetic disorders. Medical researchers have already identified genes associated with numerous conditions, allowing them to detect mutations that lead to the diseased condition, diagnose predispositions to genetic disorders, and develop new drugs. Someday gene therapies might successfully treat or cure these conditions. A better understanding of the human genome will also help scientists better assess and reduce the risks associated with exposure to radiation and harmful chemicals. Comparative analyses of the sequences will reveal information about evolutionary lineages and the migration patterns of human populations throughout history, as well as help to determine the function of numerous genes whose workings have not yet been identified. Forensic investigations will benefit from more precise identification of suspects, people wrongly accused of crimes, and crime and catastrophe victims who cannot be identified by the traditional means of fingerprinting or dental X-rays. The advancements in technology made possible by the Human Genome Project will improve the genomic research of other organisms whose applications are far-reaching. Microbial genomics research can be applied to the production of biofuels, removing pollutants from the environment and industrial processing and reducing toxic wastes. Genomics can benefit agriculture by making possible the creation of disease- and drought-resistant crops, creating healthier animals that give higher product yields, increasing the nutritional value of produce, controlling pests that destroy crops, and producing edible vaccines that are incorporated into food products.

In December 2007 the NIH launched the Human Microbiome Project, an effort to sequence the genomes of the microorganisms that inhabit the human body. In addition to collecting the sequences of the microbes, researchers will examine and describe the microbial communities from five regions of the human body:

- the mouth,
- the nose,
- the skin,
- the digestive tract,
- and the female urogenital tract.

It is hoped that the information will reveal how changes in microbial communities at different body sites may be linked to human health and chronic diseases such as diabetes, obesity, and asthma. Because the microorganisms coexist in communities and their gene products interact, a newer technique called metagenomic sequencing will be used; rather than isolate and sequence the DNA from each species individually, the collective sequences of all the microbial genomes in a sample will be determined simultaneously.

See also CHROMOSOMES; CLONING OF DNA; DEOXYRIBONUCLEIC ACID (DNA); DNA SEQUENCING; GENE EXPRESSION; GENOMES.

FURTHER READING

Toriello, James. *The Human Genome Project*. New York: Rosen, 2003.

U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program. Human Genome Project Information. Available online. URL: http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml. Last modified on October 15, 2008.

Wexler, Barbara. *Genetics and Genetic Engineering*. Farmington Hills, Mich.: Thomson Gale, 2006.

human reproduction Human beings are sexually dimorphic, meaning separate male and female forms exist. Males produce haploid gametes called spermatozoa (sperm), and females produce ova (eggs). During sexual intercourse, the man deposits sperm into the female reproductive tract. If one sperm cell finds and penetrates an egg cell, a zygote is formed. The zygote develops into an embryo, then a fetus, and, after nine months, a baby is born.

Establishment of an individual's genetic or chromosomal sex occurs at the moment of fertilization, when a spermatozoon penetrates an egg cell. Normal diploid human cells have 46 chromosomes, 22 pairs of autosomes (nonsex chromosomes) and one pair of sex chromosomes. The sex chromosomes are called the X and the Y chromosomes, and the combination of these determines gender. Females can contribute only X's when making egg cells, and males can contribute either an X or a Y. If a sperm cell containing an X chromosome fertilizes an egg, the offspring will be XX, a chromosomal female. If a sperm cell containing a Y chromosome fertilizes an egg cell, the offspring will be XY, a chromosomal male.

Biological sex refers to the type of specialized sex organs a person possesses: ovaries for females or testes for males. The physiological decision that determines the biological sex occurs during early embryonic development at approximately the seventh week of gestation, when the specialized sex organs develop. In most situations a person's chromosomal and biological sex match, but chromosomal aberrations or molecular mutations can result in discordance. Determination of a newborn's gender depends on the presence of characteristic external genitalia at birth, but humans do not sexually mature until adolescence. During puberty, a person completes the transition from sexual immaturity to becoming a fertile adult. Secondary sexual characteristics appear, the external genitalia enlarge and develop, and, internally, the gonads mature and begin to secrete hormones that complete the process of sexual maturation and produce gametes capable of participating in fertilization. Adolescent females experience menarche, the onset of menstrual cycling, in preparation for a possible pregnancy.

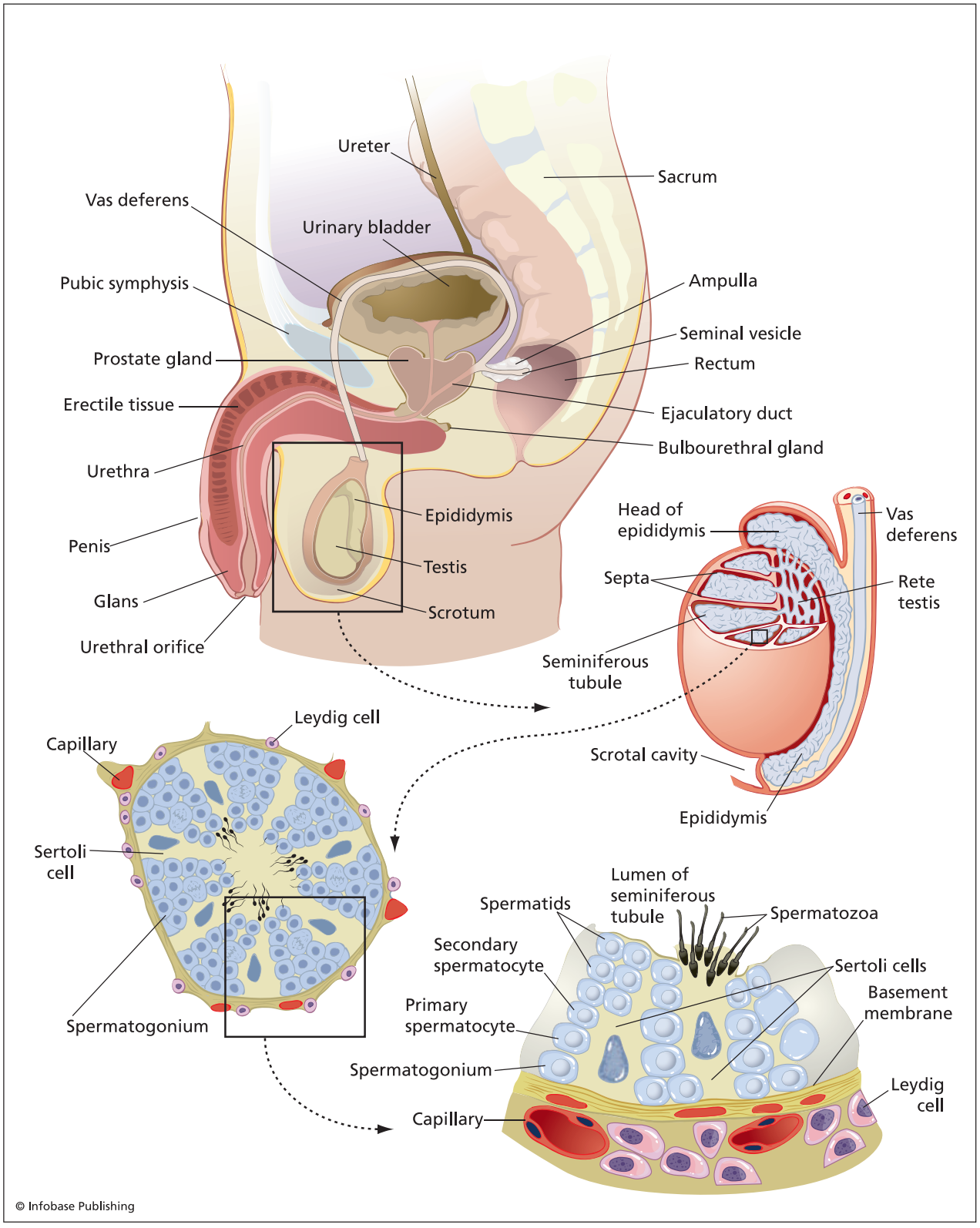
MALE REPRODUCTIVE SYSTEM

A sexually mature, fertile male must produce viable gametes, sperm cells, and successfully deliver them into the female reproductive tract. The male reproductive structures include the testes, the epididymides (singular, epididymis), vas deferentia (singular, vas deferens), urethra, seminal vesicles, prostate gland, bulbourethral glands, scrotum, and penis. The primary sex organs in males are the testes, or testicles, a pair of egg-shaped organs located in an external sac called the scrotum. The testes produce steroid hormones such as testosterone and make sperm. Testosterone is the main male sex hormone that stimulates the development of male secondary sexual characteristics, such as increased muscle mass, the growth of facial and pubic hair, and a deeper voice, and maintains testicular function after puberty. The production of sperm requires temperatures around 93°F (33.9°C), a fewer degrees lower than normal body temperature of 98.6°F (37°C). Because of this, near the time of birth, the testes descend from the pelvic cavity into the scrotal sac where they are kept slightly cooler than inside the body. Under cold conditions, contraction of muscles around the scrotal sac draws the testes closer to the body to warm them and to decrease the surface area through which heat dissipates.

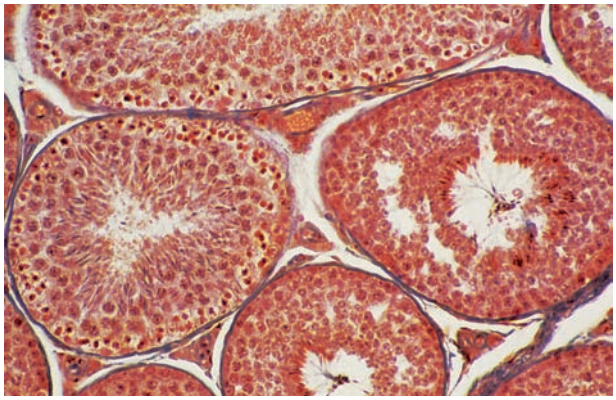
Each testis consists of approximately 250 lobules or compartments divided by septa made of connective tissue. Each contains one to three convoluted seminiferous tubules, the site for sperm production, a process called spermatogenesis. Stretched out, a single seminiferous tubule measures between 12 and 28 inches (30 and 70 cm) in length. The epithelial lining of the tubules contains two types of cells: Sertoli cells and developing sperm cells. Sertoli cells provide nourishment for the germ cells, play a role in the hormonal control of spermatogenesis, and phagocytose degraded germ cells. The base of a Sertoli cell lies against the basement membrane, and the cell extends outward toward the center of the tubule. The heads of maturing sperm are embedded in Sertoli cells, while the tails hang in the lumen of the tubule. Leydig cells, or interstitial cells, are the cells in the testes that produce steroid hormones. They are positioned within the lobules in the spaces between the seminiferous tubules, along with blood vessels.

Spermatogenesis occurs inside the lining of the seminiferous tubules. The process begins with spermatogonia, undifferentiated germ cells that constantly undergo mitosis, cell division of a diploid parent cell to form two diploid daughter cells. During mitosis, each chromosome duplicates itself and one member of each identical pair passes to a daughter cell, resulting in progeny identical to the parent cell; thus, mitosis constantly replenishes the supply of spermatogonia. Spermatogenesis involves meiosis, a two-staged form of cell division that results in the formation of haploid daughter cells, cells that only contain half of the diploid number of chromosomes and that can fuse with a gamete made by the opposite sex to form a diploid zygote during fertilization. Spermatogonial cells that have begun meiotic division are called primary spermatocytes. During the

first stage of meiosis, one spermatogonium gives rise to two haploid secondary spermatocytes, but the chromosomes are still duplicated. In other words, only 23 chromosomes are present in each cell, but each chromosome is still linked physically to an identical copy of itself. The second meiotic division



The male reproductive system consists of sex organs, ducts, and accessory glands that function to produce sperm and deliver them to the female reproductive tract.



This cross section of a human testicle reveals numerous seminiferous tubules containing sperm cells at different stages of development. *(Andrew Syred/ Photo Researchers, Inc.)*

separates the identical copies of each chromosome, resulting in four haploid spermatids. The spermatids then undergo spermiogenesis, a process by which they differentiate into mature sperm. In humans, the complete transformation of one spermatogonial cell into four mature sperm cells takes about 64–74 days, and every day, the testes produce approximately 200 million new sperm. When viewed in cross section, one can observe the progression of stages of spermatogenesis and spermiogenesis that occurs beginning at the basement membrane of the tubule and looking inward toward the center or lumen of the seminiferous tubule.

A mature sperm cell has three parts, a head, a middle section, and a tail. The head contains the nucleus holding the 23 chromosomes. A cap called the acrosome, a pocket full of enzymes that aids in the penetration of an egg cell during fertilization, is present on one end of the head. The midsection connects the head to the tail and houses mitochondria that supply energy needed for movement. The tail, or flagellum, of the sperm contains microtubules that slide past one another, causing a wavelike motion that propels the sperm forward.

After their release into the lumen of the seminiferous tubules, mature sperm travel to the rete testis, a tubular network that empties into the efferent ductules, ciliated tubules that lead out of the testis. The epididymis is a comma-shaped structure located on the posterior side of the testis that stores sperm and secretes substances that help the sperm mature further. The vas deferens, a long tubular structure, emerges from the end of each epididymis, extends 18 inches (45 cm) into the abdominal cavity, loops over the bladder, and enlarges to form an ampulla, a reservoir for the sperm. The ampulla leads to a short ejaculatory duct that connects to the urethra, which extends approximately eight inches (about 20 cm) from the urinary bladder to the end of the penis. Both urine and semen pass through the urethra to exit the body, but not at the same time. A mechanism exists that blocks the flow of urine during ejaculation, when rhythmic contractions force sperm out of the urethral opening.

During sperm transport, different sex accessory glands secrete substances that make up the seminal plasma, the fluid that carries the sperm. The majority of the volume of seminal plasma comes from paired seminal vesicles that lie at the base of the bladder and secrete a thick alkaline fluid containing the sugar fructose. Short ejaculatory ducts carry contents from the seminal vesicles to the ampulla of the vas deferens. The prostate gland is a doughnut-shaped structure that is located below the bladder and surrounds the upper portion of the urethra. Alkaline fluid flows from the prostate through several tiny ducts that empty into the urethra. Another pair of glands, the pea-sized bulbourethral glands (also called Cowper's glands), lie at the base of the penis and secrete mucous that lubricates the urethra, facilitating the flow of semen during ejaculation.

The penis is the copulatory organ of the male, meaning it functions in getting the sperm from the duct system of the male reproductive tract into the female reproductive tract, a requirement for internal fertilization. Three columns of erectile tissue run along the length of the penis, in addition to blood vessels and nerves. The two columns on the sides of the penis are called corpora cavernosa, and the third column, the corpus spongiosum surrounds the urethra on the ventral side. At the end of the penis, the corpus spongiosum forms a cap, the glans penis, that is covered by the prepuce, or foreskin. Circumcision is the surgical removal of the foreskin, a practice usually performed for religious or cultural rather than medical reasons.

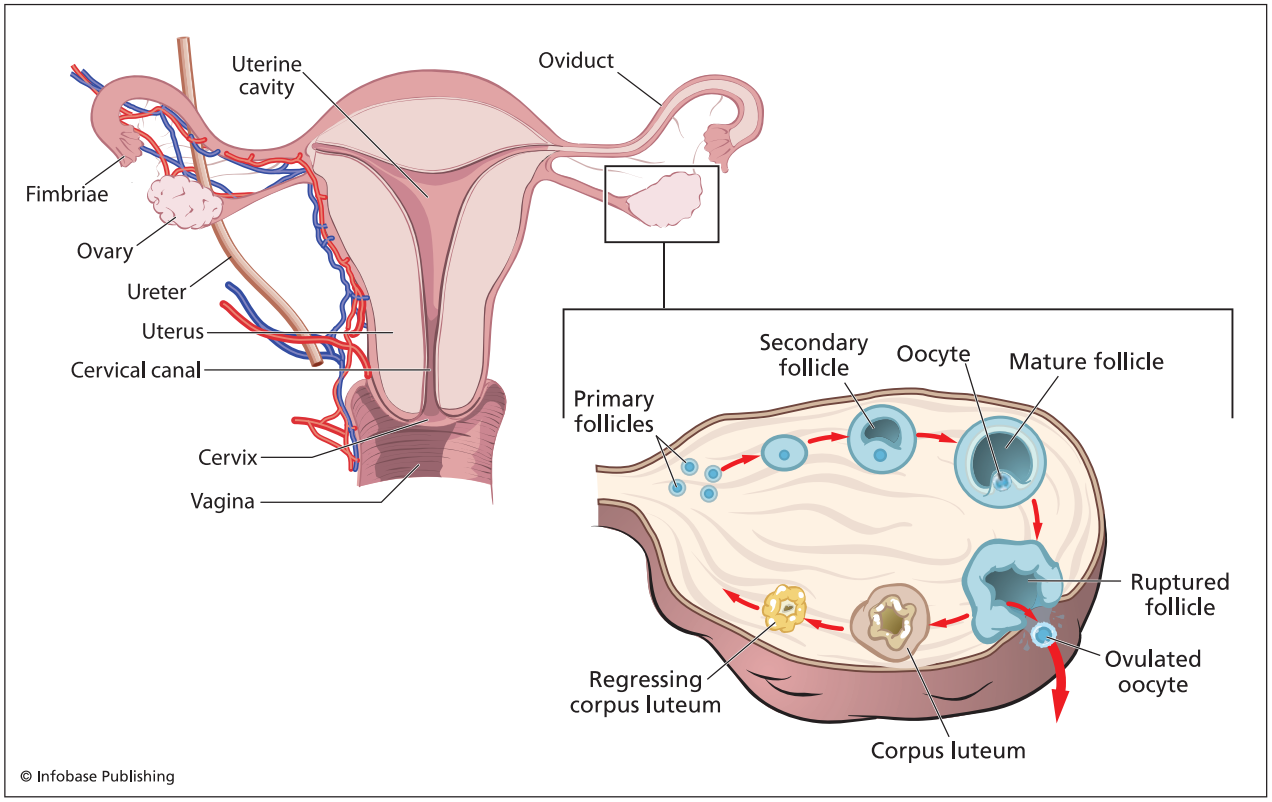
When an adult male becomes sexually aroused, the spongy erectile tissue fills with blood, becoming engorged and stiffening the penis. The purpose of an erection is to allow the male to insert his penis into the female vagina during intercourse. Continued stimulation leads to emission, the accumulation of sperm and seminal plasma in the urethra. Peristalsis, rhythmic waves of contractions of smooth muscles surrounding the reproductive ducts, transports the sperm, which is expelled from the distal end of the penis during ejaculation. Approximately 300–400 million sperm cells in about 0.7 teaspoons (3.5 mL) of semen are ejected during ejaculation, but only one sperm cell is necessary for conception to occur. Once a male has deposited his sperm into the female reproductive tract, his role in reproduction is complete.

FEMALE REPRODUCTIVE SYSTEM

The female reproductive system must not only make gametes, but also house the sperm for up to two days, transport the early embryo to the uterus, implant it, nourish the embryo and fetus during the nine months of pregnancy, deliver the baby, and produce and secrete milk to feed the newborn. The female reproductive organs include the ovaries, uterine tubes, uterus, vagina, external genitalia, and mammary glands. A group of ligaments supports the internal abdominal reproductive organs and holds them in place. Ovaries are the primary reproductive organs in females. The paired structures, located in the pelvic cavity on either side of the uterus, produce ova and secrete the female steroid sex hormones estrogen and progesterone. A thin surface epithelium covers the ovarian cortex, the portion of the ovary that is made of dense connective tissue and contains the primary germ cells, the oogonia. Internal to the cortex is the ovarian medulla, the region containing the blood vessels, lymphatic vessels, and nerves.

Oogenesis, the production of female gametes, begins during fetal development. After initiating meiosis, an oogonium becomes a primary oocyte. Meiosis halts in the middle of the first meiotic division and does not resume until after puberty. In order to be fertilized, the immature germ cell must resume oogenesis and be released into the female reproductive tract. Following sexual maturation, the process of ovulation, the release of a mature ovum from an ovary, occurs approximately once per month. Just prior to ovulation, the primary oocyte completes the first meiotic division, resulting in a haploid secondary oocyte and a polar body. The majority of the cytoplasm, including organelles such as the mitochondria, remains with the secondary oocyte, and the polar body acts mainly as a repository for extra genetic material. The polar body either degenerates or completes the second stage of meiosis, forming two polar bodies. The secondary oocyte initiates the second meiotic division but stops before the process is complete until fertilization occurs.

A layer of granulosa cells surrounds the primary oocytes, forming structures called primordial follicles that lie around the edge of the ovarian cortex. After puberty, some of the primordial follicles develop into primary follicles, and, on average, every 28 days some of the primary follicles mature into secondary follicles. At this stage, the follicles contain a large, fluid-filled antrum, and the oocyte rests to one



The female reproductive system produces egg cells, provides a nourishing environment for a developing fetus, and delivers the baby at the end of gestation.

side within a mass of granulosa cells called cumulus cells. A clear membrane called the zona pellucida lies between the oocyte and the innermost layer of granulosa cells called the corona radiata. The theca, the capsule that surrounds the follicle, contains the cells that produce ovarian hormones.

Female babies are born with all the immature germ cells they will ever have. By the fourth month of development, the ovaries contain about 5 million primary germ cells, or oogonia. The number of follicles decreases to about 2 million prior to birth, and about 200,000–400,000 by puberty. Only 400 will ever mature into ova. The process of follicular degeneration is called atresia, and it can occur at any stage of follicular development. The most mature follicles, Graafian follicles, protrude as lumps from the ovary. Only one normally reaches this stage each month. During ovulation, the follicle ruptures, releasing the oocyte and its surrounding mass of cumulus cells into the abdominal cavity. If more than one follicle fully matures simultaneously, more than one can be fertilized, and a multiple birth can result.

The infundibulum, a funnel-like structure at the opening of each oviduct, also called fallopian tube or uterine tube, surrounds a large portion of the ovary and accepts the ovum at the ostium, the opening. Extensions called fimbriae embrace the ovaries and help ensure that the egg actually enters the uterine tubes rather than remain loose in the peritoneal cavity. Muscular tissue that encircles the oviducts contracts to help move the ovum toward the uterus. In addition, the internal lining of the oviducts secretes mucous and has cilia that beat to aid in transport. Fertilization, the fusion of a haploid sperm cell with a haploid egg cell to form a diploid zygote, normally occurs in the oviducts. The zygote begins rapidly dividing by mitosis, while continuing to travel toward the uterus.

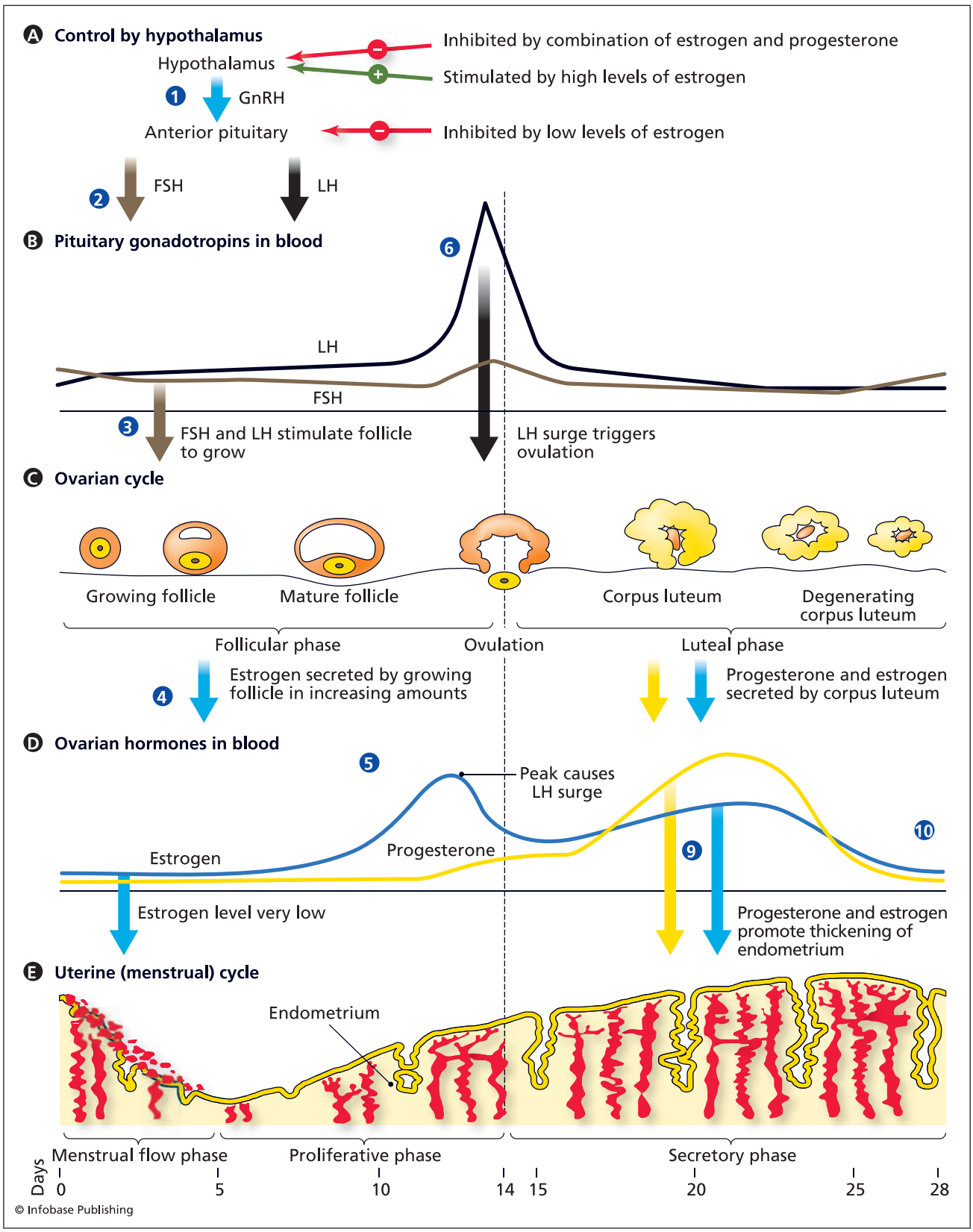
The uterus is a thick, muscular organ with dimensions of approximately 3 × 2 × 1 inches (7.5 × 5 × 1.75 cm). The uterine fundus is the region where the oviducts join on either side near the top. The cervix is a narrower region adjacent to the vagina, the canal that leads to the vestibule, the opening to the exterior environment. The uterus consists of three layers of tissue: the thin outer membrane called the perimetrium, the thick layer of smooth muscle called the myometrium, and the innermost layer, the endometrium, in which a developing embryo implants. In nonpregnant women, the structure of the endometrium changes in response to hormonal cues, leading to a cyclical series of monthly physiological changes, the menstrual cycle. Prior to ovulation, the endometrium proliferates in preparation for receiving a developing embryo, but, in the absence of pregnancy, the uterus discharges blood, secretions, and endometrial tissue in a process called menstruation, and a new cycle begins.

The vagina serves as a portal for the exit of menstrual discharge, as a receptacle for deposition of semen from the penis during sexual intercourse, and as part of the birth canal during parturition. The epithelial cells that line the inner region secrete mucous that lubricates the vagina. The muscular vaginal walls can stretch to accommodate the penis during coitus and during childbirth.

MENSTRUAL CYCLE

The ovaries and the uterus both play key roles in the female reproductive system, but to reproduce successfully, they need to work together to coordinate the timing of their functions. If ovulation occurred too close to menstruation, the uterine lining would not be prepared to receive an embryo for implantation. Hormones regulate the cycle of events that happen in the ovary, the ovarian cycle, with the menstrual (uterine) cycle.

The ovarian cycle has two phases, the follicular and the luteal phase. The cycle begins when the hypothalamus secretes gonadotropin releasing hormone (GnRH), which in turn stimulates the anterior pituitary gland to secrete small amounts of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). As its name suggests, FSH stimulates the development of up to 25 follicles in the ovary. The follicular cells are the cells responsible for the production and secretion of estrogen, so as the follicles grow, the circulating levels of estrogen increase. During most of the follicular phase, the levels of estrogen are low, and low levels of estrogen inhibit the anterior pituitary from releasing more FSH and LH. In addition, developing follicular cells secrete inhibin, a protein that inhibits FSH secretion. As the follicular cells secrete increasing levels of estrogen, the hypothalamus secretes more GnRH. In response, the levels of LH and FSH rise sharply, an event termed the LH surge. As follicular cells develop, their sensitivity to LH also increases due to an increase in the number of LH receptors. Usually, only one follicle fully matures to the Graafian stage. In response to the LH surge, the primary oocyte of the mature follicle completes the first meiotic division and a large fluid-filled sac develops in the follicle and finally ruptures, causing ovulation of the secondary oocyte about one day later. Ovulation occurs on or near day 14 of a 28-day cycle and marks the end of the follicular phase.



Hormones coordinate cyclical physiological events in the ovaries and the uterus in preparation for reproduction. These are referred to as the menstrual and ovarian cycles.

LH induces the transformation of the ruptured follicle into a corpus luteum, hence the name luteal phase for the second phase of the ovarian cycle. The remaining granulosa cells of the corpus luteun secrete increased levels of progesterone and some estrogen. The combined high levels of these two hormones inhibit the hypothalamic release of GnRH, and they also decrease the number of GnRH receptors in the anterior pituitary. Together, these events cause the levels of LH and FSH to decrease to very low levels. In the absence of pregnancy, the corpus luteum disintegrates, so the production of estrogen and progesterone declines sharply. The return of low levels of estrogen and progesterone remove the inhibitory effect of the combined high levels of those hormones on the hypothalamus, and GnRH is released. The anterior pituitary responds by releasing FSH, which stimulates the development of a new group of follicles in the ovary, completing the cycle and initiating another one.

Meanwhile, the uterus must prepare for possible implantation in case fertilization of the ovulated oocyte occurs. Estrogen and progesterone both affect physiological events in the uterus. The onset of menstruation (a woman's period) marks the first day of a new ovarian cycle and the menstrual flow phase of the uterine cycle. The increasing levels of estrogen produced by the developing follicular cells in the ovary cause the endometrial lining to proliferate, thus the next phase of the uterine cycle is called the proliferative phase. Cells rapidly divide to replace the cells lost during menstruation, forming spiral-shaped uterine glands. Spiral arteries supply a rich source of nutrients to the endometrial cells. After ovulation, when the corpus luteum forms, the progesterone it secretes induces the secretory phase of the uterine cycle, and the uterine glands thicken and start to secrete a fluid rich in the carbohydrate glycogen. By day 21 of the menstrual cycle, the uterine lining is suitable for accepting an embryo for implantation. In the absence of pregnancy, as the corpus luteum disintegrates and the levels of estrogen and progesterone both drop near the end of the luteal phase, the spiral arteries that supply the uterine glands with nutrients constrict. Without oxygen, the cells of the spiral glands die and the functional layer of the uterine lining sloughs off, and the menstrual fluid flows out of the uterus through the cervix and vagina. A new cycle begins.

PREGNANCY, BIRTH, AND LACTATION

After a male deposits sperm into the vagina of a female, the sperm swim through the cervix and uterus into the oviducts, sometimes aided by contractions of the uterus caused by prostaglandins in the semen and oxytocin released from the female posterior pituitary gland. The environment of the female reproductive tract is acidic, but the alkaline character of the semen keeps the sperm alive during their journey. Ejaculated sperm cannot penetrate the zona pellucida of the ovum until they undergo a process called capacitation, which is stimulated by conditions in the female reproductive tract. Acrosomal enzymes are released, allowing penetration of the cervical mucous, the cumulus mass cells, and the oocyte cell membrane. Sperm have 24 hours after ovulation to reach an egg, but sperm themselves can survive for up to six days in the female tract. After a sperm penetrates the secondary oocyte, the second meiotic division resumes, forming an ootid and another polar body. The nuclear contents of the sperm cell fuse with the nuclear contents of the ovum, regenerating the diploid state and forming a zygote. Biochemical changes prevent more than one sperm cell from fertilizing an ovum.

The zygote undergoes a series of rapid cell divisions called cleavage. Within one week after fertilization, the embryo has traveled as far as the uterus and has developed into a blastocyst, a sphere of cells with a hollow interior. The embryo settles into the endometrial lining, which is ideal for implantation during the secretory phase of the uterine cycle. The trophoblast, the outer layer of the embryo, secretes a hormone called human chrorionic gonadotropin that travels via blood circulation to the ovaries, where it acts to maintain the corpus luteum, which would start disintegrating in the absence of a pregnancy. The corpus luteum continues to secrete progesterone and estrogen, hormones necessary for maintenance of the uterine lining throughout the pregnancy, and the mother ceases to ovulate or have menstrual cycles until she is no longer pregnant. After the placenta develops, it begins to produce estrogen and progesterone, and, at about three months, it produces sufficient quantities of the hormones without assistance from the corpus luteum.

Gestation of a human fetus takes nine months, divided into three trimesters. After implantation, the endometrium grows over the blastocyst. Membranes that protect the developing baby also develop. The amnion encloses the entire embryo, and the chorion helps the trophoblast to form the placenta, a disk-shaped organ through which diffusion occurs. The placenta is very vascular, and without any direct mixing of maternal and fetal blood, oxygen and nutrients from maternal circulation diffuse out of maternal vessels into fetal vessels. Carbon dioxide and waste products diffuse out of fetal circulation, and maternal vessels carry them away. Other substances such as drugs, alcohol, and some pathogens can diffuse across the placenta and enter fetal circulation, thus the mother must be vigilant about her

health. An umbilical cord connects the placenta to the fetus. Two umbilical arteries bring fetal blood to the placenta, and one umbilical vein carries blood from the placenta back to the fetus. Organogenesis, the development of body organs, occurs mainly during the first trimester. By the end of the first trimester, the embryo is called a fetus.

During the second trimester, the fetus becomes distinctly recognizable as a human, and even develops fine structures such as fingernails and eyebrows. The uterus grows large enough to form an obvious protrusion from the lower abdomen. The corpus luteum disintegrates and the placenta completely assumes the job of producing progesterone.

The last trimester is characterized by rapid fetal weight gain. The large uterus compresses the mother's internal organs and can cause heartburn, constipation, frequent urination, and back pain. After approximately 40 weeks from the first day of the last menstrual period, the fetus is ready for life outside of the mother's womb.

The mechanisms that initiate labor are not fully understood but involve numerous hormones. Estrogen levels rise significantly during the last trimester and, as a result, the numbers of oxytocin receptors on the uterus increase. Made by the fetus and the mother's pituitary, the hormone oxytocin induces uterine contractions and stimulates the placenta to secrete prostaglandins, which also stimulate contractions. The stress associated with labor causes the release of even more oxytocin, forming a positive feedback loop that ends after birth, or parturition. Labor includes all the physical activities that lead up to birth. During the first stage of labor, the cervix effaces (thins out) and dilates (opens up) wide enough to allow passage of the baby. The uterus contracts in a rhythmic manner to force the baby down and out through the birth canal. The mother assists by bearing down to push the baby out. After the baby is born and the umbilical cord is cut, the mother still must deliver the placenta. Situations that are dangerous for the mother or the fetus sometimes

FETAL DEVELOPMENT

By the End of Month...	Length	Weight (Average)	Milestones
1	0.25 inch (0.64 cm)		heart, digestive system, backbone, and spinal cord form
2	1.13 inches (2.9 cm)		facial features and teeth forming, heart is functioning, embryo can move, penis appears in boys
3	2.5–3.0 inches (6.4–7.6 cm)	0.5–1.0 ounce (14–28 gm)	appendages all apparent, most organs and tissues are present, eyes almost fully developed, physician can hear heartbeat using a special instrument, nails and earlobes form
4	6.5–7.0 inches (16.5–18 cm)	6–7 ounces (170–198 gm)	develops sucking and swallowing reflexes, sex is identifiable, recognizably human but unable to survive outside womb, skin is pink and transparent
5	8–10 inches (20–25 cm)	1 pound (454 gm)	hair grows on head and lanugo on body, internal organs maturing, mother can feel the fetus move, eyebrows, eyelids, and eyelashes appear
6	11–14 inches (30–35.6 cm)	1.75–2 pounds (794–907 gm)	eyes can open, fetus hiccups occasionally, vernix (a waxy protective coating) covers skin
7	14–16 inches (35.6–40.6 cm)	2.5–3.5 pounds (1,134–1,588 gm)	organs continue to mature, fat layers form, skin is reddish and wrinkly
8	16.5–18 inches (41.9–5.7 cm)	4–6 pounds (1,814–2,722 gm)	rapid brain growth, significant weight gain, all body organs mature except for lungs, strong kicks, fingernails extend beyond fingertips
9	19–20 inches (48.3–50.8 cm)	7–7.5 pounds (3,175–3,402 gm)	lungs mature, baby is ready to live outside of womb

necessitate a cesarean section, the delivery of a fetus by surgical incision of the abdominal walls.

As mammals, human mothers have the ability to produce and secrete milk from their breast tissue. During pregnancy, the breasts enlarge, and the duct systems and secretory units of the breast tissue mature. The drop in progesterone levels following parturition leads to the secretion of the hormone prolactin from the anterior pituitary. Prolactin stimulates milk production by the mammary glands in the breasts, but it takes a few days after birth for milk production to begin. Until that time, the mammary glands secrete colostrum, a fluid rich in antibodies and nutrients. The antibodies help the newborn fight infection while his or her immune system is developing. When the baby suckles at the nipple, prolactin is released from the anterior pituitary and oxytocin is released from the posterior pituitary. Oxytocin stimulates the release of milk from the breast. Breast milk contains all the nutrients essential for a newborn during its first year of life. As long as a mother continues to breastfeed, the suckling will stimulate the continued production and secretion of more milk.

See also ANATOMY; ANIMAL FORM; ASSISTED REPRODUCTIVE TECHNOLOGY; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; ENDOCRINE SYSTEM; PHYSIOLOGY; REPRODUCTION; SEXUAL AND REPRODUCTIVE HEALTH.

FURTHER READING

Jones, Richard E., and Kristin H. Lopez. *Human Reproductive Biology*. 3rd ed. San Diego, Calif.: Academic Press, 2006.

Mader, Sylvia S. *Human Reproductive Biology*. 3rd ed. New York: McGraw Hill, 2004.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 2. Detroit, Mich.: U*X*L, 2000.

Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

Humboldt, Alexander von (1769–1859) German *Naturalist*

Science is often separated into several disciplines that seem related only because they all employ the scientific method. For example, biology is the science of living beings and life processes, and geology is the scientific study of Earth, its origins and evolution, the materials that compose it, and the processes that act on it. At first glance, the two disciplines may not seem related in scope, but they are. Earth's structure in a particular location affects the life-forms and the lifestyles of the organisms it supports. In return the life-forms may alter the structure or the physical and chemical composition of their habitat, particularly over long periods of time. A polymathic scientist and explorer named Alexander von Humboldt embraced a philosophy of unification of the natural sciences long before the rest of the scientific world. His aspiration was to discover the natural connections among all natural phenomena. He explored geographic regions that were practically unknown to the scientific world in search of natural laws that related organisms, landforms, and climate.

PLANS FOR A CAREER IN POLITICS OR MINING

Friedrich Wilhelm Karl Heinrich Alexander von Humboldt was born on September 14, 1769, in Berlin, the capital of the kingdom of Prussia. His father, Alexander Georg von Humboldt was an army officer and an aristocrat. His mother was Maria Elizabeth von Hollwege, a wealthy woman. As a child, Alexander was often sick and confined to his home. He especially appreciated the time he spent with his father strolling around and exploring nature at the family's countryside estate in Bradenburg, Schloss Tegel. Alexander's father died when he was only 10, leaving his mother in charge of his and his older brother Wilhelm's education. The boys were taught mostly by private tutors and studied the classics, history, languages, mathematics, politics, and economics. Their mother hoped they would obtain positions in the government when they grew up, but Alexander enjoyed botany and natural history and preferred to spend his free time collecting rocks and insects that he brought home to study and sketch.

Alexander's mother enrolled him at the University of Frankfurt an der Oder in 1787 to prepare him for a government career. In 1789 he enrolled at the University of Göttingen to study law, but he became more interested in other subjects, such as geology, mineralogy, and mining. One of his professors introduced him to Georg Forster, a scholar and explorer who had accompanied Captain James Cook on a Pacific Ocean voyage. In 1790 Forster brought Humboldt on a European tour down the Rhine and introduced him to many influential scientists. Forster's tales of travel fascinated Humboldt, who resolved to undertake his own adventures someday.

Following his mother's wishes, Humboldt enrolled in the Hamburg School of Commerce to study economics, business, and politics in the fall of 1790. However, a career in politics was his mother's desire, not his. To obtain the training necessary to

embark on a scientific career, Humboldt entered the Mining Academy at Freiberg in southern Germany in 1791. His mother approved, expecting him to work in civil service afterward. The curriculum was both physically and mentally rigorous. The mining students labored in the mines all morning and studied geology and mineralogy in the afternoon. After successfully completing his studies, Humboldt was offered the enviable position of inspector of the mines in 1792. In this position he established the first mine-laborer training school. He financed this project from his own pocket. In southern Prussia he actively participated in the mine inspections, giving him the opportunity to travel. He also collected plant specimens and mineral samples from deep within the mine shafts and tunnels. From these botanical pursuits, he wrote his first scientific piece on plant distribution, "Freiberg Flora," which won him a gold medal and recognition from scientists. Yet he did not ignore his obligations to Prussia's mining department. His efforts increased by sixfold the mineral output of mines thought to be depleted. As a result, he was offered a promotion to counselor of mines in 1794. Humboldt also performed research on what he called animal electricity (nerve conduction) around this time. In 1797 he published two volumes of 4,000 experiments, firmly establishing himself as a scientist. In 1795 he was offered the position of director of mines in Silesia, but he declined. He was making other plans.

EMBARKS ON EXOTIC EXPEDITION

Humboldt's mother died of breast cancer in November 1796, leaving him a large fortune. Without hesitation, he quit the mines and started planning his own scientific expedition. The Napoleonic Wars made travel difficult, so his plans were continually delayed. In 1799 Humboldt met up with Aimé Bonpland, a French physician and botanist. Because of Humboldt's reputation for mining successes, King Charles IV of Spain permitted him free travel on all royal vessels and sanctioned safe passage and exploration of Spain's Central and South American colonies in hopes that Humboldt would find gold and diamonds as he did in Prussia. This permission gave him access to exotic countries such as Cuba, Mexico, Venezuela, Colombia, Ecuador, Peru, Chile, Argentina, and the Philippines.

The men spent a few months preparing and gathering all the necessary supplies, instruments, notebooks, and equipment. They departed from La Coruña, Spain, on June 5, 1799, aboard the frigate *Pizzaro*. At the time, he recorded in his journal his hope to find and demonstrate a unity of nature, a relationship between all of the natural sciences. Humboldt wasted no time during the oceanic voyage. He immediately began collecting data on marine life and sea water chemistry and measuring air and ocean temperatures and the position of the Sun. When typhoid fever broke out on the ship, instead of arriving in Havana, the crew was forced to forgo Cuba and continued directly to their final destination of Cumaná, Venezuela, where they landed in early July.

Though the typhoid epidemic was unfortunate, landing at Cumaná was exhilarating for the two young explorers. From the moment they stepped off the ship they were thrilled at the abundant display of exotic tropical life. After spending three months attempting to classify all the unfamiliar flora and fauna, they penetrated the rain forest on the backs of mules. Again they were amazed at the greenness and the richness of the life that surrounded them. They collected as many biological specimens as they could pack on their mules. As Humboldt directed his energies toward the rocks and minerals of the region, he was relieved to find them similar to those in Europe. Damage from earthquakes gave evidence to the violent subterranean forces at work shaping the Earth, and Humboldt began to ponder the geological relationships between the continents.

In November 1799 Humboldt and Bonpland sailed for Caracas, the capital of Venezuela. On the way they witnessed an enormous meteor shower. In Caracas they rented a house for the duration of the rainy season and tried to organize the multitude of specimens already gathered. They catalogued them and wrote letters home detailing their explorations to this point. So far they had collected more than 1,600 plant specimens.

Rumors of a natural canal supposedly connecting the Orinoco and the Amazon river basins, called Casiquiare, caught Humboldt's interest. He wondered if it really existed. If so, it would be the only natural canal of its kind and would have not only scientific implications, but economic and political as well. Local Indians and missionaries confirmed the tales, and they pointed Humboldt in the right direction. He wanted to chart the waterway, and thus, in February 1800, Humboldt and Bonpland left the capital city and crossed the coastal mountain range called the Cordillera. On the way Humboldt noted the garnet crystals embedded within the mountain rocks. They then crossed the llanos, the tropical grasslands full of crocodiles, snakes, and cattle herds. This was not a leisurely tourist excursion, but a physically demanding, tiring journey.

While traveling, they stopped at the ranching station of Calabozo, where Humboldt was fascinated by electric eels. He wanted to examine an eel closely but could not get hold of one without receiving an electric shock. Humboldt accidentally stepped on an eel and suffered fierce pain in his knees and joints

for the remainder of the day. To assist him, the local Indians corralled horses and mules and drove them into a swamp full of the eels. The poor animals were repeatedly stung until the eels ran out of juice, facilitating its capture and rigorous analysis.

By the end of March 1800, they had traveled 108 miles (174 km) and reached the end of the llanos. In San Fernando, on the banks of one of the Orinoco's tributaries, the Apure River, they were joined by a Spaniard named Nicolás Soto. The abundant wildlife continued to amaze the explorers. Flamingoes, crocodiles, spoonbills, monkeys, jaguars, and capybaras (rodents the size of large dogs) greeted them along the riverside. Based on information they obtained from the locals, they headed onward to the Orinoco with a group of hired canoe rowers. En route, Humboldt analyzed the waters and noted geological landmarks. Along the Orinoco they stopped at an island where hundreds of Indians were waiting to harvest turtle eggs from which they could extract oil. Humboldt estimated there were 30 million eggs buried in the sand.

Natives who heard about the European explorers' travel plans warned them against proceeding. They told them tales of one-headed monsters and other monstrosities. Despite this, Humboldt exchanged his guides and persisted up the Río Negro. A missionary priest who knew the way accompanied the explorers. They never encountered the foretold monsters, but the mosquitoes were vicious and the waters were putrid and full of dead animals.

The explorers reached the Casiquiare in the middle of May, finally verifying the rumors of the existence of the natural canal. Humboldt used survey tools to measure and map the exact location of the entrance of the river. The next day, they turned around and began retracing their route. On the way Humboldt visited an ancient burial ground of the extinct Atures Indians, from which he took some unauthorized souvenirs, including bones from the graves. In the village of Uruana he noted the use of hallucinogenic plants and the custom of eating dirt. After covering 1,500 miles (2,413.5 km) on foot and by canoe in search of the Casiquiare, they had to rest for one month in Angostura before crossing the llanos again. Both Humboldt and Bonpland contracted typhoid and needed time to regain their strength. They reached Cumaná again in November 1800, having charted the longitude and latitude of 55 locations.

Humboldt and Bonpland spent the winter touring Cuba and organizing thousands of plant and animal specimens for shipment back to Europe. They also visited the local sugar plantations, tobacco, cotton, and indigo fields, and many factories. While he enjoyed the comforts of being in the most developed Spanish colony, Humboldt was appalled at the exploitation of slaves to boost the Cuban economy. He published *Political Essay on the Island of Cuba* (1828), describing the island's geography, landforms, geology, and climate as they related to the island's economy and population.

Humboldt and Bonpland next sailed into Cartagena, Colombia. From there the men hiked 20 miles (32 km) to Turbaco to explore the local gas volcanoes. Then they traveled through the rain forests until they passed the village of Honda, 50 miles (80.5 km) west of Bogotá. The elevation was approximately 9,000 feet (2,743 m), and Humboldt noticed a change in the vegetation with the increase in altitude. They reached Bogotá, where they were warmly greeted. Bonpland caught a fever, and they remained for two months while he recovered. Humboldt spent time examining the collections of an eminent botanist who resided nearby. He also took several short trips during which he found rock salt, coal fields, and fossilized mastodon bones.

In September 1801, Humboldt and Bonpland left Bogotá for Quito, Ecuador. They had to travel across the Andes Mountains. The geography was much different from that which they had experienced in South America thus far. Over the mountains there were cliffs, icy lakes, and peaks and valleys. They passed ancient Inca Empire ruins, which intrigued Humboldt, who was interested in anthropology. They climbed a volcano named Puracé and faced obstacles such as mudslides, downpours, and lightning. Once they reached Quito in January 1802, they were surrounded by huge, active, smoky volcanoes. The residents hosted several receptions for the weary travelers and provided them with comfortable living quarters.

In Quito, Humboldt investigated all of the nearby volcanic mountains. He climbed them, examined their geological structures, analyzed their gaseous exhalations and the composition of the surrounding atmosphere, and timed their seismic waves. While there, Humboldt climbed the Chimborazo, then thought to be the highest mountain in the world. (Today, the highest is known to be Mount Everest, at 29,035 feet, or 8,850 m). The ascent was dangerous and difficult as much of the party suffered horribly from altitude sickness. They were rewarded for their determination by a spectacular sight never before viewed by human eyes from their record-setting altitude of 19,286 feet. Encouraged and exhilarated by their achievement, the men made their way back down, stopping periodically so the tireless Humboldt could chip away rock samples for later examination.

Humboldt and Bonpland, now accompanied by Carlos Montúfar, a young Ecuadorian scholar from an aristocratic family, made their way to Lima, Peru, by late October. Again, they needed to organize their

data and pack up rock and plant specimens for transport back to Europe. While in Lima, they enjoyed watching the planet Mercury pass in front of the Sun. Humboldt also collected guano, which the locals used as fertilizer. Guano is made primarily from bird droppings. Humboldt shipped some back to Europe for chemical analysis, and it was found to be rich in phosphates. A few decades later, tons of guano were exported to Europe, increasing food production and improving the economy of South America.

On Christmas Eve the trio departed from Callao, Peru, on a ship for Guayaquil, Ecuador. During this trip Humboldt studied the cold currents to the west of Peru. Today this phenomenon is called the Peru Current, also known as the Humboldt Current, and it has a significant impact on the regional economy. They next departed for Acapulco, Mexico. In Mexico Humboldt mapped the exact location of Acapulco, which had been too far east on the current maps. He also toured the Guerrero Mountains on mule and horseback and observed the geological outcroppings. He recorded the longitude and latitude of several significant landmarks. He spent time in the government archives in Mexico City. While visiting the mountain region of Guanjuato, Humboldt explored the silver mines and collected mineral specimens. A special mule train was required to transport the numerous specimens. He also visited the location of a volcano formed only 44 years before, named Jorullo. The lava fields were still smoldering and Humboldt measured gases and shocked the Indians by climbing into the crater itself.

Humboldt, Bonpland, and Montúfar left Mexico in January 1804 and went to Washington, D.C., where their reputation preceded them. Everyone wanted to meet the men who had climbed Chimborazo and lived to tell about it. Humboldt was anxious to meet President Thomas Jefferson. As president of the American Philosophical Society, Jefferson was well aware of Humboldt's scientific exploits as well as his fame as an adventurous explorer. The two men formed a strong friendship and corresponded for the rest of their lives. The impression Humboldt left on Americans before sailing home is evidenced by the numerous cities, mountains, bays, and parks named after him.

BACK HOME

The party arrived in France on August 1, 1804. To Humboldt's amusement, he learned that the French thought he had died of yellow fever during his travels. The rumors increased his fame even more. It is reported that during his lifetime, he was second in fame only to Napoleon, who incidentally, was jealous of Humboldt's popularity and publicly belittled him by comparing him to his wife, calling them both flower-collectors. Suspecting Humboldt was an enemy spy, Napoleon ordered him out of France. He rescinded this order but had Humboldt followed by the secret police.

Though the American expedition was over, Humboldt's greater task of communicating all he had learned and observed lay ahead of him. He took trips to Italy and Berlin, but settled in Paris where, surrounded by other scientists, libraries, publishers, and engravers, he began to write his manuscripts. The vast amount of information he had gathered placed Humboldt in a position no previous scientist had occupied. He was a true polymath, a veritable walking encyclopedia of knowledge. He had amassed large amounts of data on magnetism, geology, meteorology, climatology, geography, mineralogy, zoology, botany, astronomy, anthropology, and more. Humboldt and Bonpland had collected more than 60,000 plant specimens, 10 percent of which were unknown in Europe. In addition, from his research, the new field of plant geography was founded. Plant geography is concerned with how the climate and history of the planet affect the locations of plant populations. He described a systemic distribution of vegetation as one ascended from sea level upward. At the equator, a zone of palms characterized the altitudes from sea level to 3,000 feet (914 m). Ferns existed up to 4,900 feet (1,494 m), followed by oaks to 9,200 feet (2,804 m). Shrubs predominated to 10,150 feet (3,094 m), then herbs to 12,600 feet (3,840 m), and grasses and lichens existed up to 14,200 feet (4,328 m). From his observations of plants occurring at different mountain altitudes, Humboldt concluded that the populations of plants could be predicted given the climate and altitude. From these studies, Humboldt achieved his goal of finding unity in nature. Physical factors such as height above sea level, weather and climate, and soil type all affected the types of vegetation that grew in an area.

Humboldt started his scientific career as a neptunist, that is, a follower of Abraham Gottlob Werner. Werner taught mineralogy at Freiberg and was the leader of the neptunists, who believed that all rock was formed from sediment or precipitate of a universal ocean. Werner also believed and taught that basalt was of aqueous origin. Empirical studies, in particular those of the volcanoes surrounding Quito, convinced Humboldt that basalt was of igneous origin. He believed that volcanic activity was responsible for shaping the Earth. He also correlated the linear pattern of volcanoes to underground fissures and looked for correlations between geological structures and geographical elements.

With respect to Earth's magnetism, Humboldt found that the intensity declined as one moved from the poles toward the equator. In Paris, Humboldt met

with French chemist Joseph Gay-Lussac to study the law of magnetic declination. He returned to research on geomagnetism later in his life.

Investigations on climate and meteorology also filled many of Humboldt's notebooks. He found there was an overall decrease in temperature with an increase in altitude. He also was the first to chart isotherms and isobars. Isotherms are plots on a map that connect areas that have the same mean temperature. Isobars are lines on a map that connect points with equal air pressure.

The first work published from his travels was *Aspects of Nature,* which contained a variety of essays on plants, the llanos, and volcanic structure. Intended to share the wonders of nature with the general public, the book became one of his most popular. Humboldt estimated that it would take five or six years to complete approximately 17 scientific volumes about his South American field studies. In the end, it took him 30 years and 30 volumes. The series was titled *Voyages to the Equinoctial Regions of the New Continent Made During the Years 1799 to 1804* (1807–39). The volumes covered topics such as plant geography, astronomy, and botany, and they even included social and political essays. In addition to the many scientific volumes, Humboldt also wrote separate volumes intended for the general public. Remarkably, this series included 1,400 illustrations, which cost more to publish than the expedition itself and resulted in the depletion of the remainder of the fortune from his mother's inheritance. In need of a regular income, Humboldt accepted an appointment as privy councilor to King Friedrich Wilhelm and moved back to Berlin in 1827. He became the king's primary adviser on scientific and artistic matters. When the king died in 1840, his successor also depended on Humboldt, who was appointed to the state council.

DIAMONDS IN THE URALS

Russia's finance minister requested Humboldt's expertise soon after his return to Berlin. Humboldt was invited to explore the Ural Mountains at the czar's expense in hopes of finding valuable mineral deposits. Even though he was in his 60th year, Humboldt was thrilled at the opportunity to explore this virtually unknown territory. He was anxious to explore the Earth's magnetism using Russia as his field laboratory. He recruited a biologist and physician named Christian Gottfried Ehrenberg, a chemist and mineralogist named Gustav Rose, and his own valet Karl Seifert to accompany him. They set out by horse and carriage on April 12, 1829.

The men traveled through Eastern Europe to reach St. Petersburg and Moscow, and then on to the Urals. Every so often Humboldt measured and recorded the magnetic intensity of Earth and took astronomical observations. There were no inns en route, so they slept in the carriages on the frozen Siberian plains. On June 15 they reached Ekaterinburg, a town in the central Urals that they used as a base. Already they had collected 14 boxes of mostly rock and mineral specimens to ship back to St. Petersburg and Berlin. From this town they hiked out to the mines and studied mineral deposits. They examined specimens of iron, copper, gold, and platinum as well as others. Humboldt had learned during his previous travels that diamonds were often found where gold and platinum existed. Soon after he suggested they search the area, diamonds were successfully located. As a result, Humboldt was credited with discovering Russia's first diamond field.

After exploring the Urals, the men continued across the hot, mosquito-infested, grassy steppes of Siberia until they reached the Russian border with China. Then they turned around. By the time they arrived at Moscow in November 1829, they had traveled 12,000 miles (19,312 km). Rose published some mineralogical and geological findings, and Humboldt published three volumes of *Central Asia* in 1843. The first two volumes described the Asian mountain ranges, and the third covered magnetic and climatological observations. Significantly, Humboldt was able to convince the Russian government to establish several magnetic and meteorological observation stations. Eventually, the British did also. This effort was the first major international scientific collaboration. From information gathered at these stations, Humboldt developed the principle of continentality, which describes the moderating effect of proximity to large water bodies on regional climate.

THE COSMOS

A few years later Humboldt began composing his capstone, *Cosmos: A Sketch of the Physical Description of the Universe,* an attempt to describe the workings of the entire universe for the general public. Published in five mammoth volumes (1845–62), it included scientific descriptions of the physical earth, but also descriptions of the heavens and of all life. Humboldt explained the interdependence of all the natural sciences, a principle that pervades modern sciences but was novel at the time. Geology affects climate. Climate affects life-forms. Life affects the environment and leaves imprints on nascent rock forms, and so on. This was the beginning of ecology, the study of the relationships between organisms and their environments.

Alexander von Humboldt was not able to complete the *Cosmos* series. He died on May 6, 1859, at age 89, just two weeks after sending the fifth volume to his publisher. He was given a state funeral

and buried at Schloss Tegel, his family's countryside estate. He bequeathed his estate to his devoted servant, Seifert. Humboldt has been called the most learned man of his time. He was awarded several honorary doctorate degrees and elected to membership in all of the prestigious academic organizations. The Royal Society of London awarded Humboldt the Copley Medal in 1852.

Humboldt's work instigated advancements in several fields in the Earth sciences such as oceanography and geomagnetism. Plant geography helped stimulate British biologist Alfred Wallace to develop the theory of evolution, and Humboldt's South American travels inspired British biologist Charles Darwin to do the same. Humboldt's observations on the complementary jigsaw puzzle piece pattern of the South American east coast and the African west coast influenced Alfred Wegener's proposal of the continental drift theory. Humboldt was skilled at recognizing the economic benefits of local biological and geological features such as diamonds, guano, and the Peru Current; thus his studies affected politics and economics as well as science. No doubt this pleased Humboldt, as he was a man for whom no boundaries existed between disciplines. He is considered a pioneer in ecology, not only for the enormous amount of data he collected during his explorations, but also for unifying biology with physical geography, geology, and climatology. Only a man who wholly understood nature could describe nature as a whole.

See also BIOGEOGRAPHY; ECOLOGY.

FURTHER READING

De Terra, Helmut. *Humboldt: The Life and Times of Alexander von Humboldt, 1769–1859*. New York: Alfred A. Knopf, 1955.

Gaines, Ann. *Alexander von Humboldt: Colossus of Exploration*. New York: Chelsea House, 1997.

Helferich, Gerard. *Humboldt's Cosmos: Alexander von Humboldt and the Latin American Journey That Changed the Way We See the World*. New York: Gotham Books, 2004.

hydrothermal vents Hydrothermal vents are fissures in the ocean floor that spout mineral-rich hot water. Found in both the Atlantic and Pacific Oceans, most hydrothermal vents are found at an average depth of about 7,000 feet (2,100 m) in areas of the seafloor spreading along the Mid-Ocean Ridge system, an extensive underwater mountain chain. As the tectonic plates that form Earth's crust slowly spread, cracks form in the ocean floor. Water from the ocean floor seeps into the cracks, where magma (molten rock) from underneath Earth's surface rises and heats water that has seeped into the cracks. The superheated water rises back up and forcibly spews out of the cracks, forming a hydrothermal vent.

Surprisingly, these harsh conditions in the deep, dark ocean support thriving communities containing abundant life. Because sunlight can penetrate only about 300 feet (91 m) of water, and photosynthetic organisms form the foundation of most food webs, until the late 1970s biologists believed the ocean floor to be relatively sterile, inhabited by only a few scavenger life forms that lived off dead organic material that fell to the ocean floor. The startling discovery of tiny ecosystems containing many unusual life forms, including microorganisms, tubeworms, mussels, clams, shrimp, and crabs, around hydrothermal vents has provided scientists with information about the origin of life and Earth's earliest creatures.

DISCOVERY OF LIFE AT HYDROTHERMAL VENTS

In 1977 the National Science Foundation funded the Galápagos Hydrothermal Expedition, headed by Richard Von Herzen and Robert Ballard of Woods Hole Oceanographic Institute (WHOI); Jack Corliss, Jack Dymond, and Louis Gordon of Oregon State University; John Edmond and Tanya Atwater of the Massachusetts Institute of Technology; Tjeerd van Andel of Stanford University; and Dave Williams of the U.S. Geological Survey. The crew on board the ship *Knorr* was searching for hydrothermal vents in the Galápagos Rift off the South American coast west of Ecuador. As the rift spreads, molten magma rises from underneath the Earth's surface to fill the gap, heats water that has seeped in, and forcibly expels it. Evidence of hot water was interesting to the marine geologists and geophysicists, who were studying hydrothermal circulation to gain a better understanding of the flow of heat energy from inside the Earth through the crustal plates. Geologists believed that mountains on land were formed from buckling under pressure, but that, underwater, heat played a more prominent role in sculpting mountains.

The crew used a two-ton steel cage on a sled, nicknamed ANGUS (Acoustically Navigated Geophysical Underwater System), equipped with cameras and strobe lights, to survey the ocean floor. On February 15, 1977, as the *Knorr* towed ANGUS near the ocean floor at a depth of 8,250 feet (2,500 m), taking photographs every 10 seconds, the crew noticed a spike in temperature that lasted about three minutes. The next morning, they collected and developed the film from ANGUS, expecting to see photographic evidence of a fissure or a conical vent in the lava from the pictures taken at the same time

as the temperature anomaly occurred. Surprisingly, the photos revealed hundreds of clams surrounded by turbid, milky water. Unfortunately, because the crew set sail never expecting to see a thriving colony of living organisms in near-freezing temperatures on a bed of hardened lava, no biologists were aboard the ship. *Alvin,* WHOI's small submersible used for biological and physical research of the ocean's bottom, dove into the astonishing clam bed, where the temperature registered 61°F (16°C). They retrieved samples of not only the clams, but also the water for chemical analysis. Onboard, the crew opened the water bottles, which released a powerful stench of rotten eggs, a telltale odor for hydrogen sulfide (H_2S), a gas poisonous to most living creatures.

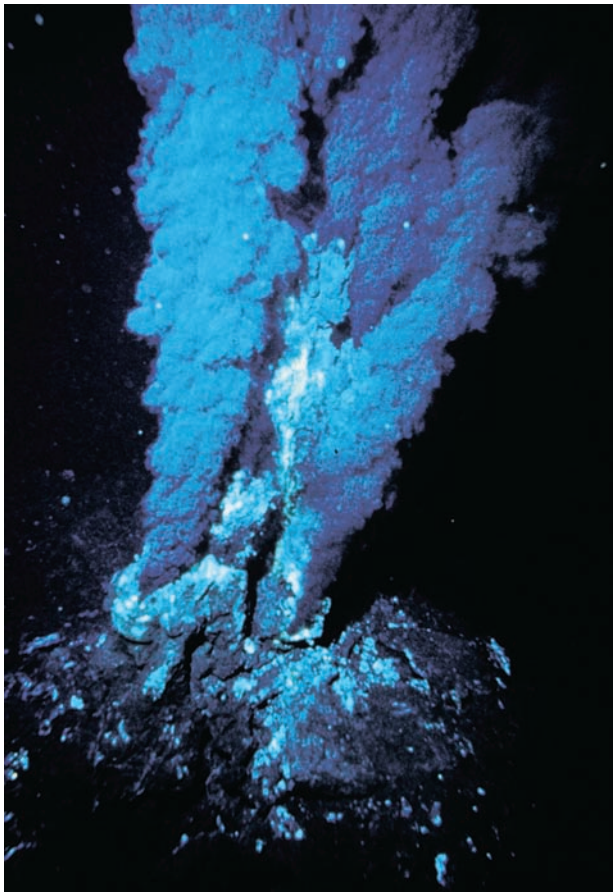
Over the next five weeks, 21 more dives on the rift revealed other colonies of living creatures, including scavenging white crabs, albino lobsters, pink fish, red tubeworms, and flowerlike animals. One location contained only empty clamshells; previously existing warm water vents that supported life in the small, defined surrounding area were no longer present. Though different organisms dominated different sites amid the barren basaltic rocks, all contained H_2S, which acted as the ultimate source of energy for the little ecosystems. On the ocean's surface and on land, most life depends on sunlight that photosynthetic organisms utilize to make carbohydrates from carbon dioxide (CO_2), but sunlight cannot penetrate to the ocean floor. Chemosynthetic bacteria were extracting energy from the H_2S and using it to synthesize organic compounds that other animals could metabolize for energy. The high concentrations of sulfur around these vents would kill many organisms, but the life-forms that inhabited the hydrothermal vents were uniquely adapted to survive in such an environment.

The discovery of abundant life in isolated oases on the otherwise desolate environment of the ocean floor was both shocking and remarkable. Seawater trickled down into the vents, became superheated, and brought up from within Earth dissolved minerals that allowed life to exist in extreme conditions similar to those that dominated during the early days of Earth's formation. The implications were astounding—life may have originated under these conditions, and such environments might exist elsewhere in the universe. Since this was supposed to be a geophysical and geochemical research cruise, the crew was not properly equipped to collect biological samples, and they resorted to stuffing Tupperware and soup tureens with the unique specimens that might provide insight into the origin of life.

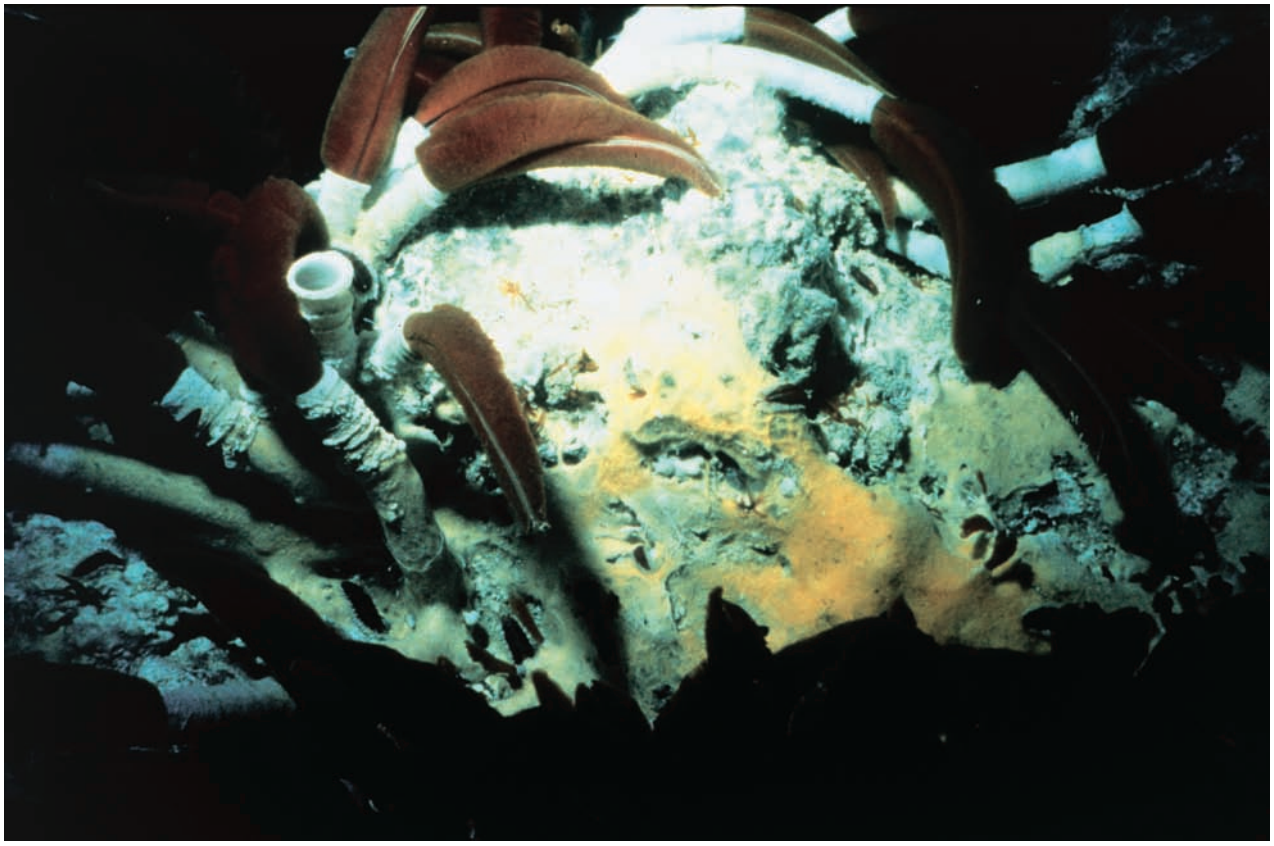
Funding for additional explorations was easy to obtain. In 1979 Ballard took crews of marine biologists to the Galápagos Rift, where *Alvin* helped them retrieve numerous interesting organisms, some from entirely new phyla and some that were living fossils. Microbiologists identified more than 200 strains of prokaryotic organisms. One key characteristic of many of these microorganisms was their ability to survive in such high temperatures, up to 230°F (110°C). Chemists have determined that nutrients at the vent sites were present in concentrations 300 to 500 times greater than outside the vent areas.

BLACK SMOKERS

Amidst the excitement accompanying the biological discoveries, an inexperienced scientist on a French expedition in 1978 to the East Pacific Rise off Mexico's Baja California had collected a geological sample from an unusual chimneylike structure. Unaware of its peculiarity, he packed it away with numerous other samples to be analyzed later. Months passed before researchers identified the sample to be a sulfide of zinc never before found on volcanic seafloor terrain. During the Galápagos Hydrothermal Expedition, the crew had observed strange tubular depos-



Black smokers are vents in geologically active areas of the seafloor, particularly near mid-oceanic ridges, that discharge mineral-rich, hot fluids. *(OAR/National Undersea Research Program [NURP]; NOAA)*



Vestimentiferan tube worms grow in hydrothermal vent communities and depend on chemosynthetic prokaryotes for nutrition. *(OAR/National Undersea Research Program [NURP]; College of William & Mary)*

its, but they crumbled when the crew tried to take samples. In 1979 Ballard was in the East Pacific Rise when divers spotted cylindrical chimney structures, one of which was spouting what looked like black smoke. This could not have been smoke underwater, but instead was a rich suspension of minerals, mostly iron and sulfide, which combine to form iron monosulfide, a chemical compound that colored the discharge black. The temperature registered 91°F (32°C), warmer than any water at the Galápagos Rift. After hauling *Alvin* up that night, the scientists saw that the tip of the temperature probe was charred and melted and wondered how hot the temperature had really been.

The following day Ballard went down in *Alvin* with a more durable temperature probe, and as he came within about 10 feet (3 m) of a chimney, the temperature measured an unbelievable 662°F (350°C). Observations over the next 12 days revealed both white and black smokers, some up to 30 feet (9 m) tall. White smokers expel cooler water that contains barium, calcium, and silicon, elements that give the discharge a whitish appearance. The chimneys were composed of almost pure crystalline zinc sulfide, and geochemists determined that the seawater maintained its unique chemistry by recycling through fissures and hydrothermal vents. The superheated water rising from the vents carried dissolved metals that precipitate, or form, solids when they hit the colder ocean water. These precipitated metals collected over time to form these chimneylike structures.

HYDROTHERMAL VENT COMMUNITIES

Since 1977 biologists have identified more than 300 animal species from hydrothermal vent communities. Most of the animals live on the outside of the chimneys, where the temperature is much lower than where the fluids discharge from the vent. Even so, the temperatures are much higher than elsewhere in the cold, deep ocean. Giant vestimentiferan tube worms, *Riftia pachyptila,* are the most evident of the organisms that live in the communities supported by hydrothermal vents. The worms grow up to eight feet (2.4 m) long and about 0.8 inches (2 cm) in diameter but have no digestive system, an unusual trait for such a large animal. At the posterior end of the worm, a structure called an opisthosome anchors the worm to its protective chitinous tube covering, thus the animal is sessile. Scientists discovered that

Spider crabs are one of many types of crabs known to inhabit vent sites. *(OAR/National Undersea Research Program (NURP))*

endosymbiotic prokaryotic microorganisms living in the body of the worms use H_2S present in the volcanic gases as an energy source to fuel the synthesis of organic compounds in a process called chemosynthesis. This process is similar to photosynthesis except chemosynthesis uses chemicals rather than light as the source of energy for fixing carbon from CO_2. The microorganisms are chemolithotrophic, meaning they oxidize reduced inorganic compounds (such as H_2S) as their energy source. The worm brings in H_2S from the seawater through a respiratory gill plume at its anterior end, and it binds to hemoglobin, a protein that colors the worms red at their tips. The circulatory system of the worm transports the H_2S bound to hemoglobin to the endosymbionts. The circulatory system also carries the required CO_2 and molecular oxygen (O_2) to the bacteria, which synthesize the organic compounds that fulfill the nutritional and energetic needs of the worm. Though sunlight does not serve as the main energy source to fuel these communities, the organisms still depend on the process of photosynthesis to generate the O_2 that is dissolved in seawater and is necessary to oxidize the H_2S.

Clams also depend directly on the chemosynthetic bacteria. Vescomyid clams are similar to the more familiar clams and have some ability to filter-feed, but, like tube worms, they also depend directly on endosymbiotic chemosynthetic microorganisms that live in their gills to satisfy their nutritional needs. The feet of these clams are red due to the presence of hemoglobin, which binds the H_2S for the endosymbionts to use in chemosynthesis. Bathymodiolid mussels are another common vent animal that lives in a mutualistic relationship with chemosynthetic microbes. Because they are better at filter-feeding than the clams, the mussels can exist farther away from the vents. Major predators of vent sites include zoarcid fish, which eat shrimp, and tube worms and octopuses, which eat crabs, clams, and mussels. Several species of crabs including spider crabs with leg spans approaching 31.5 inches (80 cm) and different species of shrimp also inhabit the areas surrounding hydrothermal vents. Scavengers called dandelions, colonies of individuals similar to the Portuguese man-of-war, are often found at vent sites that are no longer active.

New types of archaeans have also been found in the hydrothermal vents. In 1967 Dr. Thomas Brock of Indiana University discovered prokaryotic organisms that attached themselves to microscope slides left in a hot spring at Yellowstone National Park. The water temperature measured 176°F (80°C), yet these microorganisms thrived. Since this remarkable finding, scientists have identified numerous thermophiles, organisms with optimal growth temperatures higher than 113°F (45°C) and hyperthermophiles, organisms with an optimal growth temperature above 176°F (80°C). Fluids discharged from hydrothermal vents can exceed 752°F (400°C), yet the water does not boil due to the extreme pressure, and thus the water surrounding hydrothermal vents supports the growth of these organisms. (In comparison, the average temperature of the ocean floor is 35.6°F or 2°C.) Thermophiles and hyperthermophiles belong to the domain Archaea, which includes organisms that are extreme in other respects in addition to their optimal growth temperatures. Dr. Derek R. Lovley from the University of Massachusetts and his colleagues discovered archaeans taken from fluid samples of hydrothermal vent sites that "breathe" iron. The organisms appear to use iron during cellular respiration, rather than oxygen as aerobic organisms use, and they produce the hard, black mineral magnetite as a by-product. One organism that they found thrived at 250°F (121°C). In 2005 J. Thomas Beatty, a microbiologist at the University of British Columbia reported the discovery of bacteria that can undergo photosynthesis in the absence of sunlight at depths of 7,800 feet (2,377 m) in the Pacific. Evidence suggested the bacteria harvest geothermal radiation, infrared and visible light from magma at a geothermal vent for energy. Most of the energy from geothermal vents occurs in the form of heat, but some is released as light. In the presence of sulfur provided from the vent discharge, these microbes use the photons to fix

carbon from CO_2 into organic compounds. Due to the limited amount of light energy, these organisms grow very slowly.

In addition to hydrothermal vent sites, other extreme environments also provide unique conditions for establishing communities in habitats previously believed to be inhospitable. For example, scientists have since identified new bacterial and archaean species living at depths of more than 10,000 feet (3,048 m) in areas of the Mediterranean, where extremely salty conditions prevent most life forms from growing. The existence of such extreme communities not only provides insight into the origin of life on Earth and early biological evolution, but also the possibility of life elsewhere in the solar system.

See also ARCHAEA; BROCK, THOMAS; COMMUNITY ECOLOGY; ECOSYSTEMS; INVERTEBRATES; MARINE BIOLOGY.

FURTHER READING

American Museum of Natural History Expeditions Black Smokers Web site. Available online. URL: http://www.amnh.org/nationalcenter/expeditions/blacksmokers/black_smokers.html. Accessed on January 14, 2008.

Ballard, Robert D. "Notes on a Major Oceanic Find." *Oceanus* 20 (1977): 35–44.

Woods Hole Oceanographic Institute and National Science Foundation. Dive and Discover's Deeper Discovery: Hydrothermal Vents. Available online. URL: http://divediscover.whoi.edu/vents/index.html. Accessed on January 14, 2008.

Woods Hole Oceanographic Institute, National Science Foundation, and National Oceanic and Atmospheric Administration. The Discovery of Hydrothermal Vents 25th Anniversary. Available online. URL: http://divediscover.whoi.edu/ventcd/index.html. Accessed on January 14, 2008.

Hyman, Libbie Henrietta (1888–1969) American *Invertebrate Zoologist* Libbie Hyman was an invertebrate zoologist well known for her comprehensive treatise on invertebrate animals and her taxonomic expertise on hydras and flatworms.

Libbie Henrietta Hyman was born on December 6, 1888, in Des Moines, Iowa. She was the only daughter of Joseph and Sabina Neumann Hyman, recent Jewish immigrants who also had three sons. The family moved to Sioux Falls, South Dakota, for a while, then back to Iowa, where they settled in Fort Dodge. She attended public school and especially enjoyed subjects concerning the natural world, but at home she performed most of the housework. According to Hyman, her parents never encouraged her academic pursuits. In Libbie's free time she collected wildflowers, which she categorized following botanical classification schemes outlined in *Elements of Botany* by Asa Gray.

In 1905, after graduating as valedictorian from high school in Fort Dodge, Iowa, Hyman obtained a job gluing labels on cereal boxes in a factory. When a former high school teacher ran into her, she set in motion the actions necessary for Hyman to receive a one-year scholarship to the University of Chicago. She entered the botany program in 1906, but a cruel laboratory assistant forced her to change majors to zoology. She received her bachelor's degree in 1910, and then pursued her doctorate in zoology under Charles Manning Child. After earning her Ph.D. with a dissertation titled *An Analysis of the Process of Regeneration in Certain Microdrilous Olicochaetes* (a type of annelid worm) in 1915, she continued working as Child's assistant. For financial support during graduate school, Hyman assisted in teaching the introductory zoology courses. Unimpressed with the available laboratory manuals, she wrote two of her own, which the University of Chicago Press published. *A Laboratory Manual for Elementary Zoology* (1919, second edition in 1929) and *A Laboratory Manual for Comparative Vertebrate Anatomy* (1922, second edition in 1942 titled *Comparative Vertebrate Anatomy*) were both tremendously successful, and the royalties from these books and other writing endeavors supported her from 1931, when she left Child's lab, until her death. Her passion, however, was invertebrates, and she thought about writing a laboratory manual on this subject, but colleagues convinced her to write a badly needed advanced text on the subject instead.

After leaving Chicago in 1931, Hyman traveled to Europe, stopping at the marine station at Naples. In order to be near the library of the American Museum of National History, she then settled in New York, where she was made an honorary research associate and given an office in 1937. Under these circumstances, she began writing a comprehensive, six-volume treatise on invertebrates. Her goal was to stimulate study on all aspects of invertebrates, including morphology, physiology, embryology, and other biological issues. She took drawing lessons to illustrate her work and prepared her own tissue sections to demonstrate histology. The first volume of *The Invertebrates,* published in 1940, was subtitled *Protozoa through Ctenophora.* Praised as authoritative and highly successful, it was followed by the second volume, *Platyhelminthes and Rhynchocoela.* The third volume, *Acanthocephala, Aschelminthes, and Entoprocta,* came out in 1951; volume four, *Echinodermata,* in 1955; volume five, *Smaller Coelomate Groups,* in 1959; and volume six, *Mollusca,* in 1967. No other single-author biological treatise compares in scope or utility to *The Invertebrates.*

In 1941 Hyman moved into a house in Millwood, in Westchester County, New York, so she could have a garden. The commute was too long, though, and gardening distracted her from writing her treatise. So the dedicated Hyman moved back to a hotel apartment in New York City in 1952.

All of Hyman's scientific fame does not rest on her authorship. Her early research in Child's laboratory mostly "bolstered Child's ideas," in her own words, but in the 1920s she also published several papers describing techniques for studying protozoans, flatworms, and coelenterates. She researched the physiology of hydras and planarians, invertebrates often used but misidentified by biologists. After recognizing that no zoologist had ever performed a careful taxonomic study of hydras and flatworms, she became an expert in their taxonomy and for 30 years was considered the foremost authority.

Hyman belonged to and served in numerous academic societies: American Microscopical Society, Marine Biological Laboratory (Woods Hole), American Society of Zoologists (vice president in 1953), Society of Systematic Zoology (president in 1959), American Society of Limnology and Oceanography, Society of Protozoologists, and the American Academy of Arts and Sciences. She also served as editor for *Systematic Zoology* from 1959 to 1963. Her treatise on invertebrates brought her many honors. The National Academy of Sciences elected Hyman to membership in 1961 and awarded her the Daniel Giraud Elliot Medal in 1951. She received the Gold Medal from the Linnean Society of London in 1960. The American Museum of Natural History gave her the Gold Medal for Distinguished Achievement in Science in 1969.

Libbie Hyman developed Parkinson's disease. Beginning in 1961, her health deteriorated. While writing volume six of *The Invertebrates* she became wheelchair-bound and knew she could not complete her project. She died on August 3, 1969, in New York City. This modest woman remains the most influential comparative invertebrate zoologist of the 20th century.

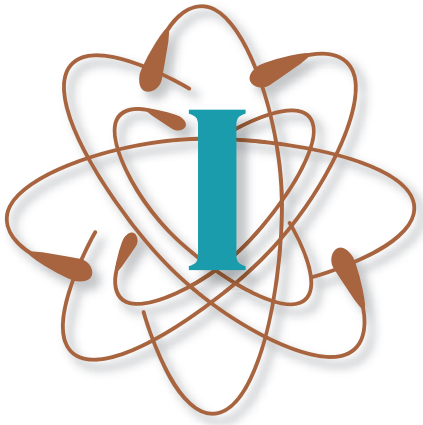
See also INVERTEBRATES.

FURTHER READING

Hyman, Libbie H. *The Invertebrates*. 6 Vols. New York: McGraw Hill, 1940–1967.

Hyman, Libbie H., and G. Evelyn Hutchinson. "Libbie Henrietta Hyman (December 6, 1888–August 3, 1969)." In *Biographical Memoirs. National Academy of Sciences*. Vol. 60. Washington, D.C.: National Academy Press, 1991.

Jenner, R. A. "Libbie Henrietta Hyman (1888–1969): From Developmental Mechanics to the Evolution of Animal Body Plans." *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 302, no. 5 (September 15, 2004): 413–423.



immune system disorders A properly functioning immune system surveys the body's fluids and tissues, seeking out potentially pathogenic foreign invaders for destruction and removal. Sometimes the immune system overreacts to harmless substances that have entered the body, and such an inappropriate response can cause discomfort or even harm to the person. Other times, the immune system fails to distinguish self versus nonself and attacks its own tissues and cells. Infections, medical treatments, and congenital defects can also lead to disorders or dysfunctions of the immune system.

HYPERSENSITIVITIES

The term hypersensitivity applies to either an exaggerated or an inappropriate response of the immune system. The reactions are part of the normal specific immune response, but in the case of hypersensitivities, they result in injury to the host. Because hypersensitivities involve acquired, specific immunity, they require an initial sensitizing exposure before observation of the detrimental effects. Of the four total types of hypersensitivities, three involve antibodies and the fourth is cell-mediated.

Commonly known as allergies, type I hypersensitivity symptoms occur within 15 minutes of exposure to the responsible antigen (subsequent to a sensitizing exposure). The antigens that stimulate these reactions are referred to as allergens and are usually harmless agents such as pollen, animal dander, venom from an insect sting, certain drugs, fungal spores, or food substances. These substances are not harmful in themselves, but, to someone who is allergic to them, they can cause conditions such as asthma, eczema, hay fever, or hives, and, in more serious cases, anaphylactic shock and death. When an allergic individual initially encounters the allergen, the B cells of the immune system produce the immunoglobulin IgE, which then binds to receptors located on mast cells that are embedded in the body's tissues or on circulating basophils. Because the affinity of the IgE antibodies to the receptors on the mast cells and basophils is so high, the IgE can remain bound anywhere from a few weeks to indefinitely, and, as long as it is bound, the person is considered sensitized to the antigen. Binding of the allergen to the IgE molecules during a second exposure prompts the mast cells and basophils to which IgE is bound to secrete chemical mediators such as histamines, leukotrienes, and prostaglandins. Acute inflammation occurs, causing symptoms that range from mildly uncomfortable to potentially fatal.

An allergic reaction can occur locally or systemically. Local reactions are less harmful and the symptoms depend on the site of entry. Airborne allergens stimulate the release of chemical mediators such as histamine from the mast cells in the eyes, nose, and upper respiratory tract, causing the blood vessels to dilate and the capillaries to become more permeable. This can lead to hay fever symptoms including a runny nose, sneezing, watery eyes, and congestion. By the same mechanism ingestion of a food allergen can lead to increased acid secretion from the gastric mucosa leading to nausea, cramping, and diarrhea. Antihistamines can bring some relief by blocking histamine receptors. In bronchial asthma, allergens stimulate the release of chemical mediators from mast cells in the lower respiratory tract. As a result, fluid flows from the blood into the tissues, bringing about edema and inflammation. The smooth muscles of the airways contract, mucous production increases, and breathing becomes restricted,

making breathing difficult. Anti-inflammatory agents such as steroids can reduce the inflammation, bronchodilators help relax the muscles surrounding the airways, and expectorants loosen the mucous so it can be coughed up. If the hypersensitivity reaction is systemic, blood vessels throughout the body become more permeable, excessive amounts of fluid can leave circulation, and blood pressure could drop dramatically. Without sufficient oxygen being brought to the vital organs, anaphylactic shock can be fatal. These overpowering reactions are most often caused by a bee sting or the injection of antibiotics.

Allergies to specific substances can be diagnosed by a skin prick test, in which minute amounts of the substance are scratched onto the skin. The appearance of local redness and swelling, called a wheal and flare response, indicates a positive result. Avoidance of the allergen is the most effective treatment, though in some cases desensitization is effective. Desensitization through repeated injections with increasing quantities of the allergen acts by increasing the amount of circulating IgG specific for the allergen. If sufficient amounts of circulating antibody are present, they will bind the allergen, neutralizing it before it can find IgE antibodies bound to mast cells and stimulate them to release inflammatory mediators.

Type II hypersensitivities are referred to as antibody-dependent cytotoxic reactions. Circulating antibodies (either IgG or IgM) bind to cells or particles, activating complement to form a membrane attack complex and lyse the cells, or marking the particle for phagocytosis. The need for matching blood types is based on the potential for cytotoxic reactions. Blood cells of different types (A, B, AB, O) express different glycoproteins on their surfaces. If an individual receives a blood transfusion with cells that express a glycoprotein that his or her own cells do not express, then antibodies will bind the foreign glycoproteins on the ill-matched blood cells. Complement will lyse those cells, leading to severe complications, including kidney damage and toxic effects from the large amounts of heme released from hemoglobin of the lysed red blood cells. The reason why people have preformed antibodies that recognize foreign blood cell glycoproteins is unknown.

Hemolytic disease of the newborn also results from type II hypersensitivity reactions. A protein called the Rh factor is present on the surface of the red blood cells of some individuals (Rh^+) but not others (Rh^-). If a mother does not have the Rh factor but her fetus does, during childbirth the mother will come into contact with the antigenic Rh factor from the fetal blood and develop a specific immune response to it. During a subsequent pregnancy with an Rh^- fetus, the mother's immune system will attack the fetus's blood cells. IgG antibodies will cross the placenta and bind to the fetal blood cells, and complement will lyse the cells, leading to anemia, jaundice, edema, an enlarged spleen and liver, and potential death. Hemolytic disease of the newborn can be prevented by passive immunization. During childbirth, pregnant women that are Rh^- are given anti-Rh antibodies purified from donated serum. The presence of these preformed antibodies prevents the mother's immune system from becoming sensitized to the Rh antigen by binding and neutralizing any fetal blood cells that enter her circulatory system.

Serum sickness and the Arthus reaction are type III hypersensitivities, also called immune complex reactions. The Arthus reaction, named after the French bacteriologist Nicholas Maurice Arthus, is characterized by local inflammation that results when complexes of antigen and antibodies are deposited in tissue spaces and in blood vessel walls, activating complement and phagocytosis by neutrophils. This acute response usually occurs when an injection containing a vaccine or drugs is given in the same site as a previous injection. Serum sickness is similar and occurs when a foreign antigen is injected into an individual and IgG and IgM bind to the antigens. Because the antibodies can bind more than one antigen at a time, extensive crosslinking occurs, the immune complexes travel through the bloodstream and lodge in the kidneys, joints, and skin. Symptoms include fever, swollen lymph nodes, hives, joint swelling, and renal lesions.

Type IV hypersensitivities are the only class of hypersensitivities that are cell-mediated rather than antibody-mediated. Also called delayed hypersensitive responses, these result when an antigen sensitizes a set of T lymphocytes, causing them to release cytokines and other chemicals. This is what happens when a host rejects a tissue transplant. Antigens found on the surface of the donor cells and in the donor tissues attract the T cells to the graft site, where they release cytokines and attack the transplanted donor tissue. Drugs that inhibit the production and activity of lymphocytes are administered to suppress the recipient's immune system. The tuberculin skin test is another common example of a delayed hypersensitive reaction. Used to determine if a patient has been exposed to *Mycobacterium tuberculosis,* the causative agent of tuberculosis, a small amount of a purified protein from the bacteria is injected just underneath the skin surface. If the individual has been previously exposed, he or she will have T cells sensitized to recognize and respond to that antigen. The T cells migrate to the area, bind to the purified protein antigen, and recruit white blood cells such as macrophages. Fluid and cells build up, leading to redness and swelling at the site

of injection between 24 to 48 hours later. Another manifestation of a type IV hypersensitivity is allergic contact dermatitis, in which small allergens combine with proteins in the skin to produce an immune response. Reactions such as those induced with poison ivy, metals in jewelry, and latex are all examples of contact dermatitis.

IMMUNODEFICIENCIES

When the immune system cannot or does not respond vigorously enough, an immunodeficiency is said to exist. Immunodeficiencies are divided into two general categories: primary or congenital (meaning the child is born with the disorder) and secondary, which are acquired after birth as the result of an infection, aging, or a malignancy. Inborn deficiencies can affect any or several components of the immune system: the B cells, the T cells, phagocytes, or complement. A deficiency in B cells results in lower than normal levels of immunoglobulins, a condition called agammaglobulinemia (complete absence) or hypogammaglobulinemia (low levels). The complete absence of immunoglobulins is very rare. A child born with a B cell deficiency will exhibit recurrent bacterial infections by the age of six months. Treatment includes the administration of preformed immunoglobulins and antimicrobial drugs. Deficiencies in T cell development or expression are more dangerous than B cell deficiencies. Thymic aplasia, more commonly known as DiGeorge syndrome, results when the embryonic tissues from which the thymus develops do not mature. Without a cell-mediated branch of the specific immune response, children with this syndrome cannot efficiently fight viral, fungal, or protozoal infections. Therapy often calls for transplantation of thymus tissue. The most dangerous immunodeficiencies involve both branches of the immune system and are called severe combined immunodeficiencies (SCIDs). Depending on when the developmental error occurred, the child might have a complete absence of lymphocytes or very low numbers. Infections begin to develop within days of birth. A bone marrow transplant is the recommended therapy for SCIDs.

Infection, disease, chemotherapy, or radiation can cause secondary immunodeficiencies. Acquired

EXAMPLES OF AUTOIMMUNE DISORDERS

Disease	Type of Reaction	Target	Characteristics
Graves disease	Type II (cytotoxic)	thyroid stimulating hormone receptors	The thyroid gland overproduces thyroid hormone and becomes greatly enlarged.
myasthenia gravis	Type II (cytotoxic)	acetylcholine receptor on muscle cells	Muscles become progressively weaker. Can lead to respiratory failure.
multiple sclerosis	Type II (cytotoxic) and IV (cell-mediated)	myelin of nerve cells	Neurons in the brain cannot receive, process, or send information efficiently. Early symptoms include muscle weakness, loss of coordination, numbness, loss of vision, and fatigue. Later symptoms can include muscle spasticity, tremors, pain, paralysis, and the inability to think clearly.
rheumatoid arthritis	Type III (immune complex)	joints	Chronic inflammation of joint synovium that leads to damage of cartilage and bone in the joints.
systemic lupus erythematosus	Type III (immune complex)	systemic: joints, skin, lungs, heart and circulatory system, nervous system, kidneys, gastrointestinal tract, eyes	Symptoms include fever, fatigue, rashes, muscular pain, weight loss, arthritis, kidney dysfunction, swollen spleen and lymph nodes. Characterized by the presence of antibodies against nuclear antigens such as double-stranded DNA, single-stranded DNA, and histone proteins.
insulin dependent diabetes mellitus	Type IV (cell-mediated)	pancreatic islet beta cells	Causes increased blood glucose levels, excessive urine production, vision problems, and weight loss.

immunodeficiency syndrome (AIDS) is a well known example of an immunodeficiency that results from infection with the human immunodeficiency virus (HIV). The virus specifically attacks the helper T cells, making the body more susceptible to opportunistic infections and certain cancers as the disease progresses. Cancers that target bone marrow or lymphoid organs also can lead to immunodeficiencies, as can chemotherapy and radiation treatment for cancers. Patients who have received transplanted tissues or organs often require immunosuppressive drugs that prevent them from attacking their transplanted donor tissue. Unfortunately, these medications render the patient more susceptible to infections they could otherwise easily fight off.

AUTOIMMUNE DISORDERS

An autoimmune disorder results when the immune system recognizes an antigen on the host's own tissues as being foreign and causes damage to those tissues. Lymphocytes normally undergo clonal deletion, a process that occurs during development in the thymus when cells that specifically recognize self-antigens are destroyed or inactivated. The ability to distinguish, and therefore not attack self-antigens is called immune tolerance or self-tolerance. The loss of this specific nonreactivity leads to autoimmune diseases that can result from cytotoxic, immune complex, or cell-mediated hypersensitive reactions. Some studies suggest that an individual can be genetically predisposed to autoimmune disorders. Autoimmunities are often classified based on the tissues or organ systems that are affected, and the symptoms depend on the affected tissues or organs.

See also ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS); HOST DEFENSES; INFECTIOUS DISEASES.

FURTHER READING

Cassell, Dana K., and Noel Rose. *The Encyclopedia of Autoimmune Diseases*. New York: Facts On File, 2003.

National Institutes of Health. National Institute of Allergy and Infectious Diseases home page. Available online. URL: http://www3.niaid.nih.gov/. Last updated on January 14, 2008.

Wise, Darla J., and Gordon R. Carter. *Immunology: A Comprehensive Review*. Ames: Iowa State University Press, 2002.

infectious diseases A variety of factors can lead to a diseased state, a condition in which all or part of the body does not function properly. Diseases may be caused by the inheritance of mutated genes, malnutrition, wear and tear of a body tissue or organ, exposure to environmental factors such as toxic chemicals or radiation, the body's immune system attacking its own tissues, or simply aging. Infectious diseases are diseases caused by the invasion and growth of a pathogenic microorganism in a host. Although people often use the terms *infectious* and *communicable* interchangeably, not all infectious diseases are communicable. A communicable disease is one that can be spread from one host to another. For example, otitis media is a diseased state of the middle ear that can be caused by a bacterial infection, but one person cannot catch an ear infection from another person—ear infections are not contagious.

BACKGROUND INFORMATION ON INFECTIOUS DISEASES

Pathogenic microorganisms are bacteria, viruses, fungi, protozoa, or worms that are capable of causing disease. Just because someone comes into contact with a potentially pathogenic microorganism does not necessarily mean they will become ill. The microorganism must first gain entry into a host tissue in which it can grow and multiply. Certain types of pathogens can infect only certain parts of the body. For example, a virus that infects the respiratory tract may not be able to grow inside the digestive tract. The portal of entry also varies depending on the particular pathogen. Microbes can enter the body through ingestion, inhalation, through a wound in the skin or mucous membranes, directly into the blood during an injection or bug bite, or through sexual intercourse. The potential host has defense mechanisms to prevent microorganisms that successfully gain entry from establishing an infection (see HOST DEFENSES), but if a sufficient number of microbes enter, they may be able to evade the host defenses. Many pathogenic microbes have specialized structures, secrete enzymes, or produce chemicals that help them elude the host defenses and increase the likelihood of causing disease. Molecules on the exterior of the microorganism may specifically recognize and attach to molecules on the surface of a host tissue, or slimy polysaccharide capsules may prevent host phagocytes from ingesting and destroying the pathogen. Enzymes may digest host tissues so the microorganism can penetrate deeper. Once the pathogen has found its way to the infection site, it grows and multiplies, living off nutrients supplied by the host. As the number of pathogens increases, the host may show signs and symptoms of illness. In normal, healthy individuals, the immune system eventually catches up and takes over, destroys all the pathogens, and the host's body eventually regains its health.

During infection, the host may shed the pathogenic microorganisms into the environment. The

portal of exit, or how the microorganism leaves the body, depends on the site of the infection. In the case of a respiratory tract infection, a sneeze or cough expels microorganisms from the body. If another person is nearby and inhales droplets or dust particles that contain the pathogenic microbe, the process may begin again in the new host. Communicable gastrointestinal diseases are transmitted by the fecal-oral route. The host excretes pathogenic microbes during bowel movements, and the microbes may spread through contaminated water supplies or unclean fingers to food during meal preparation or directly to the mouth. Microbes may also be shed with dead skin cells, saliva, semen or vaginal secretions, or from a bleeding wound.

The immune system of the human body effectively fights pathogens that cause many communicable diseases, but sometimes medical intervention is necessary. Antimicrobial drugs target the microbial pathogen and either destroy it or prevent it from growing and multiplying.

EPIDEMIOLOGY

The Centers for Disease Control and Prevention (CDC), one of the major components of the U.S. Department of Health and Human Services, maintains reliable data for morbidity and mortality of communicable disease in the United States. Morbidity is the relative incidence of a disease, and mortality is the number of deaths in a given time or place due to a disease. The CDC classifies certain diseases as notifiable, defined by the CDC as diseases for which regular, frequent, and timely information regarding individual cases is considered necessary for the prevention and control of the disease. The most common notifiable diseases, according to the *Summary of Notifiable Diseases—United States 2005*, the most recent year for which complete data is available, are reported in the table Summary of Notifiable Diseases in the United States (2005). It should be noted that not all notifiable diseases are communicable, but all are infectious.

Comprehensive global data regarding the morbidity of communicable diseases is difficult to obtain because many of the most prevalent diseases are endemic to developing countries lacking organized health care systems with medical care reaching the whole population. The World Health Organization (WHO), the health authority of the United Nations, monitors worldwide health situations including those relevant to communicable diseases. WHO provides the most reliable information, but even its data is not comprehensive. Due to social and economic factors, the worldwide profile for infectious diseases differs from that of the United States. According to the WHO and the Global Health Council, a nonprofit, U.S.-based organization created in 1972, more than 90 percent of the deaths due to infectious disease are caused by a handful of diseases: lower respiratory infections, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), diarrheal diseases, tuberculosis, malaria, and measles. Most of these diseases have been brought under control in industrialized nations with vaccines, antibiotics, and medical treatment.

SUMMARY OF NOTIFIABLE DISEASES IN THE UNITED STATES (2005)

Rank	Disease	Total Reported Cases
1	Chlamydia	976,445
2	Gonorrhea	339,593
3	Salmonellosis	45,322
4	AIDS	41,120
5	Syphilis	33,278
6	Varicella	32,242
7	Pertussis	25,616
8	Lyme Disease	23,305
9	Giardiasis	19,733
10	Shigellosis	16,168
11	Tuberculosis	14,097

LEADING INFECTIOUS CAUSES OF DEATH

Worldwide, lower respiratory tract infections such as pneumonia are the leading cause of death due to infectious diseases in children under the age of five, with about 75 percent of pneumonia deaths occurring in infants less than one year old. According to the WHO, pneumonia kills more than 2 million children each year, more than AIDS, malaria, and tuberculosis combined. Even in industrialized countries, pneumonia is a leading cause of death among the elderly. Pneumonia is primarily a bacterial infection of the lungs, and when antibiotics are available, it can usually be treated effectively. *Streptococcus pneumoniae* and *Haemophilus influenzae* are the leading culprits. Viruses, fungi, and parasites also cause pneumonia, though less frequently, and these cases are less likely to be fatal. In the body of a person who has pneumonia, the alveoli, the terminal sacs of the bronchioles that fill with air during inhalation, become inflamed and filled with fluid. This causes coughing, shortness of breath, chest pain, and fever, and may be accompanied by muscle aches.

X-rays and analysis of sputum samples can confirm a diagnosis when listening to the lungs with a stethoscope reveals abnormal breath sounds such as crackling. Pneumonia can be transmitted through direct contact with infectious secretions, and the risk of infection increases with HIV infection, malnutrition, and malaria. Vaccinations are available for both *Haemophilus influenzae* and *Streptococcus pneumoniae,* which also causes meningitis, blood infections, and other health problems.

According to the WHO, an estimated 40 million people are living with HIV/AIDS, with 95 percent of cases in developing countries and sub-Saharan Africa being the most affected. According to the CDC, approximately 1.2 million people in the United States were living with HIV/AIDS in 2005, the last year for which comprehensive data is available. AIDS refers to the progressive decline in immune function in HIV-infected individuals, whereas HIV/AIDS refers to cases of infection with the causative retrovirus whether or not the case has progressed to AIDS. Transmission occurs by sexual intercourse or oral sex with an infected individual, injection drug use, mother-to-child transmission, and contact or transfusion with infected blood. The virus infects white blood cells called T-lymphocytes that play an important role in the specific immune response. Over time, the number of these white blood cells decreases as they are destroyed by the virus, and eventually the person is no longer able to fight infections. At this stage the person has AIDS and can easily contract diseases and infections that healthy people can resist. Tuberculosis is the major cause of death among people infected with HIV. No cure and no vaccine are available for HIV/AIDS. Since the mid 1990s, advances in treatment have slowed the progression of infection with HIV to AIDS and decreased the mortality rate of AIDS. Unfortunately, these treatments are largely unavailable in developing countries, the regions of the world most affected by HIV/AIDS. Prevention strategies include providing access to condoms, access to voluntary testing, treating other sexually transmitted diseases, preventing mother-to-child transmission, educating intravenous drug users and the community about HIV transmission, and improving access to medical care and support.

Diarrhea kills about 2.2 million people globally (source: WHO), mostly children and mostly in developing countries, where water treatment and sanitation are lacking. Ingestion of contaminated water is the most common cause of transmission, but transmission can also occur via flies, utensils, or directly by the fecal-oral route. If someone's hands come into contact with an infected person's stools (such as through assisting in hygienic care), and the hands come into contact with the mouth, the microorganism can gain entry into the digestive tract. Thus, prevention strategies include access to safe drinking water, improved sanitation, hygienic practices, and educating people on the spread of infections. Certain bacteria, viruses, and parasites can cause diarrhea. The bacterium *Vibrio cholerae* causes cholera, one of the most severe diarrheal diseases, characterized by profuse watery diarrhea, vomiting, and leg cramps. Dehydration can occur rapidly and cause death. Treatment includes rehydration and administration of sugar and salts to replace lost electrolytes. Other microorganisms that cause diarrhea include rotavirus, *Escherichia coli, Salmonella, Shigella,* and *Giardia.*

Tuberculosis (TB), formerly known as consumption, white plague, or white death, has been around for at least 7,000 years and has killed more than 2 billion people in the last few centuries alone. The WHO estimates that similar numbers of people are currently infected with the TB bacillus; this is approximately one-third of the world's population. A latent TB infection means the bacteria are present in the body but are dormant; the disease TB results when the germs actively multiply and divide in the host, causing symptoms. Only 5–10 percent of these people will become ill with TB; the risk increases when someone is infected with HIV. Between 1.5 and 2 million people die annually from TB, more than 90 percent of whom live in developing countries. Caused by *Mycobacterium tuberculosis,* TB is primarily an infection of the respiratory tract, but the bacterium can also invade other body tissues such as the brain, kidneys, or spine. The symptoms of TB include general malaise, weight loss, fever, night sweats, coughing, coughing up blood, and chest pain. The bacteria are spread in sputum released into the air when someone coughs or sneezes and the germs may remain in the air for several hours. Diagnosis of TB usually consists of the Mantoux tuberculin test, which is performed by injecting a small amount of tuberculin fluid under the skin of the forearm. The appearance of a red, raised bump 48 to 72 hours after injection indicates the person has been infected, but the infection may be latent. In order to confirm TB disease, a physician will order a sputum analysis to look for the presence of *Mycobacterium* or a chest X-ray to look for characteristic lesions called tubercles in the lungs. A strict regimen of antibiotics can generally cure TB; however, drug-resistant strains of the bacterium have been documented in every country. Drug resistance is due in part to patients not taking all of their medicines. Particularly worrisome are multidrug-resistant TB (MDR-TB), which is resistant to the two powerful anti-TB drugs isoniazid and rifampicin, and extensively drug-resistant TB (XDR-TB), a strain that is resistant to isoniazid, rifampicin,

quinoline, and at least one of a second-line TB treatment (i.e., kanamycin, capreomycin, or amikacin).

Malaria is a potentially fatal vector-borne illness caused by a parasite transmitted to humans through the bite of an infected *Anopheles gambiae* female mosquito. The disease is most common in warmer regions of the globe, where the *Anopheles* mosquito thrives. The CDC estimates that 350–500 million cases of malaria occur annually worldwide, and approximately 1 million people die from it. Four species of the causative protozoan infect humans: *Plasmodium vivax, Plasmodium ovale, Plasmodium falciparum,* and *Plasmodium malariae*. *Plasmodium* species undergo a complex life cycle. The mosquito injects threadlike cells called sporozoites into a human during a blood meal. The sporozoites travel to the liver and infect the liver cells. A single sporozoite can generate 30–40,000 daughter merozoites within six days of infecting a liver cell. This release as the liver cell bursts causes the first symptoms, sweating and high fever. The merozoites infect red blood cells, grow larger, and upon rupture 48 hours later, release 8 to 24 daughter cells into the bloodstream. By an unknown mechanism, millions of red blood cells rupture simultaneously, releasing toxins that cause characteristic symptoms such as high fever, chills, and flulike symptoms. An infected person may also experience anemia due to the loss of red blood cells, and, as the liver breaks down the damaged red blood cells, the skin may appear jaundiced. After several cycles of reinfection of red blood cells, some merozoites transform into male or female gametocytes that a mosquito may pick up during a feeding. Fertilization occurs inside the mosquito, eventually forming more sporozoites for the mosquito to release into the next human bite victim. Diagnostic testing consists of examination of a blood smear under the microscope. A trained pathologist can recognize the parasites in the blood cells. Treatment is most effective when started early in the course of infection. If untreated, or treated with an inappropriate drug for the particular strain, then the parasites can persist in the blood or remain latent in the liver for decades. Malaria parasites are becoming increasingly resistant to antimalarial drugs, so prevention is also important in reducing the effects of this devastating illness. Keeping mosquitoes away by insecticide sprays around living quarters, wearing insect repellent, and sleeping under insecticide-treated bed nets are all effective preventative strategies. Because the parasites are present in the blood of an infected person, transmission of malaria can also occur by blood transfusion, organ transplantation, or sharing syringes contaminated with blood.

Though measles (rubeola) is no longer endemic in the United States due to effective vaccination programs, the problem persists in developing countries. In 2003 measles killed more than 500,000 people worldwide, with more than half of those deaths occurring in Africa. Measles is caused by a virus from the Paramyxoviridiae family. The virus is highly contagious even before the infected person exhibits any symptoms and easily spreads through mucous by sneezing or coughing. After an incubation period of 10–12 days following exposure, lesions in the oral cavity called Koplik spots—red spots with white centers—appear. These are a diagnostic indicator for the disease. Other symptoms include a high fever, cough, runny nose, and a blotchy rash over the entire body. Four days after the rash, the person is no longer contagious. Complications that accompany measles can also be devastating: pneumonia, diarrhea, middle ear infections, and malnutrition. No specific treatment for measles exists, but supportive care includes hydration, medications to reduce fever, and treatment for the complications.

INFLUENZA

Though not a leading cause of death worldwide, influenza (the flu) consistently debilitates and kills significant numbers of people each year. In the United States, between 5 and 20 percent of the population catches the flu each year, and approximately 36,000 people die. Influenza is caused by a virus that infects the upper respiratory tract. Symptoms include the sudden onset of a high fever, muscle aches, fatigue, a headache, a nonproductive cough, sore throat, and rhinitis. Secondary complications include bacterial pneumonia, ear infections, sinus infections, and dehydration. People with other health concerns, the elderly, and the young are at greater risk for infection and for having more severe symptoms. A person can get the flu by inhaling germs discharged when an infected individual coughs or sneezes or by touching something that an infected individual has contaminated and then touching their own nose or mouth.

Getting a flu shot in the late fall of each year is the best means of preventing the flu, and when more people get vaccinated, the flu cannot spread as rapidly throughout the population. Influenza viruses come in three types, A, B, and C. Types A and B cause yearly epidemics during flu season, which typically lasts from November through May in the Northern Hemisphere. Human subtypes of A are called H1N1 and H3N2, named after two types of specific proteins on the surface of the virus (H for hemagglutinin and N for neuraminidase). Type B influenza is not categorized. Influenza viruses rapidly mutate, giving constant rise to new strains. Virologists collect data and strains from across the globe in an attempt to predict which strains may cause an epidemic the following year. Based on their analysis, they include

strains of A(H1N1), A(H3N2), and B in the vaccination (type C is not included). A newer alternative to getting a shot is the nasal-spray flu vaccine. Whereas the injectable vaccine contains inactivated viruses, the nasal spray version contains active but weakened viruses. Once someone comes down with the flu treatment usually consists of rest, fluids, and over-the-counter medications to relieve symptoms.

Type A influenza viruses also affect many other animals. Subtypes H5 and H7 can cause widespread outbreaks in birds. Occasionally, a bird can transmit avian influenza to other animals, including pigs and humans. If a new subtype that can infect not only humans but that can *spread* between humans emerges, a potentially devastating pandemic could result. One way this could happen is if both avian and human viruses infected pigs at the same time, gene mixing (called antigenic shift) could occur, creating a virus that has avian surface proteins and genes that allow it to replicate inside human cells. This combination would be particularly dangerous because most humans would not have any protective immunity against the avian surface proteins, and thus the virus would spread rapidly and could cause millions of deaths. Such a worldwide outbreak occurred in 1918. Dubbed the Spanish influenza, 40–50 million people died, making the 1918 pandemic one of the deadliest in history. Because of the potential for an avian influenza virus strain to mutate into a strain that is communicable between humans, epidemiologists carefully monitor it. One strain in particular, H5N1, has this potential. H5N1 first infected humans in 1997 and has killed more than half of the 100 infected people since then. Since 2003 severe outbreaks of avian H5N1 in chickens in Asia have led to the mandated slaughtering of millions of birds to prevent it from developing into a pandemic influenza.

See also ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS); ANTIMICROBIAL DRUGS; GERM THEORY OF DISEASE; HOST DEFENSES; PROKARYOTIC CELLS; SEXUAL AND REPRODUCTIVE HEALTH; VIRUSES AND OTHER INFECTIOUS PARTICLES.

FURTHER READING

Centers for Disease Control and Prevention home page. Available online. URL: http://www.cdc.gov. Last updated on January 24, 2008.

Grady, Sean M., and John Tabak. *Biohazards: Humanity's Battle with Infectious Disease*. New York: Facts On File, 2006.

Turkington, Carol, and Bonnie Lee Ashby. *The Encyclopedia of Infectious Diseases*. 2nd ed. New York: Facts On File, 2003.

World Health Organization home page. Available online. URL: http://www.who.int/en/. Accessed on January 25, 2008.

Zimmerman, Barry E., and David J. Zimmerman. *Killer Germs: Microbes and Diseases That Threaten Humanity,* Revised and updated ed. Chicago: Contemporary Books, 2003.

Ingenhousz, Jan (1730–1799) Dutch *Physician and Plant Physiologist*

Jan Ingenhousz discovered photosynthesis, the process by which plants and other photoautotrophs utilize energy from sunlight and from atmospheric carbon dioxide to synthesize carbohydrates. All life-forms on Earth depend on photosynthesis, either directly or indirectly. Ingenhousz observed that the green parts of plants absorbed carbon dioxide from the air and released oxygen, functions that supported animals, which take in oxygen and release carbon dioxide. Ingenhousz was also a proponent of inoculations as a means of inducing protective immunity and was the first to describe Brownian motion.

Jan Ingenhousz was born in Breda, Netherlands, on December 8, 1730, to Arnoldus and Maria Beckers Ingenhousz. His father was a leather merchant who later became a pharmacist. Jan studied classical languages at the Breda Latin School. Ingenhousz followed the tradition of most Roman Catholics from the Netherlands by attending the Catholic University of Louvain in Belgium, from which he graduated with a degree in medicine in 1753. He then entered Leiden University to continue his medical studies, but he stayed there less than one year. Though no formal record exists, Ingenhousz may also have studied for some time in Paris and at Edinburgh.

After moving back home, Ingenhousz practiced medicine and performed chemistry and physics experiments. When his father died in 1764 he used his inheritance to move to England, where he met the chemist Joseph Priestley and the American philosopher, inventor, and scientist Benjamin Franklin. Priestley earned scientific renown by identifying and discovery numerous gases and for increasing the understanding of photosynthesis. Most of Franklin's fame was due to his discovery that lightning was an electrical phenomenon, but his reputation extended beyond science to politics as a leading statesman who contributed to the foundation of the United States of America.

SMALLPOX INOCULATIONS

Smallpox is a highly contagious disease caused by a Variola virus. Although the World Health Organization declared the disease eradicated in 1980, during the American Revolutionary War in the late 1770s, smallpox claimed the lives of 125,000 people in the Thirteen Colonies. The name of Edward Jenner, an English doctor, is associated with creating the small-

pox vaccine. After noticing that milkmaids did not get smallpox, he vaccinated the first patient in 1796 by injecting a bit of cowpox fluid extracted from cowpox blisters of a milkmaid. As hoped, Jenner's vaccination procedure did artificially induce immunity as observed in the dairymaids, as subsequent injections with the live smallpox virus did not cause disease in inoculated individuals. Vaccination was safer, but inoculation, which is the purposeful infection of a person with the virus so as to make the person immune or reduce the severity of the disease if contracted, had been used since 1000 B.C.E. A man named Daniel Sutton revived the practice of inoculation in England. Some people objected to inoculation for religious reasons, claiming the practice defied the will of God. In 1766 Ingenhousz began inoculating people with smallpox by Sutton's methods at Foundling Hospital, where the practice was mandatory. Ingenhousz also started his own private inoculation practice and traveled to towns where outbreaks occurred. In 1768 King George III of the United Kingdom sent Ingenhousz to Austria to inoculate the royal family there. His success in doing so pleased the Austrian empress Maria Theresa, who, in appreciation, appointed Ingenhousz court physician. He continued traveling and inoculating relatives of the imperial family in return for a handsome lifelong salary that he used to support his science experiments.

PHOTOSYNTHESIS AND RESPIRATION

Scientists knew that the flame of a candle in a sealed jar would eventually burn out; likewise, a living animal would die in a sealed container. They explained this as the "good air" being used up. In the 1770s, while studying various gases, Priestley showed that plants could restore the fresh air in the containers by removing the "bad air" (now called carbon dioxide) and replacing it with "good air" (now called oxygen) such that the air could once again support a burning candle or the respiration of a living animal. Ingenhousz extended these studies to show that the release of oxygen required light in the visible range of the spectrum and that only the green parts of plants possessed this capability of restoring the air. These findings laid the foundation or scientific understanding of the process now termed photosynthesis.

This well-researched process converts light energy into chemical energy stored as organic compounds used as food. The chloroplast is the structure capable of undergoing photosynthesis in plants and algae. Chloroplasts are specialized membrane-bound organelles that contain the pigment chlorophyll, which absorbs the red and blue light of the visible spectrum. Because green light is reflected, most photosynthetic organisms appear green in color, or at least have green parts. When chlorophyll absorbs energy in the form of light, electrons of the chlorophyll molecules jump to higher energy levels and begin a cascade of falling step by step to sequentially lower energy levels in a chain of molecules. Water molecules (H_2O) that supply the electrons from their hydrogen atoms are split, and the remaining oxygen atom is released and combines with a second oxygen atom to form molecular oxygen, O_2 gas. The hydrogen atoms donate electrons to the chlorophyll molecules, and the remaining protons are used to create a proton gradient. The energy harvested from the sunlight, now in the form of a chemical gradient, is used to drive the synthesis of high energy molecules that the cells use to synthesize carbohydrates, or sugars. Ingenhousz showed that the carbon in plant material originated from CO_2 in the atmosphere. In summary, during photosynthesis, organisms use sunlight, CO_2, and water to make sugars, and they release oxygen in the process.

$$6CO_2 + 12H_2O + \text{sunlight} \rightarrow C_6H_{12}O_6 \text{ (sugars)} + 6O_2 + 6H_2O$$

Ingenhousz made the connection that the plants were using carbon dioxide to generate oxygen and that the process mirrored respiration in animals. Plants released oxygen into the environment, animals took the oxygen in and released carbon dioxide, which the plants took in, and the cycle continued. For these studies Ingenhousz is credited with the discovery of photosynthesis. Though Priestley is often credited for this discovery, Ingenhousz published his book *Experiments upon Vegetables, Discovering Their Great Power of Purifying the Common Air in the Sunshine, and of Injuring It in the Shade and at Night, to Which Is Joined, a New Method of Examining the Accurate Degree of Salubrity of the Atmosphere* in 1779, before Priestley demonstrated an understanding of this biochemical process. Ingenhousz also showed that plants, like animals, undergo respiration.

As a physician, Ingenhousz recognized the importance of clean, fresh air to the health of living organisms. After discovering that plants were responsible for restoring the purity of air, he invented an apparatus that made and administered the pure air to patients, the earliest record of oxygen therapy.

The Royal Society of London elected Ingenhousz to membership in 1769. In 1778 and 1779 he delivered the Bakerian Lectures, the premier lecture in the physical sciences of the Royal Society. His topics included "Electrical Experiments to Explain How Far the Phenomena of Electrophorus May Be Accounted for by Dr. Franklin's Theory of Positive and Negative Electricity" and "Improvements in Electricity." Ingenhousz also discovered and described Brownian motion, the random movement

of particles in solution by molecules bumping into one another, while observing powdered charcoal suspended in alcohol.

See also PHOTOSYNTHESIS; PLANT FORM AND FUNCTION; PRIESTLEY, JOSEPH.

FURTHER READING

Ingenhousz, Jan. *Experiments upon Vegetables, Discovering Their Great Power of Purifying the Common Air in the Sunshine, and of Injuring It in the Shade and at Night, to Which Is Joined, a New Method of Examining the Accurate Degree of Salubrity of the Atmosphere*. London, 1779.

inheritance Until the 1860s, when an Austrian monk named Gregor Mendel performed a systematic investigation into the transmission of characteristics between generations of garden pea plants, not much was known about how traits were passed from parent to offspring. Even after Mendel presented his now landmark results, they went unnoticed for 35 years, when other scientists came across his paper and recognized its significance. Since then, science has uncovered much about the mechanisms of inheritance with the discovery of genes, their relationship to chromosomes, how they encode proteins that confer distinct characteristics to an organism, how those proteins are expressed, how genes mutate or change, and how they are duplicated and passed on to offspring in the gametes.

INHERITANCE OF TRAITS

The deoxyribonucleic acid (DNA) of organisms contains within its sequence of four alternating nucleotides all of the genes necessary for directing the growth, maintenance, and reproduction of an organism. A gene is a physical entity composed of a stretch of DNA, located at a particular location on a chromosome of an organism. As the functional unit of inheritance, each gene encodes for either a protein or a ribonucleic acid (RNA) molecule. The gene products, especially proteins, perform all the work of a cell, everything from serving as structural building components to catalyzing biochemical reactions. Different species have characteristic numbers of chromosomes; for example, mosquitoes have six chromosomes (three pairs), rice plants have 24 chromosomes (12 pairs), humans have 46 chromosomes (23 pairs), and bacteria have a single chromosome. Organisms that reproduce sexually produce gametes, eggs or sperm, that contain only half of the normal number of chromosomes. Cells that contain two of each type of chromosome are diploid, and cells that contain only a single set of chromosomes are haploid. The gametes, which are haploid, are sometimes called sex cells. All the other cells of the body are diploid and are called somatic cells.

During gametogenesis, the process of making gametes, a parent cell undergoes meiotic division to create four haploid cells. In spermatogenesis, the process of making male gametes, each of the haploid cells matures into a sperm cell. In oogenesis, the process of making female gametes, a single haploid mature ovum, or egg, results. At fertilization, an egg and sperm cell unite, and their haploid genomes combine to restore the original diploid number of chromosomes in the resulting zygote. The zygote undergoes numerous rounds of mitotic division, creating embryonic cells that are all also diploid.

Each chromosome type contains a certain collection of genes, and since a cell contains a pair of each type of chromosome, each cell contains a pair of genes for each trait. The chromosomes that encode for the same traits are called homologous chromosomes. Each copy of a gene is called an allele. Many forms of an allele can exist in a population, but only a single form exists at each gene locus on a chromosome. The two copies in a cell can be the same type, or they can be different types. The term *homozygous* means the two copies of a gene are the same, and the term *heterozygous* means the two forms are different. The law of segregation, initially described by Mendel, states that during gamete formation, the two alleles separate, or segregate, and end up in different gametes. Though at the time, the events of meiosis were unknown, the law of segregation explains the behavior of chromosomes during the first meiotic division. When the members of a pair of chromosomes separate, they travel to opposite ends of the parent cell, and ultimately end up in different daughter cells. Since both members of a pair of chromosomes carry the same collections of genes, this action also separates the members of a pair of alleles for a gene. Each gamete receives only one of the two alleles for a particular trait.

The genotype refers to the combination of alleles an individual possesses. An individual can contain two identical copies of an allele, a condition described as homozygous, or possess two different forms of the allele, a condition described as heterozygous. The phenotype is the outward expression of a characteristic. The combination of alleles an individual possess determines how the characteristic appears. The phenotype of homozygous individuals is only determined by a single form of an allele, since the individual possesses two identical copies. In heterozygous individuals, the dominant allele determines the organism's appearance. One allele (the dominant one) takes over, and the other allele (the recessive one) has no observable effect; expression

(continues on page 476)

EPIGENETICS: REGULATION ABOVE THE GENOME

by Alexandra C. Silveira, Ph.D.
University of Alabama at Birmingham

Epigenetics is the study of modifications that regulate gene expression without changing DNA sequence. Like the genetic information programmed in the genome, epigenetic information is also heritable; however, unlike the DNA sequence, epigenetic changes are reversible and are continually being added or taken away in response to stimuli.

The term *epigenetics* was first used by the British embryologist and geneticist Conrad Waddington in 1942 to describe events regulating differences between genotype, the information encoded by DNA, and phenotype, the expression of traits. As early as the 1960s, scientists began to uncover the exact nature of these differences, leading to the classification of three different types of epigenetic regulation: DNA methylation, histone tail modification, and the expression of microRNA transcripts.

TYPES OF EPIGENETIC MODIFICATIONS

The best-studied epigenetic modification is DNA methylation, the addition of a methyl group (-CH_3) on cytosine, a DNA nucleotide. In vertebrates, a group of proteins known as methyltransferases catalyze the addition of methyl groups to cytosines in CpG islands, areas of DNA where there are several repeats of coupled cytosines and guanines. CpG islands often occur in promoter sequences, the initial regulatory regions of genes, and methylation of these areas frequently marks genes that are inactive. Methylation regulates gene expression by inhibiting the recruitment of certain binding factors to sites on a promoter or by recruiting chromatin modifying transcriptional repression complexes.

Histone modifications involve the addition or subtraction of chemical modifications to the amino-terminal tails of histones, the proteins that organize DNA. One such modification is methylation, like that seen for DNA. Methylation, along with phosphorylation and acetylation, are the best-studied histone tail modifications. Other known modifications include ubiquitination, SUMOylation, ADP ribosylation, deimination, and proline isomerization. Ubiquitination is the covalent addition of the small protein ubiquitin to another protein. Addition of ubiquitin can label a protein for degradation or modify its function. To date, not much is known about histone tail ubiquitination; however, recent studies show ubiquitination regulates some histone tail methylation. Like ubiquitination, SUMOylation involves the addition of a ubiquitin-related protein, the small protein SUMO (small ubiquitin-related modifier). SUMOylation is thought to antagonize histone tail acetylation, resulting in transcriptional repression. ADP ribosylation is the addition of an adenine-ribose-P-P-ribose group to histone tails. Both deimination and isomerization result in the modification of the amino acid proline. The sum of the genetic information encoded for in these modifications is referred to as the histone code.

Some histone tail modifications at certain sites result in DNA that is more "loose," or rather a less tightly packed conformation, which allows transcriptional machinery to access the DNA; removal of these same groups results in a more compact and less transcriptionally permissive chromatin structure. Epigenetic changes can also act to recruit other, nonhistone proteins. In this way some modifications serve as activating marks to signal transcription. Each of these modifications is in turn regulated by various sets of proteins. As in the case of DNA, there exist histone methyltransferases. Another example is that of histone acetylases, which add an acetyl group (-$COCH_3$) to histone tails, and histone deacetylases, which remove acetyl groups. Histone modifications play a role in transcriptional regulation affecting differentiation, mitosis, spermatogenesis, and DNA repair.

The third type of epigenetic change was most recently discovered in 2001 when three separate groups identified microRNAs, single-strand RNA sequences that vary in length from 21 to 24 nucleotides. These small RNA sequences are encoded in an individual's genome and, like genes, their expression is controlled by heritable epigenetic modifications such as DNA methylation or histone tail additions. MicroRNAs control gene expression by binding to a complementary region on messenger RNA transcripts to consequently inhibit translation, target transcripts for degradation, or both. Currently, scientists estimate that about 30 percent of genes are regulated by microRNA.

Epigenetic modifications not only have direct effects on DNA and RNA, they can also regulate each other. For example, methylation of DNA can recruit histone modifying complexes and control the transcription of microRNAs. Similarly, the expression of microRNAs could conceivably influence the expression of proteins that regulate other epigenetic changes. Likewise, different histone tail modifications can facilitate the addition of further modifications that can act in concert to affect changes.

EPIGENETICS AND DEVELOPMENT

Epigenetic modifications encode information that is necessary for the development of different cell types, a process known as differentiation, from the single DNA template contained within a fertilized egg. By looking at epigenetic profiles, snapshots of the epigenetic modifications, scientists investigate how these modifications specifically regulate cellular differentiation.

At the first stages of development, undifferentiated embryonic stem cells have less compacted chromatin, making these cells more transcriptionally permissive. Histone tail modification silences gene families that drive development

(continues)

(continued)

and differentiation, such as the homeobox gene family, during this period. While cells differentiate, certain regions of the chromatin begin to compact—in particular, regions of DNA that contain nonfunctional genes flanking the centromeres, known as pericentric heterochromatin. As cells differentiate, genes that are important for maintaining the totipotent nature of stem cells, such as *Oct4* and *Nanog,* can become silenced through histone modifications and DNA methylation; silencing of these genes ensures that cells remain differentiated. By regulating the expression of tissue specific genes, other epigenetic modifications mark the final differentiation of a cell type. Parent cells pass on epigenetic changes to daughter cells. This epigenetic inheritance is explained by the maintenance methylase theory: Methyltransferases methylate a newly synthesized strand of DNA using the original strand of DNA as a template much like DNA replication machinery does during DNA synthesis.

EPIGENETICS AND ADULT CELLS

Epigenetic modifications are also responsible for genomic imprinting, a phenomenon regulating less than 1 percent of genes in which methylation directly or indirectly silences a maternally or paternally inherited gene, resulting in expression of a single, parent-specific allele (form of a gene). For example, in differentiated cells only the paternal copy of the *Insulin-like growth factor II* gene is expressed while the maternal copy is silenced.

Epigenetics also regulate X-inactivation, the silencing of one copy of the two X-chromosomes in female cells. Inactivation involves reduced acetylation, also known as hypoactelyation, of histones H3 and H4 by methylation of an amino acid at a specific position on histone H3 and by DNA methylation. During oocyte formation inactivation is reversed so that each egg contains a single, active X-chromosome.

In addition to the regulation of chromatin and specific genes, epigenetic modifications play an important part in maintaining the stability of the genome by regulating transposons, segments of DNA that can move around in the genome of a single cell. Methylation and histone modification silence these transposons, thereby preventing them from moving in the genome, a process that can result in gene mutation.

EPIGENETICS AND TWINS

Modifications of DNA and histone tails also affect phenotypic differences between individuals having the same genetic background—twins. Monozygotic twins, more commonly known as identical twins, share the same DNA sequence; however, despite sharing nearly the exact same DNA, individual twins exhibit unique traits. For example, in cases where one identical twin has schizophrenia, there is a 50 percent chance that the other twin will also have schizophrenia (rather than the 100 percent chance predicted by genetics alone).

Scientists have traditionally attributed variance in twins to the environment. However, studies done by Klaus Gärtner and colleagues in genetically identical animals demonstrated a "third component" aside from genes and environment—this "third component" is epigenetic regulation. In the case of genetically identical mice, epigenetic regulation of the *Agouti* gene can cause individual mice to have various coat colors ranging from yellow to brown. Arturas Petronis and colleagues found that human twins also exhibit subtle differences in the methylation of cytosines. Variations in methylation may further explain schizophrenic dissimilarity in twins. Studies demonstrate reduced expression of two proteins, glutamic acid decarboxylase and reelin, through methylation of their promoters in the brains of schizophrenic patients.

Unique epigenetic states can arise through various mechanisms. In some cases, at twinning unequal cell division might occur. Even normal cell division can result in differences in methylation states with up to 3 to 5 percent change occurring per mitosis.

EPIGENETICS AND THE ENVIRONMENT

While some epigenetic changes remain unexplained, others directly result from environmental pressures. This could, in part, also explain some of the differences in twins that *are* attributable to the environment.

Dietary studies done in mice with variations of the *Agouti* gene found that feeding mothers foods rich in donor methyl groups resulted in varied methylation and shifting of coat color. Most recently, a group studying the *Agouti* gene showed that maternal diets could affect later generations. Other researchers discovered that differences in the expression of the *Agouti* gene affected the expression of several other genes; those mice with a yellow coat were found to be larger, obese, hyperinsulinemic, more susceptible to cancer, and shorter lived than their nonyellow siblings. These variations have been linked to alterations in the methylation of transposons that insert into the promoter element of the *Agouti* gene to affect transcription.

In 2005 researchers Marcus Pembrey and Lars Olov Bygren reported a similar phenomenon in humans. Their study found that the diet of grandparents affected their grandchildren in a sex-specific manner: The diet of a paternal grandfather affected the longevity of his grandson, and the diet of a maternal grandmother affected the longevity of her granddaughter.

Other data demonstrate that prenatal and postnatal exposure to environmental pressures can influence susceptibility to diseases and chronic conditions including depression, cancer, cardiovascular disease, diabetes, and obesity. This data supports the theory that diseases affecting a person in adulthood can arise from fetal exposures.

EPIGENETICS AND CANCER

Epigenetics is especially well studied in cancer. Researchers have shown that different tumor types have distinct patterns of methylation, or methylation signatures. A separate study done by Hilal Özdağ and colleagues presented evidence that epigenetic status can be used to distinguish tumor samples from normal counterparts by looking at a histone-modifying enzyme. Publications also report that loss of specific acetylation on histone H4 is an early alteration in cancer.

In addition to distinct epigenetic characteristics, most cancers share other

general traits. Cancer cells often exhibit global hypomethylation, resulting in the expression of genes that are normally silenced, a phenomenon known as loss of imprinting. Pockets of hypermethylation on regions controlling the expression of tumor suppressors result in the loss of tumor suppressor expression early in cancer development. These tumor suppressor genes encode proteins that are involved in DNA repair, halting an aberrant cell cycle, activating embryonic pathways, and controlling cell death. Cancerous cells also exhibit general histone hypoacetylation and consequently more compacted chromatin in regions that could affect the expression of tumor suppressing or promoting genes.

Epigenetic modifications in cancer can come about through the altered expression or function of proteins that are responsible for epigenetic regulation. Some evidence suggests that two different DNA methyltransferases, DNMT1 and DNMT3b, are overexpressed in multiple cancer types. Other cancers abnormally express proteins that modify the histone tails, such as the histone deacetylase proteins HDAC1 and HDAC2, or possess amplifications or deletions of the genes that encode histone methyltransferases.

MicroRNA expression has also been linked to cancer. Studies have shown several different microRNA transcripts, *miR-125b, miR-145, miR-21,* and *miR-155,* to be reduced in breast cancer. Further evidence indicates a role for microRNAs in other cancers, including lymphomas, hepactocellular carcinomas, leukemia, and lung, colorectal, and brain cancers. Some microRNAs are thought to be oncogenic, that is, their increased expression represses tumor suppressor genes leading to cancer formation or a more aggressive phenotype as is the case with *mir-17-92,* which has increased expression in lung cancer and several lymphomas. Other microRNAs are thought to function as tumor suppressors such as *let-7,* a microRNA that has been found to be poorly expressed in lung cancer. Scientists observed that reintroduction of *let-7* into cancerous lung cells inhibited the ability of these cells to grow in culture.

By studying epigenetic changes in cancer, scientists can better diagnose and treat the disease. Studies have already shown that in lung, colorectal, and brain cancers, hypermethylation of certain proteins is linked to tumor aggressiveness. In prostate cancer, overall changes in the levels of individual histone modifications can help predict the clinical outcome.

Examination of epigenetic changes might also help in predicting the responsiveness of certain cancers to chemical treatment. For example, methylation of a DNA-repair protein, MGMT, in human brain tumors makes these cells resistant to apoptosis induced by chemotherapeutic agents that alkylate DNA. Improved understanding of epigenetic modifications has also led to the development and FDA approval of two demethylating agents for the treatment of preleukemic disease. In addition, many HDAC inhibitors are in early trials for the treatment of cancer, and the FDA has approved one such drug, SAHA, for the treatment of cutaneous T-cell lymphoma.

THE FUTURE OF EPIGENETICS

In a recent review, leading scientists in the field of epigenetics, Aaron Goldberg, C. David Allis, and Emily Bernstein, describe epigenetics as "a bridge between genotype and phenotype." Despite the many developments in the field, this bridge has yet to be fully crossed. To better understand the process of phenotypic expression, some advocate the mapping of the epigenome, similar to the studies done mapping the genome. This would include looking at CpG islands, characterizing genes that are epigenetically silenced, and identifying how changes in expression affect development and disease. Others are interested in the emerging field of microRNAs where much work has yet to be done. The hope is that the study of epigenetics can one day help scientists to understand fully the complicated networks that encode genetic information.

FURTHER READING

Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell.* 5th ed. New York: Garland Science Taylor and Francis Group, 2007.

Allis, C. David, Thomas Jenuwein, Danny Reinberg, and Marie-Laure Caparros. *Epigenetics.* Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2007.

Esteller, Manel. "Cancer Epigenomics: DNA Methylomes and Histone-modification Maps." *Nature Reviews Genetics* 8 (April 2007): 286–298.

Gibbs, W. Wayt. "The Unseen Geneome: Beyond DNA." *Scientific American* 289, no.6 (December 2003): 106–113.

Goldberg, Aaron D., C. David Allis, and Emily Bernstein. "Epigenetics: A Landscape Takes Shape." *Cell* 128, no. 4 (February 23, 2007): 635–638.

Jirtle, Randy L., and Michael K. Skinner. "Environmental Epigenomics and Disease Susceptibility." *Nature Reviews Genetics* 8 (April 2007): 253–262.

Özdağ, Hilal, Andrew E. Teschendorff, Ahmed Ashour Ahmed, Sarah J. Hyland, Cherie Blenkiron, Linda Bobrow, Abhi Veerakumarasivam, Glynn Burtt, Tanya Subkhankulova, Mark J. Arends, V. Peter Collins, David Bowtell, Tony Kouzarides, James D. Brenton, and Carlos Caldas. "Differential Expression of Selected Histone Modifier Genes in Human Solid Cancers." *BMC Genomics* 7 (April 25, 2006): 90.

Quina, A. S., M. Bushbeck, and L. Di Croce. "Chromatin Structure and Epigenetics." *Biochemical Pharmacology* 72 (2006): 1,563–1,569.

Reik, Wolf. "Stability and Flexibility of Epigenetic Gene Regulation in Mammalian Development." *Nature* 447 (May 24, 2007): 425–432.

Spivakov, Mikhail, and Amanda G. Fisher. "Epigenetic Signatures of Stem-Cell Identity." *Nature Reviews Genetics* 8 (April 2007): 263–271.

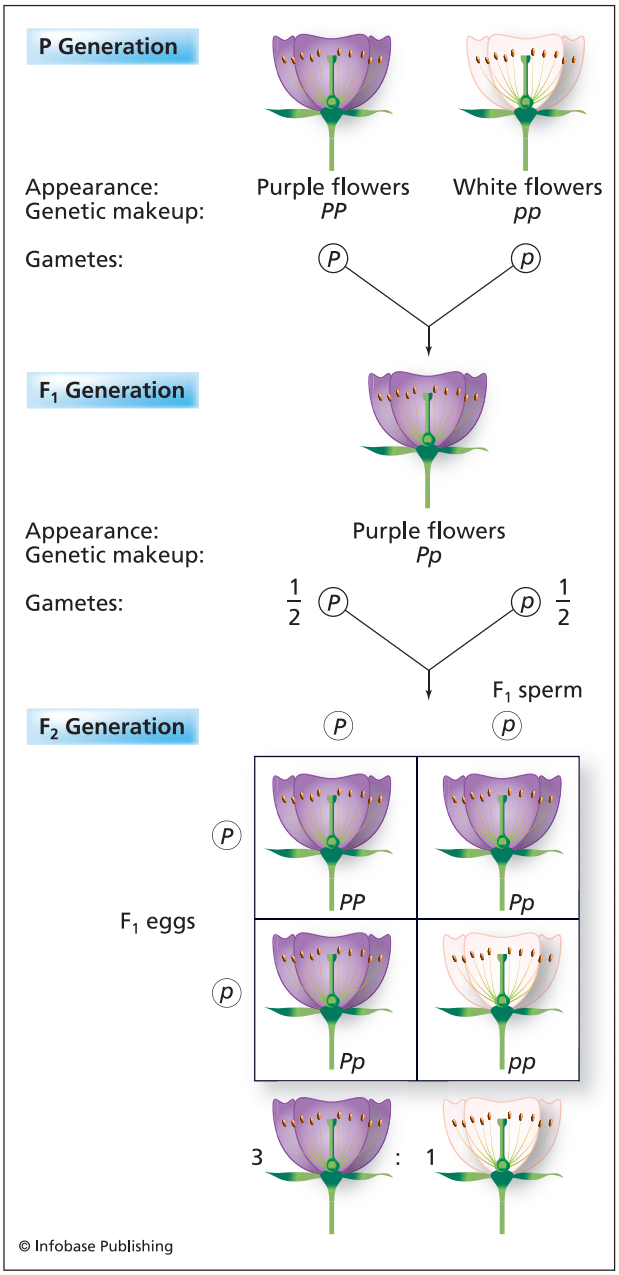
Watters, Ethan. "DNA Is Not Destiny." *Discover* 27, no. 11 (November 2006): 33–37, 75.

Wong, Albert H. C., Irving I. Gottesman, and Arturas Petronis. "Phenotypic Differences in Genetically Identical Organisms: The Epigenetic Perspective." *Human Molecular Genetics* 14, no. 1 (2005): R11–R18.

Zhang, Baohong, Xiaoping Pan, George P. Cobb, and Todd A. Anderson. "MicroRNAs as Oncogenes and Tumor Suppressors." *Developmental Biology* 302 (2007): 1–12.

(continued from page 472)
of the dominant allele masks the presence of the recessive allele. For example, pea plants might have purple or white flowers. If a plant has two copies of the allele that encodes purple flowers (represented by *P*), the genotype is *PP* and the phenotype is purple. If the organism is homozygous for the recessive allele (*pp*), the one that encodes white flowers, then the phenotype will be white flowers. In a heterozygous individual, one with a genotype of *Pp*, the phenotype would be purple, because *P* is the dominant allele. By convention, in simple dominance, the dominant allele is depicted by an uppercase letter, and the recessive allele is depicted by a lowercase letter.

If an organism is homozygous for a particular allele, then all the gametes made by the individual will contain the same allele. If the individual is heterozygous, or has two different forms of the allele, then the probability that a gamete has one form is 50 percent. Punnett squares facilitate the prediction of frequencies of genotypes from a cross between two organisms. In the following example, in the first generation, a true-breeding homozygous purple-flowered plant (*PP*) is crossed with a true-breeding homozygous white-flowered plant (*pp*). True-breeding plants are homozygous for a specific trait and can be obtained by self-fertilizing a plant for many generations. When a true-breeding plant is crossed with itself, all the progeny will have the same genotypes and therefore the same characteristics as the parent. The first generation is called the parental generation, or simply P. The cross is *PP* (purple) × *pp* (white). The purple parent can make only gametes that contain *P* alleles, and the white parent can make only gametes that contain *p* alleles. So, all the progeny that result from this first cross will have the genotype *Pp* and a purple phenotype, since heterozygous individuals outwardly express the dominant phenotype. The progeny generated from the first cross are hybrids and are called the first filial generation, or simply F_1. When the F_1 plants fertilize other F_1 plants, the cross is represented as *Pp* × *Pp*. Each parent can contribute either a *P* or a *p* to a gamete. By chance, 50 percent of the gametes can have a *P* allele, and 50 percent of the gametes will have a *p* allele. These probabilities are exhibited along the left side and above the Punnett square. The eggs are shown along the left side, and the sperm are shown on top. Half of the rows (one of two rows in this square) represent the 50 percent of eggs that contain a *P* allele, and the other half (the other row of the two) represent the 50 percent of eggs with the *p* allele. Similarly, across the top, half of the columns (one of the two columns in this square) represent sperm cells that contain a *P* allele, and half of the columns represent sperm cells that contain a *p* allele. By allowing the hybrid F_1s to pollinate each other, the possibility exists for either type of egg to be fertilized by either type of sperm. As demonstrated by examining the boxes where a certain type of sperm fertilizes a certain type of egg, four possibilities exist. One-fourth of the squares are homozygous dominant (*PP*), and one-fourth of the squares are homozygous recessive (*pp*). Two-fourths, or one-half of the squares, represent heterozygous progeny with the genotype *Pp*. One of the two heterozygous squares represents the union of a sperm with a dominant allele and an egg with a



The law of segregation states that two alleles for a given gene separate during gamete formation. Punnett squares facilitate the calculation of genotypic frequencies based on this principle.

recessive allele, and the other one represents the union of a sperm with a recessive allele and an egg with a dominant allele. In either case, the heterozygous progeny will exhibit the dominant phenotype. Thus, the genotypic ratio in the second filial, or F_2, generation is $1PP : 2Pp : 1pp$. The phenotypic ratio of the F_2 is 3 dominant (purple) : 1 recessive (white). The data is more likely to approach these expected ratios as the quantity of offspring increases.

If the parental genotypes are known, one can use probability to predict the outcome of a cross. The probability of an event can be calculated by the following formula:

$$\text{Probability of an event} = \frac{\text{number of one kind of possible outcome}}{\text{total number of all possible outcomes}}$$

The outcome can be described in words, decimals, fractions, ratios, or percentages. In the above example, if one wanted to predict the percentage of individuals with a homozygous genotype from the cross $F_1 \times F_1$, the calculation would be

$$\text{Probability of a homozygous genotype} = \frac{\text{2 homozygous genotypes}}{\text{4 total possible genotypes}}$$

$$= \frac{1}{2}$$

There is a 1 in 2 chance that a homozygous genotype will result from the heterozygous cross $Pp \times Pp$. There are two possible homozygous genotypes in the offspring, *PP* or *pp,* of four total possible genotypes: *PP, Pp, pP,* and *pp*. (Since the dominant allele could come from either the male or the female parent, both possibilities must be considered.) Saying there is a 50 percent chance the offspring will have a homozygous genotype is another way to report this information.

To determine the probability of two events occurring simultaneously, the probabilities of the separate events are multiplied. For example, if a boy drew a card from a complete deck, the probability that he would draw a spade is ¼. If he replaced the card and then drew again, the probability he would draw a heart this time would also be ¼. To determine the probability that he would draw a spade on his first draw and a heart on his second draw, the separate probabilities are multiplied.

$$\frac{1}{4} \times \frac{1}{4} = \frac{1}{16}$$

So, the probability that he would draw a spade on the first draw, then a heart on the second (after replacing the first card and shuffling), would be 1 in 16. To calculate the probability that one event or the other would occur, the probabilities of the separate events are added. So, the probability of drawing either a spade (¼) or a heart (¼) is 1 in 2, or 50 percent.

$$\frac{1}{4} + \frac{1}{4} = \frac{2}{4} = \frac{1}{2}$$

If an organism displays the dominant phenotype, the underlying genotype could be either homozygous dominant or heterozygous. Performing a testcross will reveal information that will allow one to infer the correct allele combination that is present. In a testcross, the individual with the unknown genotype is crossed with an individual that exhibits the recessive phenotype, and therefore can contribute only recessive alleles to offspring, for the trait of interest. By definition, an individual with a recessive phenotype must possess two recessive alleles. If the testcross produces any offspring with the recessive phenotype, then the unknown individual must have contributed a recessive allele, and therefore must be heterozygous, because both parents must contribute a recessive allele in order for the offspring to exhibit the recessive phenotype. Probability predicts that in a testcross, a heterozygous parent will produce gametes with an equal chance of possessing either the dominant or the recessive allele, and since the homozygous recessive individual parent can produce only eggs with the recessive allele, 50 percent of the offspring should receive both recessive alleles, and 50 percent should receive one dominant allele (from the unknown parent) and one recessive allele (from the testcross parent).

Another principle of inheritance is the law of independent assortment, which states that the genes of different alleles segregate independently of one another. For example, the alleles responsible for flower color in peas will move into gametes in a manner unrelated, or independent, of how the alleles for the gene for plant height divide into gametes. This law applies only to genes that are encoded on different chromosomes or are sufficiently far apart from one another on the same chromosome. When Mendel proposed this law of independent assortment, biologists did not know the physical nature of traits and certainly not that traits are encoded by genes that reside on chromosomes and that chromosomes are what separate during meiosis. Today biologists recognize that the law of independent assortment describes the behavior of individual chromosomes during the first meiotic division, when one member of a homologous pair moves to one end of a cell and the homolog moves to the other end of the cell. Independent assortment describes the manner in which the chromosomes line up and move to one

end or the other as being completely random, so the genes encoded on separate chromosomes sort independently.

This information is useful when examining a cross in which two or more characteristics are of interest to the breeder. A dihybrid cross is a specific type of cross in which organisms that are heterozygous for two different genes are mated. If two plants that are true-breeding for two different characteristics, with each having a different allele pair than the other parent at the site for that gene, the offspring (F_1) of those parents will be heterozygous for both genes. For example, a true-breeding pea plant that has purple flowers and round seeds (encoded by the dominant allele *R*) will be homozygous for both genes and have the genotype *PPRR*. A true-breeding plant that has white flowers and wrinkled seeds will also be homozygous for both genes and have the genotype *pprr*. The purple, round parent will be able to make gametes that have only dominant alleles for both genes—*PR*. The white, wrinkled parent will be able to make gametes that have only recessive alleles for both genes—*pr*. Thus, all the F_1 plants will have dominant phenotypes, being purple and round, and have heterozygous genotypes—*PpRr*. When the F_1 produce gametes, each allele for a gene has an equal chance of being placed into any gamete; thus, there are four possible gametes (*PR*, *Pr*, *pR*, and *pr*) each occurring at equal frequencies (1:1:1:1). Setting up a Punnett square helps visualize this scenario.

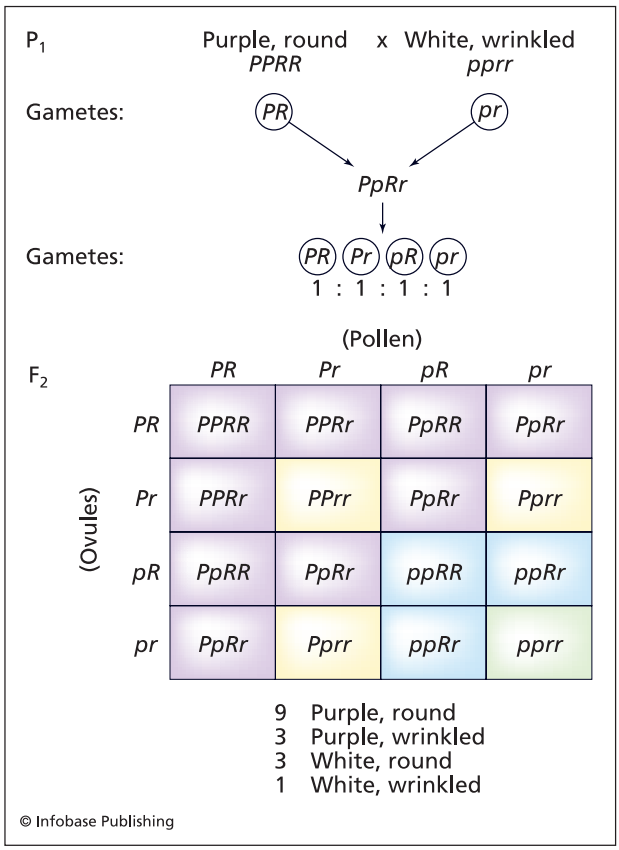
The genotypes of the ovules are depicted alongside each row on the left side of the square, and all the possible genotypes in the pollen are depicted at the top of each column. As in a single gene cross, the boxes within the larger square represent the union of two gametes and show the genotype of the resulting offspring. The resulting phenotypic ratio of

9 (purple and round) : 3 (purple and wrinkled) : 3 (white and round) : 1 (white and wrinkled)

masks the underlying genotypic ratio of

1 (*PPRR*) : 2 (*PPRr*) : 1 (*PPrr*) : 2 (*PpRR*) : 4 (*PpRr*) : 2 (*Pprr*) : 1 (*ppRR*) : 2 (*ppRr*) : 1 (*pprr*).

A horticulturist could refer to these ratios to answer questions such as: what is the probability of creating a white flowered pea plant with wrinkled seeds from crossing parents heterozygous for both traits? The answer would be 1 in 16. Or, what are the chances that the F_2 offspring will have the same genotype as the F_1 plants (*PpRr*)? Since four of the 16 boxes in the Punnett square have this genotype, the answer would be 4 in 16, or one-fourth, or 25 percent.



A dihybrid cross, a cross between two individuals that are heterozygous for two different genes, results in a 9:3:3:1 ratio.

COMPLEX PATTERNS OF INHERITANCE

Though understanding the concepts of simple dominance, the law of segregation, and the law of independent assortment is a big step in comprehending how traits are passed from one generation to the next, most traits are not encoded by a single gene, and rarely are there only two possible alleles that affect a given trait. Though multiple different possible alleles could exist at a specific gene on a chromosome, only one gene can exist at a time on one chromosome, thus each individual possesses only two copies. In the case of multiple alleles, one still simply considers the relationship of the two alleles that are present; is one dominant and one recessive? If so, the situation is still relatively simple to analyze.

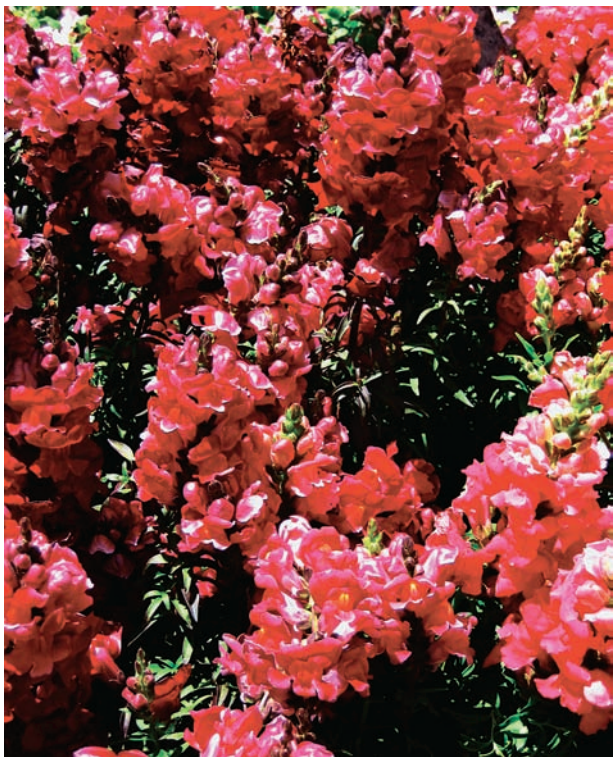
For some traits, a single gene controls the phenotype, but determination of the phenotype does not follow the concepts of simple dominance and recessiveness. In incomplete dominance, neither allele dominates over the other, though often one of the alleles is partially dominant. In these cases both alleles of a heterozygote influence the phenotype. A dominant allele does not mask a recessive allele. For example, the alleles that control flower color in

snap dragons are red (C^R) or white (C^W). Capital letters with superscripts are used to represent allele combinations in which one is not dominant over the other. A flower on a plant with the genotype C^RC^R will be red, and the genotype C^WC^W will produce white flowers, but a heterozygous (C^RC^W) plant will be pink, a phenotype intermediate between red and white.

In codominance both alleles are fully expressed. Neither dominates, nor does blending occur to generate an intermediate phenotype. Red blood cells in humans express a set of molecules on their surface. The phenotype for these molecules is determined by the combination of two possible alleles, *M* and *N*. Individuals with the genotype *MM* express only type M molecules on the surface of their red blood cells, and individuals with the genotype *NN* express only type N molecules on the surface of their blood cells. The blood cells of heterozygous individuals (*MN*) will express both type M and type N equally.

Polygenic inheritance is slightly more complex. Numerous genes play a role in the outward expression of the phenotype for polygenic traits. Traits such as eye color, height, and skin color are examples of polygenic traits in humans. Epistasis occurs when one gene changes the phenotypic expression of another independently inherited gene. For example, in mice one gene controls whether the coat color is black or brown, but a completely separate gene determines whether the hair will contain any pigmentation. If the animal hair contains no pigmentation, it does not matter what alleles are present for the first gene, as the fur will be white anyhow.

To further complicate matters, genetics alone does not determine the phenotype of an individual organism. The environment often plays a significant role. Often, the genotype sets the parameters, or the limits of phenotypic expression, and the environment determines to what extent a phenotype is expressed. The nutrition available can determine the extent to which an organism reaches it greatest potential height. The acidity of the soil can affect the color of flowers. Temperature affects fur color in arctic foxes.



Pink snapdragons can result from incomplete dominance of the alleles for flower color. *(Kimber Rey Solana, 2007, used under license from Shutterstock, Inc.)*

SEX-LINKAGE

Characteristics that are encoded by genes found on the sex chromosomes exhibit unique patterns of inheritance. In humans, 22 of the 23 pairs of homologous chromosomes are autosomal (nonsex chromosomes), and the final pair consists of the sex chromosomes, called X and Y chromosomes, that are responsible for determining whether an embryo develops as a female or as a male. Females have two X chromosomes, and males have one X and one Y chromosome. Though considered a pair, the X and Y chromosomes contain different genes, and the X chromosome in particular encodes for many genes that have nothing to do with gender. Genes that are on the sex chromosomes are said to be sex-linked. Such genes can differentially affect females and males due to the fact that females have two X chromosomes, and therefore two copies of all the genes located on the X chromosome, whereas typical males have only one copy.

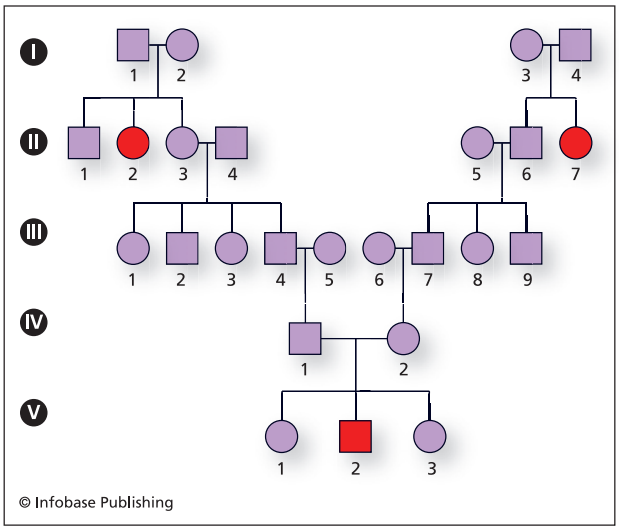
Male offspring receive their Y chromosome from their father and their X chromosome from their mother. Female offspring receive one X chromosome from each parent. If a male has an allele for a recessive sex-linked condition (meaning the gene is located on the X chromosome), such as red-green colorblindness or hemophilia, he will express the phenotype associated with that allele, since he has only a single X chromosome. A female must have two copies of the recessive allele to have the condition, since females have two X chromosomes. If only one of a female's X chromosomes has the recessive allele and the other has the dominant allele, she is considered a carrier and will have the normal phenotype. Males that are affected will pass the recessive allele to all of their daughters, since they have only one X chromosome and all daughters receive a copy of that chromosome. Half of the gametes produced

by female carriers will have the recessive alleles, thus there is a 50 percent chance that a female carrier's son would inherit the condition from her. Because females have two X chromosomes, they are likely to have at least one normal dominant allele for rare sex-linked disorders and thus will rarely be affected. If a dominant allele causes a sex-linked condition, all of the daughters of an affected male will have the condition.

PEDIGREE ANALYSIS

A pedigree is a chart of a family's genetic history. Pedigree analysis helps an individual determine the chance of inheriting, or passing on to their children, a specific genetic trait. To perform a pedigree analysis, a geneticist collects information about a family's medical history, including as much as is known about the number and gender of children from each set of parents and who exhibited symptoms of a particular condition. Boxes represent males, circles represent females, and shaded shapes indicate that that individual was affected by a particular disease or condition. Horizontal lines indicate matings, and vertical lines indicate offspring with the oldest sibling on the left, and the youngest on the right. If a couple has several children, the vertical lines extend down from a horizontal line that indicates siblingship. Generations are labeled with Roman numerals, and individuals with Arabic numerals.

To illustrate, consider the following pedigree for a family affected by the autosomal recessive condition, cystic fibrosis. Autosomal means the responsible gene is not located on one of the sex chromosomes, and recessive means an individual must have two recessive alleles to have the disorder. Cystic fibrosis disrupts normal function of the exocrine glands, and the symptoms include digestive problems, respiratory problems due to mucus buildup in the airways, and excessive loss of salt in the sweat.



Pedigrees are useful tools for determining the mode of inheritance and probabilities associated with genetic disorders.

The female V-3 might seek genetic counseling before starting a family in order to determine her risk of having a child affected by cystic fibrosis. After she reports her family medical history, including the facts that both sides of the family have had affected members, to the genetic counselor, the counselor constructs a pedigree. Cystic fibrosis is known to be an autosomal recessive condition, but if the genetics were not understood, one could determine the mechanism of inheritance by examining the pedigree. Since both males (V-2) and females (II-2 and II-7) in her family have had the condition, it is unlikely that the trait is sex-linked. Since the condition skips generations, it cannot be encoded by a dominant allele, because as the allele is passed down, anyone having at least one of the dominant alleles would have the condition. One of the woman's brothers has cystic fibrosis and neither of her parents has the disorder, therefore both parents must be carriers of the mutant recessive allele, in other words, they are heterozygous. The expected genotypic ratio of offspring born to two heterozygous parents is 1:2:1. In other words, there is a 25 percent chance that a child would have two normal dominant alleles, a 50 percent chance that a child would be heterozygous (a carrier), and a 25 percent chance that a child would have the homozygous recessive genotype, and therefore have cystic fibrosis. Since the woman (V-2) knows she does not have the disorder, she must have at least one dominant allele, and the chance that she is a carrier is two out of three. Without knowing the medical history of her husband's family, one cannot accurately predict the likelihood that she would give birth to an affected child, though the probability that he is a carrier could be deduced from the allelic frequency in a certain population. If she were a carrier, she would pass the cystic fibrosis allele to 50 percent of her children. In order for any of her children to have the condition, her husband would also have to be a carrier (or have the condition himself). If both parents carry the alleles for an autosomal recessive disorder, with each pregnancy, there is a 25 percent chance that the child will be affected.

See also CHROMOSOMES; GENETIC DISORDERS; GENETICS; GENOMES; MENDEL, GREGOR; MORGAN, THOMAS HUNT; REPRODUCTION; SEX DETERMINATION.

FURTHER READING

Griffiths, Anthony J. F., Susan R. Wheeler, Richard C. Lewontin, and Sean B. Carroll. *Introduction to Genetic Analysis*. 9th ed. New York: W. H. Freeman, 2008.

Hartwell, Leland, Leroy Hood, Michael L. Goldberg, Ann Reynolds, Lee M. Siler, and Ruth C. Veres. *Genetics: From Genes to Genome*. 3rd ed. New York: McGraw Hill, 2008.

Richards, Julia E., and R. Scott Hawley. *The Human Genome: A User's Guide*. 2nd ed. San Diego, Calif.: Academic Press, 2004.

integumentary system An integument is a structure that covers and protects an organism, such as skin on a human, a shell of a diatom, or the husk of a corn cob. The integumentary system of animals comprises the skin and its accessory organs, including the hair, nails, sweat glands, and sebaceous (oil) glands. The chief functions of the skin are to cover and protect the body's tissues against water loss and injury, to prevent the entry of pathogenic microorganisms, and to house sensory receptors that gather information about the external environment. The accessory organs perform a variety of roles, such as the production of sweat, which helps maintain a constant body temperature, and the production of certain chemicals such as sebum that prevents the skin from drying out.

SKIN

Because it is composed of two or more tissue types and performs a specialized function, the skin is technically an organ. With a surface area of approximately 20 square feet (almost 2 m^2), it is the human body's largest organ. The skin consists of two distinct layers—the epidermis and the dermis. The outer layer, the epidermis, is made of stratified squamous epithelial cells. Squamous cells are flat and thin, and stratified simply means more than one layer exists. The innermost layer of the epidermis contains stem cells (also called basal cells) that synthesize keratin and melanocytes that produce melanin. Keratin is the substance that makes up calluses and nails, coats hairs, and forms a tough protective coating over the surface of the skin. The basal cells constantly divide, giving rise to new cells that push the older cells toward the surface. As they move outward toward the surface of the skin, the cells become keratinized, or hardened and flattened. Melanin is a pigment that can be yellow, reddish brown, dark brown, or black. The amount of melanin produced by the melanocytes determines one's skin color. A freckle results from an excess of melanin in one spot. The inner layer of epidermal tissue also plays a role in the synthesis of vitamin D. The penetration of a small amount of ultraviolet radiation from sunlight catalyzes the conversion of a molecule derived from cholesterol and found in the lower epidermis into vitamin D. (The exposure of too much ultraviolet radiation, however, is dangerous because it causes mutations that can lead to skin cancer.) The outermost layer of epidermis contains 20 to 30 layers of mostly flattened and dead cells that have not yet sloughed off. After these cells die, their keratin remains, forming a water-resistant, protective covering.

The dermis is much thicker than the epidermis and is composed mainly of fibrous connective tissue containing collagen and elastic fibers. The hair follicles, sweat glands, sebaceous glands, nerve endings, sensory receptors, and capillaries are all embedded in the dermis. Capillaries supply nutrients and oxygen to the dermis. The epidermis does not contain its own capillaries, but small extensions of the dermis that contain capillaries project into the innermost layer of the epidermis to allow for exchange of nutrients and gases with the metabolically active basal cells. The patterns of these extensions form the whorls and ridges of fingerprints. Sensory receptors specific for temperature, pressure, and touch detect and relay information about the external environment to the central nervous system, which processes the information and initiates an appropriate response. Nerves whose free ends penetrate into dermis act as pain receptors. Skin covering different parts of the body has different numbers of the specialized types of receptors, causing some areas to be more sensitive to certain kinds of stimuli such as changes in temperature or degree of pressure. The dermis also contains some adipose tissue, which stores fat, insulates the body, and cushions against injury.

Below the dermis lies the subcutaneous layer, which technically, is not part of the skin. Consisting mostly of adipose tissue and some connective tissue, the subcutaneous layer also contains nerves, arteries, and veins that service the skin.

ACCESSORY ORGANS AND STRUCTURES

The accessory organs and structures of the skin, including the sweat glands, sebaceous glands, hair follicles, and nails, are all derived from epidermis. The sweat glands are tubules that lie in the dermis, but extend through the epidermis to an opening at the skin's surface or sometimes empty into a hair follicle. They function in homeostasis by helping the body maintain a constant internal body temperature. When the body temperature rises, the sweat glands secrete sweat, which cools the body as it evaporates on the surface of the skin. As the temperature returns to normal, the sweat glands constrict and become inactive. The sweat glands also play a minor role in waste excretion by eliminating excess urea and salt with sweat.

Hairs that project from the skin originate at follicles that extend down into the dermis and some-

times into the subcutaneous layer. Capillary beds associated with the follicles supply nutrients. The sebaceous glands produce and secrete into the follicles an oily substance called sebum that helps prevent the skin and hair from drying out. During puberty, an increase in steroid hormone production stimulates the synthesis of excess sebum, which can combine with dead skin cells to clog follicles, causing blackheads. Bacteria that normally inhabit the skin infect and inflame the blocked follicles, leading to the formation of pimples.

At the base of a hair follicle, epithelial cells multiply, causing the hair to grow outward, and produce keratin, which coats the hair as it grows. The shape of the hair shaft, the region that projects from the skin, determines whether the hair is straight or wavy, and melanin made by melanocytes near the follicle determine the hair color. Because melanin production naturally decreases with age, hair turns gray as one gets older. When someone is frightened or cold, arrector pili muscles associated with each hair follicle involuntarily contract (goose bumps), causing hairs to stand on end. In animals, raising the hair increases the amount of air trapped near the skin, an action that insulates the body and prevents heat from escaping.

Human fingernails and toenails are homologous to the claws of birds and reptiles and the hooves of horses and cattle. Composed of keratinized cells, nails project from a root that is embedded in the skin and covered by a cuticle. Their main function is to protect the ends of fingers and toes from injury. Just as hair grows when epithelial cells at the base of the follicle multiply, nails grow as cells at the root divide and push the older cells outward.

See also ANATOMY; ANIMAL FORM; BIOLOGICAL MEMBRANES; CANCER, THE BIOLOGY OF; HOMEOSTASIS; INVERTEBRATES; PHYSIOLOGY; SENSATION; VERTEBRATES.

FURTHER READING

American Academy of Dermatology home page. Available online. URL: http:/www.aad.org. Accessed on January 14, 2008.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 1. Detroit, Mich.: U*X*L, 2000.

invertebrates

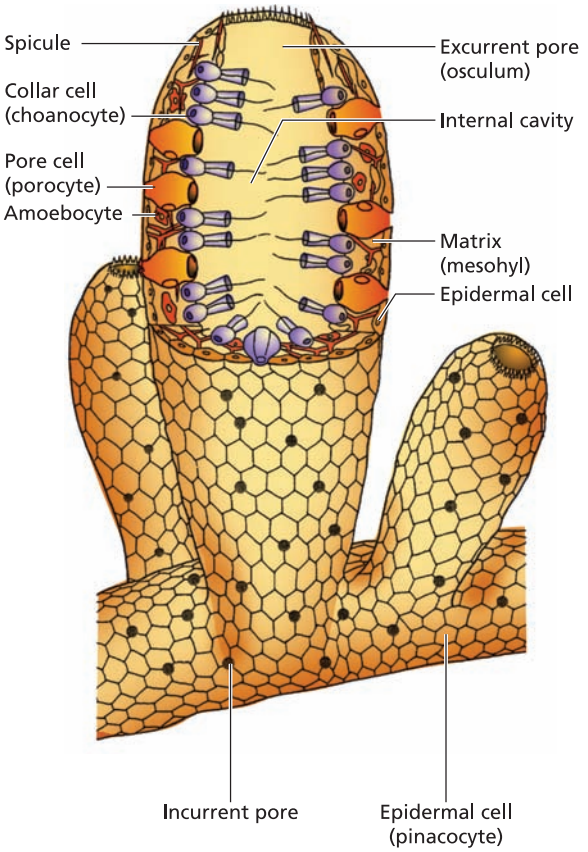
Simply defined, invertebrates are animals without backbones or bony skeletons. Invertebrates comprise more than 95 percent of identified animal species, translating into more than 1 million species and possibly millions more yet to be discovered. This group of animals includes an enormously diverse range of organisms: aquatic and terrestrial, free-living and parasitic, and from microscopic to creatures several meters in length. Performing a variety of beneficial and necessary roles, they recycle nutrients, aerate the soil, participate in food webs, and pollinate plants. Animals are classified into approximately 35 phyla. Some of the most common invertebrate phyla are Porifera, Cnidaria, Ctenophora, Platyhelminthes, Nematoda, Rotifera, Mollusca, Annelida, Arthropoda, and Echinodermata. The phylum Chordata also contains some invertebrate species.

PORIFERA

Phylum Porifera, more commonly known as the sponges, contains the simplest of animals. Classified as plants until the late 1700s, the estimated 5,000 species of sponges exhibit no symmetry, and their cells are not organized into true tissues or organs. Most poriferans are marine, though approximately 150 species are found in freshwater environments. Their bodies may be tall, extending upward from a fraction of an inch to 6.5 feet (1 cm to 2 m), or spread out over a flat surface, and they may contain branches or lobes. Pigments in the dermal cells give bright, colorful appearances to many sponges. Structurally, sponges resemble holey sacs consisting of masses of specialized cells embedded in a gelatinous matrix called mesohyl. A layer of cells called pinacocytes cover the external surface and some of the internal surfaces. A skeleton consisting of spiny, crystalline needles called spicules provides structural support, as do fibrils of the protein collagen found in the intercellular matrix. Spicules are made of calcium carbonate, silica, or a flexible protein fiber called spongin.

Because adult sponges are sessile, meaning they are permanently attached to a surface such as a rock or coral, they obtain nutrition by filter-feeding. Cells called choanocytes that line the internal body cavity are surrounded by a collar and have flagella that beat, causing water to pass through the body wall via tiny openings called ostia. The collar cells trap plankton in hairlike projections and then transport the captured food by phagocytosis into their cytoplasm, where digestion occurs. Waste materials are excreted into the body cavity and leave the sponge through larger openings called oscula. Irregularly shaped, wandering amoebocytes pick up nutrients released by the collar cells and carry them throughout the mesohyl to nourish the rest of the sponge's cells.

Sponges have the ability to reproduce asexually by growing buds that detach and float away and by fragmentation, more properly called somatic embryogenesis, a process by which an entire new organism may grow from a piece broken off a parent



Sponges, animals of the phylum Porifera, exhibit a simple asymmetric body plan with no specialized tissues or organs.

sponge. Freshwater species and some marine species can form gemmules in very cold or dry conditions. These structures consist of clusters of amoebocytes surrounded by a protective coat that can endure until the environmental conditions improve, at which time they develop into new sponges. Most sponges are hermaphroditic, meaning they possess both male and female reproductive structures and produce both sperm and eggs from either choanocytes or amoebocytes. Self-fertilization rarely takes place because eggs and sperm are produced at different times. Water currents carry sperm out of a parent sponge to nearby neighbors, whereas eggs stay inside the mesohyl until fertilization occurs. The zygotes develop into flagellated larvae that leave the parent and settle on a suitable surface where they mature into a sessile adult.

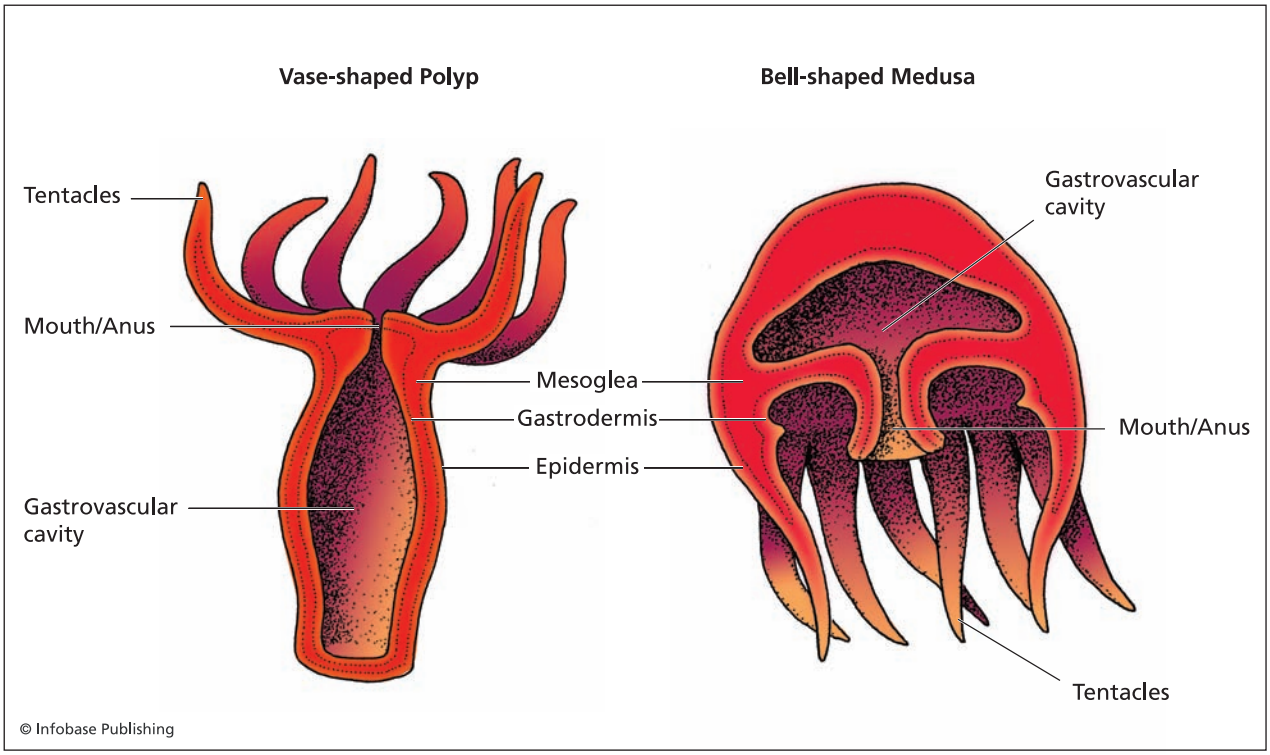
CNIDARIA

Cnidarians are slightly more complex than poriferans in that they have radial symmetry and specialized tissues; however, they possess no organs. They exhibit two main body forms, the free-floating medusa (such as jellyfish) and the sessile polyp (such as hydra and sea anemones). Both types have a central gastrovascular cavity with a single opening that acts as both a mouth and an anus, but the polyp resembles a hollow cylinder and remains attached to a rock or other surface, while the medusa assumes an inverted bell shape and moves about by contracting its body and drifting with the water currents. Some cnidarians exist as medusas, others as polyps, and still others pass through both forms during their life cycle.

The body of cnidarians is composed of two layers of cells separated by a gel-like matrix called mesoglea. The ectoderm forms the outer layer, the epidermis, and the endoderm gives rise to the inner layer, the gastrodermis. Tentacles surround the opening to the gastrovascular cavity, which also acts as a hydrostatic skeleton. When the mouth is closed, contraction of the body pushes against the water-filled cavity and allows the animal to change its shape and therefore move. Though cnidarians have no brain, they do possess a noncentralized nerve net. The ends of the tentacles contain stinging cells called cnidocytes, a distinguishing characteristic of the animals in this phylum. The cnidocytes contain nematocysts, little barbed spears used to pierce prey and inject venom to stun or kill their prey. The organism then uses its tentacles to move the captured prey into its gastrovascular cavity where digestion begins extracellularly. Cells secrete enzymes into the cavity to break the food into smaller parts that can be transported into cells for further digestion.

Three main classes make up the more than 9,000 identified cnidarian species: Hydrozoa, Schyphozoa, and Anthozoa. Hydrozoans are mostly marine, but some live in freshwater. Most hydrozoans alternate between sexual and asexual means of reproduction during their life cycles, a strategy exhibited by *Obelia*. This organism lives in colonies made up of branched polyps that form by buds that do not separate and that specialize in different functions. Feeding polyps have tentacles for capturing prey, and reproductive polyps produce medusas though asexual budding. After being released, the medusas produce sperm and eggs that unite, forming a zygote that develops into a free-swimming, ciliated, planula larva. After settling on the substratum, the larva develops into a polyp that can form a new colony by budding, completing the *Obelia* life cycle.

The hydra, a freshwater hydrozoan, exists only in the polyp stage and can move by tumbling. Though hydras are atypical, they are easy to grow and maintain in the laboratory and therefore are well studied. They propagate asexually by budding when environmental conditions are favorable—small outpockets of



Cnidarians exhibit two body forms, medusa and polyp.

the body wall develop, detach, and form new hydras. In unfavorable conditions, they reproduce sexually by forming tough, dormant zygotes that can persist until conditions improve, and then young hydras hatch.

The Portuguese man-of-war is an example of a more complex colonial marine hydrozoan that belongs to the order Siphonophora. A member of the genus *Physalia,* the man-of-war, also known as the bluebottle, is really a colony made up of several different types of individuals with specialized functions that support the whole colony. A transparent, aerodynamic, muscular sac is a single individual that keeps the colony afloat by producing and secreting its own gas. The long, purple tentacles are polyps that detect and capture prey. The sensation of small crustaceans or surface plankton nearby stimulates the nematocysts to discharge, stinging the prey. To humans, the feeling resembles a bee sting; however, the stings from Portuguese man-of-war can be fatal to infants, the elderly, or those who are allergic to them. Many beaches display warning signs when the numbers are high, since the toxins on the tentacles can sting even if the tentacles become detached or if the organism is dead. Other specialized polyps perform a digestive role, by secreting enzymes to digest whatever food the tentacles drag toward them. As hermaphrodites, these hydrozoans produce both sperm and eggs. After fertilization, larvae form and develop into new colonies by asexual budding.

The class Scyphozoa includes jellies that live in coastal regions and the open ocean. The coastal species usually have an inconspicuous polyp stage and exist in the medusa form most of their lives. Jellyfish that live in the open ocean often do not have a polyp stage at all and can range in size from as small as a grape to as large as a small automobile with tentacles extending up to 76 feet (70 m). They hunt for food, capture prey with their tentacles, and produce potent toxins in their nematocysts.

Members of Anthozoa, the largest class of cnidarians, exist only as polyps. The most familiar anthozoans are the brightly colored, flowerlike sea anemones and corals. Anthozoans possess a stalklike body with tentacles extending from the top like a head of hair. Sea anemones range from 0.2 to 4 inches (5–100 mm) in diameter and reproduce by pulling themselves into two halves that grow into two new organisms. As carnivores, they obtain their nutrition by ensnaring small fish and other marine life with their tentacles. Corals usually live in shallow waters of warm regions in colonies called reefs, made by the secretion of calcium carbonate that forms a skeletal network linking polyps to their neighbors. Even after the coral dies, the skeleton remains, leaving behind reefs that form a foundation for living polyps and for numerous other species that populate reef ecosystems. The vibrant colors of many corals actually are due to dinoflagellates that live in symbiosis with the corals.

CTENOPHORA

Members of the marine phylum Ctenophora, more commonly known as the comb jellies or sea gooseberries, resemble cnidarians, but they have a pair of anal pores like animals with bilateral rather than radial symmetry. Ctenophores also have a third layer of tissue between their ectoderm and endoderm, another characteristic of bilaterally symmetric animals, but the correctness of their phylogentic position is still an active area of research. Comb jellies swim by means of eight rows of comblike plates constructed from fused cilia that beat in unison to propel the organism. They range in size from less than one to four inches (1 to 10 cm) and have nerves that extend from a sensory organ to the combs. Most comb jellies also have a pair of long retractable tentacles. Unlike cnidarians that possess stinging cells, the tentacles of ctenophores contain colloblasts, structures that shoot out a sticky thread that captures food and brings it to the mouth. Zoologists have identified approximately 100 ctenophoran species.

The purple tentacles of this feeding Portuguese man-of-war sting, while the blue tentacles are for digestion. *(George Lower/Photo Researchers, Inc.)*

PLATYHELMINTHES

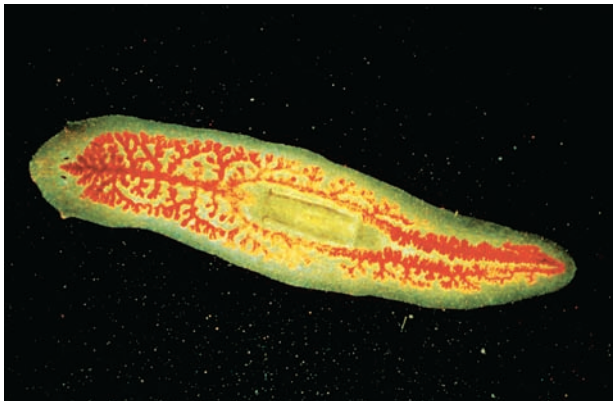
The phylum Platyhelminthes belongs to an early branch of bilaterally symmetric animals, called acoelomates, that lack a body cavity, are triploblastic (meaning they have three germ layers: ectoderm, mesoderm, and endoderm), display forward movement, and exhibit some degree of cephalization. Also known as flatworms, platyhelminthes include more than 20,000 species that live in marine, freshwater, and moist terrestrial habitats. Some are free-living, and others are parasitic. Sizes range from microscopic to more than 65 feet (20 m) in length. Having a third germ layer, the mesoderm, allows for the development of more complex organ systems, but, like radially symmetric animals, platyhelminthes only have one opening to their gastrovascular cavity. Most flatworms are hermaphroditic. Four platyhelminth classes include Turbellaria, Monogenea, Trematoda, and Cestoda.

The class Turbellaria consists mostly of free-living, marine flatworms less than 0.2 inches (5 mm) long that are difficult to maintain in captivity. Many freshwater varieties are found in ponds and streams. Members of the freshwater genus *Dugesia* (planarians) are carnivores that lack specialized organs for respiration or circulation. Some planarians can grow up to 20 inches (0.5 m) long. Because they are flattened, most cells come into contact with water in their surroundings, so the exchange of gases and ammonia waste can occur directly with the environment. The solute concentration inside the planarian cells is greater than that of the freshwater environment, so special adaptations are necessary to rid the organism of the excess water that enters by osmosis. Ciliated cells called flame cells located along the sides of the animal function to maintain the osmotic balance by swishing water through ducts that open to the external environment. To feed, planarians pin down protozoans and dead or dying animals with a muscular pharynx that protrudes from the middle of the ventral side, and then secrete enzymes to begin the digestive process. After softening, the food is ingested through the mouth into the gastrovascular cavity where digestion is completed. The gastrovascular cavity branches extensively, reaching all tissues of the body for the exchange of nutrients through the intestinal wall. Cephalization, the concentration of sensory and neural organs in an anterior head, is accompanied by a pair of light-sensitive eyespots (ocelli) and lateral flaps involved in smell. Planarians reproduce asexually by constricting behind the pharynx and stretching until they split into halves, each of which regenerates into a complete organism. They are hermaphroditic and sexually reproduce by simultaneously exchanging sperm with another individual. Turbellarians lay their fertilized eggs in clusters that are protected by a

capsule. In two to three weeks time, most planarian eggs hatch into young that resemble the adult form, but some turbellarians pass through a larval stage. Cilia located on the ventral surface beat to achieve locomotion, causing the worm to slide along a path of secreted mucous. Other turbellarians move by undulating through the water by muscle contraction.

The largest class of flatworms is Trematoda, the parasitic flukes. Most are endoparasites, meaning they live inside their hosts, while some are ectoparasites and live outside of their hosts. A thick, outer covering protects endoparasites from the harsh conditions of the host interior. Because they obtain their nutrition from the tissues and fluids of other living organisms, flukes have simple digestive systems. They attach to their host using suckers and bring in nutritious host fluids through muscular pharynxes. Trematodes have complex life cycles that often involve different hosts. For example, flukes of the genus *Schistosoma* that cause schistosomiasis, a disease marked by blood loss and tissue damage, penetrate the skin of human hosts when people enter water contaminated with cercariae, tadpolelike trematode larvae. After traveling to and through the circulatory system, they settle in blood vessels of the human intestinal wall, often causing intestinal ulceration, bleeding, anemia, dysentery, and liver damage. Eggs penetrate the host intestine and leave the body with feces. In a contaminated body of water, the eggs hatch and release miracidia, free-swimming, ciliated larva. The miracidia infect an intermediate host, a snail, in which they form sporocysts before being released as cercariae into the water, where the larvae seek a human to serve as its final host.

Class Monogenea consists of small, hermaphroditic, parasitic flukes that have a distinguishing structure called an opisthaptor. This bulbous organ aids in attachment of the adult worms to the gills of their host fish. The monogenetic flukes lay eggs that hatch into ciliated larvae that swim until they find a new host, to whom they attach and mature into the adult stage.



The muscular pharynx is apparent in the center portion of this stained planarian. *(Michael Abbey/Photo Researchers, Inc.)*

The scolex of the tapeworm has suckers and a crown of hooks that the tapeworm uses to attach to the host intestines. Proglottid segments are also visible in this photo. *(Centers for Disease Control and Prevention/Dr. Mae Melvin)*

Tapeworms, belonging to the class Cestoda, are parasitic flatworms that can grow up to 40 feet (12 meters) long. Their body consists of a head, or scolex, that has several suckers and hooks that function in attachment to the host intestine, and a string of sections called proglottids. Each proglottid contains both male and female reproductive organs. Mature proglottids containing thousands of fertilized eggs can break off of the posterior end and leave the host in feces. Food is absorbed directly through the skin of the tapeworm, and their life cycles typically involve at least two hosts. *Taenia saginata* is a tapeworm that infects humans through ingestion of undercooked beef or pork from an infected source. The tapeworm larva encloses itself in a cyst within the muscle of the cattle or pig, and if the meat does not reach a high enough temperature during cooking, the larva can survive and infect the human digestive tract. A person can usually eliminate the tapeworms by taking medication, though in extreme cases surgery may be necessary.

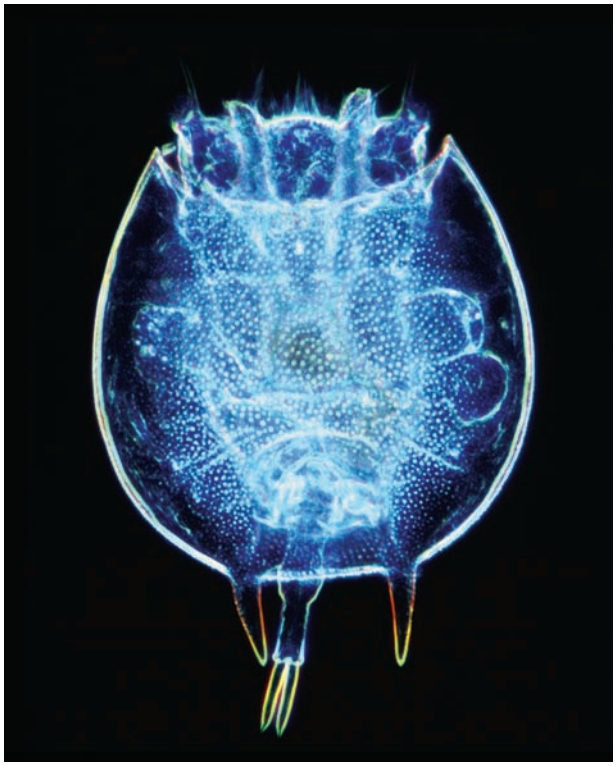
NEMATODA

The phylum Nematoda, commonly known as roundworms, consists of more than 15,000 known species and is one of the earliest branches of animals that have a pseudocoelom, a false body cavity without a well-defined mesodermal lining. Nematodes have elongated, cylindrical bodies ranging from mostly microscopic to 43 feet (13 m) in length (a species that parasitizes the sperm whale) and have a one-way digestive tract. Food ingested through the mouth is

crushed in the pharynx before being digested in the gut, which leads to the anus, through which waste is excreted. Fluid that flows throughout the pseudocoelom distributes nutrients as well as oxygen to body cells and carries away carbon dioxide. A thick, collagen-containing cuticle and a layer of fused epidermal cells protect the worm and surround a length of muscle that causes a whipping motion when it contracts and expands. The muscles branch toward two nerves, one that runs the length of the dorsal side and one that runs along the ventral side, that connect at a nerve ring near the head. Most roundworms are free-living and can be found in marine, freshwater, or terrestrial habitats where they hunt protozoa, algae, and other microorganisms for food, but many of the most recognizable species are animal or plant parasites. Plant parasites infect the leaves, stems, or roots, causing the plant to wither. Some common roundworms that infect humans are *Ascaris lumbricoides, Trichinella spiralis,* and members of the genus *Necator*. Infection with *Ascaris* occurs when a human accidentally ingests *Ascaris* eggs, usually after coming into contact with contaminated soil or eating uncooked vegetables that have eggs on them. The eggs hatch in the host small intestine, and the juveniles penetrate into the blood vessels through the intestinal wall. The bloodstream carries the juveniles to the lungs where they mature further, and then the host coughs them up and swallows them. Once in the small intestine, the *Ascaris* juveniles complete their maturation, and male and female adults mate. Fertilized eggs pass out of the host in the feces, completing the cycle. Encysted *Trichinella spiralis* larvae (the organism responsible for the disease trichinosis) may be ingested with undercooked meat, usually pork. Acid in the stomach releases the larvae from the cysts, and they travel to the small intestine where they mature and mate. Eggs develop into immature worms that enter the lymphatic system or bloodstream and migrate throughout the body until penetrating a muscle cell, where they can curl up and become enclosed in a cyst. Symptoms can include headaches, nausea, diarrhea, vomiting, and fever, or in more dangerous cases, chills, coughing, muscle pain, the inability the coordinate muscle movements, difficulty breathing, and even death. *Necator* species, also known as hookworms, live in tropical regions and can enter a host by boring through the soles of feet to enter the bloodstream. After maturing in the small intestine, eggs pass out of the body with feces and develop into the infective larval stage.

ROTIFERA

Rotifers are multicellular animals possessing a body cavity that is partially lined with mesoderm. Ranging from microscopic to 0.12 inches (3 mm) long, these pseudocoelomates mostly inhabit freshwater, but some live in the sea or moist soil. They have a digestive tract with a separate mouth and anus. Fluid that fills that pseudocoelom functions as a hydrostatic skeleton in addition to a circulatory system for transporting dissolved gases, nutrients, and waste material. A circular arrangement of cilia, called a corona, around the mouth, pulls water into the pharynx, which has jaws for grinding microorganisms that serve as food. Rotifers have trunks that vary in shape dependent upon its living conditions and a posterior tail or foot that can have up to four toes. The foot aids in attachment and in creeping and is reduced in swimming forms. Some species of the phylum Rotifera reproduce by parthenogenesis, when an unfertilized egg develops into a new individual. In some species, females produce more females by this mechanism. In other species, when conditions are unfavorable, the unfertilized eggs develop into both females and males whose only function is to produce sperm. In unfavorable conditions, fertilized eggs develop into dormant zygotes that are resistant to desiccation. When conditions improve, development and maturation resume. Adults are also able to withstand temperature variations and dry conditions.



Rotifers are multicellular, microscopic, free-swimming aquatic invertebrates. *(Laguna Design/Photo Researchers, Inc.)*

MOLLUSCA

Phylum Mollusca includes more than 100,000 animals such as snails, clams, octopuses, and squids, organisms that are bilaterally symmetrical and have a true coelom that develops entirely within the mesoderm. Most mollusks are marine, but some inhabit freshwater, and some snails and slugs are terrestrial. Mollusk bodies have three parts: a fleshy mantle, a visceral mass, and a foot. The heavy outer mantle envelops the visceral mass, the central region that contains the internal organs. The foot is a muscular organ used for locomotion. Mollusks have specialized organ systems for digestion, circulation, respiration, excretion, and reproduction. A protective exoskeleton composed of one or two outer shells is made from calcium carbonate and protein. Except for bivalves, mollusks with a two-valved hinged shell, all mollusks have a radula, a structure located in the mouth that has thousands of backward-curved teeth. The radula helps the animal scrape food off rocks during feeding and is also used to attack prey.

As is characteristic of all coelomates, the digestive tubes of mollusks are completely surrounded by mesoderm. This arrangement prevents nutrients from directly diffusing into the body cells, a situation remedied by a circulatory system. Most mollusks have a three-chambered heart and an open circulatory system for bringing nutrients and oxygen to body tissues and carrying away carbon dioxide and waste products. Gills located in the mantle cavity of most mollusks respire by extracting oxygen from fluid that passes through them. Terrestrial snails have a thin membrane lining their mantle cavity in place of gills. The membrane must be kept moist in order to allow for gas exchange, explaining why snails are most active after it rains or at night. After waste products diffuse into the fluid of the coelom, cilia sweep the fluid into nephridia, tiny excretory organs that reabsorb the sugars, salts, and water before excreting the waste-laden fluid out through a pore into the mantle cavity. Most mollusk species have distinct males and females, but some are hermaphroditic, and others can switch back and forth between sexes. Fertilized eggs develop into trochophore larvae, free-swimming forms that drift with ocean currents and propel themselves through the water by beating cilia. In octopuses, squids, freshwater snails, and some freshwater mussels, a juvenile stage mollusk hatches from the egg, skipping the trochophore larvae stage.

Though all mollusks share in common a three-part body plan, variations suited for different habitats have evolved. The class Gastropoda includes marine, freshwater, and terrestrial snails and slugs. During embryonic development, one side of the visceral mass grows faster than the other in a process called torsion. The result is placement of the visceral mass and shell over the body of the snail, a coiled digestive tract, and positioning of the mantle cavity and anus over the head. Most gastropods have one spiraled shell and a pair of tentacles extending from their head with eyes at the ends. Land species secrete mucous from their foot, upon which they slide using a rippling motion. Herbivorous gastropods use their radulae to scrap algae from surfaces or to saw leaves from plants. Carnivorous gastropods use their radulae to poke holes into the shells of other mollusks, as a harpoon, or to tear apart the flesh of their prey.

The giant squid is the largest known mollusk and can grow to lengths of 60 feet (18 m). *(AP Images)*

Clams, oysters, scallops, and mussels are bivalves, mollusks that have a two-part hinged shell formed by calcium carbonate secretions from the mantle, a membrane that lines the shell. Some bivalves are marine and some are freshwater, and they do not have distinct heads or radulae. Their body consists of a visceral mass and a muscular foot but no distinct head. They have a nerve ganglion above their foot and often have sensory cells that respond to light and touch located around their mantle. Thick adductor muscles hold the two shell halves together very tightly when contracted. Bivalves are generally sessile, but some contract and relax their adductor muscles to skitter along the seafloor. Bivalves obtain their nutrition by filter-feeding. Two tubes, an incurrent and an excurrent siphon extend from either end of the body. Beating cilia on the gills brings in water through the incurrent siphon. Small marine organisms and other organic material adhere to a sticky substance on the gills, which are located in the mantle cavity and are also responsible for respiration. The cilia then move the food particles toward the mouth. When a foreign object such as a grain of sand irritates the area between a bivalve's mantle and shell, the animal secretes thin coats of nacre around the object, forming a pearl.

Cephalopods include squids, octopuses, cuttlefish, nautiluses, and devilfish. Their bodies consist of large heads with a modified foot divided into numerous tentacles that have suction cups or hooks for capturing prey and a funnel that expels water from the mantle. Octopuses have eight tentacles, squids have 10 tentacles, and nautiluses have between 80 and 90 tentacles. The only cephalopod that has maintained an external shell throughout its evolution is the chambered nautilus, whereas squids and cuttlefish have internal shells, and many octopuses lack any shell. Cephalopods have closed circulatory systems, well-developed nervous systems, and sense organs and eyes similar to those of vertebrates. They vary in size from about an inch (2–3 cm) to extremely large. The giant squid is capable of growing to 65 feet (20 m), making it the largest invertebrate animal. Movement occurs by jet propulsion—water is drawn into the mantle cavity, then quickly expelled through an anterior siphon used in steering. Squids and octopuses can also shoot out ink when threatened, coloring the surrounding water to mask their escape from potential predators. As predators themselves, cephalopods hunt smaller mollusks, crustaceans, and worms as prey. The tentacles bring the food to the mouth, beaklike jaws tear the food apart, and the radula pulls the food pieces into the mouth.

ANNELIDA

The phylum Annelida, more commonly known as the segmented worms, is the earliest phylogenetic branch that contains segmented animals. Found in marine and freshwater habitats as well as moist soil, annelids are coelomates with well-developed circulatory, digestive, and excretory systems. A primitive brain fashioned from a pair of cerebral ganglia exists in an anterior segment, and a ventral nerve cord runs the length of the organism. Other segments may be specialized for functions such as reproduction, feeding, or sensation. The majority of the segments that repeat along the length of the worm each contain digestive, excretory, circulatory, and locomotor structures. Internal body walls called septa separate the annelid segments though nerve cords, the digestive tract, and the circulatory system can penetrate these partitions. Paired, external, bristlelike structures called setae provide traction for crawling or burrowing, and some annelids have fleshy parapodia for locomotion. The presence and number of these structures is used in classification.

The largest class of annelids, Polychaeta, includes more than 10,000 species of mostly marine segmented worms. Polychaetes range in length from less than 0.04 inches (1 mm) to 9.8 feet (3 m), with 2–4 inches (5–10 cm) being average. Each segment

These sessile polychaetes have their featherlike tentacles extended. *(OAR/National Undersea Research Program [NURP])*

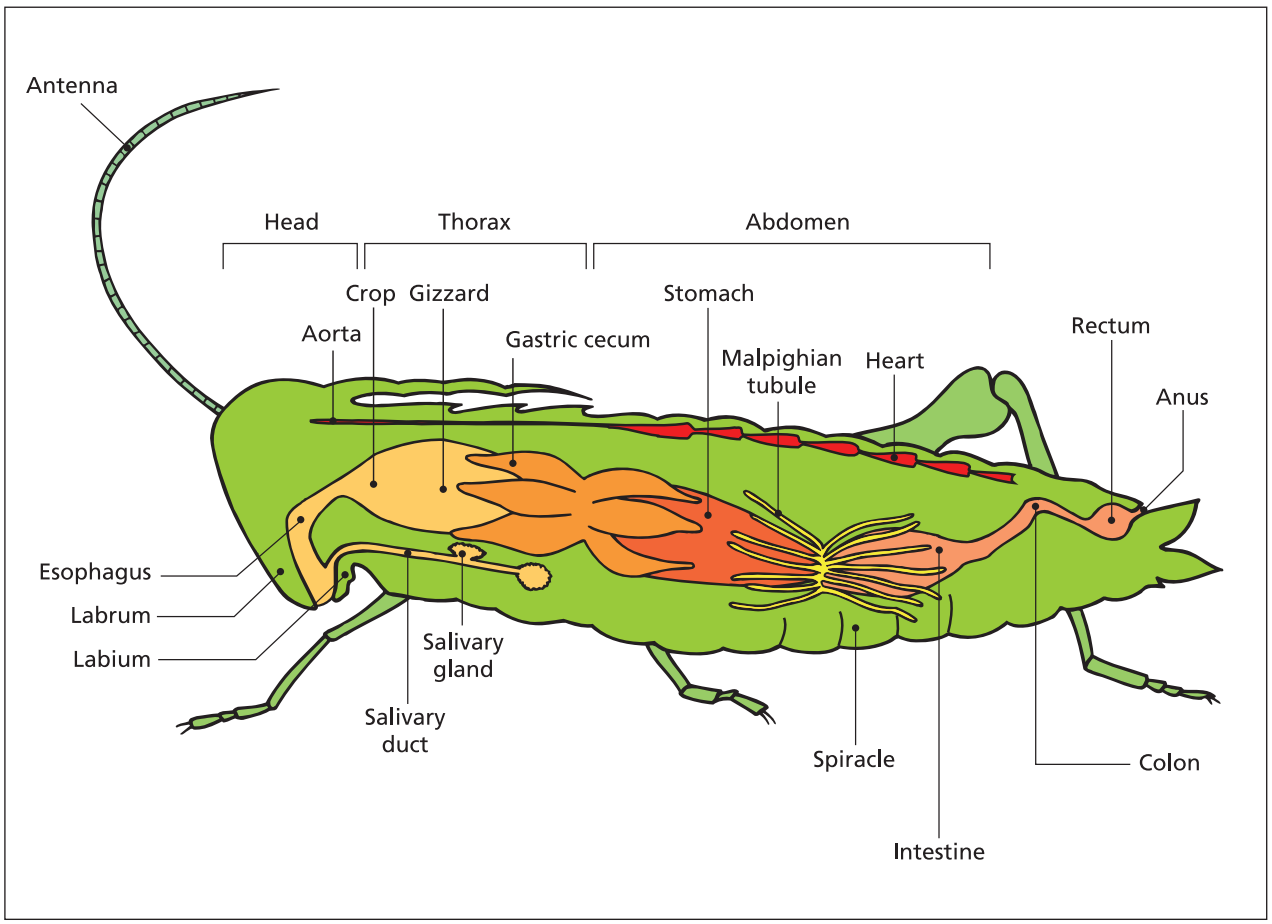
of a polychaete possesses a pair of ciliated, paddle-like parapodia used for swimming, burrowing, and crawling. The presence of these appendages also increases the surface area across which gas exchange can occur. Some polychaetes burrow, but others, such as the feather duster, live permanently stuck in tubes formed from hardened secretions and sand bits with only their head exposed. Featherlike tentacles coming from the head filter food suspended in the seawater. Some polychaetes filter water that is pumped through their body, and others capture prey in jaws that extend from their pharynx.

Earthworms belong to the class Oligochaeta, consisting of more than 3,000 annelid species with no parapodia and few setae. They have no eyes, but they do have light-sensitive organs and other sensory structures for detecting touch and moisture. Earthworms eat by tunneling through the soil, taking it in, storing it in a pouched enlargement called a crop, and grinding it with their gizzard. Nutrients are absorbed through the intestinal walls into blood circulation, while the indigestible material passes out of the body through the anus as castings. As the worms tunnel through the soil, they aerate it, leaving castings behind, making it rich and fertile for farming. Earthworms respire through their skin, which must be kept moist. The brain coordinates the muscular activity necessary to achieve wavelike movement, and the hydrostatic skeleton created by the fluid within the coelom of each body segment gives the muscles something to pull against. First, setae of several rear segments anchor to the ground, then anterior muscles that encircle the worm contract, causing the worm to elongate. Setae in the front then anchor into the ground while the circular muscles relax and longitudinal muscles contract, shortening the worm and pulling it forward. Oligochaetes are hermaphroditic and mate by joining with another worm to exchange sperm. After mating, the clitellum, a thickened ring of pale cells that encircles part of the body, secretes a mucous cocoon that slides off the worm and protects the fertilized eggs until young worms hatch two weeks later. Some earthworms also reproduce asexually by fragmentation and regeneration.

The class Hirudinea includes leeches, annelids with flattened bodies, continuous internal segments, and neither parapodia nor setae. Most inhabit freshwater, but some live on moist land. Many leeches are predators of other small invertebrates or scavengers, and some are parasites that obtain their nutrition by sucking the blood from other animals.

ARTHROPODA

The phylum Arthropoda includes more than 1.2 million species (a conservative estimate), many yet to be identified, making it the most diverse invertebrate phylum. Jointed appendages are an evolutionary advantage shared by all arthropods. Most arthropods are less than 0.04 inches (1 mm) long, but some grow up to 12 feet (3.6 m) across. This large group of invertebrates results from three major evolutionary lineages: two comprised of animals with jaws, subphylums Uniramia and Crustacea, and one with fangs or pincers, subphylum Chelicerata. Other than jointed appendages, characteristics shared by most arthropods include segmentation, a distinct head often with compound eyes, a hard exoskeleton (the cuticle), an open circulatory system, excretion through Malpighian tubes, wings, and respiration by gills, tracheae, or book lungs. After the larval stage the body segments of arthropods fuse to form three main body parts: the head, the thorax, and the abdomen. Some species, such as the crab, have a cephalothorax, a combined head and thorax. Many arthropods have compound eyes, and some have both compound eyes and single-lens eyes. Compound eyes are multifaceted and contain numerous independent focusing units. Each sends information to the brain, which then combines the data to form an image. A rigid exoskeleton made from the polysaccharide chitin and protein protects the softer internal body from injury and water loss and gives muscles a site for attachment. Larger arthropods require thicker exoskeletons to handle the work of larger muscles, but exoskeletons cannot expand as an organism grows. The creature must periodically molt, a process termed ecdysis. Specific hormones such as ecdysone initiate the growth of a new exoskeleton, and when it is formed, the old one breaks. The exoskeleton of an animal that emerges from a discarded exoskeleton is soft and takes a few hours or days to harden, leaving the animal temporarily vulnerable. Arthropods have open circulatory systems; a heart pumps fluid called hemolymph through small arteries into open spaces surrounding the body tissues. These spaces are collectively referred to as the hemocoel and are separate from the coelom, which becomes greatly reduced as the animal matures. The respiratory system of terrestrial arthropods includes spiracles, valved structures through which air enters a network of hollow tubes called tracheae that distribute oxygen throughout the body. In order to eliminate waste products of metabolism without losing water, arthropods have excretory units called Malpighian tubules that open into the digestive tract. Hemolymph bathes the tips of these long extensions around the gut, and small molecules diffuse through them into the gut. The epithelial tissues of the rectum reabsorb the water, ions, and valuable nutrients while leaving the nitrogenous waste products behind to be excreted through the anus with feces.



The insect body, as depicted in this female short-horned grasshopper, has three main regions: the head, the thorax, and the abdomen.

The enormous subphylum Uniramia consists of mostly terrestrial arthropods with chewing mouthparts called mandibles, or jaws. Three main classes include Insecta (insects), Diplopoda (millipedes), and Chilopoda (centipedes). With more than 1 million identified species, and potentially a million or more unnamed, insects are the largest group of animals. The body structure consists of three main sections, the head, thorax, and abdomen. Insect heads possess a large pair of compound eyes, three light-detecting ocelli, and a pair of antennae adapted for sensing smell and touch. Three fused segments compose the thorax, to which three pairs of jointed walking legs are attached. Wings are modified extensions of the exoskeleton. One or two pairs of wings emerge from the thorax in most insects, but some species such as fleas and lice are wingless. Flight confers a distinct advantage to insects in escaping predators, locating food, finding new habitats, and seeking mating partners. The abdomen consists of nine to 11 segments.

The head contains mandibles that are adapted for different food sources, depending on the species. Other mouthparts, the labrum, labium, and maxillas, can hold and tear pieces of plants to be chewed by the jaws. The crop stores the chewed food, passes it to the gizzard where it is ground up, then to the midgut to complete the digestive process. Food molecules diffuse into the fluid of the coelom and then into circulation. The circulatory system includes a series of muscular pumping organs running along a long dorsal blood vessel. Because the system is open, hemolymph leaves the vessels and directly bathes the body tissues. Respiratory structures called spiracles allow the entrance of air into a highly branched network of trachea that direct air throughout the body. Wings develop from saclike outgrowths of the thorax and are made up of chitin. Downward strokes power flight. In insects that have more than one pair of wings, only one is used to power flying, and the other is used either for stability during flight or as a protective wing cover during rest. Female insects have pouches called spermatheca that can store sperm inserted into the vagina by a male during mating. When she is ready to release eggs, she seeks out a location near an appropriate food source. Fertilization occurs as the eggs pass out of her body.

Insect life cycles are complex and involve a metamorphosis. A complete metamorphosis is a developmental process characterized by a remarkable change in body structure, in contrast to an incomplete metamorphosis that is much less dramatic. In complete metamorphosis, a fertilized egg develops into a wingless, wormlike larval form that specializes in eating, growing, and storing energy. It molts as it grows, and then wraps itself into a protective cocoon called a chrysalis to complete the pupal stage of its development. The larval tissues are broken down, and an adult forms from cells that were quiescent in the larva. An adult form better suited for dispersal and reproduction emerges from the cocoon. In species that undergo incomplete metamorphosis, the eggs hatch into nymphs, juvenile forms that closely resemble the adult form but are smaller. The nymph grows and molts several times before reaching its full size.

Organisms belonging to the classes Diplopoda (millipedes) and Chilopoda (centipedes) have heads attached to bodies made up of numerous segments. Millipedes have two pairs of legs per segment and up to 100 or more segments. Centipedes have one pair of legs per segment, and as many as 177 segments, in addition to a pair of antennae and three pairs of appendages modified as mouthparts. Centipedes are carnivorous, and millipedes are herbivorous.

The subphylum Crustacea includes crabs, lobsters, crayfish, shrimps, barnacles, and water fleas. Most species are marine, but some are found in freshwater environments, and a few species are terrestrial. Crustaceans have mandibles like insects, two pairs of antennae, three pairs of appendages used for chewing, walking legs attached to the thorax, and gills. Larger species respire with gills, and smaller crustaceans exchange gases across their cuticle. A unique, free-swimming larval form called a nauplius that has three pairs of branched appendages molts several times before becoming an adult. Many smaller crustaceans such as krill, water fleas, ostracods, fairy shrimps, and copepods, serve an important role as food for other marine organisms. Decapods are larger crustaceans such as lobster, shrimp, crab, and crayfish that have five pairs of legs. They have a fused cephalothorax covered by a hard, protective carapace. The front legs are modified into large, strong pincers called chelipeds. The second and third pairs have smaller pincers at their ends. Swimmerets, smaller appendages at the posterior end, aid in swimming and reproduction, and uropods (also called pleopods), flattened appendages at the end of the abdomen are used for swimming. Many crustaceans also have a telson, or tail spine at the end of their abdomen. Contracting this sectioned tail allows lobsters to move backward quickly. Most lobsters are carnivores and they can easily crush prey with their claws. The smell of rotting flesh also attracts lobsters, a feature exploited by fishermen.

Barnacles, once mistaken for mollusks, are a type of crustacean that are free-swimming as larvae, then attach to a rock or other surface such as the hull of a ship, a whale, or a turtle shell, and live as sessile adults. They gather plankton for food with jointed feathery appendages called cirri that project from between plates that protect the organism's body. This protective shell, called a carapace, allows barnacles to withstand harsh weather conditions associated with intertidal zones of rocky coasts. Unlike most crustaceans, barnacles are hermaphroditic.

The subphylum Chelicerata is divided into three classes—one major class, Arachnida (spiders, scorpions, ticks, and mites), and two smaller classes, Merostomata (horseshoe crabs), and Pycnogonida (sea spiders). The body of an arachnid consists of a head with six eyes and a cephalothorax. Appendages at the mouth, called chelicerae, are often specialized into fangs. Another pair of anterior appendages, the pedipalps, assists the arachnid in capturing prey. Four pairs of walking legs are attached to the cephalothorax. Spiders are carnivores but can ingest only liquid food. After capturing prey in a web, they kill or paralyze it by injecting it with poison through their fangs. Then they secrete a mixture of digestive enzymes that liquefy its tissues, and then they suck the food into their stomach for further digestion. Because they eat so many insects, including potential pests, spiders are an important part of terrestrial ecosystems and beneficial to humans. On their abdomen, spiders have spinnerets from which they secrete sticky strands of silk that harden into fibers used to construct webs. Respiration is accomplished using book lungs, structures with stacks of thin blood-filled plates of tissue. Air enters the body through openings on the underside of the abdomen and flows over the book lungs. Oxygen enters by diffusion and circulates throughout the body. Male spiders store sperm in modified pedipalps, and then use them to insert the perm into a female during mating. Afterward, female spiders lay between 20 and 50 fertilized eggs at a time into a silk cocoon. Though spiders are feared by many people, only two known species found in the United States are dangerous—the black widow and the brown recluse.

The chelicerae and pedipalps of other arachnids, scorpions, ticks, and mites, are modified. Scorpions have segmented abdomens, and their pedipalps are greatly enlarged and pincerlike for grasping prey. They also possess an extension from their abdomen called a metasoma, a segmented tail ending in a needlelike stinger that is usually curled forward over the scorpion body. The largest group of arachnids,

the mites (including chiggers and ticks), are distinguished by an unsegmented body composed of a fused head, thorax, and abdomen. Mites are usually less than 0.04 inches (1 mm) long and can be aquatic or terrestrial. Aquatic types are often herbivorous, while terrestrial mites are often predators. Plant mites can pass fungal or viral infections to plants during feeding, and blood-sucking ticks can pass microbial parasites such as the bacteria responsible for Lyme disease to humans.

Members of the marine class Merostomata, the horseshoe crabs, possess a large shield that covers their cephalothorax and a spiky posterior extension called a telson. Their compound eyes are smaller than most chelicerates.

Members of the class Pycnogonida live in the ocean at depths up to 23,000 feet (7,000 m). Sea spiders resemble terrestrial spiders to some degree, but they possess a long proboscis through which they suck the juices of small soft-bodied invertebrates for food. They may have three or four or more pairs of walking legs.

ECHINODERMATA

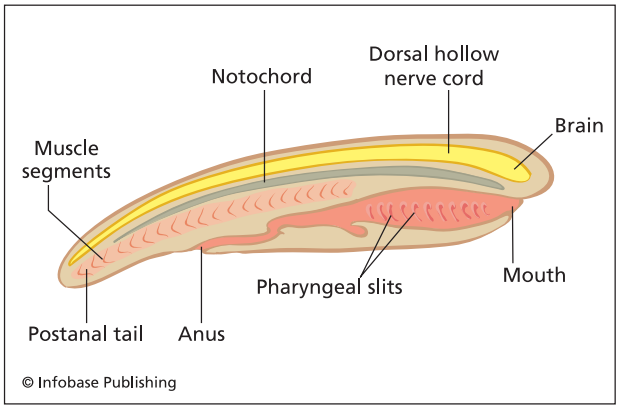
Members of the phylum Echinodermata are marine coelomates that follow the deuterostome pattern of development, in which the blastopore becomes the anus rather than the mouth, as it does in protostomes. Some well-known echinoderms include sea stars, sea urchins, sand dollars, and sea cucumbers. In addition to being the first deuterostomes, the echinoderms were also the first to have an endoskeleton. Though in adults the layer of skin covering the endoskeleton is sometimes worn away, in young echinoderms, the individual, hard, calcareous plates called ossicles that compose the endoskeleton are completely enclosed in living tissue. Often fused, the ossicles offer protection, act as muscle attachment sites, and in most species project spines through the skin. Echinoderms are bilaterally symmetrical as larvae, but most exhibit five-part radial symmetry as adults. They have no head, but they do have a central ring of nerves with branches extending into each of the arms. Numerous branched canals and thousands of tube feet make up a water-vascular system that circulates throughout the body and function in movement. The tube feet of some species, which extend through tiny openings in the ossicles, have terminal suckers that can be used to creep along the seafloor or pull apart bivalves. Some gases and wastes are exchanged through the walls of the tube feet, but the fluid-filled coelom serves as a simple circulatory and respiratory system. Many echinoderms also have small fingerlike projections called skin gills that function in respiration and excretion. During feeding the stomach of sea stars partially protrudes, secretes digestive enzymes, and then ingests the liquefied prey. Most echinoderm species have separate sexes and reproduce sexually by releasing gametes into the water. Sea stars, however, can regrow a new arm if one breaks off, or even an entire organism from an arm if part of the central disk is included.

Echinoderms are the most numerous of the marine invertebrate phyla. The carnivorous sea stars, more commonly known as starfish, may be the most familiar, but the brittle stars and sea baskets are the most abundant. They all live on the seafloor and generally feed by filtration of the ocean sediment. A unique characteristic of the sea lilies and feather stars is the positioning of their mouth on their upper rather than lower surface. Both are mostly sessile and attach to the ocean floor—the lilies by stalks that can grow up to two feet (60 centimeters) in length. Roughly spherical sea urchins and flattened sand dollars both have hard endoskeletons and protruding spines. Some sea urchins produce toxins that can paralyze prey, and others eat seaweed. Sea urchins live on the ocean floor and sand dollars live in coastal areas. The ossicles of sea cucumbers are small and not fused, so their bodies are soft. Though their bodies are cylindrical and elongated, sea cucumbers do have the five rows of tube feet characteristic of echinoderms. They feed by trapping small marine creatures in sticky tentacles that surround the mouth. When threatened, sea cucumbers release sticky extensions from their anus to trap their predators. Sea daisies were discovered in 1986 off the coast of New Zealand. They are considered echinoderms because they exhibit five-part radial symmetry, but their relationship to other echinoderms has not yet been established.

INVERTEBRATE CHORDATES

The phylum Chordata includes both invertebrate and vertebrate members and is characterized by the presence during development of a flexible, dorsal rod called a notochord that provides skeletal support. Muscles attached to the notochord can pull the animal body from side to side, helping it swim in aquatic environments. In addition to a notochord, chordates have a single, dorsal, hollow nerve cord that later develops into the brain and spinal cord, a series of pharyngeal slits that develop into gills or other structures in terrestrial chordates, and a postanal tail that extends beyond the anus and aids in swimming in many aquatic chordates. These structures are present at least during embryonic development in all chordates though sometimes are absent in the adult stage.

Chordates are divided into three subphyla: Vertebrata, Urochordata (tunicates), and Cephalochordata (lancelets). In vertebrates, a backbone replaces the notochord during embryonic development. Tunicates have a nerve cord, a notochord, and a postanal tail as free-swimming larvae but not as adults. Adult



Chordates share four distinguishing characteristics: 1) a notochord; 2) a dorsal, hollow nerve cord; 3) pharyngeal slits; and 4) a postanal tail.

tunicates are sessile, have prominent pharyngeal slits, and filter feed. Beating cilia bring water through an incurrent siphon into the body where it circulates and leaves through an excurrent siphon. Food is filtered from the water as it passes through the pharynx, it enters the stomach, and undigested material leaves through the excurrent siphon. Tunicates are hermaphroditic and can also reproduce by budding, resulting in colonies of genetically identical individuals. In lancelets, these structures persist into adulthood. Only a few centimeters long, they are shaped like blades and are usually found burrowed in the mud of shallow ocean waters, but they can swim using coordinated muscular contraction, as in fishes. Muscles shaped like the letter V repeat along the segmented body. Beating cilia draw in water from which the lancelet filters protists for food that travels down the digestive tube, and then the water exits through the gill slits. Lancelets reproduce sexually.

See also ANIMAL FORM; BIODIVERSITY; BIOLOGICAL CLASSIFICATION; CIRCULATORY SYSTEM; DIGESTIVE SYSTEM; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; MUSCULOSKELETAL SYSTEM; NERVOUS SYSTEM; NUTRITION; REPRODUCTION; RESPIRATION AND GAS EXCHANGE; VERTEBRATES; ZOOLOGY.

FURTHER READING

Attenborough, David. *Life in the Undergrowth*. Princeton, N.J.: Princeton University Press, 2005.

Brusca, Richard C., and Gary J. Brusca. *Invertebrates*. 2nd ed. Sunderland, Mass.: Sinauer, 2002.

Moore, Janet. *An Introduction to the Invertebrates*. 2nd ed. Cambridge: Cambridge University Press, 2006.

Pechenik, Jan A. *Biology of the Invertebrates*. 5th ed. New York: McGraw Hill, 2005.

Tudge, Colin. *The Variety of Life: A Survey and a Celebration of All the Creatures That Have Ever Lived*. Oxford: Oxford University Press, 2000.

Ivanovsky, Dmitri (1864–1920) Russian *Botanist, Microbiologist* Dmitri Ivanovsky was a Russian botanist who made a discovery that caused biologists to question the very definition of life. While investigating tobacco mosaic disease, he discovered the first filterable pathogen, later termed a virus, an infectious agent now known to be responsible for numerous diseases including polio, the common cold, chicken pox, the flu, acquired immunodeficiency disorder, and some cancers.

Dmitri Iosifovich Ivanovsky was born on November 9, 1864, in Gdov, Russia. He enrolled in the natural sciences department at St. Petersburg University in 1883. In 1887 he embarked on studies in collaboration with a fellow student on a disease called wildfire that affected tobacco plants in the Ukraine and Bessarabia. Though their conclusions that the affliction was noninfectious in nature and resulted from overtranspiration were wrong, the investigations awakened in Ivanovsky an interest in plant pathology. This work formed the basis of his dissertation, titled "On Two Diseases of Tobacco Plants."

After earning a degree of candidate of science in 1888, Ivanovsky stayed at St. Petersburg to prepare for a teaching career. In 1890 the department of agriculture requested that Ivanovsky examine a new disease affecting tobacco plants in the Crimea. The plants did not grow well and the leaves blistered and became mottled, creating a mosaic pattern. He identified the disease as tobacco mosaic disease, which had been characterized only four years earlier by the German botanist Adolf Mayer. To examine the cause of the disease, Ivanovsky ground up plant tissue and passed the sap through a filter fine enough to trap any bacteria that might have been present. He expected the filter to remove the bacteria and leave the fluid noninfectious, but the resulting clear fluid retained the ability to transmit disease. He concluded that extremely tiny bacteria or bacterial endospores were present. Ivanovsky's results, presented to the St. Petersburg Academy of Sciences in 1892, were the first record of a filterable pathogen.

In order to further pursue his scientific career, Ivanovsky needed additional education. In 1895 he defended a dissertation on the process of alcoholic fermentation by yeast to earn his master of botany degree. The following year he accepted a position teaching plant anatomy and physiology at the Technological Institute.

In 1899 the Dutch botanist Martinus Wilhelm Beijernick, a former colleague of Mayer, reported that extracts from infected plants retained the ability to transmit the disease to healthy plants after filtration, unaware of Ivanovsky's findings from 1892. He also concluded that bacterial spores or perhaps

a toxin was present in the extracts and proceeded to test this experimentally. Endospores form in some bacterial cells to withstand harsh environmental conditions, such as heat. Beijernick heated the filtered extracts to 194°F (90°C) and found that the material remained infectious, eliminating spores as a possible explanation. In addition, after inducing the disease in a healthy plant from an extract, the fluids from the second plant could then pass on the disease to other plants, indicating that the responsible agent multiplied in the plant tissue. He layered infectious extracts onto a block of agar, a semisolid gelled substance, and 10 days later material from inside the agar block was infectious. From this experiment, Beijernick concluded that since a particle could not have diffused through the agar block, the disease-causing substance was a dissolved particle or a characteristic of the fluid itself. He proposed a contagious living fluid was responsible for tobacco mosaic disease and called it a virus, which means poison in Latin. Beijernick went further to predict that the contagious fluid must be incorporated into a living cell in order to reproduce, which he described as a passive endeavor, a prophetic hypothesis.

When Ivanovsky saw Beijernick's published work, he claimed priority for discovering that a filterable agent caused tobacco mosaic disease (Beijernick agreed). Ivanovsky also rightly disagreed with Beijernick's conclusions that a particulate agent could not have penetrated into the agar block, believing instead that the pathogen was particulate but exceedingly small. He verified his conclusions and published his ideas as part of his doctoral dissertation in 1902, "Mosaic Disease in Tobacco."

In 1908 Ivanovsky accepted a position at Warsaw University, where his research focused on photosynthesis and chloroplasts. He studied light absorption by the chlorophyll pigments and found that light destroyed chlorophyll. Based on this, he proposed that the yellow pigments in plant tissue functioned to protect the chlorophyll from damage.

Ivanovsky married E. I. Rodionova and had one son, named Nikolai. He never returned to virology, but his discovery of a filterable pathogen continues to impact the life sciences. He died on June 20, 1920, in the Union of Soviet Socialist Republics.

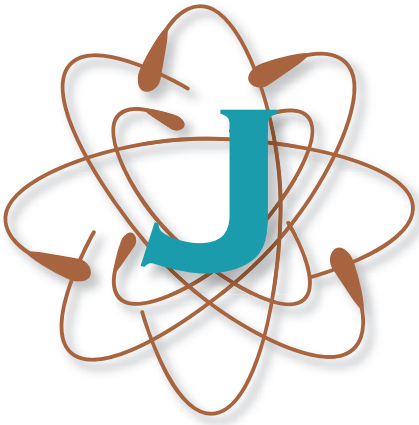
In 1932 an American scientist named Wendell Stanley crystallized Ivanovsky's filterable agent, tobacco mosaic virus (TMV), and concluded that it consisted of protein. A few years later two British researchers, Fred Bawden and Norman Pirie, demonstrated that TMV contained not only protein, but also some nucleic acid, a characteristic now known to be true for all viruses.

See also MICROBIOLOGY; VIRUSES AND OTHER INFECTIOUS PARTICLES.

FURTHER READING

Gutina, V. "Ivanofsky, Dmitri Iosifovich." In *Dictionary of Scientific Biography*. Vol. 7, edited by Charles Coulston Gillispie, 34–36. New York: Charles Scribner's Sons, 1970–1976.

Radetsky, Peter. *The Invisible Invaders: Viruses and the Scientists Who Pursue Them*. Boston: Little, Brown, 1991.



Just, Ernest (1883–1941) American *Embryologist* Ernest Everett Just was an expert in marine invertebrate zoology who made important discoveries and taught others how to do the same in the areas of fertilization, parthenogenesis, and early embryology. As an accomplished cellular physiologist, one who studies the functions and activities of living organisms at the cellular level, he recognized and called attention to the role of protoplasm in cellular development.

PREPARATION FOR A PROFESSORSHIP IN BIOLOGY

Ernest Everett Just was born on August 14, 1883, to Charles and Mary Cooper Just in Charleston, South Carolina. He was their fourth child, born into unfortunate circumstances. His father was an alcoholic who could not keep a job and died penniless when Ernest was only four years old. Several of his siblings died young, and his family moved to St. James Island, South Carolina. His mother labored in the phosphate mines to support her family, dedicated herself to improving the education of the mostly black islanders, and taught Sunday school and dressmaking in her home. She was such a devoted member of the community that the town became known as Maryville in her honor. As the oldest surviving child, Ernest shared many of the routine household chores such as cooking, cleaning, and caring for his younger siblings, but he never complained about the extra work.

Until age 12, Ernest attended the school his mother ran, the Frederick Deming, Jr. Industrial School. In 1896 he enrolled at an African-American high school, the Colored Normal, Industrial, Agricultural and Mechanical College, in Orangeburg, South Carolina, to be trained as a teacher. He received his license to teach in the black public schools in 1899, but since he was only 15 years old, he was not ready to devote his life to a career in teaching. He received a scholarship to attend Kimball Union Academy, a high school in Meriden, New Hampshire, and he worked on a ship to earn passage to New York in 1900. At Kimball, Ernest was the editor-in-chief of the school newspaper and excelled at oratory. He completed high school in three years instead of the usual four and was accepted at Dartmouth College in Hanover, New Hampshire.

Just was committed to learning and preparing for his future. After reading an essay on the development of the egg cell during his sophomore year, Just eagerly signed up for every biology course the college offered, but he also excelled at the classics, earning the highest freshman grade in Greek. The head of the biology department, William Patten, asked Just to assist him in writing and illustrating the section on frog embryonic development for a textbook he was writing, *The Evolution of the Vertebrates and Their Kin* (1912). Twice during college, Just was awarded the title of Rufus Choate Scholar, the highest academic award for an undergraduate at Dartmouth, and he was elected into Phi Beta Kappa, the nation's oldest academic honor society. He earned his bachelor's degree in biology and two minors in Greek and history, graduating magna cum laude in 1907.

Just sought a research position, but such jobs were not available to African Americans. He moved to Washington, D.C., where he accepted an instructorship in English and rhetoric at Howard University, a historically African-American private university. His pupils were fortunate to have a dedicated and enthusiastic teacher. A few years later Just was appointed an assistant professor of biology, teaching zoology and histology. In 1912 he was promoted to full professor and head of the zoology department and later

also taught in, and headed the physiology department at, the medical school. Just involved himself in other aspects of campus life as well. He organized a drama club for the students and acted as adviser to a new black fraternity, Omega Psi Phi. He remained associated with Howard University until his death.

MARINE BIOLOGICAL RESEARCH

Desirous of a graduate degree, Just asked his former professor, Patten, for advice regarding graduate training. Patten suggested he contact Frank R. Lillie, the head of the department of zoology at the University of Chicago and the director of the Marine Biological Laboratory (MBL) at Woods Hole, Massachusetts. Lillie invited Just to come to MBL, an independent research and teaching institution not affiliated with any university, as his research assistant for the summer of 1909, the first of many Just would spend at MBL. He advanced his knowledge of theoretical biology by enrolling in an invertebrate zoology course that first summer and embryology the following year. With Lillie, he researched sandworm fertilization, the process whereby male and female gametes unite to form a zygote, a fertilized egg. Impressed by Just's intelligence and dedication, Lillie recommended that Just enroll in a doctoral program in absentia at the University of Chicago.

The polychaete *Nereis* is a segmented worm that can grow up to six inches in length and lives in sandy or rocky beaches, estuaries, mud flats, and wharfs. Because they have unique breeding patterns, studying fertilization in *Nereis* was a difficult process. Just was convinced, rightly, that the condition of his research material was paramount to obtaining reliable and meaningful results. The eggs he studied needed to be fresh, and they could only live for 24 hours outside the female's body. The sandworms swarmed only at nighttime, monthly, in cycle with the Moon. Just knew when and where he needed to collect his specimens using a hand net and a lantern. Sandworm fertilization is an external process, meaning the eggs and the sperm are shed into the water, and fertilization occurs there rather than inside the body as it does in mammals. Just had to bring the fertilized cells to the lab quickly and make his observations throughout the night. Sometimes, he captured male and female worms separately and brought them back to the lab. He perfected a method for artificial fertilization by washing and drying off the male sandworms, then cutting them open to collect dry sperm cells. Since female worms only release eggs naturally in the presence of males, he would slice open a washed female to release the eggs and then mix the cell types while observing the process under a microscope.

In 1911 Just made an important discovery that cemented his status as a marine invertebrate zoologist. He was examining cell cleavage, the process of early embryonic development in which the cell membrane pinches off, followed by repeated cell division, converting the zygote into a ball of cells. He discovered that the location of sperm entrance together with the polar bodies determined the position of the line of cleavage. These results were published in "The Relation of the First Cleavage Plane to the Entrance Point of Sperm" in 1912. During the next two years, he published two additional articles on breeding habits of sandworms.

Ernest Just was a pioneering marine invertebrate zoologist who studied early embryonic development. *(Scurlock Studio Records, Archives Center, National Museum of American History, Smithsonian Institution)*

In 1912 Just married Ethel Highwarden, a German teacher and the daughter of an Ohio riverboat captain. They bought a Victorian three-story house in LeDroit Park, a residential section in northwest Washington, D.C., and filled it with three children.

During the same summer he married, Just met biologist Jacques Loeb, from the Rockefeller Institute for Medical Research. Loeb was committed to improving African-America education, specifically in the field of medicine. He became involved with the National Association for the Advancement of Colored People (NAACP), and when asked to recommend a recipient for a new award for an American of African descent "who shall have made the highest achievement during the preceding year in an honorable field

of human endeavor," he suggested Just. The governor of New York, Charles Whitman, presented Just with the first Spingarn Medal in 1915.

While the publicity and national recognition from this award did much to advance his reputation as a scientist, Just knew he needed a Ph.D. to further advance his career. In 1915 he moved to Chicago for one year, leaving his wife and first daughter behind while he completed residency and minor course requirements. Lillie accepted Just's previous publications as his doctoral thesis, and the following year Just received his doctorate in zoology from the University of Chicago.

After becoming a doctor of zoology, Just was recommended for and elected to membership in many professional societies, including the American Society of Naturalists, the American Society of Zoologists (for which he served as president), the American Association for the Advancement of Science, and the American Ecological Society. At the time, this was quite an achievement for an African-American scientist. He resumed his routine of teaching at Howard during the school year and researching at Woods Hole during the summers. Eventually, Just became a member of the corporation of the MBL and for a while served as editor of the laboratory's journal, *Biological Bulletin*. He later served as associate editor for several other scientific journals, including *Physiological Zoology* and the *Journal of Morphology*.

During the period 1917–19, Just researched the fertilization process in the sand dollar, *Echinarachnius parma*. After the sperm contacts the egg, the two cellular membranes fuse, and a series of changes that alter the outer portion of the egg cytoplasm take place. This is necessary to prevent polyspermy, the fertilization by more than one sperm, and also results in biochemical changes in the cytoplasm. Just published a series of articles in the *Biological Bulletin* (1919–20) describing his research on fertilization and activation of the biochemical changes in the egg. These investigations led Just to discoveries that contradicted some of Loeb's conclusions about parthenogenesis, the process whereby an unfertilized egg develops into an adult organism. Loeb had pioneered research in the study of parthenogenesis, and was able to induce development in sea urchin and frog eggs without any sperm by pricking them with a needle or treating them with very salty water. Loeb proposed a double theory of cytolysis and correction, where a cytolytic factor broke down the outer surface of the egg and a corrective factor prevented the cytolysis from going too far. He initiated cytolysis artificially by treating eggs with salt water and butyric acid, and then stopped cytolysis by rinsing away the butyric acid with salt water or adding magnesium. Since Loeb could re-create fertilization in the lab by chemically simulating these events, he concluded that fertilization was a nonspecific process, similar to artificial parthenogenesis. Just found that normal development could also be induced by treating the eggs in the reverse order, using the supposed corrective agent first and then the cytolytic agent, or even by treatment with very salty water by itself! Just also examined the physiological condition and degree of activation of the eggs following butyric acid treatment and concluded that Loeb had either poor laboratory technique or a general lack of knowledge about the initiation of development.

Just had been studying reproduction in marine invertebrates for over a decade, and other scientists respected his technical expertise in this area. His advice on how to discern normally developing eggs from abnormal ones was sought and trusted by embryologists at MBL and elsewhere. While this research soured the relationship between Just and Loeb, it established Just as an outstanding researcher. Lillie invited Just to collaborate with him in writing the fertilization section of E. V. Cowdry's *General Cytology* (1924) book. Lillie proudly introduced Just to philanthropist Julius Rosenwald in 1920. Rosenwald felt that because of Just's race, he was being denied the advantages that accompany an academic appointment at a major academic institution and offered a grant to Just to help fund his research. This initiated a series of grants that supported Just's research until the mid-1930s, and allowed him to relinquish his teaching responsibilities at the medical school in 1920 in order to devote more time to his research. Over time, he began to view his teaching responsibilities at Howard as a burden and looked for opportunities to do research elsewhere. Being an African American, however, eliminated the possibility of finding a research professorship at a predominantly white institution.

RESEARCH IN EUROPE

Even though MBL offered Just a place to perform his pioneering research and a break from his heavy teaching load at Howard, he faced racial discrimination there. When he was finally able to convince his wife and children to accompany him to Woods Hole during the summer of 1927, they were treated so horrifically that Just had to interrupt his research to move them back to Washington, D.C., after only a few weeks. Howard University was not a research institution, so Just could not carry out his experiments there. By 1929, frustrated with the environment at Howard and racial oppression in the United States, Just began traveling to Europe to attend scientific meetings and to study. He worked as a guest researcher at the Naples Zoological Station, extending fertilization principles he discovered while work-

ing on American marine organisms to species that inhabited the European waters. He repeated experiments he had performed on *Echinarachnius* (the sand dollar) and *Arbacia* (sea urchin) on European species *Paracentrotus lividus* and *Echinus microtuberculatus*. Two decades before, he had observed differences in breeding habits between the American and European sandworms that some scientists believed to be the same species. In Naples, he carefully examined the two and distinguished *Platynereis megalops* and *Nereis dumerilii* as distinct species. As a break from the more tedious fertilization studies that had to be carried out under a microscope, Just performed anatomical studies of the wormlike chordate *Amphioxus,* also known as a lancelet.

Over the next decade, Just visited several other European scientific facilities. In 1930 he was the first American invited as a guest professor to do research in Berlin at the highly regarded Kaiser Wilhelm Institute for Biology, where he studied the function of the ectoplasm in the freshwater protozoan *Amoeba proteus*. He worked at the Laboratoire d'Anatomie et d'Histologie Comparées at the University of Paris and the Station Biologique de Roscoff at the Sorbonne.

Just was welcomed by the scientific community and drawn to the culture of Europe. Though he returned to Woods Hole to celebrate Lillie's 60th birthday, he never returned there to work. From time to time he returned to Howard University to teach or to perform compulsory administrative tasks, but he was always anxious to return overseas. After coming to a minimally satisfactory agreement with Howard's administration concerning his salary support, which they wanted to discontinue in 1938, Just exiled himself to Paris.

Just composed a textbook, *The Biology of the Cell Surface* (1939), summarizing the results of two decades of his research. One of Just's major contributions was dispelling the assumption that all cellular activities were controlled by the nucleus of the cell. He demonstrated that the cytoplasm, the material outside of the nucleus but still contained within the cell, also performed important cellular functions. He asserted that the ectoplasm, the outer, rigid layer of protoplasm, the essential semi-fluid living substance of cells, played an important role in fertilization and embryonic development. Just found that polyspermy, the condition of penetration by more than one sperm, resulted from an improperly functioning ectoplasm. In addition to his research on fertilization, egg activation, and cell division, Just also made contributions including studies examining the effect of ultraviolet radiation on the number of chromosomes in eggs and the hydration and dehydration of cells.

In 1940 he published *Basic Methods for Experiments on Eggs of Marine Animals,* a handbook of laboratory techniques that outlined methods for handling egg and sperm cells from 28 marine species. He also stressed the need for using clean glassware and shared his knowledge about temperature and handling essential to maintaining the healthy condition of specimens in the lab.

Just divorced his wife in 1939 and married Hedwig Schnetzler, a graduate student in philosophy whom he met in Berlin and with whom he had been having an affair for eight years. In 1940 the Nazis, who had taken over France, forced Just from his laboratory, and he and his part-Jewish wife struggled to obtain the necessary papers for passage to America. He brought his then pregnant wife to Washington, D.C., where he resumed teaching at Howard and began writing a manuscript, "Ethics and the Struggle for Existence." Their daughter was born in 1940. Just's health began to fail, and in the summer of 1941, Just was diagnosed with pancreatic cancer. He died on October 27, 1941, at the age of 58, in Washington, D.C., and was buried in the Lincoln Cemetery.

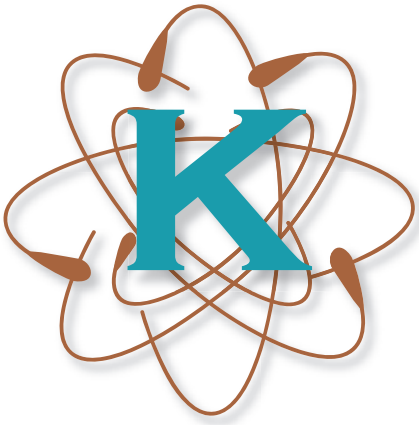
Just was a quiet, bookish, dignified man, who published over 60 scientific articles in addition to his two textbooks in his short lifetime. His contributions are still remembered and have been honored in recent years. In 1983 the 26th Southeastern Conference of Developmental Biology dedicated to Just a symposium on cellular and molecular biology of invertebrate development at the Bell W. Branch Institute for the Marine Biology and Coastal Research in South Carolina. In 1996 the U.S. Post Office issued a stamp in his honor. Recognized as a world authority on marine organisms, Just made significant advances to the field of invertebrate zoology, especially concerning fertilization and early embryonic development. The findings of this research carry over to human studies, as marine organisms are excellent simple models, and have contributed to the development of techniques such as in vitro fertilization. Whether intended or not, by choosing to pursue the rational, objective field of science in the face of discrimination, Just also made progress in the great effort to reach equality among races in America.

See also EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; INVERTEBRATES; MARINE BIOLOGY.

FURTHER READING

Brown, Mitchell C. "Ernest Everett Just." In *The Faces of Science: African Americans in the Sciences*. University of California at Irvine. Available online. URL: http://webfiles.uci.edu/mcbrown/display/just.html. Updated November 25, 2007.

Manning, Kenneth R. *Black Apollo of Science: The Life of Ernest Everett Just*. New York: Oxford University Press, 1983.



Koch, Robert (1843–1910) German *Physician, Microbiologist* Robert Koch was a German physician who made numerous contributions to bacteriology and the study of infectious diseases at the end of the 19th century. His pioneering work led to the establishment of methods for growing pure cultures of bacteria in the laboratory, the development of staining and photomicroscopy that facilitated the observation and identification of bacteria, the definition of a set of criteria for demonstrating that a particular organism causes a specific disease, and the identification of the organisms responsible for anthrax, tuberculosis, and cholera.

TRAINS TO BECOME PHYSICIAN

Robert Heinrich Hermann Koch was born on December 11, 1843, in Clausthal, Oberharz, Germany. He was the third son in a family with 13 children. He taught himself to read and write before he started primary school at the age of five and attended the public schools, where he developed an interest in biology. In 1862 he entered the University of Göttingen to study natural sciences and switched to medicine after two years. The 1860s were an exciting time to study life sciences, because the French chemist Louis Pasteur, with whom Koch would share many similar research interests, had recently asserted that yeasts were responsible for the process of fermentation and debunked the theory of spontaneous generation. One professor in particular, an anatomist named Jacob Henle, who had published an essay in 1840 asserting that living organisms caused infectious diseases, stimulated Koch's interest in research.

After graduating with highest distinction in 1866, he went to Berlin to study pathology under the tutelage of Rudolf Virchow, the German pathologist who declared that cells only arise from other cells, a major tenet of the cell theory. The following year Koch became an assistant at the General Hospital in Hamburg. After holding several positions, he succeeded in setting up a private practice in the small town of Rakwitz. When the Franco-Prussian War broke out in 1870, he volunteered his services as a field hospital physician for the German army. He returned briefly to Rakwitz in 1871, but the next year he took and passed his district medical officer examination and then served as the district medical officer for Wollstein (now Wolsztyn, Poland) from 1872 to 1880.

RESEARCH ON ANTHRAX

While at Wollstein, Koch became interested in anthrax, a deadly disease of cattle and sheep. He was busy working as a general practitioner and did not have a suitable lab or even access to scientific publications, but he performed research on anthrax in a makeshift laboratory set up in his home, which also served as the site of his medical practice. With the goal of proving that the bacteria *Bacillus anthracis* caused anthrax, he inoculated mice using slivers of wood containing bacteria he obtained from the spleens of farm animals that died of anthrax. The mice developed anthrax. When he inoculated mice with blood from the spleens of healthy farm animals, they did not get sick. Koch wondered if the fact that the bacteria had been isolated from animals affected the ability to cause disease, so he grew *Bacillus* for several generations by culturing it outside of an animal host. When inoculated into mice, the bacteria still caused anthrax. In 1876 Koch, a relative unknown, published the results of his anthrax studies, including a description of the

bacteria's life cycle and a description of their ability to form spores in certain conditions, such as in the presence of oxygen. An endospore is a resistant, dormant form of a bacterial cell that can persist in the soil for hundreds of years or more and that germinates when it comes into contact with the right environment, such as in the presence of amino acids and glucose (as in the blood or tissue of an animal). At the same time, the renowned Pasteur was also researching anthrax; he had induced the disease in animals with the bacillus and performed an overwhelmingly successful demonstration of a vaccine made from attenuated strains. Both men publicly attacked the other's methods and claims. Though each had contributed significantly to the understanding and control of anthrax, their pride and jealousy prevented them from acknowledging the other's contributions. The critical dispute between the two persisted for five years.

KOCH'S POSTULATES

Knowledge of Koch's work spread rapidly, and Koch continued to improve methods for growing bacterial cultures and staining and photographing specimens. In 1880 he accepted a position as a government adviser to the Imperial Department of Health in Berlin and eventually secured a laboratory better equipped for his bacteriological research. Two of his main goals were to establish methods for isolating and growing disease-causing microorganisms and to develop guidelines to prevent diseases from spreading. He outlined a series of four essential steps for demonstrating that a particular microorganism causes a specific disease. Today these are referred to as Koch's postulates, and they appear in every microbiology textbook and in most general biology textbooks as well.

The first postulate states that the specific organism should be shown to be present in all cases of animals suffering from a specific disease but should not be found in healthy animals. The second postulate affirms that the specific microorganism should be isolated from the diseased host and grown in pure culture in the laboratory. The third criterion requires that when organisms from the pure culture are inoculated into a healthy laboratory animal, it duplicates the disease seen in the original animal. Finally, the same microorganism must be recovered from the artificially infected host. These measures are not faultless since some agents of disease are difficult to grow in an artificial environment in the laboratory. In addition, in the case of diseases specific to humans, one cannot experimentally infect a host to qualify the third postulate. Nevertheless, Koch's postulates remain a valuable set of criteria for determining whether a particular agent is the cause of a disease.

WORK ON TUBERCULOSIS AND CHOLERA

While in Berlin Koch also devoted time to the study of tuberculosis, a lung disease that he believed was caused by a microorganism though many physicians thought it was a genetic disorder. At the time, tuberculosis killed 7 million people annually. Though many attempted to identify and isolate a responsible organism, technical difficulties in staining and cultivating the agent hindered success. Koch's patience led to his discovery that a special alkaline dye allowed the observation of a bacillus in lung tissue and that the microbe had strict nutritional growth requirements. He added coagulated blood to the growth medium. Using these methods and the postulates that he conceived, Koch demonstrated that the organism now known as *Mycobacterium tuberculosis* caused tuberculosis. He presented his results in 1882 to a spellbound audience of Berlin Physiological Society members, whom he further impressed by bringing his lab equipment and specimens to the lecture, so the audience could see for themselves the cultures, the slides, and even tissue samples from infected individuals. Three weeks later the paper, titled "The Etiology of Tuberculosis," appeared in *Berliner Klinische Wochenschrift*. The publication quickly became a classic, and the presentation is considered a landmark

Robert Koch won the Nobel Prize in physiology or medicine in 1905 for his investigations on tuberculosis. *(National Library of Medicine)*

event in medical history that led to the conception of 20th-century medicine.

The year after he published his sensational work on the tubercule bacillus, Koch traveled as head of a team of the German Cholera Commission to investigate a cholera outbreak in Egypt and later in India. In 1884 he isolated a comma-shaped bacterial species (*Vibrio cholerae*) from the cadavers of patients who died from cholera, but he was unable to produce the disease in monkeys, dogs, chicken, or mice by feeding or injecting the bacteria into the healthy animals. In a village in India he identified several ponds used as a public water source as reservoirs for the local outbreak. He used his expertise in bacteriology to formulate a set of recommendations for improved hygiene and sanitation that led to the decline of the cholera epidemics, and in return received a large monetary award in appreciation and the Berlin Medical Society held a banquet in his honor.

Despite Koch's achievements, not everyone believed cholera was caused by the bacillus. In 1892 when Hamburg experienced a cholera outbreak, Koch made recommendations to the government concerning the isolation of infected individuals, disinfection measures, and sanitation of water supplies. Max von Pettenkofer, a senior hygienist in Germany, thought Koch's advice was ridiculous and made a public display expressing his certainty that the bacillus was not the cause of cholera by drinking a live culture. Because he suffered only diarrhea, Pettenkofer claimed he was correct in that the bacillus alone was not responsible for cholera. Time and continued investigations proved otherwise.

In 1885 the Friedrich Wilhelms University in Berlin named Koch director and professor of hygiene. Koch helped plan and open, in 1891, a new institute, the Royal Prussian Institute for Infectious Disease, which today bears the name of its founder. Koch continued researching tuberculosis, in particular, a means for preventing or curing the disease. In 1890 he claimed to have developed a substance called tuberculin that supposedly exhibited both preventative and curative effects for tuberculosis. Unfortunately, despite much public hype and optimistic early trials of the substance, it proved to be too toxic and ineffective. This embarrassing incident had one beneficial outcome—tuberculin proved to be useful for diagnosing a tuberculosis infection. Infected individuals developed an inflammatory response following injection with tuberculin, whereas noninfected individuals did not. Koch's failed tuberculin vaccine forms the basis for today's tuberculosis skin tests. The diagnostic value of the substance restored his professional reputation to some degree.

LATER CAREER AND HONORS

During the last decade of the 19th century, Koch participated in many international collaborations to assist with outbreaks of infectious diseases and help other countries to develop control measures to prevent the spread of communicable diseases. In South Africa he studied rinderpest, an infectious, often fatal disease of ruminants, such as cattle, that causes fever and diarrhea. A virus causes rinderpest, a fact unknown at the time. Koch was unable to isolate and identify the causative agent, but he did succeed in limiting the outbreak by devising a means to immunize cattle by injecting them with a mixture of serum from recovered animals and blood from infected animals. He investigated many different diseases, including malaria, Texas fever, coast fever, horse sickness, sleeping sickness, rabies, and the plague. He also studied typhus and determined that human-to-human contact was a more common mode of transmission than drinking contaminated water, a finding that led to more appropriate and effective control measures.

Many universities and governments honored Koch with honorary doctorate degrees and honorary citizenships, and academic organizations sought his membership. One of the most prestigious awards he received was the Nobel Prize for physiology or medicine in 1905 for his achievements on tuberculosis. In 1906 he received the Prussian order Pour le Mérite for his work on tropical diseases. The German government, in collaboration with the American philanthropist Andrew Carnegie, established the Robert Koch Foundation, which promotes basic research in infectious diseases. The foundation recognizes scientists who have made outstanding contributions to the field of infectious diseases by presenting a Robert Koch Gold Medal annually.

In 1867 Koch married Emmy Adolfine Josephine Fraatz, with whom he had one daughter, Gertrud, born in 1868. He divorced his first wife and married Hedwig Freiberg in 1893. Robert Koch suffered a heart attack in April 1910 and died on May 27, 1910, in Baden-Baden. In his honor, on the 30th anniversary of Koch's discovery of the tubercle bacillus, the Institute for Infectious Diseases was officially renamed the Robert Koch Institute.

See also GERM THEORY OF DISEASE; INFECTIOUS DISEASES; LEEUWENHOEK, ANTONI VAN; MICROBIOLOGY; PASTEUR, LOUIS; VACCINES.

FURTHER READING

Brock, Thomas D. *Milestones in Microbiology: 1546–1940*. Washington, D.C.: ASM Press, 1999.

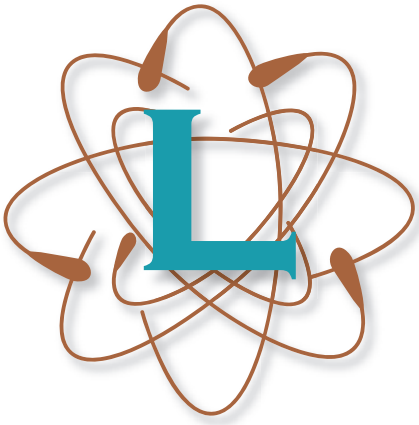
———. *Robert Koch: A Life in Medicine and Bacteriology*. Madison, Wisc.: Science Tech Publishers, 1988.

Daniel, Thomas M. *Pioneers in Medicine and Their Impact on Tuberculosis*. Rochester, N.Y.: University of Rochester Press, 2000.

De Kruif, Paul, with introduction written by F. Gonzalez-Crussi. *Microbe Hunters*. New York: Harcourt Brace, 1996.

Dolman, Claude E. "Koch, Heinrich Hermann Robert." In *Dictionary of Scientific Biography*. Vol. 7, edited by Charles Coulston Gillispie, 420–435. New York: Scribner, 1970–1976.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1905." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1905/index.html. Accessed January 14, 2008.



Lamarck, Jean-Baptiste (1744–1829) French *Naturalist* Jean-Baptiste Lamarck was an early proponent of evolution and the mutability of species. Although today his name is mostly associated with the debunked theory of the inheritance of acquired characteristics, Lamarck's support of the notion that species gradually changed over time significantly influenced the development of modern evolutionary theory.

Jean-Baptiste Pierre-Antoine de Monet de Lamarck was born on August 1, 1744, in Bazentin-le-Petit in northern France. He was the youngest of 11 children born to Marie-Françoise de Fontaines de Chuignolles and Philippe Jacques de Monet de La Marck, who was a military officer. At age 11, Jean entered the Jesuit school at Amiens with plans to become a priest. Shortly after his father died in 1759, however, Jean joined the military and fought in the Seven Years' War. He distinguished himself in his first battle in 1761, and was promoted to officer. Afterward he remained in the army and moved from fort to fort along the French border. Lamarck took advantage of these travels to familiarize himself with different types of plants.

Lamarck finally left the French army in 1768 due to injury and eventually found employment at a bank. He attended medical school and developed interests in meteorology, chemistry, and shell collecting.

BOTANICAL RESEARCH

Lamarck established his reputation within the French scientific community when he published *Flore françoise* (French flora) in 1779 (though the title page says 1778), through the patronage of the influential French naturalist Georges-Louis Leclerc, comte de Buffon, who also supported Lamarck's election to the Académie Royale des Sciences as an adjunct botanist, a position that came with a state pension. Lamarck briefly tutored Buffon's son during travels throughout Europe in 1781, giving him the opportunity to examine many new plants and minerals. A "Preliminary Discourse" introducing the three-volume *Flore françoise* summarized the fundamentals of botany and explained his methods of analysis. The major innovation of this work was the utilization of a dichotomous key for the identification of plants, beginning with the most general forms, then dividing and subdividing, always by two, based on two exclusive characteristics. Since the 1750s, botanists relied on the classification system, proposed by Swedish botanist Carl Linnaeus, based on reproductive structures. Lamarck's system was much easier to use, and with each step eliminated a large group of plants based on the presence of a certain characteristic. By following the key and noting whether certain characteristics were present or absent at each step, one could quickly and reliably identify any known plant found in France. Lamarck included the plant names given by Linnaeus and by the 17th-century French botanist Joseph Pitton de Tournefort, who was the first to distinguish between a genus and species of plant. *Flore françoise* also contained good descriptions of all known French flora. All the copies of the first printing sold within a year, and Lamarck published what was called a second edition in 1795, but it did not differ from the first edition. In 1805, because Lamarck was too busy, A. P. de Candolle (who later established his own reputation by developing a natural system of classification for plants) revised and published an updated third edition, which was reprinted in a fourth volume in 1815.

In the preliminary discourse to *Flore françoise,* Lamarck shared his view that all of nature, both living and nonliving components, should be considered together, and he observed that nonliving aspects of the environment affected living components. For example, seeds taken from one plant but grown in different environments would display different characteristics. He also made note of the process of artificial selection, in which plant growers selected plants with desirable traits for breeding purposes, and the way in which, over several generations, the offspring changed. When the first edition of *Flore françoise* appeared, Lamarck still believed that species were fixed and did not change. He suggested that these changes brought about by the environment led to the creation of new varieties. One could follow the progression of a variety by starting with the most primitive form, and then following it as it became more complex over time.

Another of Lamarck's major botanical works was *Dictionnaire de botanique* (Botanical dictionary, 1783–95), which comprised three and one-half volumes of the eight-volume work *Encyclopédie méthodique*. The accompanying *Illustration des genres* (1791–1800) contained 900 sketches and descriptions of genera classified by Linnaeus's system. The preliminary discourse to the *Dictionnaire de botanique* expounded on ideas Lamarck put forth in *Flore françoise*. He wrote about the notion of progress, about his search for a natural method of plant classification, and about his agreement with other botanists at the time, namely, that classification should be based on larger grouping of plants, such as classes rather than genera. An article followed in which he laid out the classes of plants descending from the most to the least complex. Because he viewed nature as a whole, he did the same for animals. In 1803 he published his last strictly botanical work, the two-volume *Introduction à la botanique,* which became part of the 15-volume *Histoire naturelle des végétaux,* written mostly by the French botanist Charles-François Brisseau de Mirbel. *Introduction à la botanique* included part of Lamarck's evolutionary theory.

INVERTEBRATE ZOOLOGY RESEARCH

Impressed by *Flore françoise,* Buffon supported Lamarck's appointment as an assistant botanist at the Jardin du Roi, the royal botanical garden, which was also a center for medical education and biological research. When the French government was overturned in 1793, the garden was reorganized into the Musée National d'Histoire Naturelle, which employed 12 professors of different specialties. Lamarck was made a professor of "insects and worms," in other words, invertebrates (a term that Lamarck invented), not considered a prestigious field at the time. His main responsibilities were to classify the collections of invertebrates at the museum and to give lectures on the subject, but his major accomplishment in this role was turning what was considered a subject unworthy of study into a diverse new field of biology. He felt that invertebrates were important to the study of zoology because they were more numerous and exhibited more diversity than vertebrates.

Lamarck had developed an interest in zoology through shell collecting, a hobby that by 1799–1800 led him to change his belief that species were fixed and immutable. One of Lamarck's friends, Jean-Guillaume Bruguière, was an expert on mollusks. When he died in 1798, Lamarck finished his *Histoire des vers* (History of vertebrates) for the *Encyclopédie méthodique*. The classification of mollusks unavoidably raised the question of fossil organisms. He saw that fossil mollusks differed from the living forms. Other scientists had also discovered fossils of mammals and reptiles for which no living forms were known, and they looked to Lamarck in hopes that he could resolve the issue through his studies of fossil shells. If he concluded that no living forms existed that resembled fossil forms, then that meant extinction was a real phenomenon, a view supported by the French comparative anatomist and paleontologist Georges Cuvier. Lamarck was hesitant to make sweeping statements about extinction because new species were constantly being discovered and because it was viewed as an opposing theory to transformism, the idea that species changed over time. Many believed that new species were created following a round of extinction, and that explained why current species did not resemble past species. Other explanations for not finding living life-forms similar to the fossil forms included migration and transformism. Lamarck became convinced that species changed, and the theory of transformism became an integral part of his perception of the natural world, a view apparent in his works dating from 1800 and later. Lamarck published an important work on Parisian fossils titled *Mémoires sur les fossils des environs de Paris* (1802–06). Some consider Lamarck to be the founder of invertebrate paleontology.

Lamarck's work on invertebrates led to the book *Système des animaux san vertèbras* (System of invertebrate animals, 1801). In 1800 he gave a lecture at the museum introducing the main concepts in this work. This lecture became famous because it was also his first formal presentation of his ideas on transformism. As he proposed with plants, he presented classes of animals as a progression in reduction of complexity with respect to specialized anatomical structures and physiological systems. He began by dividing

all animals into two major groupings, the vertebrates and the less complex invertebrates. In order of decreasing complexity, he arranged vertebrates into four groups: mammals, birds, reptiles, and fishes. He divided invertebrates into more classes than anyone had previously done. Again, he presented the classes in order from most to least complex: mollusks, crustaceans, arachnids, insects, worms, radiates, and polyps. By following his descent from the most advanced organisms to the simplest, Lamarck defined what he believed to be the minimal characteristics of life.

By the time he published *Philosophie zoologique* (Zoological philosophy) in 1809, he had added more divisions to the invertebrates and presented his graded series of classes from the most rudimentary to the most elaborate, as nature had constructed them. *Philosophie zoologique,* Lamarck's most widely read work, contained a complete synthesis of his animal classification system and transformism. In order of gradually increasing complexity this time, he described 10 classes of invertebrates: infusorians, polyps, radiates, worms, insects, arachnids, crustaceans, annelids, cirrhipedes, and mollusks. Between 1815 and 1822 he published a more developed account of his studies on invertebrates in the significant seven-volume work *Histoire naturelle des animaux sans vertèbras* (Natural history of invertebrates). This work was universally adopted among naturalists.

EVOLUTIONARY THEORY

All of Lamarck's studies and experiences contributed to the development of his theory of evolution. He never used the term evolution, but spoke rather of a progression or natural path leading to the creation of all living organisms. These ideas first appeared in the form of an introductory lecture he gave on invertebrates at the museum in 1800, and then in print in his *Système des animaux sans vertèbres*. According to Lamarck, nature consisted of living and nonliving components, and substances cycled between the two. Two bridges connected the living and nonliving realms: spontaneous generation and death. Lamarck proposed that spontaneous generation of the lowest life-forms (protists) occurred by means of the element of fire (through heat, sunlight, or electricity) acting on inorganic matter, stirring it up into simple life-forms. After death, living organisms, comprising plants and animals, decomposed into inorganic substances that formed nonliving components of the Earth.

In 1802 Lamarck expounded and clarified his ideas about evolution in *Recherches sur l'organisation des corps vivans* (Researches on the organization of living bodies). He organized animals into groups beginning with mammals and following what he called degradations down to the much simpler polyps. He could not think of a mechanism to propel change, however, and instead proposed a rather vague natural tendency toward increasing complexity. This may be the critical factor explaining why Lamarck's ideas on evolution were not embraced as were his other contributions to natural history, such as his work on plant and animal classification and his invertebrate research. Others viewed his ideas on evolution as unfounded speculation.

As mentioned previously, Lamarck propounded the theory of transformism, believing that nature had formed the simplest plants and animals directly, but that time and changes in the environment led to the production of all other organisms. He asserted that changes to organisms occurred slowly, such that one could perceive change only over long periods of time. Lamarck viewed evolution as a natural progression from simple to complex. Living organisms had constant and alterable "faculties." The constant faculties included processes such as digestion, respiration, and reproduction and were permanent due to their importance in sustaining continued existence. Alterable faculties consisted of less functionally important organs and structures such as those for locomotion and communication. The organism's surroundings affected change to these faculties. The movement and participation of internal fluids helped induce the formation of more complex structures, such as vessels and hollow organs that eventually developed into physiological systems. Thus, habits led to acquired change. For example, water birds developed webbed feet, shore birds developed long legs, and giraffes that fed off high tree tops developed long necks. As organisms grew more complex, they depended less on the environment. Thus, plants were simpler than animals, since they cannot move in order to find food or to reproduce like animals can. He suggested that different environmental conditions required changes in the needs of an animal, which caused changes in their behavior or habits that led to a strengthening or weakening of preexisting body parts or organs. The acquired modifications were then passed on to offspring.

Man came about in the same manner as other animals, but the complex nervous system and mental abilities of humans presented a challenge. Lamarck explored this subject further in *Philosophie zoologique,* which included a more detailed discussion of his complete synthesis of the natural sciences and his formulation of transformism. This treatise merged the subjects of natural history, classification, physiology, and even psychology. One concept Lamarck presented was that of *sentiment intérieur,* a type of internal feeling brought about by agitation of nervous fluid, a component of the highly complex nervous system. Physical needs stimulated movement

necessary to fulfill that need, and repeated similar actions would cause the nervous fluids to be agitated in the same way, causing new organs or adaptations to form over time. His other major work concerning evolution was the introduction written for his *Histoire naturelle des animaux sans vertèbres*. This work contained a summary of his evolutionary theory based on four laws.

- The first law was his notion that organic matter had a natural tendency toward increasing complexity. This law explained why evolution progressed to produce classes of plants and animals that were more complex over time.
- The second law explained how the environment influenced the development of new organs in an animal. Persistent needs led to new movements that resulted in the production of new organs.
- The third law, summarized as the use-disuse principle, described how repeated actions or lack of actions led to modifications in an animal's body over time.
- The fourth law affirmed that characteristics acquired by animals passed on to the offspring. This inheritance was necessary to explain the gradual accumulation of characteristics over time.

While Lamarck was correct in proposing that the environment played a role in slowly shaping the evolution of organisms, his idea that the organism's interactions with the environment produced heritable change never became mainstream. His ideas are often summarized as the inheritance of acquired characteristics, and Lamarckianism is often mentioned in discussions of the development of evolutionary theory associated with his discredited theory of evolution. It should be noted that Lamarck was not the first to propose the notion of the inheritance of acquired characteristics and that this concept was commonly accepted at the time. The mechanisms of heredity were still a mystery.

Today, widely accepted evolutionary theory resembles the ideas presented in 1859 by the British naturalist Charles Darwin. According to Darwin, environmental conditions simply select for the maintenance of modifications that occur randomly but that contribute to an animal's success in passing on its genes.

CHEMISTRY, METEOROLOGY, AND GEOLOGY

Lamarck's interest in sciences other than biology stemmed from his belief in the unity of nature. He viewed life as a physical and chemical phenomenon and that interactions with the environment affected living organisms. Lamarck first became interested in chemistry when he studied medicine in the 1770s. At the time, most scientists still thought that all matter consisted of four elements: earth, air, water, and fire. During Lamarck's lifetime, most scientists' beliefs evolved with the onset of the chemical revolution, which was spurred by the French chemist Antoine Lavoisier, leading to an understanding that air was not an element but rather was composed of numerous other elements. Lamarck continued to believe in the four elements, thus his work in this field was not well respected, although it did help him develop his ideas regarding organic evolution. For example, he believed only living organisms could produce chemical compounds, and, after life ended, the substances degraded and decomposed to their natural inorganic state.

In general, Lamarck's meteorological work met with as poor a reception as did his chemical ideas. Again, he was concerned with the relationships between all of nature, and thus was interested in the effect of climate on living organisms. One of his main goals was to elucidate the laws of nature that controlled climate. He suggested that the Moon was the ultimate force explaining all climate change.

Lamarck's geological inquiries resembled his other research in that he sought general principles to explain many observations and, as in other research fields, he considered the effects of biological phenomena on geological change. He was not concerned with minor details but wanted to understand the big picture and so he related his other research to his geological studies. For example, he tried to explain how geological change led to fossil shells being found on dry land when they must have been laid down under water by suggesting the Moon slowly pulled the water bodies around the surface of the globe. He thought fluids were fundamental forces of nature, living and nonliving, and he described the formation of mountains as occurring through the deposition and degradation of organic remains that water acted upon to mold into geological structures. His emphasis on fluids such as water is exemplified by the title of his book *Hydrogéologie* (*Hydrogeology,* 1802).

PERSONAL AND CAREER

In 1783 the Académie des Sciences promoted Lamarck to associate botanist, and then to pensioner in 1790. From 1795 until his death he was a resident member of the botanical section of the reorganized Institut National des Sciences et des Arts.

In 1792 Lamarck married Marie Rosalie Delaporte, with whom he already had six children. She was dying when they were married, and the following year he married Charlotte Victoire Reverdy, with

whom he had two children. She died in 1797, and he married Julie Mallet in 1798. They had no children together, and she died in 1819. Lamarck may have married a fourth time, but no documentation supporting this has been found.

Lamarck's health began to fail in 1809. After he became blind in 1818 one of his daughters transcribed his dictation, as he continued to write. Later in his life Lamarck explored the basis of human knowledge and metaphysics. In 1820 he published *Système analytique des connaissances positives de l'homme* (An analytical system of man's positive knowledge). By the time Lamarck died in 1829, the family was penniless and had to ask the Académie des Sciences to help pay for his funeral. They sold all of his belongings, including his scientific collections and books. His body was buried in a rented grave and removed after five years. The current whereabouts are unknown.

The goal of understanding nature through the discovery of constant natural laws guided all of Lamarck's scientific explorations. Although his proposed theory for the progression of plants and animals was flawed, he was a key figure in the development of modern evolutionary theory, and many prominent 19th-century biologists staunchly supported his views. Even Darwin credits Lamarck with being the first to draw attention to the subject and to suggest that organic evolution resulted from natural phenomena rather than from miraculous intervention by a divine being. Lamarck's other contributions to life science, such as the introduction of dichotomous keys in plant identification and his taxonomic achievements, are also meaningful and noteworthy.

See also Buffon, Georges-Louis Leclerc, comte de; Cuvier, Georges, Baron; Darwin, Charles; evolution, theory of.

FURTHER READING

Cuvier, Georges. "Éloge de M. de Lamarck." In *Mémoires de l'Académie Royale des Sciences de l'Institut de France*, 2nd ser., 13 (1831), i–xxxi.

Jordanova, L. J. *Lamarck*. New York: Oxford University Press, 1984.

Lamarck, Jean-Baptiste. *Zoological Philosophy: An Exposition with Regard to the Natural History of Animals*. Translated by Hugh Elliot. With introductory essays by David L. Hull and Richard W. Burkhardt, Jr. Chicago: University of Chicago Press, 1984.

Leeuwenhoek, Antoni van (1632–1723)

Dutch *Microbiologist* Antoni van Leeuwenhoek was the first to observe microscopic life. His observations of microorganisms using hand-crafted microscopes revealed a whole new world unbeknownst to man, though microbes have existed on Earth for more than 3 billion years. Though by profession he was a cloth merchant, scientific history records him as a founding father of microbiology.

A DELFT DRAPER

Antoni van Leeuwenhoek was born on October 24, 1632, in Delft, Holland. When he was about eight, Antoni was sent to grammar school 20 miles (32 km) from home at Warmond. Afterward he lived temporarily with an uncle who was both an attorney and a town clerk at Benthuizen, located about nine miles (14.5 km) from Delft. At the age of 16, Antoni was sent to Amsterdam to serve as an apprentice to a linen draper. He was a diligent, responsible worker and served as cashier and bookkeeper as well. In 1654 Antoni returned to Delft, where he spent the rest of his life.

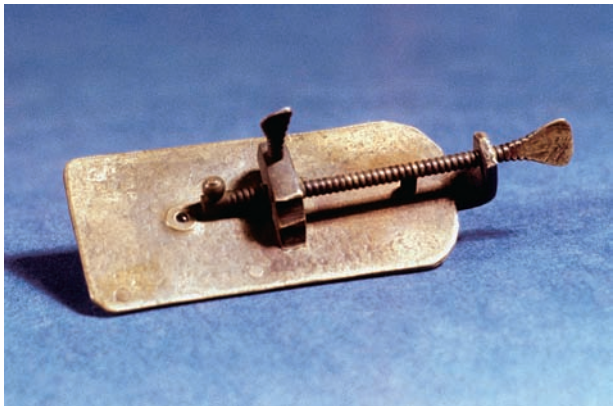
Leeuwenhoek wed Barbara de Mey shortly after his return to Delft. He was 21, and she was 24 years old. He bought a house and opened up his own draper business. The Leeuwenhoeks eventually had three sons and two daughters, all of whom died in infancy except Maria, who was born in 1656. Barbara died in 1666, leaving Leeuwenhoek alone after 12 years of marriage. In 1671 he married Cornelia Swalmius, a relative of Barbara's.

Leeuwenhoek's business was successful, and he spent most of his days inspecting fabrics and selling cloth, buttons, and ribbon. He was also employed as chamberlain to the sheriffs of Delft from 1660 to 1699. In addition, he was appointed surveyor to the court of Holland from 1669 until his death, and he was a wine gauger from 1679 onward. He gained a respectable reputation among the inhabitants of his quaint Dutch town, and he secured enough money to support his scientific hobbies.

HOBBY OF MICROSCOPY

While it is not known exactly when Leeuwenhoek became preoccupied with microscopy of biological specimens, it is certain that it was before 1673, when the first preserved correspondence concerning his microscopic examinations appeared. In the 17th century, grinding lenses for magnifying glasses was a skill common to drapers. After all, cloth merchants had to inspect the weave of their linens carefully. Leeuwenhoek developed an especially remarkable talent in this area and reportedly constructed about 550 lenses during his lifetime.

After carefully grinding a lens with a gritty material, he polished it with a fine-grain putty, and mounted it between two metal plates that had openings specially designed to hold the tiny lens, which was less than one-eighth of an inch (3 mm) wide. A series of pins with screws at one end were also attached



Leeuwenhoek's microscopes, such as the one shown here, contained a single lens that he ground himself and could obtain magnifications of up to 300×. *(HIP/Art Resource, NY)*

to the brass plates in order to move and hold the object in position in front of the lens. Leeuwenhoek usually left specimens fixed to his microscopes so he could continue to view them whenever he pleased. Compound microscopes—microscopes using more than one lens—were also in use at the time. Though the potential magnification with compound microscopes was larger than with simple microscopes, the images were often blurred or the colors distorted. Leeuwenhoek started examining objects other than cloth products with his lenses, inspecting anything he suspected might be interesting when magnified. Most of his specimens were biological, such as insect wings and eyes, pollen grains, and mold.

BEGINS CORRESPONDING WITH THE ROYAL SOCIETY

A friend from Delft, Dutch physician Regnier de Graaf, had seen Leeuwenhoek's work. He thought Leeuwenhoek's microscopes were finer than the best ones used by academicians at the time, and he asked Leeuwenhoek to record some of his investigations. After composing his own plea for the Royal Society to consider Leeuwenhoek's recorded observations, de Graaf mailed Leeuwenhoek's notes to England's most prestigious academic organization. Leeuwenhoek's notes consisted of descriptions of fungal spores, a common louse, and the stinger, mouth, and eye of a bee. This correspondence was published in the *Philosophical Transactions of the Royal Society* in 1673.

Like de Graaf, the fellows of the Royal Society were impressed and wanted to hear more from the Dutch draper. Leeuwenhoek humbly responded with more detailed notes and observations as well as illustrations prepared by a draftsman based on Leeuwenhoek's sketches. In this second letter, Leeuwenhoek also admitted to being uneducated in any language but his native tongue, Dutch. This was very unusual for a scientist at the time since most scientific correspondence was in Latin, English, or French. Furthermore, Leeuwenhoek was not familiar with scientific writing, which is usually clear and concise. His letters were long, rambling, and often included bits of personal information. Some members possibly were repulsed by this crude, uneducated man. It is just as likely that some were amused by his straightforward, unashamed manner. Most were impressed, however, as Leeuwenhoek's correspondence with the Royal Society of London continued for 50 years until his death.

DISCOVERS MICROSCOPIC LIFE

Leeuwenhoek's most famous communication transpired in the year 1676. He claimed that he saw tiny living "animalcules" swimming about in rain water, and he estimated that 1,000 of these tiny creatures could fit on the head of a pin. Leeuwenhoek explained how in 1674 he had visited a lake a few hours from Delft and taken samples of the water that he described as murky and covered with green clouds. He was amazed to see many types of tiny creatures rapidly moving in the liquid. After this discovery he started looking for these animalcules in other locations. He examined samples of snow, rain, seawater, and well water and found them everywhere. He finally wrote a long letter giving very vivid descriptions of the little animals, which we now know were mostly protozoa, namely, microscopic, unicellular, eukaryotic organisms. They appeared in a variety of shapes including round, oval, and even spiral. Some had what he described as fins or legs or little hairs. The "wretched beasties" swam around quickly using different types of motion.

Microscopes had been around for approximately 50 years when Leeuwenhoek began his studies. He was by no means the inventor of the microscope, yet he was able to obtain clear images of magnifications more than 250 times the original size. Not only was he able to achieve great magnifications, but they were viewed with good resolution. Resolution, or resolving power, is the ability to distinguish fine detail. Leeuwenhoek was able to achieve resolutions of approximately 4×10^{-5} inches (1 μm). Some of the people that read his correspondence to the Royal Society thought it impossible that such great magnifications could be reached with only a single lens. In order to magnify things, lenses must be convex in shape, meaning rounded out. Leeuwenhoek's lenses were so convex they were practically spherical. He never taught anyone how he achieved such remarkable results with his lenses nor let anyone watch him make them, so it is understandable that some doubted his claims, especially now that he was proclaiming that the world was filled with wretched

beasties that were subvisible to the naked eye. He was labeled everything from a liar to a magician, but he was merely a skilled observer driven by his natural curiosity to understand the world around him.

In response to the doubters, Leeuwenhoek provided affidavits from eight reputable men, including clergymen, attorneys, and physicians, but when the Royal Society examined specimens similar to the ones in which Leeuwenhoek said he had found the tiny creatures, they saw nothing. Finally, they asked fellow Robert Hooke to repeat Leeuwenhoek's procedures exactly. Hooke was the society's curator of experiments and was considered an expert microscopist. In 1665 he had published a text titled *Micrographia,* which included drawings and descriptions of enlarged, detailed sections of insects, fossils, cloth, and mold. Hooke also was the first to report the existence of cells in cork (from tree bark). Since *Micrographia* was not written in Dutch, Leeuwenhoek could not have read it, but he could have looked at the pictures or been told about the contents by others. This may have been his initial inspiration for examining objects other than fabrics. When Hooke repeated Leewenhoek's experiments and published that he was able to see miniscule swimming creatures in 1678, the academic establishment replaced their doubts with amazement for this newly discovered whole microscopic living world.

Whenever he was curious about something, Leeuwenhoek sought answers through exploration with his microscopes. For example, Leeuwenhoek wondered what gave pepper its characteristic biting flavor; perhaps the pepper grains were covered with bunches of tiny sharp points. He softened pepper by soaking it in water, and then he sucked the samples into tiny cylindrical glass tubes for viewing. He was astonished to find not only the animalcules that he had seen in the past, but hundreds of thousands of creatures even smaller than the previously admired protists. He estimated that 100 of them stretched end to end would not attain the width of a grain of sand. He wrote up these findings to Hooke in 1678. This correspondence began a series of papers considered to be the first in bacteriology. Bacteria are unicellular, prokaryotic (containing no nucleus) organisms that approximate 4×10^{-5} inches (1 μm) in size.

In 1680 Leeuwenhoek was elected a full fellow of the Royal Society. Though he never went to the meetings in London, he wrote over 200 letters to the society. Most of these were published in the *Philosophical Transactions of the Royal Society* over the next several decades. In 1699 he was nominated as a corresponding member of the Académie des Sciences of Paris. He became known all over the world and had frequent visitors. The queen of England and the emperor of Germany were among the numerous foreign dignitaries who visited him. In 1698 the czar of Russia, Peter the Great, requested that Leeuwenhoek visit his ship, which was docked nearby. Leeuwenhoek complied, and he brought specimens to view under his remarkable scopes.

HUMANS HOST MICROBES

Another shocking letter Leeuwenhoek wrote to the Royal Society in 1683 avowed that the tiny animalcules that he was now famous for discovering inhabited the human body. Leeuwenhoek prefaced this bold claim by informing his readers of his dental hygiene routine, which included cleaning his teeth daily by rubbing them with salt. Despite this, he still found a whitish sticky substance coating the surfaces of his teeth. When he examined this substance under the microscope, he found it was teeming with bacteria. When he examined the material from the mouths of people who did not clean their teeth regularly, he found even more creatures, including one new type that resembled a corkscrew. He commented that these creatures might be the cause of bad breath.

This spawned his interest in examining other bodily fluids. He even looked at his own loose bowel movements (1681) and found what was probably *Giardia,* a flagellated protist that attaches to human intestinal walls with a sucker and causes persistent diarrhea. *Giardia* are parasitic, meaning they live off a host, doing harm to the host in the process. However, humans are covered inside and out with many microscopic organisms that cause no harm. These are called natural flora, and humans have in fact come to depend on many of them. For example, *Escherichia coli* inhabit our intestines. They produce vitamin K and some B vitamins that can be absorbed into the bloodstream and used to benefit the human body. The bacteria benefit from humans as well. They are provided with a safe, warm environment loaded with nutrients. Microorganisms also live in the mouths and on the skin of healthy individuals. While Leeuwenhoek found that microorganisms inhabited many parts of the human body, he never suggested they cause harm. Two hundred years later, French scientist Louis Pasteur and German physician Robert Koch proposed that microorganisms cause disease.

Leeuwenhoek also examined semen and was excited to find it loaded with millions of swimming cells, spermatozoa. Sperm, for short, are not bacteria or protozoa, but, rather, are the gametes produced by adult males. Leeuwenhoek's discovery of these in 1677 shed much light on the then mysterious process of reproduction. He observed sperm in the seminal fluid from a variety of other animals, including insects, shellfish, fish, birds, amphibians, and mammals. His studies suggested that sperm cells interact with egg cells from a female to produce offspring.

He believed that the female's egg and uterus provided nourishment and shelter for the newly created organism as it grew. This research helped debunk the theory of spontaneous generation, which proposed that life arose from nonliving matter.

OTHER RESEARCH

Though he is most famous for his discovery of microorganisms, Leeuwenhoek also investigated anatomy, reproduction, and nutrient transport in plants. He described the microscopic structure of several types of anatomical structures, including what he called air vessels, intestinal tubes, chyle vessels, blood vessels, and nerve tubes. When intrigued by a question of structural anatomy or functional physiology, Leeuwenhoek studied several types of organisms, made comparisons, and then drew up generalizations about all living organisms. He studied blood and independently discovered capillaries in 1683. Not being fluent in scientific literature, Leeuwenhoek was unaware of the Italian anatomist Marcello Malpighi's discovery in 1661 that capillaries connected arteries with veins. Leeuwenhoek also studied dozens of types of insects and watched spiders spin silk. He studied life cycles of microorganisms, weevils, lice, eels, and other animals.

When he was 84 years old, the University of Louvain honored Leeuwenhoek with the presentation of a medal (similar to the giving of an honorary degree) and a tributary poem. He wrote in response that the poem brought tears to his eyes. The father of microbiology died on August 26, 1723, from lung disease, probably pneumonia, and he was buried at the Old Church of Delft. On his deathbed, he asked his daughter to deliver the gift of a black cabinet containing 26 finely crafted silver microscopes holding a variety of specimens to the Royal Society. Unfortunately, these microscopes bequeathed to the Royal Society vanished, as have most of the hundreds of others Leeuwenhoek so painstakingly crafted. Two hundred years passed before lenses of magnification and resolution comparable to Leeuwenhoek's were made again.

Today, students learn about Antoni van Leeuwenhoek's discovery of microbial life within the first few chapters of any microbiology textbook and most biology textbooks. Millions have repeated some of his basic examinations, and people continue to be amazed at what they see under the microscope. Since he was never formally educated and had no scientific training, Leeuwenhoek's views were always fresh and unspoiled. Because he understood only Dutch, he was oblivious to the current literature and engaged in few interactions with other scientists outside of his correspondence with the Royal Society. Yet he advanced the field of biology greatly due to his natural desire to learn, his finely honed skills, and his ability to describe so vividly and objectively all that he observed.

See also ALGAE; BACTERIA (EUBACTERIA); EUKARYOTIC CELLS; FUNGI; MICROBIOLOGY; MICROSCOPY; PROKARYOTIC CELLS; PROTOZOA; SPONTANEOUS GENERATION.

FURTHER READING

Adler, Robert E. *Science Firsts: From the Creation of Science to the Science of Creation.* Hoboken, N.J.: John Wiley & Sons, 2002.

De Kruif, Paul. *Microbe Hunters.* San Diego, Calif.: Harcourt Brace Jovanovich, 1954.

Dobell, Clifford. *Antony van Leeuwenhoek and His "Little Animals."* New York: Russell and Russell, 1958.

Ford, Brian J. *Single Lens: The Story of the Simple Microscope.* New York: Harper and Row, 1985.

Spangenburg, Ray, and Diane Kit Moser. *The Birth of Science: Ancient Time to 1699.* New York: Facts On File, 2004.

Waggoner, Ben. "Antony van Leeuwenhoek." Museum of Paleontology, University of California at Berkeley. Available online. URL: http://www.ucmp.berkeley.edu/history/leeuwenhoek.html. Accessed January 15, 2008.

Levi-Montalcini, Rita (1909–) Italian *Cell Biologist* Rita Levi-Montalcini discovered nerve growth factor (NGF), a protein produced by the body that controls the growth of neurons and is required for their survival. Her research opened up the field of molecular developmental biology, and 50 years later, scientists are learning the implications of NGF in cancer treatment, Alzheimer's disease management, the development of birth defects, and repair of spinal cord injury.

SECRET RESEARCH

Rita Levi and her fraternal twin sister Paola were born into an intellectual Jewish family on April 22, 1909, in Turin, Italy. Her father, Adamo Levi, was an electrical engineer and a factory manager, and her mother, Adele Montalcini Levi, was a talented painter. The twin girls grew up with their older sister and brother in a loving but traditional home where the father was the undisputed head of the family. He did not believe girls needed a university education to fulfill their intended roles as wives and mothers, so even though Rita was very bright, he enrolled her at a less academically rigorous girls' finishing school. When her former governess was diagnosed with stomach cancer, 20-year-old Rita resolved to become a physician. After convincing her father to grant permission, she worked hard to compensate

Discrimination against her Jewish ancestry forced Rita Levi-Montalcini to launch her Nobel Prize–winning research career using homemade equipment in a hidden laboratory inside her home. *(Becker Medical Library, Washington University School of Medicine)*

for her limited educational preparation. Within eight months she learned Latin, Greek, and mathematics, graduated from high school, and entered medical school at the University of Turin, where she took classes from the famous histologist Dr. Giuseppe Levi (no relation).

As an adult, Levi added her mother's maiden name to her own in order to distinguish herself from other Levis from Turin, becoming Rita Levi-Montalcini. After she graduated summa cum laude in 1936, she joined Levi's lab as a research assistant and began investigating the nervous system. He taught her a sensitive new technique for staining neurons of chick embryos using chrome silver. In 1938 Levi-Montalcini was forced to resign as a result of a racial manifesto issued by the Italian fascist dictator Benito Mussolini that prohibited Jews from holding academic or other professional positions. She left the country to work at the Neurological Institute in Brussels, Belgium, until December 1939, several months before the German army invaded Belgium.

Back in Turin, Levi-Montalcini set up a clandestine research laboratory in her home using a small binocular microscope, a stereomicroscope for operating on embryos, an incubator made by her brother, and primitive dissecting tools, including watchmaker's forceps, tiny ophthalmologic scissors, and scalpels and spatulas ground from common sewing needles. Pretending to have children, she begged farmers for fertilized eggs (claiming they would be more nutritious) and immersed herself in the development of the nervous system in chick embryos. Her former mentor, Giuseppe Levi, fled to Turin from Belgium and joined her. Levi-Montalcini had read an article by Viktor Hamburger, a German-born neuroembryologist (one who studies the development of the nervous system in unborn animals) who was working in America. Hamburger performed experiments revealing that destruction of limb buds in chick embryos greatly reduced the growth of nerves to the limb buds. He hypothesized that the absence of an inductive factor released by innervated tissues prevented the growth of motor neurons (nerve cells that transmit signals from the brain or spinal cord to muscles) and sensory neurons (nerve cells that receive information and transmit signals to the central nervous system).

In order to determine what influenced the formation and differentiation of the embryonic nerves, Levi-Montalcini repeated the experiments Hamburger described by amputating limb buds from chick embryos and observing the effects on the nerves growing from the spinal cord. After various periods of time, she sliced thin sections of the spinal cord, silver-stained them, and examined them under her microscope. She found that in the absence of a limb, the motor neurons leading to that limb disappeared. Whereas Hamburger believed they were never induced to grow in the first place, Levi-Montalcini's careful observations led her to believe that the motor and sensory neurons did grow, but then they died. She thought that after proliferation and differentiation began, a factor released by the limb buds stimulated continued growth and development. The absence of such a factor led to degeneration. Unable to publish the results in Italian journals because of the manifesto banning Jews from performing academic research, Levi-Montalcini and Levi eventually published them in Belgian and Swiss journals.

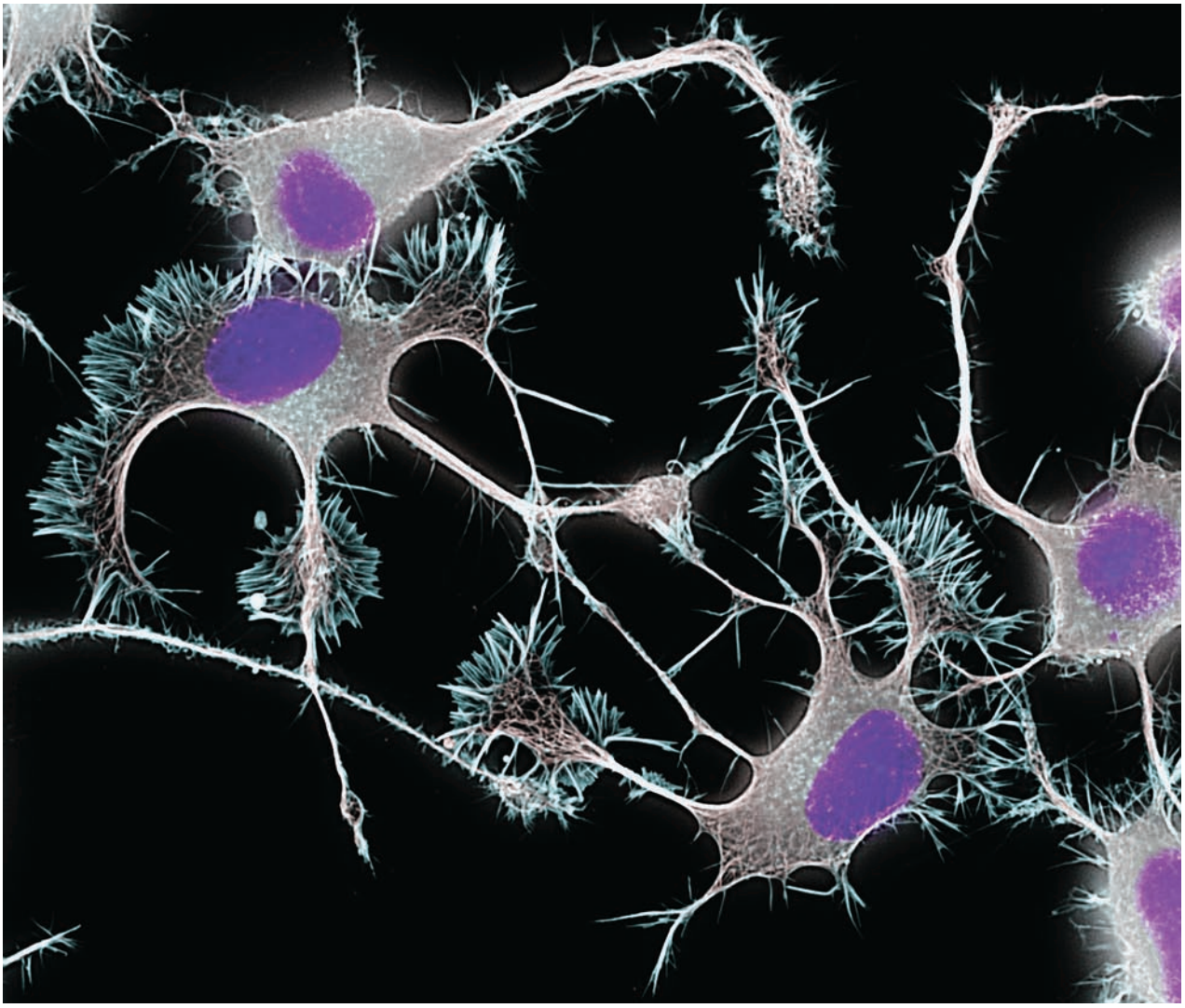
Systematic bombing of Turin by Allied forces in 1942 forced Levi-Montalcini to relocate her home laboratory to the countryside. Wartime conditions proved too dangerous for her to carry on her forbidden studies, and less than two years later she fled to Florence, where she lived under a false identity. After the Nazis were driven from the city in 1944, she worked as a volunteer physician for the Allied forces at a refugee camp for the duration of the war.

NERVE GROWTH FACTOR

After the war ended in 1945, Levi-Montalcini resumed her position as Levi's laboratory assistant at the University of Turin. In 1946 she received an invitation from Hamburger, who had read her research articles, to enter a collaborative research project on the regulation of chick embryonic development at

Washington University in St. Louis, Missouri. The research Levi-Montalcini conducted in her private home laboratory during the war had been inspired by the work of Hamburger, yet they had formulated different conclusions to explain their observations. He believed that tissues and organs in the body sent out signals inducing cells of the developing nervous system to grow and divide. Without the limb, the motor neurons did not receive a signal to proliferate. Levi-Montalcini thought a specific nutrient was needed for already proliferating nerves to survive and to continue growing. Despite their competing ideas and different approaches, the two developed a friendly working relationship. She planned to stay for less than one year, but the success of their joint research persuaded her to stay for three decades. Washington University named her an associate professor of zoology in 1956 and a full professor in 1958.

Levi-Montalcini continued to research development of the nervous system in chick embryos, and she demonstrated that a specific factor was necessary for normal nerve cell growth and differentiation, as she hypothesized. By scrutinizing microscope slides of sections taken from various stages of development, she reconstructed the process step by step, counting new nerve cells and noting their locations. In 1947 she finally recognized the big picture, which involved the programmed migration of nerve cells to predetermined destinations and then the demise and removal of certain cells by the embryonic immune system. Neurons degenerated in the course of normal development, not only following the ablation of a limb bud, and the evidence of their previous exis-



Following the addition of nerve growth factor (NGF), the initially spherical cells have formed long branching extensions called neurites (in gray and green) that will form the axons and dendrites that connect nerve cells and transmit nervous impulses. The nuclei are indicated by purple. *(Dr. Torsten Wittmann/Photo Researchers, Inc.)*

tence was quickly cleaned up. Her intuition told her that a factor or hormone made in the limbs acted as a feedback signal necessary to sustain the growth of new neurons.

In the 1950s, at Hamburger's suggestion, Levi-Montalcini surgically attached fragments of mouse tumors to chicken embryos and was elated to observe nerve fibers chaotically growing on, all around, and into the tumors. She surmised that the tumors produced and released a potent chemical growth factor, a substance that stimulates cell growth. When she attached the tumor to an external membrane that was connected to the embryo only through blood circulation, the nerves still grew, demonstrating that the signaling factor was not conveyed along nervous pathways, but was a humoral factor, meaning it was transported by the blood.

A friend from medical school named Hertha Meyer had fled to Rio de Janeiro during the war and was an expert at in vitro tissue culture. In vitro experiments are performed on tissue growing in glass or plastic dishes in the laboratory rather than in animals and are useful because a scientist has more control over the experimental conditions and fewer unknown factors complicate interpretation of the results. Using such a technique would speed up Levi-Montalcini's experiments and help her to verify definitively the presence and role of a nerve growth factor, NGF. She smuggled two tumor-laden mice through customs in her coat pocket and visited the Institute of Biophysics in Brazil from 1952 to 1953. In her friend's laboratory, Levi-Montalcini put a piece of the mouse tumor near to, but not touching, a bit of chicken nerve tissue. Within a few hours, dense halo-shaped growths of nerve fibers appeared around the chicken tissue, radiating outward from it like rays from the sun, confirming that a humoral factor secreted by the tumor acted upon the nerve tissue. When she allowed the culture to grow for two or three days, she noticed the new nerves elongated in the direction toward the tumor tissue.

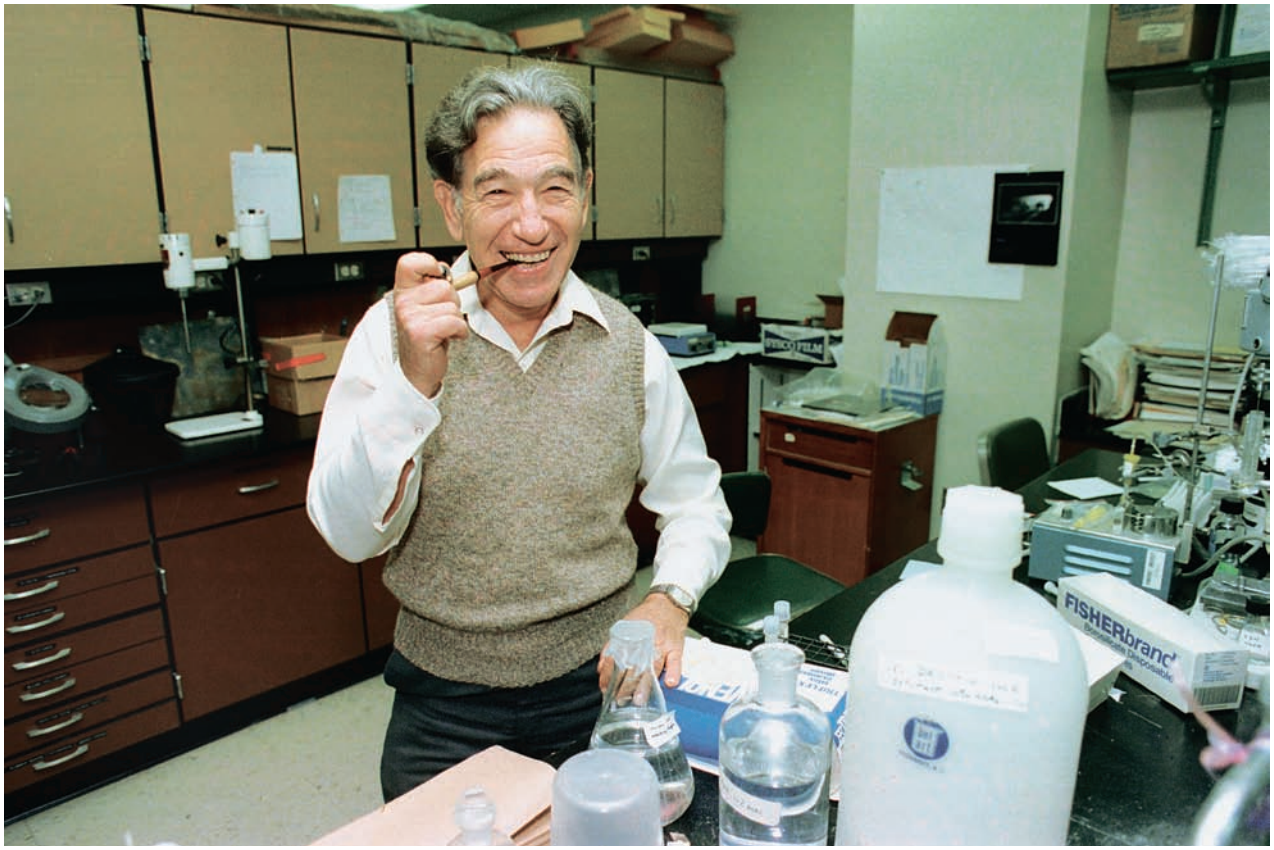
After returning to St. Louis, Levi-Montalcini paired up with an American postdoctoral biochemist, Stanley Cohen, to isolate the growth factor. Levi-Montalcini spent an impatient year growing enough mouse tumors for Cohen to extract a large enough quantity of the factor for identification. Future Nobel laureate (in physiology or medicine in 1959) Arthur Kornberg, a biochemist who was working at Washington University at the time, recommended they use snake venom to purify their fractions by removing excess nucleic acids. Fractions treated with the snake venom turned out to be powerful stimulators of halo formation, suggesting that the venom either neutralized an inhibitor in the tumor extract or contained a factor with properties similar to the growth factor they were investigating. The latter proved true; astonishingly, the snake venom was 3,000 times richer in NGF than the tumor extract. This accidental discovery led them to purify NGF from mouse salivary glands, correctly thinking that because salivary glands are the mammalian counterpart to the snake glands, they might also be a rich source. With a cheap and abundant supply, Cohen successfully isolated NGF, identified it as a protein (a molecule made up of amino acids), and determined its molecular weight and other physicochemical characteristics.

Over a six-year period, Levi-Montalcini characterized NGF with respect to its biological properties, while Cohen analyzed its chemical properties. Injection of NGF into newborn rodents induced the formation of many new neurons. Using immunological techniques, they showed that NGF was important in the differentiation and survival of certain types of nerve cells. Antiserum to snake venom inhibited the in vitro formation of the fibrillar halos. They made NGF-specific antibodies, proteins manufactured by the immune system that recognize and bind to specific molecules, and the specific antibodies also prevented the formation of the halos. When injected into newborn rodents, the NGF-specific antibodies bound to the natural NGF that was present, and the developing nerves suffered almost complete atrophy.

Due to budget constraints at Washington University, Hamburger could not offer Cohen a permanent position. In 1959 Cohen left for Vanderbilt University, and an Italian named Piero Angeletti assisted Levi-Montalcini in investigations on the structure of NGF.

In 1961 Levi-Montalcini returned to Italy, and with the help of Angeletti, she established a laboratory at the Higher Institute of Health in Rome, where she continued performing neurobiological research. The Italian National Research Council (CNR) transformed her research unit into the Laboratory of Cell Biology in 1969. She spent half of each year in Italy and the other half in the United States until 1977, when she retired from Washington University and became a professor emerita. She retired as director of the Laboratory of Cell Biology in 1979 and became a guest professor at the CNR's Institute of Neurobiology in Rome and a guest researcher at the Laboratory of Cell Biology.

Levi-Montalcini joined numerous scientific organizations, including the American Academy of Arts and Sciences, the Belgian Royal Academy of Medicine, the National Academy of Sciences of Italy, the European Academy of Sciences, Arts, and Letters, and the Academy of Arts and Sciences of Florence. In 1968 the National Academy of Sciences elected her a member, only the 10th woman elected since



Stanley Cohen, the American biochemist with whom Rita Levi-Montalcini shared the 1986 Nobel Prize in physiology or medicine, performed most of the biochemical characterization of nerve growth factor. *(AP Images)*

its foundation in 1863. In 1974 she became the first female member of the Pontifical Academy of Sciences in Rome. As scientists realized the importance of NGF in the 1980s, they reviewed Rita Levi-Montalcini's research from three decades earlier, leading to her selection for the highly regarded Nobel Prize in physiology or medicine in 1986, shared with Stanley Cohen, for their discovery of growth factors. In 1987 President Ronald Reagan awarded her the highest distinction bestowed upon American scientists, the National Medal of Science. Several universities, including the University of London and Harvard University, have granted her honorary degrees, and she has declined many more.

IMPACT OF LEVI-MONTALCINI'S WORK

Since her discovery of NGF in 1952, medical researchers have learned a great deal concerning NGF's mechanism of action and potential significance. NGF belongs to the neurotrophin family of proteins that induce proliferation and survival of neurons. After secretion, the protein seeks out specific receptors, molecules located on the surface of cells that recognize and bind NGF. Only certain cells, called target cells, have the specific receptors for NGF and react to its presence. Binding of NGF to its receptor triggers a series of biochemical changes within the cell that lead to activation of proteins that induce the nerve cells to grow axons. When a growing axon reaches a target cell, a synapse is formed, allowing the nerve cell to communicate with the target through the release of neurotransmitters, chemical messengers released by one neuron that diffuse across the synapse and bind specific receptors on a post-synaptic cell.

In 1971 Levi-Montalcini's postdoctoral fellow Ruth Hogue Angeletti and a biochemist named Ralph Bradshaw at Washington University determined the amino acid sequence for NGF. The gene that encodes NGF was identified and sequenced in 1983, enabling the protein to be synthesized using biotechnological methods and its uses to be explored more easily. Damage to nerve tissue, whether caused by injury from stroke, trauma, disease, or aging, is particularly dangerous, since the body has natural mechanisms for inhibiting the regeneration of nervous tissue, such as molecules that inhibit the growth of axons. Medical researchers currently are investigating treatments using NGF for brain and spinal cord injuries and neurodegenerative diseases such as Alzheimer's, Lou Gehrig's, and Parkinson's.

Levi-Montalcini's ground-breaking research on neuroembryonic development revealed an important clue to the mystery of cellular growth and differentiation. Following her lead, scientists have identified and studied numerous other factors that perform similar functions in a variety of cell and tissue types. In addition to pioneering a molecular movement in developmental biology, the discovery of NGF has led to many new, effective treatments for a variety of maladies. Therapeutic use of NGF to slow the progression of neurodegenerative diseases or to stimulate the growth of motor neurons in spinal cord injury patients may be among the most optimistic goals, but NGF has also been effective in speeding healing from burns, diminishing the negative effects of chemotherapy and radiation therapy, healing bedsores, and eradicating corneal ulcers.

See also NERVOUS SYSTEM.

FURTHER READING

Levi-Montalcini, Rita. *In Praise of Imperfection: My Life and Work*. New York: Basic Books, 1988.

McGrayne, Sharon Bertsch. *Nobel Prize Women in Science*. Washington, D.C.: Joseph Henry Press, 1998.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1986." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1986/index.html. Accessed January 22, 2008.

Linnaeus, Carl (1707–1778) Swedish *Naturalist*

In the 18th century Carl Linnaeus created a system for classifying all living organisms, facilitating communication among biologists. He went further in establishing a means for naming all the organisms and providing very detailed descriptions for distinguishing each type of living thing from all others. The system Linnaeus developed is still used today as a starting point for classifying and naming newly discovered species.

CHILDHOOD AND EDUCATION

On May 23, 1707, Carl Linnaeus was born in a small town in southern Sweden. His father, Nils Ingemarsson (son of Ingemar), was a clergyman and an amateur botanist. As a child, Carl spent much time in his father's flower garden. After being admonished by his father for repeatedly asking the names of the same plants, Carl worked hard to remember the names of the plants in his father's gardens and the flowers with which he amused himself in place of toys as a child. This was a difficult task, as plants were then given names consisting of long phrases of Latin words strung together.

When Carl was seven, his parents hired a tutor for him, but Carl preferred roaming the nearby meadows rather than studying with his stern teacher. When he was nine, his parents sent him to Växjö, where he remained through high school. He was an average student who obtained the nickname of "little botanist" from his schoolmates. Carl enjoyed natural history and Latin more than the classes that were necessary to prepare for the ministry, which his parents expected him to enter. In high school, he was introduced to Dr. Johan Rothman, who lectured on logic and physics. Rothman became aware of Carl's interest in botany, which was closely linked to medicine, as pharmaceuticals were synthesized from plants. Many physicians had their own gardens from which they treated their patients. To his father's dismay, Carl's teachers agreed that he was not cut out for the ministry, but Rothman offered to take Carl in and mentor him during his last year in Växjö. Rothman offered Carl private lessons in botany and medicine and introduced him to the current system for plant classification proposed by Joseph Pitton de Tournefort. This system was based on the shape of the corolla, which is the outer portion of a flower.

In 1727 Linnaeus enrolled as a medical student at the University of Lund, his father's alma mater. His impressions of the university were bleak. There was only one professor of medicine, no botany classes were offered, and the equipment and instruction were lacking. He rented a room at the home of Dr. Kilian Stobaeus, as did another student, David Koulas, who served as Stobaeus's assistant. Stobaeus had a marvelous library that he kept locked. Koulas sneaked library books for Linnaeus to read, and, in return, Linnaeus tutored Koulas in physiology. One night Stobaeus caught Linnaeus reading borrowed library books in his room. He was upset, but he came to appreciate the budding botanist's desire to learn about the natural world. He allowed Linnaeus free access to the library and eventually free room and board as well.

Linnaeus went home to Reshult during the summer and was visited by Rothman. When Rothman learned that there were no botany classes at Lund, he encouraged Linnaeus to transfer to the University of Uppsala. The University of Uppsala had a good reputation, but when Linnaeus arrived he found the situation not much better than that at Lund. There were two professors of medicine, Lars Roberg and Olaf Rudbeck. Both were old, and Rudbeck did not even lecture anymore, but left the teaching of his classes to an assistant, Nils Rosén, who was out of the country obtaining his degree at the time. Linnaeus spent much of his time in the botanical gardens, which were also in a state of disrepair. One day he was approached by a man who began quizzing him about botany. The man, Dr. Olaf Celsius, dean of the cathedral and professor of theology, was impressed with Linnaeus's

answers, and when he learned that Linnaeus had a collection of over 600 pressed flowers, he invited him to his home. Celsius offered him a room in exchange for his assistance on a book he was writing about biblical plants.

That spring, Linnaeus met an older medical student named Peter Artedi. Peter had an excellent academic reputation and enjoyed natural history as much as Linnaeus. The two struck up an extraordinary friendship and spent much time studying together. They even split up the natural world so each could focus their studies on certain subjects and then share their knowledge with each other. Friendly rivalry kept them motivated.

BEGINS WORK CLASSIFYING PLANTS

It was customary for pupils to present their favorite professors with a poem on New Year's Day. Instead, Linnaeus presented Celsius with a scientific discourse about plant pollination. In it, he explained the roles of plant reproductive structures. In this informal report, he discussed the theory of plant sexuality and compared plant reproduction to animal reproduction. He compared removing the anthers (the male reproductive structures that produce pollen) to castration, the surgical removal of testicles. He likened pollination by related plants to incestuous relationships and having more than one stamen or pistil to bigamy. While his graphic descriptions offended some, Celsius was so impressed he showed it to Rudbeck, who in turn asked Linnaeus to lecture for him in botany and hired him to tutor three of his sons.

Though Linnaeus was busy tutoring, lecturing, and tending to his own studies, he somehow found time to devote to his love of botany. During his college days he began composing some of his most renowned works. Linnaeus organized all the known plants into 24 classes based on the number and position of their stamens. Then he further organized them by the number of pistils they contained and the form of the fruit they bore. For several years, he continued to revise and add to these manuscripts.

In March 1731, Nils Rosén returned to campus, having earned his doctorate in Holland. He expected to take over the botany classes that Linnaeus had been teaching, but Rudbeck let Linnaeus keep them, angering Rosén. Linnaeus was a popular lecturer, which only increased Rosén's jealousy and fueled a decades-long animosity between the two.

SURVEYS LAPLAND AND OBTAINS MEDICAL DEGREE

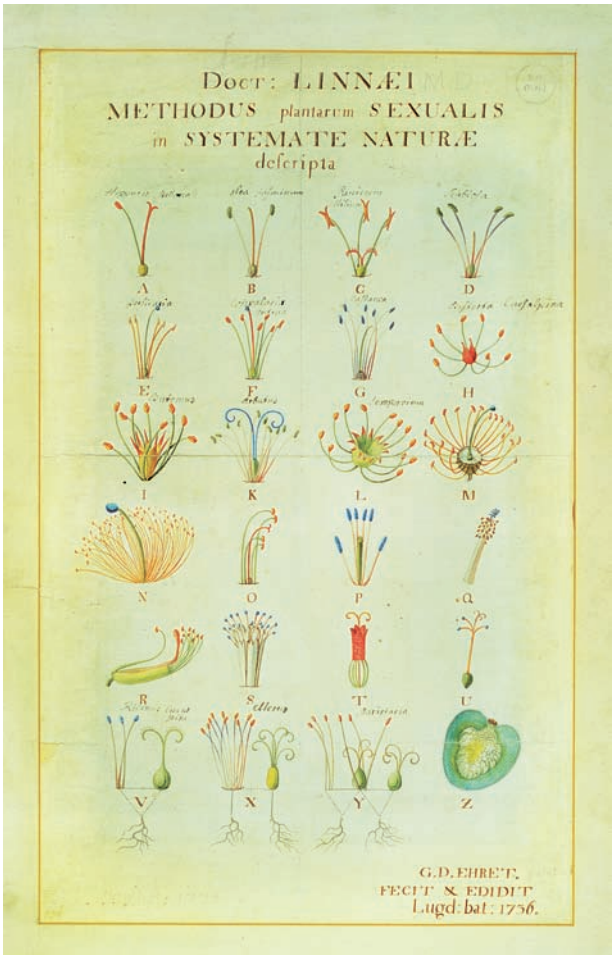
The following autumn, Linnaeus was in need of a change in scenery and applied for a small grant to journey to Lapland, an area encompassing the extreme northern portions of Europe, including parts of Sweden, Finland, Norway, and northwest Russia. The Royal Society of Science at Uppsala awarded him money to survey the area for its natural resources. In May 1732, he set off on a four-month-long dangerous expedition. He faced bitter cold, lack of food, and hazardous travel conditions, yet he was amazed at the region's enormously rich wildlife. He gathered much information on little known plants of the region, minerals, and animals as well as Lap customs. He even became a specialist on reindeer habits and discovered over 100 new plant species. Some of his observations from this influential journey were published in *Flora lapponica* (The plants of Lapland) in 1737. Others were submitted to the Royal Society but only published in parts. Reports say that the hardships that he claimed to endure on this journey were exaggerated, and at least one of his side trips was completely invented, yet this expedition remains one of the most famous in Sweden.

In 1734 Baron Nils Reuterholm, the chaplain to the provincial governor of Dalarna, invited Linnaeus to survey his province as he did Lapland two years earlier. During a Christmas visit to the home of fellow student Claes Sohlberg, he toured the copper mines in the capital of Falun. Sohlberg's father was the inspector of these mines, which were of interest to Linnaeus since he was now lecturing in mineralogy at Uppsala in addition to botany. While in Falun he also met his future wife, Sara Lisa, the daughter of Dr. Johan Moraeus. Two weeks after meeting they became engaged, but Moraeus insisted they wait at least three years before marrying.

Sohlberg's father offered to pay Linnaeus to take his son traveling across Europe and to tutor him. Until this point, Linnaeus apparently saw no reason to speed up the completion of his degree. He was earning just enough money to live, was a respected lecturer at the university, and had written several papers on natural history. Yet he took advantage of this sojourn with Sohlberg to Europe, and in 1735 he ventured off to the Netherlands, where degrees were easily obtained. He had previously written a thesis on the cause of fever, and within two weeks he passed a written and oral examination and received his doctor of medicine degree from the University of Harderwijk.

PUBLISHES *SYSTEMA NATURAE* AND OTHER POPULAR BOOKS

They spent three years traveling throughout Holland, where Linnaeus published numerous botanical papers and met several influential botanists and physicians. Many helped support him, not only financially, but also by introducing him to other colleagues and benefactors. In Leiden, he met Jan Frederick Gronovius who was so impressed with



Linnaeus classified plants into 24 categories based on a system of sexual characteristics, illustrated in this color engraving created by Linnaeus. *(Natural History Museum, London, UK/The Bridgeman Art Library)*

Linnaeus's manuscript *Systema naturae* (*A General System of Nature*) that he published it for him in 1735. *Systema naturae* presented an outline for classifying the three natural kingdoms: plants, animals, and minerals. The plants were classified according to their sexual systems. For the rest of his life Linnaeus would update and publish new editions of this work. The 12th volume was published in 1768 and included over 2,300 pages in three volumes. Another popular publication of this time was his *Genera plantarum* (The genera of plants) in which he classified and described all the known plants of the time, approximately 1,000 species. Sohlberg's father never paid Linnaeus, and the two young men drifted apart. Linnaeus found himself dependent upon his growing popularity and his patrons to support himself.

He ventured to England and soon met a wealthy businessman named George Clifford. Clifford was an enthusiastic botanist as well as the director of the Dutch East India Company. He invited Linnaeus to live with him to oversee his gardens and serve as his personal physician.

While in Holland, Linnaeus by chance encountered his old friend Peter Artedi. Unfortunately, Artedi drowned late one night soon after, and Linnaeus took it upon himself to complete and publish the major effort Artedi was working on at the time, *Ichthyologia,* about the natural history of fishes.

Linnaeus published *Hortus cliffortianus* (The horticulture of Clifford's garden) in 1737. It contained detailed descriptions of all the plants in Clifford's garden and included illustrations of dissected plants in addition to exacting descriptions of the plants' growth habitats. While this was an extremely tedious and laborious task, Linnaeus was in his element, having the opportunity to classify many plants that he had never seen before. In 1738 he returned to Sweden, but in order to marry Sara Lisa, he still needed a job.

On the advice of his future father-in-law, Linnaeus set up a medical practice in Stockholm. At first, potential patients were hesitant to seek medical advice from a young, inexperienced, plant-loving man, but eventually he established a positive reputation, and soon the queen herself was seeking Linnaeus's medical aid. Count Carl Tessin befriended the young doctor and recommended him for the post of physician to the Admiralty. A scientific society, which was to become the Royal Academy of Sciences, was established around this time, and Linnaeus served as the first president. Within one year, Linnaeus was a highly respected physician and had proven he could support Sara Lisa. They were married in June 1739, and their son, Carl, was born in January 1741. They later had three daughters who survived into adulthood.

JOINS FACULTY AT UPPSALA

Professor Roberg eventually retired, and Linnaeus was appointed to replace him. Before moving to Uppsala, however, he went on another expedition to survey the potentially economically profitable natural resources on the Baltic islands of Öland and Gotland. He was hoping to find plants that could be used as dyes and clays that could be used to manufacture porcelain, but he made many other discoveries instead. He learned of a new crop plant—hay seed—and about many local medicinal remedies and farming methods as well as how to catch seals and how to prevent sand from drifting. He also examined the rock formations, mineral springs, and quicksand.

Afterward, Linnaeus and his family moved to Uppsala, where they would reside for the remainder of their lives. In October 1741, Professor Linnaeus gave his first lecture at the University of Uppsala. Interestingly, it was about the importance of exploring and learning about the natural history of one's home-

land. Linnaeus was in charge of botany, dietetics, and *materia medica* (the uses and sources of drugs). He was a very popular lecturer, both publicly and privately. He was captivating, interesting, clear, and even humorous. He encouraged and enjoyed teaching his students, and they enjoyed learning from him.

The botanical gardens had been neglected for many years. There were less than 300 cultivated plants at the time. Linnaeus received funding from the university senate to repair them as well as build greenhouses and renovate his own home on the garden's grounds. He also enticed Clifford's gardener to come work for him. Over the next decade, the number of specimens in the gardens increased to over 3,000. Many were gifts from people he had met during his travels and from his students' explorations. Matters seemed to go well for Linnaeus, both personally and professionally, though some of his fellow faculty members complained about the behavior of the students during his regular Saturday afternoon explorations. In addition, Linnaeus was shunned by some for his explicit sexual comparisons of plants and animals.

Linnaeus remained prolific in his writing. His classic physician's reference on pharmacology, *Materia medica,* was published in 1749. He published over 170 dissertations on practically every subject of natural history, including ants, birds, stones, fossils, crystals, lemmings, grasshoppers, and buckwheat to name a few. In 1753 *Species plantarum* (The species of plants) was published. This work introduced binomial nomenclature, a system that greatly simplified botanical taxonomy and communication between botanists. In binomial nomenclature, each species is given a two-word Latin name, the first being the genus, the second being the species. The genus is capitalized, while the species name is not. To illustrate this system's utility, consider the following example. Under the old system, one plant was named *Plantago foliis ovato-lanceolatis pubescentibus, spica cylindrical, scapo tereti* (translated "a plantain with pubescent ovate-lanceolate leaves, a cylindric spike and a terete scape"). Using binomial nomenclature, the same plant is referred to simply as *Plantago media.* The generic names are shared among closely related species. The species names distinguish one plant from all other plants in the same genera. Linnaeus often coined names from the surnames of respected colleagues or others he admired. *Species plantarum* included detailed descriptions of the approximately 8,000 known plants at the time, all classified according to his sexual system. His new system was adopted over the next few decades.

In Lapland, Linnaeus had observed fishermen searching through hundreds of shellfish to find a single pearl. He noted the potential economic value if one could force their synthesis. He tried to do so by placing a tiny bit of limestone on a wire and inserting it through a small hole into a mussel. He returned the mussel to a riverbed for six years, and when he retrieved it, a large beautiful pearl was inside. In 1762 he sold the idea of this process to the Swedish government for a modest price but also for the promise to be able to name his successor at Uppsala. This was his opportunity to secure a position for his son. One wonders if he remembers how he felt when his own parents tried to choose his career, as it is said that the younger Carl was not as interested in botany as he was in women. The money was enough to pay off a debt he had taken to purchase a summer home, Hammarby. Having lived off the kindness of others for so many years during his younger days, he hated being in debt. In 1761 he was knighted by the king and took the name Carl von Linné, by which he is remembered in Sweden.

Shortly thereafter, his health began to fail. Despite this, his wife persuaded him to continue taking on many responsibilities, including rector of the university. He suffered his first angina attack in 1773. In 1774 he suffered his first stroke while giving a lecture and was left partially paralyzed. He began to suffer memory loss, which must have saddened him and his admirers greatly, as he had demonstrated such a tremendous capability for remembering names of obscure plants throughout his entire life.

Linnaeus died following a series of strokes in Uppsala, Sweden, on January 10, 1778. He was buried in the Uppsala cathedral. His family inherited his collections, which his son cared for until his own death six years later. Then his sister sold them to James Smith, an English naturalist. Although the action troubled Swedish national sensitivity and infuriated Linnaeus's former pupils, Smith admired and respected Linnaeus and founded the Linnean Society of London for the cultivation of the science of natural history. Many of his amazing collections, manuscripts, and correspondence are owned today by the society, and others are housed in various museums in Stockholm, Uppsala, and London.

Biologists consider Linnaeus the father of taxonomy, the science of classification. While his sexual system of classification for plants is no longer used, it brought about many great advances in botany. Biologists still use the method of binomial nomenclature that he developed. The challenges he undertook were tedious, and his contributions to natural history were enormous. Many botanists at the time sought to distance themselves from him, and some even ridiculed him, but, upon meeting him and observing his unparalleled expertise, they came to respect and admire him. His students revered him, and many eventually became professors themselves.

See also BIOLOGICAL CLASSIFICATION; BOTANY; PLANT DIVERSITY; PLANT FORM AND FUNCTION.

FURTHER READING

Blunt, Wilfrid. *The Compleat Naturalist: A Life of Linnaeus*. Princeton, N.J.: Princeton University Press, 2001.

Goerke, Heinz. *Linnaeus: A Modern Portrait of the Great Swedish Scientist*. New York: Scribner's, 1973.

The Linnean Society of London home page. Available online. URL: http://www.linnean.org/. Accessed January 15, 2008.

Lorenz, Konrad (1903–1989) Austrian *Naturalist* Konrad Lorenz helped found the field of animal behavior by describing imprinting, a behavior in which young animals learn to identify and then bond with their parent during a limited time period after birth. For instance, when the mother goose is absent, goslings recognize any moving object as their mother. Lorenz raised goslings that bonded with him and followed him around as if he were their parent. His groundbreaking research on imprinting, explorations into innate versus learned behaviors, and determination that behaviors evolved by natural selection contributed to the recognition of animal behavior as a unique subfield of the biological sciences.

EARLY INTEREST IN ANIMALS

Konrad Lorenz was born on November 7, 1903, in Vienna, Austria. His father (Adolf) was a successful orthopedic surgeon. Rumors claim that Adolf Lorenz was at one time a single vote short of winning the Nobel Prize in physiology or medicine for his development of a "bloodless" hip-joint operation. As a child growing up in Altenberg, Austria, Konrad loved animals. Two events that influenced his early decision to become a zoologist included the opportunity to observe spotted salamander larvae undergo metamorphosis and the imprinting of a one-day-old pet duckling on Konrad. When he was 10 years old, he questioned the relationships between birds and reptiles and between earthworms and insects. In school a Benedictine monk named Philip Heberdey introduced Konrad to Darwinian evolution and natural selection, a subject in which Konrad developed an intense life-long interest. With a friend, Bernhard Hellmann, Lorenz studied the development and evolutionary history of various aquatic creatures as well as the aggressive behavior of certain fish.

In 1922 Adolf Lorenz sent Konrad to enter the premedical program at Columbia University in New York, mostly to separate him from Margarethe (Gretl) Gebhardt, a young woman whom the older Lorenz felt was an unsuitable mate. The two had known each other since early childhood, but Gretl was a few years older and had to quit school to care for her mother and younger sister after her brothers were killed during World War I. Konrad went to Columbia, where he met Thomas Hunt Morgan, the father of modern genetics, an opportunity he later cherished, but he felt the program at Columbia was unsatisfactory and the premedical coursework would be a waste of time since the University of Vienna would not accept it. He returned to Austria against his father's will and joined the anatomy department at the University of Vienna to complete his premedical training.

One of his professors at the Anatomical Institute, Ferdinand Hochstetter, made Lorenz his assistant. He also introduced Lorenz to the comparative method for studying anatomy and embryology. Lorenz applied this approach to studying behavioral characteristics. During the course of his independent research, he realized how little was known about animal behavior. One scientist who seemed to be the leading expert on animal behavior was a German zoologist named Oskar Heinroth. After reading Heinroth's work, Lorenz committed to making the study of animal behavior his own life's work.

In 1927 Lorenz married Gretl, who was studying to become a medical doctor at the university, and, coincidentally, she was a student in the anatomy class that Lorenz taught. In his free time Lorenz kept a diary on his pet bird, a jackdaw. Without Konrad's knowledge, Gretl and Konrad's friend Hellmann conspired to submit his diary for publication. They first sent it to Heinroth in Berlin, and in 1927 the *Journal of Ornithology* published "Beobachtungen an Dohlen" (Observations on jackdaws), and the following year Lorenz received his doctor of medicine degree. Afterward he continued assisting Hochstetter, but his interest in animals dominated his studies. He began and maintained a colony of tame jackdaws at home, where he could immerse himself in his research. He eventually entered the Ph.D. program at the University of Vienna, and he obtained a second doctorate degree in zoology in 1933.

Konrad and Gretl Lorenz had two children within their first two years of marriage, Thomas and Agnes. Gretl completed her medical degree and worked as a gynecologist, freeing Konrad from financial stress while he embarked on his research career. He secured a reputation before an income, and without a research station, many animals, including jackdaws, monkeys, and rats, shared their home. At times, the young Lorenz children slept covered by a cage to protect them from the wandering animals.

GOOSE SUMMERS

During the years from 1935 to 1938, Lorenz studied the behavior of greylag geese, the animal with

which his name is indelibly associated. After years of owning pet birds, he had drawn many hypotheses concerning their behavior, in particular, behavior patterns predetermined by instincts. During these years, he methodically studied the geese, confirming observations he had made beginning in his childhood. Lorenz obtained his geese from the true wild species, raised from eggs incubated in the wild and obtained immediately before hatching. He chose geese for his studies because they could socially imprint on humans without trying to court or mate with them, they were simple to study, and they exhibited social behaviors similar to man. This last quality allowed Lorenz to draw analogies about characteristics based on observed behavioral similarities, such as the exhibition of characteristics associated with grief at the loss of a partner.

Lorenz is most famous for his work on imprinting with geese. Imprinting is a behavior with both innate and learned components that takes place during a sensitive period, the only time when the behavior can be learned. Within a genetically programmed time frame after birth or hatching, a young gosling identifies the first moving object it sees as its mother, even if the foster mother is a different species. Lorenz studied this behavior by allowing greylag goose eggs to hatch in the absence of a mother goose, but in his own presence. After the goose eggs hatched, the young birds imprinted on Lorenz as their parent. He mothered 10 goslings while in Altenberg—he called to them, swam with them every morning, and lectured around their sleep-wake schedule. When Lorenz examined imprinting behavior in mallard ducks, he had to squat and quack for the ducklings to accept him as their mother. Imprinting is an important behavioral evolutionary adaptation, as learning to recognize and stay close to its parent helps the offspring by providing it with protection and allowing it to learn feeding and escape behaviors that aid in its survival. Lorenz also found that imprinting influences the offspring's sexual behavior later in life, with the animals choosing mates similar to the type of organism upon which they imprinted.

While living as a member of the goose colony, Lorenz made numerous observations about their relationships, their life cycles, and their responses to various stimuli, rituals, and behaviors. He saw many similarities between the behavior of geese and humans, thus many of his descriptions utilized human characteristics or behavioral traits. In other words, he anthropomorphosized the geese, an act with which many scientists disagreed. His methods included extensive filming of the geese; this allowed for frame-by-frame analysis of sequences of actions. Films also allowed separate researchers to compare behaviors and describe them independently so that perceived differences could be noted and analyzed. His student Alfred Seitz performed much of the filming. One of Lorenz's observations included the existence of hierarchical systems between individual geese, within a family, and within a whole colony.

While Lorenz is most famous for his description of imprinting, he also undertook important work on release mechanisms. Lorenz collaborated with future Nobel Prize corecipient Nikolaas Tinbergen on the phenomenon of fixed-action patterns. Tinbergen and Lorenz met at a symposium in Leiden in 1936. Tinbergen was a lecturer at the University of Leiden, and he had been studying behaviors in herring gulls that were similar to those demonstrated by Lorenz's geese. The two men got along well and began a long and fruitful collaboration and friendship. Lorenz credits the successful collaborations with Tinbergen to Tinbergen's knack for designing unobtrusive experiments on whole animals and to his own keen observational skills. Fixed-action patterns are behaviors that are not learned but are innate. Upon exposure to a specific stimulus, the animal exhibits a sequence of instinctive behaviors that typically must be carried out to completion. Lorenz introduced the concept of a releasing mechanism. He found that the perception of a certain stimulus called a releaser or a sign stimulus initiated a response by an innate releasing mechanism, a neural network specifically adapted to stimulate release of the genetically programmed behavioral response, or the fixed-action pattern. For example, the two men examined egg-retrieval behavior by placing an egg near the nest of a sitting bird. The bird would bend her neck outward and roll it toward her using the underside of her beak, while using the side of her head to steady it. The sight of a displaced egg near the nest triggered the retrieval motions even though the goose had never "learned" this behavior. While carrying out these studies, Lorenz and Tinbergen made another important discovery by taking away the egg after the goose initiated the egg-rescuing behavior. The goose still carried out the physical actions necessary to roll the now missing egg toward the nest. She did not, however, use the side of her head to steady the imagined egg. From this observation, Tinbergen and Lorenz concluded that the two actions were separate and resulted from different environmental cues. Other examples of fixed-action patterns include postures used in fighting and surrendering, mating dances, and scuttling for cover from predators. Yawning is an example of a fixed-action pattern exhibited by humans. Seeing another individual yawn acts as a trigger to stimulate a person to yawn, an action that is difficult to stop once initiated. Lorenz observed that sometimes an animal performed a behavior even in the absence of a stimu-

lus. For example, he once saw his pet jackdaw fly upward and snatch an "invisible" insect out of the air with its beak, then return to its perch, beat the insect, and then swallow it. Lorenz thought perhaps the need to perform the instinctive action built up, waiting to be released. He compared an animal's need to perform a fixed-action pattern to a buildup of hydraulic pressure, which eventually may cause an explosion in the absence of a release outlet.

CONTROVERSY AND WAR

In 1937 Lorenz returned to the University of Vienna as an unpaid lecturer in comparative anatomy and animal psychology. A complicated chain of events and the assistance of the German behavioral physiologist Erich von Holst led to Lorenz's appointment in 1940 of professor of psychology at the Albertus University of Königsberg, in Germany. His new residence had no room for the geese, so they ended up at the Königsberg Zoo, and eventually they became scattered and lost.

In 1940 Lorenz published a paper with the translated title "Disorders caused by the domestication of species-specific behavior." In this paper he claimed that man has undergone a process of self-domestication that has resulted in genetic changes affecting human behavior. People were aware that domestication of animals caused genetic changes that resulted not only in physical differences, but also in behavioral changes. Lorenz's description of human self-domestication was controversial because he claimed this led to negative effects. His argument was as follows: animals bred in captivity are encouraged to grow fat and are oversexed to facilitate breeding, thus they become more bestial, greedy for food and sex. Lorenz expressed his worry that man also was undergoing genetic decay, that man was becoming more infantile, less responsible, and less altruistic. Terminology he used in his paper (i.e., annihilate, race-preserving, socially inferior human material, extermination) fed into the Nazi ideology of racial purity. Lorenz claimed he was anti-domestication, not anti-Semitic and that poor translation from the original was partly responsible, but this paper remains an unflattering smudge on this scientist's record. In 1973 the *New York Times* reported that Lorenz expressed his regret over its publication.

In 1941 Lorenz was called up to serve in the German army. His wife and three children (they had a daughter named Dagmar in addition to Thomas and Agnes) returned safely to Austria, though a box containing some of Lorenz's precious papers was lost en route. The Russians captured him in 1942, and he spent time working in Russian war hospitals, then war camps in Oritschi and then Armenia. Lorenz later admitted that he only realized the evil of Nazism around 1943–44, when he witnessed the inhumane treatment of transported inmates from a concentration camp.

When he was finally released in 1948 he had to find a job. The Austrian Academy of Sciences gave him some money to support a small research station in Altenberg. He also lectured, and then the Max Planck Society offered to provide him with a stipend and a research station adjunct to Erich von Holst's department in Buldern, Germany. Lorenz did not have full status as a faculty member of the Max Planck Institute, but, as head of a new subdepartment, he received a small budget beginning in 1951. Eventually, the University of Münster made Lorenz an honorary professor.

In 1952 Lorenz published *King Solomon's Ring,* a book intended for nonscientists and scientists of all ages. The book shared many stories about Lorenz's interactions with his jackdaws, other animals, and a few about his geese. He wrote using plain language and included many illustrations. He told of several humorous experiences, including an incident when Lorenz was waiting for Seitz to set up the camera equipment for filming some scenes with greylag geese. Annoyed by the mallard ducks hanging around interfering with his shots, Seitz said, "Ranganganganga, ranganganganga," to call them away. Then he quickly apologized to the ducks and said "quahg, gegegegeg, quahg, gegegegeg." Lorenz laughed at his busy assistant, who had just accidentally addressed the ducks in the language of the geese! The book provided an outlet for Lorenz to share with the world his love for animals. The director of the Zoological Institute of Munich, Karl von Frisch, read *King Solomon's Ring* and was so impressed that he became determined to learn all he could about the developing field of ethology, or animal behavior. Frisch later shared the Nobel Prize with Lorenz and Tinbergen for his own work on honey bees, and now all three are considered founders of the field.

For many decades, Lorenz and two of his colleagues had dreamed of opening a research institute dedicated to the study of animal behavior. Gustav Kramer was an ethologist who was interested in how birds navigated long distances, and von Holst was a physiologist who had painstakingly demonstrated that behavioral physiology consisted of more than reflex circuits. In 1955 Lorenz, von Holst, and Kramer founded the Max-Planck-Institut für Verhaltensphysiologie (Max Planck Institute for Behavioral Physiology) in Seewiesen, Bavaria. Located on a lake, the facility was much larger than the one in Buldern, and several postdoctoral scientists visited. Kramer was officially one of the codirectors

of the institute, but he did his research far away at Tübingen. Lorenz and von Holst regularly argued over details and science and ethics, but their passion seemed to keep the institute flourishing. Tragedy changed the role Lorenz played in the running of the institute. Kramer died while mountain climbing in 1959, and von Holst died in 1962, leaving all the administrative duties to Lorenz.

While serving as director at the institute, other scientists began to question Lorenz's early work on imprinting. Thus, one major line of research at the institute was a detailed, extensive study on imprinting. With his guidance, researchers gathered data and observations to support assumptions he had made decades before. Efforts were made to reduce the number of variables examined in each experiment, and behavior in different species was analyzed and sorted carefully. One criticism of his previous work was that some of his results were not repeatable, but Lorenz's workers showed at least in one case that this was due to the lack of maternal care given to the goslings in the duplicate study. His students went on to show that when goslings were raised in such a manner that only their physical needs were met, they would exhibit disturbed and abnormal behaviors as an adult, much like behavior found in human beings. Emotional needs must be met in order to raise a socially developed goose.

Other studies that were reinvestigated included the effect of imprinting on sexual behavior as an adult. When male mallard duck eggs hatched while in a sheldrake nest, the mallards grew up and tried to mate with female sheldrakes. Female mallards, however, only sought out males of their own species for mating. In situations in which male drakes were raised only in the presence of other males, they grew up to court other males. Researchers at the institute also examined courting behaviors with other species. The results of the reinvestigations proved to vindicate Lorenz's earlier work in addition to providing a stimulation for new research.

While at Seewiesen, Lorenz made many comparisons between animal and human behavior. Many scientists thought this was improper. In 1966 he published a book, titled *On Aggression,* that discussed the aggressive behavior of many animals, including material on aggression in man. In the book, Lorenz identified aggression as the fighting instinct between members of the same species and that aggressive behavior amounted to an adaptation that ensured the strongest individuals survived to reproduce. He also pointed out that humans were the only animal that killed their own species on purpose. Critics claimed that the book gave people reason to accept aggression and violence as a product of biology, and thus to accept it.

In 1973 Konrad Lorenz, Nikolaas Tinbergen, and Karl von Frisch received the Nobel Prize in physiology or medicine for their discoveries concerning the organization and elicitation of individual and social behavior. These three pioneers of ethology were the first to apply the scientific method, using systematic observation and experimentation in the study of animal behavior. Lorenz retired from the Max Planck Institute for Behavioral Physiology in Seewiesen that same year. He returned to his home in Altenberg, where he directed the department of animal sociology at the Austrian Academy of Science. He continued his studies at a research station set up for him by the Max Planck Society for the Advancement of Science. In 1978 he published yet another book, *The Year of the Greylag Goose,* which he claimed was not a scientific book but a book that derived from his pleasure in observing living animals. He supported the Austrian Green Party, a political party that campaigns for ecological issues, environmental protection, and the rights of minorities.

Konrad Lorenz died of kidney failure on February 27, 1989, in Altenberg. His quest for scientific laws that governed animal behaviors and his recognition of structured patterns helped establish ethology as a science. His observations on imprinting and his development of the concept of innate releasing mechanisms to explain fixed-action patterns appear in all animal behavior texts and most general biology textbooks today. Some of his work and ideas about the evolution of behaviors are now considered old-school, but he is a notable figure in the field of life science for pioneering the scientific study of animal behavior and for popularizing the field among the general public.

See also ANIMAL BEHAVIOR; ETHOLOGY; FRISCH, KARL VON; TINBERGEN, NIKOLAAS.

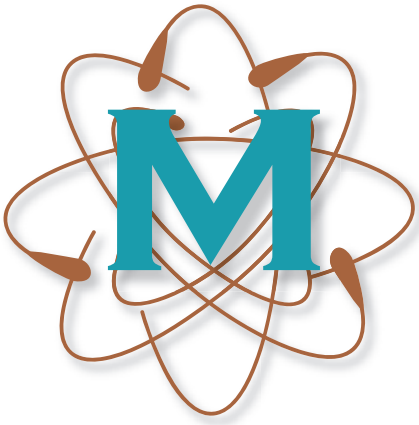
FURTHER READING

Evans, Richard I. *Konrad Lorenz: The Man and His Ideas.* New York: Harcourt Brace Jovanovich, 1975.

Lorenz, Konrad Z. *The Foundations of Ethology: The Principal Ideas and Discoveries on Animal Behavior.* Translated by Konrad Z. Lorenz and Robert Warren Kickert. New York: Simon & Schuster, 1981.

Nisbett, Alec. *Konrad Lorenz.* New York: Harcourt Brace Jovanovich, 1976.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1973." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1973/index.html. Accessed January 25, 2008.



MacLeod, Colin Munro (1909–1972) Canadian-American *Chemist* Working in collaboration with Oswald Avery and Maclyn McCarty in 1944, Colin MacLeod showed that DNA transferred genetic information from one bacteria type to another.

Colin Munro MacLeod was born in Port Hastings, Nova Scotia, Canada, on January 28, 1909. He graduated from high school ahead of his peers at age 15 after skipping three grades, and, though he was accepted at McGill University, the school would not admit him until he turned 16 years old. During that year, he served as a substitute teacher for sixth grade. In college he played varsity hockey and entered the medical school after two years.

After earning his medical degree and training as a resident for two years at Montreal General Hospital, he joined the staff of the Rockefeller Institute for Medical Research Hospital in 1934 as an assistant resident physician and assistant in medicine, where he researched pneumococcal bacteria with Oswald Avery. They examined bacterial transformation, a phenomenon first discovered by the British researcher Frederick Griffith in the 1920s. Griffith showed that crude extracts prepared from a culture of a virulent, encapsulated, smooth (S) strain of *Streptococcus pneumoniae* that had been killed by heating converted a nonvirulent, nonencapsulated, rough (R) strain of bacteria into a virulent S strain. MacLeod and Avery had been trying to identify the responsible factor from the extract, but techniques for purifying chemical substances were crude at the time, and, though many scientists recognized the biological importance of identifying the responsible factor, they gave up in frustration. MacLeod's tenacious efforts led to the description of many properties of the transformation extracts, and to the improvement of methods used to remove proteins from solutions containing polysaccharides, making possible the reproducible preparation of purer extracts. Maclyn McCarty joined Avery's lab in 1941 and performed the biochemical characterization of the active transforming substance.

In 1944 Avery, MacLeod, and McCarty published their seminal paper in the *Journal of Experimental Medicine* demonstrating that purified DNA was the chemical substance responsible for transformation of R strain pneumococcal bacteria into S strain bacteria. Other scientists were hesitant to relinquish the paradigm that protein was the genetic material and DNA was simply an accessory molecule that aided in the replication of the proteinaceous genes, but subsequent experiments performed by others eventually confirmed the findings of Avery et al. Even without having received timely recognition for the momentous discovery, MacLeod's career flourished.

Though he had been appointed chairman of the department of microbiology at the New York University School of Medicine in 1941, during World War II, MacLeod continued his association with Avery's lab and also consulted for the government on war-related science and health issues. His research and personal involvements reflected issues of primary and timely importance to public health and to a country involved in war. He studied outbreaks of microbial diseases common to young adults living in communal conditions like army barracks, worked on the development of an antipneumococcal vaccine, introduced virus-handling laboratory exercises for medical students, studied antibacterial agents such as sulfonamides, and helped shape public policy for scientific

research. MacLeod's numerous involvements led to his membership on the Army Epidemiological Board, later called the Armed Forces Epidemiological Board, for which he served in the demanding role of president from 1947 to 1955. From 1946 to 1949 MacLeod also served as a member of the first study section for the National Institutes of Health, the Antibiotics Study Section. The National Academy of Sciences elected MacLeod a member in 1955, and the following year he accepted an endowed professorship at the University of Pennsylvania. He returned in 1960 as a professor of medicine at New York University, where he continued to research genetic transformation and provided advice and counsel for the Southeast Asia Treaty Organization in fighting cholera. In 1961 MacLeod was named chairman of President John F. Kennedy's Life Sciences Panel of the President's Science Advisery Committee, and, the following year, he served as a member of the committee itself. From 1963 to 1966 he acted as the first deputy director of the Office of Science and Technology, Executive Office of the President (now called the White House Office of Science and Technology Policy). After leaving that role, he became vice president of medical affairs for the Commonwealth Fund of New York City. MacLeod died on February 11, 1972.

See also Avery, Oswald; deoxyribonucleic acid (DNA); Griffith, Frederick; McCarty, Maclyn.

FURTHER READING

Avery, Oswald T., Colin M. MacLeod, and Maclyn McCarty. "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III." *Journal of Experimental Medicine* 79 (1944): 137–158.

McDermott, Walsh. "Colin Munro MacLeod (January 28, 1909–February 11, 1972)." In *Biographical Memoirs. National Academy of Sciences.* Vol. 54. Washington, D.C.: National Academy Press, 1983.

Malpighi, Marcello (1628–1694) Italian *Physician, Anatomist* Marcello Malpighi was one of the first scientists to study anatomy at the microscopic level. He made numerous discoveries, and, as a result, many anatomical structures are named in his honor.

Marcello Malpighi was born on March 10, 1628, in Crevalcore, Italy. Not much is known about his childhood. He obtained doctorates in medicine and philosophy from the University of Bologna in 1653. After accepting a teaching appointment there, he started studying anatomy and medicine. From 1656 to 1659 he was a professor of theoretical medicine at the University of Pisa. Though he disagreed with the standard teachings there, his return to the University of Bologna was due to personal reasons.

At Bologna he became proficient with microscopes and began studying tissues at the microscopic level. Malpighi carefully examined and described tissues of the spinal cord, brain, kidneys, spleen, skin, and tongue. He also made substantial contributions to the field of developmental biology by presenting detailed descriptions of developing insect larvae and animal embryos.

In 1628 a physician named William Harvey had proposed that blood circulates throughout the body by the action of the heart, which was little more than a muscular pump. This theory of blood circulation opposed teachings dating back to the Greek philosopher Aristotle. Physicians believed that blood was made in the liver, carried through the veins, and cooled by the brain. As blood traveled through the blood vessels, different body parts absorbed it as needed. Four years after William Harvey's death, Malpighi discovered factual evidence that substantiated Harvey's theory of blood circulation. Though many ridiculed Harvey for suggesting blood circulated throughout the body, Malpighi's discovery of capillaries proved Harvey correct. Malpighi had been examining lung tissue from frogs under the microscope in 1661 when he observed the small tubes. This demonstrated that blood did not pour out of the blood vessels into different parts of the body, but the blood remained enclosed. Thus, he concluded that capillaries connected arteries and veins, completing the circulation of blood.

Today, physiologists know the path of blood flow throughout the body and understand many of its important functions. The circulatory system is composed of a closed network of tubes that transport the blood that is pumped through the vessels by the heart. Arteries carry the blood away from the heart, and veins return the blood to the heart. Larger arteries branch into smaller arterioles, which lead to capillaries, the tiny vessels that connect arteries with veins. Capillaries are extremely thin, allowing gases and other molecules to easily diffuse through their walls. Oxygen and nutrients pass into the body tissues where they are utilized. Metabolic waste products such as carbon dioxide diffuse into the capillaries, which then converge into slightly larger vessels called venules. The venules lead to veins, which carry the waste materials away from the body's tissues. Because oxygen gives blood its red color, oxygen-rich arterial blood appears red. As the blood reaches the capillaries, the oxygen diffuses into the body tissues, thus, by the time it reaches the veins, it has lost its red color and appears blue.

Many of his colleagues at Bologna did not accept Malpighi's views and were jealous of his successes, so Malpighi accepted a professorship at the University of Messina in 1662. In Sicily, Malpighi discovered taste buds and suggested they were nerve endings. He also described the microanatomy of the brain, optic nerve, and fat tissue. In 1666 he discovered red blood cells and proposed that they were responsible for the red color of blood. Others did not understand the relevance of his research to medicine and were hesitant to denounce the "old medicine."

In early 1667 Malpighi once again returned to Bologna, where he continued his histological examinations. Under the microscope, he observed the liver, brain, spleen, kidneys, bone, and the inner layer of skin. Many structures that he first identified now bear his name. The epidermis has two main layers. The outer layer consists of dead cells that are constantly flaking off. The inner living layer that replaces these cells is called the Malpighian layer, which is subdivided into three layers: the basal layer, the spinous layer, and the granular layer. Malpighian corpuscles refer to both renal corpuscles, which are filtering structures in the nephrons of the kidneys, and to splenic lymphoid nodules or white nodules, which are masses of tissue in the spleen that contain many white blood cells and are surrounded by blood vessels. He observed the microanatomy and development of many insect larvae, the silkworm in particular. Structures of the excretory system in insects are called Malpighian tubules. He also studied the development of chick embryos (work published in 1673), and he was the first to discover human fingerprints. The Royal Society published Malpighi's comprehensive work *Anatomia Plantarum* (Plant anatomy) in 1671. One incorrect statement he made was that plants have capillaries as do animals.

In 1668 the Royal Society of London named Malpighi a foreign member, and much of his research was published as letters in the society's *Philosophical Transactions*. In 1684 his home was burned, and he lost his microscopes, books, and manuscripts. Pope Innocent XII brought him to Rome as a papal physician in 1691. During the last years of his life he taught medicine at the Papal Medical School and wrote up much of his life's work in a treatise that he gave to the Royal Society.

In 1654 Malpighi married Francesca Massari, his anatomy professor's younger sister, but she died the following year. He died in Rome on September 30, 1694. For his pioneering work on the microanatomy of tissues, Malpighi is considered the father of histology.

See also ANATOMY; CIRCULATORY SYSTEM; HARVEY, WILLIAM.

FURTHER READING

Adelmann, Howard, ed. *The Correspondence of Marcello Malpighi*. Ithaca, N.Y.: Cornell University Press, 1975.

Margulis, Lynn (1938–) American *Microbial Evolutionist* Lynn Margulis developed the symbiotic theory of evolution, which explains the relationship between prokaryotic and eukaryotic cells and describes the emergence of new species by a mechanism called symbiogenesis. Her colleagues consider her either revolutionary or eccentric. Renowned sociobiologist Edward O. Wilson has called her the most "successful synthetic thinker of modern biology," while the prestigious journal *Science* has named her "Science's unruly Earth mother." Whether or not her unconventional ideas ever become mainstream, Margulis is definitely one of today's most creative scientific theorists.

CHILDHOOD AND EDUCATION

Lynn Alexander was born on March 5, 1938, in Chicago, Illinois. She was the first of four daughters born to socialites Morris Alexander, a lawyer, and Leone, who ran a travel agency. She applied for early acceptance to the University of Chicago when she was only 14 years old and enrolled at age 16

Lynn Margulis is best known for proposing the serial endosymbiotic theory and contributing to James Lovecock's Gaia hypothesis. *(Courtesy of Chelsea Green Publishing)*

with hopes of becoming a writer. While there, she met Carl Sagan, then a graduate student in physics. Sagan later became a world famous astronomer and astrobiologist and is credited with popularizing the natural sciences. The two married in 1957, the same year Margulis received her bachelor of arts degree.

The newlyweds began their lives together working on graduate degrees at universities 90 miles apart. Carl pursued a doctorate degree in planetary science at the University of Chicago, and Lynn became a master's candidate in zoology and genetics at the University of Wisconsin at Madison, where she initiated her efforts to reconstruct evolution by studying genetics. Not one to follow convention, she began by exploring cytoplasmic genetics, a subject largely ignored by mainstream biologists. Embryologists and botanists were the first to note that non-nuclear factors of inheritance affected the use of oxygen in cellular respiration and in coloring of leaves. Offspring inherit these factors from the female parent—from the cytoplasm of the egg. Today cell biologists know that the cytoplasmic organelles mitochondria (which carry out cellular respiration) and chloroplasts (which carry out photosynthesis and give plants their green color) contain their own DNA, which encodes proteins that participate in the biochemical processes that occur within these structures. When Margulis read a comment by the American geneticist Thomas Hunt Morgan stating, "From the point of view of heredity, the cytoplasm of a cell can safely be ignored," her interest and determination to learn more about cytoplasmic inheritance intensified.

After completing her master's degree, the Sagan family moved to Oakland in 1960, when Carl accepted a postdoctoral fellowship at the University of California at Berkeley. Lynn entered the doctoral program there. In 1963 Hans Ris and Walter Plaut, Margulis's former teachers from Wisconsin, published a photograph that revealed the presence of DNA inside chloroplasts. This vivid demonstration of non-nuclear DNA surprised many biologist, but not Margulis.

She completed her doctoral dissertation research in 1963 but did not officially receive her Ph.D. in zoology until 1965 due to problems convening her doctoral committee. Her dissertation, titled "Unusual Pattern of Thymidine Incorporation in the Cytoplasm of Euglena," was published in the *Journal of Protozoology*. The family moved to Waltham, Massachusetts, where Carl accepted a job at Harvard University. They had two sons—Dorion, born in 1959, and Jeremy, born in 1960. Lynn worked part-time in labs and as a lecturer in the department of biology at Brandeis University. Her marriage ended in divorce in 1964. She accepted an adjunct position in the biology department at Boston University, where she remained until 1988. She married Thomas N. Margulis, an X-ray crystallographer, in 1967. They had two children, Zachary and Jennifer, and divorced in 1980.

SERIAL ENDOSYMBIOTIC THEORY

Margulis is most well known for her serial endosymbiotic theory (SET), which explains the origin of eukaryotic cells, providing new insight into evolutionary processes. All life-forms consist of cells, which can be divided into two major types: prokaryotic and eukaryotic. Prokaryotic cells do not contain membrane-bound nuclei and are generally less structurally complex than eukaryotic cells. Members of the domains Bacteria and Archaea are unicellular, prokaryotic organisms. Members of the domain Eukarya comprise all organisms, unicellular and multicellular, whose cells contain a nucleus, other membrane-bound organelles, and a complex cytoskeleton. Symbiosis is the living together of two or more organisms of different species. When both species benefit from the close relationship, the proper term is *mutualism,* in contrast to parasitism, when one species benefits and the other is harmed, or *commensalism,* when one species benefits and the other is neither harmed nor benefited. The term *symbiosis* is often used to imply mutualism.

In 1967 Margulis published a paper proposing an endosymbiotic origin for eukaryotic cells, "Origins of Mitosing Cells." More than a dozen scientific journals refused her submission before the *Journal of Theoretical Biology* accepted it. Margulis proposed that eukaryotic cells, which she defined as higher cells that divide by mitosis, originated from symbiotic mergers between free-living prokaryotic cells. Beginning about 2 billion years ago, thermophilic, fermenting prokaryotic organisms participated in multiple unions with other bacteria. Smaller bacteria lived inside larger bacteria and eventually developed into subcellular structures including mitochondria (the organelles responsible for cellular respiration), chloroplasts (the organelles responsible for photosynthesis), and the basal bodies of flagella (for motility). Biologists were hesitant to acknowledge her ideas initially, as Margulis did not have solid evidence to support her hypothesis. Since then, an abundance of molecular, biochemical, cytological, and paleontological evidence confirms Margulis's hypotheses regarding the origin of mitochondria and chloroplasts. Margulis's contention that an ancient spirochete contributed the genetic information for encoding the cilia or flagella found on some types of eukaryotic cells remains controversial. She published a more comprehensive account of her ideas in the 1970 book *Origin of Eukaryotic Cells: Evidence and Research Implications for a Theory of the Origin and*

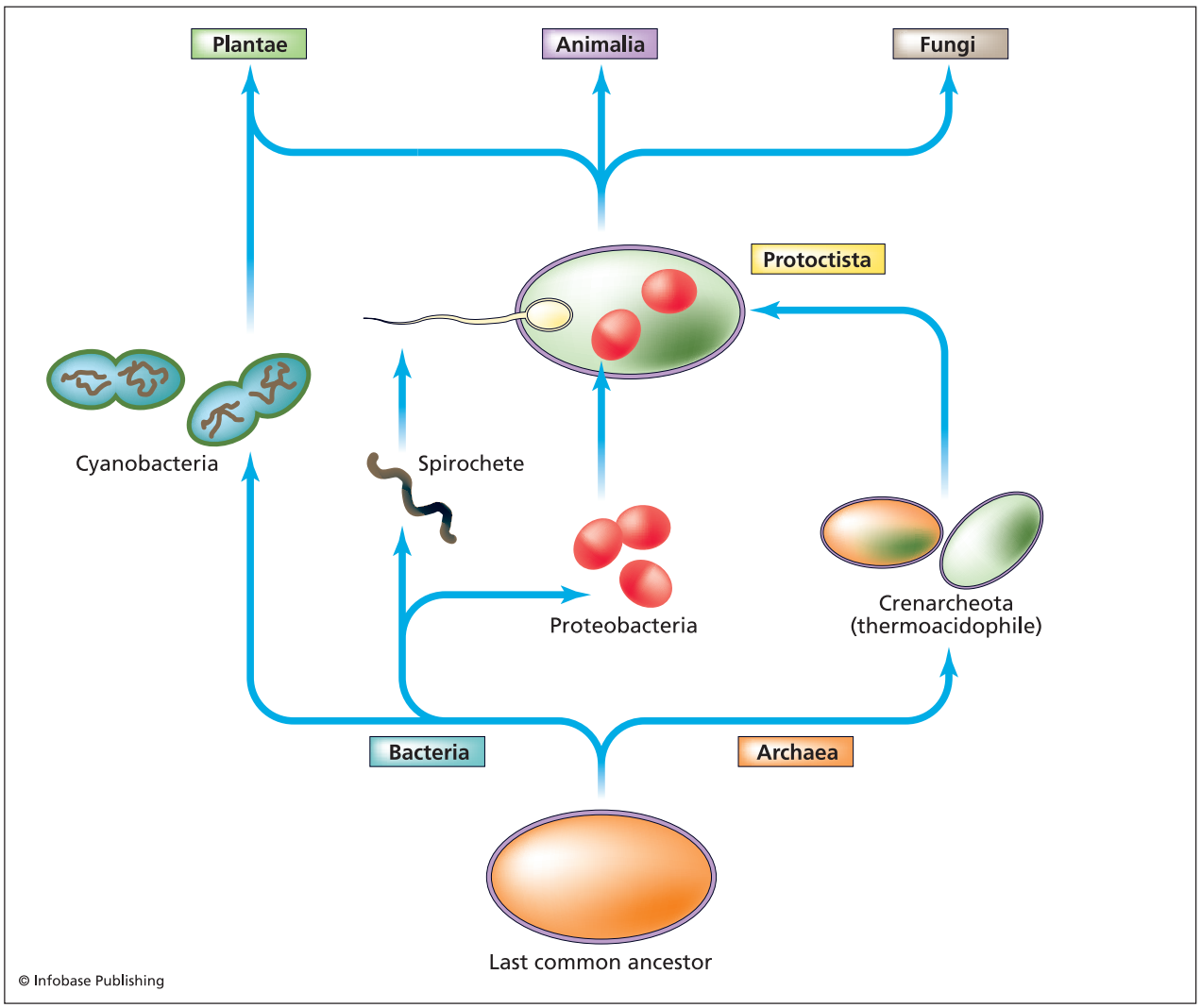
Evolution of Microbial, Plant and Animal Cells on the Precambrian Earth.

It should be noted that Margulis was not the first to propose such mechanisms to explain the emergence of eukaryotic organelles. Andreas Schimper, a 19th-century German botanist, thought that chloroplasts were the product of symbiosis. The 19th-century German pathologist Richard Altmann suggested the same for mitochondria. Russian Konstantin Merezhkovsky coined the term *symbiogenesis,* meaning the merging of two organisms into one single new organism. Others also supported this idea, but the acceptance of endosymbiosis as an evolutionary mechanism did not become mainstream until the mid to late 20th century.

Margulis's original ideas about endosymbiosis and the origin of eukaryotic cells have grown into the SET, which maintains the following: The first life-forms on Earth were prokaryotic organisms that probably resembled modern archaeans more closely than they resembled any other extant life-form. A thermoacidophilic (heat- and acid-loving) archaean and a motile bacteria, perhaps an ancient spirochete, formed a symbiosis that evolved into motile cells that contained genetic material bound within a nuclear membrane. Nuclei do not have symbiotic origins but resulted from the merger of the two types of bacteria. Margulis continues to work on the details of this development. Over millions of years, the genes that encoded the motile appendages evolved into the organizing centers and filaments that move chromosomes to opposite sides of the cell after DNA replication and during cell division. These early ancestral eukaryotes then ingested cells capable of aerobic respiration. The endosymbionts grew and multiplied inside the host symbionts, eventually developing into the cellular organelles called mitochondria. These evolved into the ancestor from which the protozoan and, eventually, animal lineages emerged. Some of the aerobic-respiring, motile, unicellular organisms engulfed photosynthetic bacteria (ancient cyanobacteria) that developed into photosynthetic plastids found in present-day algae and plants. Accordingly, SET maintains that major evolutionary changes occur as a result of multiple unions of bacterial cells.

Given her outlook on the relationships between living organisms, Margulis was bothered by the fact that most biologists were content to categorize all life-forms as an animal, a plant, or a microbe. She felt the subject of classification was inconsistent, contradictory, and difficult to teach. Formally, biologists divided the living world into five kingdoms. In 1866 the German biologist and physician Ernst Haeckel proposed a three-kingdom system (Animalia, Plantae, and Protista, with Protista including all types of microorganisms). The distinction of prokaryotic cells that lacked nuclei and eukaryotic cells that contained nuclei arose in a 1937 paper written by the French marine biologist Edouard Chatton. In 1956 the American botanist Herbert F. Copeland recognized the uniqueness of bacteria and proposed a four-kingdom classification system: Monera, Protoctista, Plantae, and Animalia. Copeland's new kingdom Protoctista included all the protozoans, some algae, slime molds, and fungi. In 1969 the American ecologist Robert H. Whittaker proposed a classification system based mainly on cell type and nutritional modes: Monera, consisting of all prokaryotic organisms; Protoctista, consisting of organisms that were unicellular but eukaryotic; Fungi, consisting of multicellular saprophytic (obtaining nourishment from the breakdown and decay of organic products) organisms; Plantae, consisting of multicellular autotrophic organisms; and Animalia, consisting of multicellular heterotrophs. Whittaker's five-kingdom classification system became standard in biology textbooks. Fungi, some algae, slime molds, unicellular organisms such as protozoans, and all prokaryotic cells fall under the realm of microbiology, however, and Margulis felt this informal grouping of all the microbes misrepresented evolutionary history. With this in mind, she set out to write *Five Kingdoms* with Karlene Schwartz, who is now a biology professor at Boston University.

Five Kingdoms presents an evolutionary perspective of taxonomy. The third edition (1998) includes 96 phyla distributed among the five kingdoms. Margulis and Schwartz organized life-forms onto a tree of life consisting of three major levels. The bacteria, defined as cells without nuclei (though now this definition includes both bacteria and archaeans) exist at the bottom level. They are distinct not only in that they lack nuclei, but in that they did not result from stable symbiotic associations that led to evolutionary change. Bacteria are the basic units of life, and members of the other four kingdoms are simply composites that resulted from multiple unions of different bacteria. Protoctista includes all unicellular eukaryotic microorganisms that emerged from symbiotic relationships that resulted in cellular nuclei that divided by mitosis and mitochondria. They stipulate in their book that this kingdom included algae, kelps, amoebas, diatoms, and slime molds. The last three kingdoms branch from the top of the tree of life, all having emerged from the early protoctists. They are distinguished by their ecological strategies: Fungi absorb decaying organic matter, Animalia ingest their nutrition, and Plantae are primary producers. The scientific community readily accepted the text. Noted ecologist and evolutionary biologist Francisco Ayala from the University of California said of the book, "The book is current and comprehensive, not



In *Five Kingdoms,* Margulis and Schwartz suggested that the five kingdoms of life can be organized into three hierarchies: Bacteria, which form the basic unit of life; Protoctista, which emerged as a result of bacteria that formed symbioses and eventually merged into single heritable units; and the more complex Fungi, Plantae, and Animalia, which resulted from multiple symbiogenetic events.

because it lists every species and genus . . . but in the artful combination of illustrative taxa and relevant biology." In 1999 *American Scientist* declared it to be "One of the 100 (or so) books that shaped a century of science."

Around the time of the first edition's publication, American microbiologist Carl Woese was beginning his campaign to divide all prokaryotic cells into two distinct domains, that is, taxonomic categories higher than kingdoms. Woese based his proposal of a three-domain (Archaea, Bacteria, and Eukarya) system for classification on differences in the sequences for small ribosomal RNA, following a neo-Darwinist perspective for the progression of evolution, something to which Margulis does not give much consideration. The molecular data demonstrated that the bacteria and archaeans differed from each other as much as or more significantly than either group differed from eukaryotes. Margulis disagrees, believing that, having formed by symbiogenesis, eukaryotic cells differ much more significantly from prokaryotic cells than bacteria and archaea do from each other. One point they do agree on is that prokaryotes dominate the history of life on Earth.

Scientists generally accept that symbiosis played a major role in the development of eukaryotic cells. Margulis's work also stimulated a renewed interest in other symbiotic relationships. For example, almost all herbivorous animals have microorganisms living inside their digestive tract to help break down cellulose, a component of plant cell walls. Some species of slugs, such as *Elysia viridis,* do not need to eat

during their adult life due to the symbiotic presence of inherited green algae that carry out photosynthesis from within the animal. Lichens are another classic example of symbiogenesis. Approximately 25,000 "species" of lichens exist; they are symbiotic associations of fungi with either green algae or cyanobacteria. The fungi and its photosynthetic partner live and reproduce as a single morphological and functional unit.

A less well-accepted component of Margulis's proposed scheme for evolution is that competition is secondary to symbiosis as a driving force of evolution. Since the British naturalist Charles Darwin published *On the Origin of Species* in 1859, evolutionists have subscribed to the view that competition for limited resources favors those organisms that are best adapted to a particular environment. Such organisms have a higher fitness, meaning they reproduce more successfully, passing on their beneficial adaptations to their offspring. Genetic variation resulting from random mutation and recombination events provides the raw material upon which natural selection acts. Margulis proposes that symbiosis provides more fuel to the evolutionary engine than the neo-Darwinist mechanism of arbitrary genetic mutation. Darwinian evolution suggests that speciation occurs as numerous genetic changes accumulate to the point at which the new species can no longer reproduce with its ancestral lineage. Margulis claims that new species arise from the acquisition and inheritance of entire new genomes. Evolution results not simply from a struggle for existence and competition for resources, but from symbiotic organisms working together successfully and forming a permanent merger.

Margulis put forth her theory of species evolution in *Acquiring Genomes: A Theory of the Origins of Species* (coauthored with her son, Dorion Sagan), published in 2002. She claims that speciation occurs by the merger and acquisition of gene sets during prolonged symbiotic associations between different organisms. She criticizes neo-Darwinism by declaring that evolution cannot depend on the gradual accumulation of random genetic variations alone. Rather, selection acts upon the symbiotic association as a single body. The book describes numerous well-documented examples of symbiogenesis to thwart critics from arguing that though some symbiotic associations do seem prolonged or have resulted in the development of some lower species, symbiogenesis is not an important evolutionary mechanism for larger species such as mammals. Some critics have claimed that Margulis and Sagan's statements are overinflated. For example, one of the 20th century's leading evolutionary biologists, Ernst Mayr, wrote in the foreward of the book, "There is no indication that any of the 10,000 species of birds or the 4,500 species of mammals originated by symbiogenesis," and he cautioned that the authors "sometimes arrive at interpretations others of us find arguable." Other critics, such as the German biologist Axel Meyer, have called the authors' claims exaggerated, admitting that some species may have originated by symbiosis but stating that "these events only account for some of the exceptions to the rule." Margulis and Sagan present individuals of a species as consortiums or communities of multiple species. Just as cooperation between species is necessary for the health of an ecological community, so are the mutually beneficial interactions between the species living in close association within an individual. Though biology has focused on genes as the units of inheritance, Margulis and Sagan demonstrate that associations can also be passed on from one generation to the next, and this can ultimately result in the assimilation of the genome of one organism into another's. They argue that acquired microbes are the units of inheritance, or the raw material of evolutionary change. The inheritance of the acquired microbial genomes is what eventually gave rise to the numerous diverse and complex life-forms present today.

Scientists have observed symbiotic relationships that support Margulis's proposal that symbiosis drives evolution. For example, certain bacteria live within aphid cells. The bacterial genome contains genes that the aphids need but cannot synthesize on their own. The aphids supply raw materials that the bacteria need to synthesize their cell membranes. Neither can live independently of the other. Evidence of organisms acquiring genomes from other organisms also exists. For example, certain types of viruses insert their genomes directly into the host chromosomes, and then when the host replicates its genome, the viral genome is also replicated.

GAIA HYPOTHESIS

James Lovecock, an English atmospheric chemist, first concocted the comparison of the planet Earth to one enormous living system in the 1960s. He claimed, in the early 1970s, that living organisms optimized the environment they inhabited, a notion that biologists ridiculed. Lovecock also observed that Earth's methane levels were 30 orders of magnitude higher than physical and chemical forces alone predicted. The presence of oxygen should have prevented such accumulation by reacting with the methane to form carbon dioxide. Lovecock asked Margulis if biological processes might contribute to the maintenance of measurable levels of methane in the atmosphere. Being aware of the diverse metabolisms of different types of bacteria, she responded that certain bacteria that inhabit cow rumens, swamps, sludge from sewage, and the intestinal tracts of animals could very

well play a role. These bacteria, called methanogens, constantly released methane into the atmosphere, so despite its rapid conversion to carbon dioxide, the levels would remain detectable. Their discussions led to the collaborative 1975 publication "The Atmosphere as Circulatory System of the Biosphere: The Gaia Hypothesis" in *CoEvolution Quarterly*.

Gaia was the ancient Greek goddess of Earth. The Gaia hypothesis equated the planet, including all of its living (biotic) and nonliving (abiotic) components, to one self-regulating living system. According to the Gaia hypothesis, the surface of the Earth functions like a physiological system, giving the planet lifelike properties, such as the ability to regulate the atmospheric composition, the temperature, and the pH of the oceans, with the goal of sustaining the biosphere, the portion of Earth consisting of living organisms. Lovecock did not claim Earth was an organism, as the Earth did not bring in or make its own nutrition as organisms do. Rather, Gaia comprises a network of interacting ecosystems that function to recycle matter on a global scale. The activities of the living organisms regulate the abiotic components of the Earth, such as the surface rock and aspects of the atmosphere, which in turn supports life. One of Margulis's former graduate students, Greg Hinkle, once described Gaia as symbiosis as seen from space, an analogy that reflects the physical association of all living organisms on Earth, since they are all in contact with the same air and water. Many scientists thought this proposed likeness was too spiritual with its goddess implication, or at least too metaphorical. Margulis contradicts this, stating that "My Gaia is no vague notion of a Mother Earth who nurtures us. The Gaia hypothesis is a science." While Margulis does not actively research Gaia, she provided the biological support for Lovecock.

HONORS AND ACHIEVEMENTS

Over a period of 22 years at Boston University, Margulis made her way up the ranks to full professor. In 1989 she joined the department of botany at the University of Massachusetts at Amherst, where she currently is a distinguished university professor in the department of geosciences. The U.S. National Academy of Sciences elected her to membership in 1983, the Russian Academy of Natural Sciences in 1997, and the American Academy of Arts and Sciences in 1998. President William Clinton presented her with the National Medal of Science in 1999, and the Library of Congress archives her papers.

In addition to the books already mentioned, Margulis has coauthored several books with her son Dorion Sagan: *Dazzle Gradually*; *Slanted Truths*; *Microbial Communities in the Archaean and Proterozoic Eons*; *Origins of Sex: Three Billion Years of Genetic Recombination*; *Microcosmos: Four Billion Years of Evolution from Our Microbial Ancestors*; *What Is Life?*; and *What Is Sex?* She has published many others as well: *Symbiosis in Cell Evolution*; *Mind, Life and Universe: Conversations with Great Scientists of Our Time* (coauthored with Eduardo Punset); and *Early Life*. Her most recent book is a novel titled *Luminous Fish*, a fictional portrayal of the lives of scientists, their relationships, their dedication to their research, and other characteristics she has found to be common among scientists.

Margulis has spent much of her career defending her unconventional ideas, but, as a result of her steadfast determination and optimistic attitude, cell biologists now accept two of three postulates to her SET—those for the origin of mitochondria and photosynthetic plastids. Her research currently focuses on the symbiotic relationships involving spirochetes, which she hypothesizes developed into structures that function in movement (such as cilia and flagella). She predicts that the current biological focus on DNA as the fundamental unit of evolution will give way to the concept of bacteria as the unit of evolution. Knowing that new ideas in science typically generate criticism, Margulis remains optimistic in the face of constant criticism and judgment by her peers. Her characteristic need to question that which others assume continues to force other scientists to rethink previously accepted "facts," which benefits science, by its very nature.

See also ARCHAEA; BACTERIA (EUBACTERIA); COMMUNITY ECOLOGY; EUKARYA; EUKARYOTIC CELLS; EVOLUTION, THEORY OF; HISTORY OF LIFE; PROKARYOTIC CELLS.

FURTHER READING

Harding, Stephan. *Animate Earth: Science, Intuition, and Gaia*. White River Junction, Vt.: Chelsea Green, 2006.

Margulis, Lynn. *Luminous Fish*. White River Junction, Vt.: Chelsea Green, 2007.

———. *The Origin of Eukaryotic Cells: Evidence and Research Implications for a Theory of the Origin and Evolution of Microbial, Plant and Animal Cells on the Precambrian Earth*. New Haven, Conn.: Yale University Press, 1970.

———. *Symbiotic Planet: A New Look at Evolution*. New York: Basic Books, 1998.

Margulis, Lynn, and Dorion Sagan. *Acquiring Genomes: A Theory of the Origins of Species*. New York: Basic Books, 2002.

———. *What Is Life?* New York: Simon & Schuster, 1995.

Margulis, Lynn, and Karlene V. Schwartz. *Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth*. 3rd ed. New York: W. H. Freeman, 1998.

Sagan, Lynn. "On the Origins of Mitosing Cells." *Journal of Theoretical Biology* 14, no. 3 (March 1967): 255–274.

marine biology Marine biology is the subdiscipline of biology concerned with life-forms that inhabit the sea. Water covers more than 71 percent of the surface of Earth, giving it the nickname the blue planet. More than 90 percent of Earth's living space is in the ocean, and until approximately 500 million years ago, all life-forms existed there. The health of the planet and its inhabitants depends on the cycling of water and the minerals and nutrients it carries.

Whereas marine science includes all of the sciences as they relate to the sea, marine biology is the study of organisms that live in the sea. However, in order to understand all aspects of a particular life-form, a life scientist, including a marine biologist, must also examine both the abiotic and the biotic factors of the environment. A marine animal may have specific adaptations that allow it to thrive in the deep ocean in the absence of light and in colder temperatures than animals that live in shallow waters. The oceans contribute to global air circulation patterns and the climate of different geographical areas, which in turn affect the life-forms that inhabit different areas. Thus a marine biologist must study not only biology, but have a basic understanding of other natural sciences, including physical science and Earth science.

Marine life encompasses a huge variety of life-forms, representing all of the domains and kingdoms of life. Prokaryotic life, including both archaean and bacterial, is abundant in the sea. Phytoplantkon that drift near the surface of many large water bodies consists mostly of photosynthetic bacteria and algae, primary producers that form the foundation of many marine food chains. Zooplankton are often slightly larger than phytoplankton and include protozoans and small metazoans (multicellular organisms) upon which larger marine life-forms feed. Plants are rare undersea, but many large seaweeds, a type of macroscopic algae, fill a similar ecological function in the sea as plants do on land—as primary producers, organisms that are autotrophic, meaning they can make organic molecules from inorganic carbon

The ocean is home to numerous diverse species. Here, a moray eel (lower left) peruses a coral reef, where assorted fish and a hawksbill turtle hunt for food. *(Alexis Rosenfeld/Photo Researchers, Inc.)*

sources. Marine plants, like seagrasses, typically inhabit shallow waters, and members of the kingdom Fungi are also found in the sea. Marine invertebrates include diverse animals such as sponges, corals, shellfish, squids, jellyfish, shrimp, worms, and sea cucumbers. Of the vertebrate animals, certainly countless species of fish live in the oceans, but so do mammals (such as whales, dolphins, and seals), and reptiles (sea turtles and marine crocodiles). Amphibians are conspicuously absent from marine ecosystems, but marine life does include many seabirds (such as gulls, albatross, or penguins) that depend on marine animals for food.

MARINE BIOLOGICAL RESEARCH

To become an independent researcher, a marine biologist must have a graduate degree, and most likely a doctorate degree. A variety of employment opportunities are available for people trained in marine biology. Governmental agencies, state fisheries, natural history museums, commercial industries, zoos and aquariums, water parks, nonprofit conservation or biological advocacy organizations, and research institutions all hire marine biologists. Research in this field covers a broad spectrum of topics ranging from microbiology to invertebrate zoology and at all levels of biological organization from the molecular and cellular to the physiological and ecological. A marine biologist might examine the distribution of populations at different depths or compare phylogenic relationships between different species of fish or the burrowing activities of marine worms and clams to see how their behavior affects the decomposition of organic materials in the sediment. A consulting firm might hire a marine ecologist to develop, implement, and monitor strategies to improve conservation efforts in a coastal tourist community. A commercial fishery might hire an ichthyologist to identify and keep records on numbers of different species caught onboard a fishing vessel. In order to examine the impact of sea-level rise on saline wetlands, a marine biologist might collect and analyze samples to observe changes in vegetation in saline wetlands in response to sea-level rise.

Some marine organisms are good model organisms for studying aspects of biology that are relevant to all types of life forms. For example, embryologists and students have learned much about fertilization, early animal development, and the molecular regulation of development by studying sea urchins. The phenomenon of cellular immunity, important to health and medicine, was first discovered in starfish. Studies of the giant squid, which has unusually large neurons, have advanced knowledge of nervous impulse transmission. Unexpected discoveries in marine science have shed light on other areas including evolution and the history of life. The discovery of life near hydrothermal vents in the ocean floor has suggested mechanisms by which ancient life-forms may have obtained energy and nutrients in the harsh conditions present on the early Earth. Biotechnology has also benefited from unexpected marine discoveries. Genes cloned from bacteria and dinoflagellates that glow, or emit light, serve as molecular markers for following gene expression.

BRIEF HISTORY OF MARINE BIOLOGY

Early marine studies involved pulling nets through the water behind a boat or dragging buckets of mud from the seafloor to the surface. Introduced in the early 1700s, the diving bell, a large container open at the bottom and filled with pressurized air supplied from leather hoses, allowed men to work in shallow water for short periods of time. A century later, diving suits that were connected via hoses to an air supply aboard a ship increased the mobility of a submerged person. In the 1940s, the introduction of scuba gear freed divers from the restrictions of any cable or connections, allowing horizontal and vertical movement within a safe range of depths. Because water pressure on the body increases with depth, divers whose bodies were not protected in an enclosed pressure-controlled environment could descend only about 100 feet (30 m). When submersible vehicles, such as the bathysphere and bathyscaph, allowed men to dive much deeper, explorers were amazed at the previously unseen colorful scenery and abundance of interesting organisms. Today, scientists skillfully maneuver remote-controlled robots that have real-time video capabilities for recording observations and coordinated mechanical arms for recovering samples from the bottom of the ocean floor from the safety of a research vessel. Sonar reveals information about the seabed, and photographs taken from satellites in space expose marine geological formations.

The Scottish naturalist Sir C. Wyville Thomson directed the first purely scientific oceanographic expedition aboard the HMS *Challenger* from 1872 to 1876. His crew systematically collected massive amounts of biological, chemical, and geological data from the world's oceans and discovered thousands of new species, giving rise to oceanography as a new field of science. Methods for studying the depths of the oceans were limited to unsophisticated techniques such as dredging and trawling, but man yearned to venture into the mysterious deeps for direct observation. In the early 1930s, the American zoologist William Beebe was one of the first scientists to watch the unique marine life forms in their own environment by dangling from a ship in a hollow steel bathysphere. As more information about the sea became available, in a movement led by

Henry Bigelow, marine scientists began to recognize the need for comprehensive physical, biological, and chemical analysis in understanding the oceans and maintaining their health.

Technological advances opened up new possibilities for undersea exploration. Frenchman Jacques-Yves Cousteau invented an underwater breathing device (SCUBA) that allowed divers to move about freely, and he popularized marine biology by improving underwater photography, bringing vivid imagery of underwater scenery into people's homes. Publicity given to underwater exploration by explorers such as Cousteau and Beebe piqued the interest of countless others, inspiring many to join the ranks of marine scientists. The wonders of the ocean abyss inspired Eugenie Clark to become a world-renowned ichthyologist and the first to study the behavior of sharks. Also interested in marine science from an early age, Sylvia Earle has used SCUBA as an integral part of her research program to study algae, aquatic ecology, whales, and even underwater habitation by humans. She has increased societal awareness of the need to preserve the oceans and its shores through her projects, writing, and media involvement. Continued advancements in deep-sea exploration technology enabled Robert Ballard to discover oases of life on the otherwise barren ocean floor, research that has contributed to understanding the evolution of the Earth and its inhabitants.

See also Beebe, William; Bigelow, Henry; Clark, Eugenie; Earle, Sylvia; Thomson, Sir C. Wyville.

FURTHER READING

Herdman, Sir William A. *Founders of Oceanography and Their Work: An Introduction to the Science of the Sea.* New York: Longmans, Green, & Company, 1923.

Reed, Christina. *Marine Science: Decade by Decade.* New York: Facts On File, 2007.

Woods Hole Oceanographic Institution (WHOI) home page. Available online. URL: http://www.whoi.edu/. Accessed January 22, 2008.

Yount, Lisa. *Modern Marine Science: Exploring the Deep.* New York: Chelsea House, 2006.

McCarty, Maclyn (1911–2005) American *Bacteriologist, Biochemist, and Geneticist* Maclyn McCarty was part of the team from the Rockefeller Institute for Medical Research Hospital (now part of Rockefeller University) that demonstrated genetic information was carried on DNA, findings that initiated the era of molecular biology in 1944.

Maclyn McCarty was born in South Bend, Indiana, on June 9, 1911. His family moved frequently due to his father's occupation. Though moving around interrupted his schooling many times, McCarty was a good student and decided on a career in medical research even before entering junior high school. He set up a chemistry lab in his basement and, with three friends, McCarty formed the Amateur Research Chemists Club during his high school years. He graduated from Kenosha High School in Wisconsin in 1929. He attended Stanford University as a biochemistry major and learned how to extract, fractionate, and analyze biochemical preparations while engaged in a senior project examining the effect of a high protein diet on liver size in rats. McCarty broadened his laboratory experiences while in medical school at Johns Hopkins University by purifying heparin preparations. After obtaining his medical degree in 1937, he interned for three years at the pediatric unit of the Johns Hopkins Hospital. McCarty studied pneumococcal pneumonia cases and gained valuable experience performing serological typing of bacterial pneumococcal strains. At the time, pneumonia was a leading cause of death and the age of antibiotics still lay in the future.

Research positions were scarce in the years leading up to World War II. In 1940 McCarty took a job examining the effect of sulfonamide drugs on pneumococcal infections in Dr. William Tillet's laboratory at New York University. Tillet helped arrange for McCarty to join Oswald Avery's laboratory at the Rockefeller Research Institute as a fellow of the National Research Council in September 1941.

Avery's lab had been studying the nature of genetic transformation since Frederick Griffith first described the phenomenon in 1928. Crude extracts prepared from a heat-killed, virulent, smooth (S) strain of *Streptococcus pneumoniae* transformed a nonvirulent, rough (R) strain into a virulent, smooth strain. When McCarty joined the project, Colin MacLeod and Avery were working to improve the reproducibility of their extract fractionation procedure. McCarty quickly found an aspect of the project that required his biochemical expertise. McCarty's major contributions to the project included preparing an enzyme that would break down DNA and purifying the transforming material from the crude extract made from the virulent strain. He prepared bacterial extracts and used enzymes to destroy classes of biomolecules, including proteins, polysaccharides, and RNA. When treated in this manner, the transformation activity was not significantly affected. When he used an extract treated with an enzyme known to degrade calf thymus DNA, the transforming activity was destroyed. These results were surprising because not much was known about the biological activity of DNA, and most scientists believed protein was the molecular carrier of genes. Also, the struc-

ture of DNA was not yet known, and most people believed it was too simple for the task of carrying genetic information. The Rockefeller team collected sufficient evidence to verify the identification of DNA as the transforming substance. They subsequently delineated a protocol for purifying their DNA preparations so they were free of all other components and performed detailed assays to demonstrate all proteins, carbohydrates, and RNA were eliminated. In addition, serological tests confirmed the absence of antigenic proteins and polysaccharides from pneumococcus. In 1944, just a few years after McCarty joined the lab, Avery, MacLeod, and McCarty published a landmark paper showing that DNA was the genetic material.

While at the Rockefeller Institute Hospital from 1942 to 1946, McCarty served as a lieutenant commander in the U.S. Naval Reserve, assigned to active duty at the Rockefeller Institute Hospital Naval Medical Research Unit. Rockefeller appointed McCarty head of the Laboratory of Bacteriology and Immunology at Rockefeller in 1946. Research from his lab led to the identification of the structural components of the cell wall of streptococcal bacteria and a better understanding of rheumatic fever, a disease caused by group A *Streptococcus* that leads to destruction of the heart valve. McCarty also served as a vice president of the university (1965–78), physician in chief at the Rockefeller University Hospital (1960–74), and board chairman of the Public Health Research Institute of the City of New York (1985–92).

Though no one from the Rockefeller team that demonstrated genes were made of DNA ever received a Nobel Prize, in 1994 the Lasker Foundation honored McCarty with the Albert Lasker Special Achievement Award in Medical Science, possibly the highest scientific honor in the United States. The National Academy of Sciences elected him to membership in 1963.

McCarty's first marriage to Anita Alleyen Davies ended in divorce, and he married Marjorie Fried. He had two sons, Richard and Colin, and one daughter, Dale. McCarty died at age 93 on January 2, 2005, in New York.

See also Avery, Oswald; biomolecules; deoxyribonucleic acid (DNA); Griffith, Frederick; MacLeod, Colin Munro.

FURTHER READING

Avery, Oswald T., Colin M. MacLeod, and Maclyn McCarty. "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III." *Journal of Experimental Medicine* 79 (1944): 137–158.

McCarty, Maclyn. *The Transforming Principle: Discovering That Genes Are Made of DNA*. New York: W. W. Norton, 1985.

McClintock, Barbara (1902–1992) American *Geneticist*

Maize geneticist Barbara McClintock first described movable segments of chromosomes, or what she called controlling elements, in 1950, but nobody paid attention to her announcement. Nearly three decades later, after scientists observed the same phenomenon in several other organisms, the significance of her finding became apparent. In 1983 McClintock received the Nobel Prize in physiology or medicine for her discovery that pieces of DNA could move from one position on a chromosome to another.

CHILDHOOD

Eleanor McClintock was born on June 16, 1902, in Hartford, Connecticut. Her father, Thomas, was a new doctor, and her mother Sara, was a professional pianist, a writer, and a painter. When Eleanor was a few months old, her parents changed her name to Barbara, which they believed sounded stronger, a characteristic they already recognized in their daughter. Due to a combination of personal and financial reasons, Barbara's parents sent her to live with Thomas's sister, Carrie, and her husband William King, a fish merchant in Massachusetts. She often accompanied her uncle on his horse and carriage excursions to sell fish, and when he bought a new car she enjoyed watching him tinker with the motor.

At age six, Barbara rejoined her family, and they moved to Brooklyn, New York. Brooklyn was not very crowded in the early 1900s, and Barbara developed a love for nature while chasing butterflies in the open fields. She attended the public school, but her parents were not traditional in that they believed school should play only a minor part in their children's lives. Barbara was allowed to skip school, for example, if the conditions were favorable for ice-skating, and once, when she came home crying claiming her teacher was "ugly inside and out," her parents let her stay home for the rest of the school year. Thomas and Sara McClintock noticed that Barbara was different from other girls; she was a thinker and loved to play sports with the neighborhood boys. She also discovered, on her own, a way of running that Buddhist monks believed gave them extreme powers of concentration.

At Erasmus Hall High School, Barbara enjoyed math and science and performed well academically. At home, she often fought with her mother. During her 14th summer, Barbara moved out and got a job through an agency that helped teenage girls find

employment and rooms to rent. She returned home in the fall to attend school. Though her mother did not think college was an appropriate pursuit for a young woman, Barbara entered Cornell University in the fall of 1919.

At Cornell, McClintock was in her element. She loved her classes but participated in extracurricular activities as well. Her freshman women's class elected her president, she played the banjo in a jazz band, and she dated frequently. By her third year, she socialized less and immersed herself in her studies, particularly in genetics. The professor who taught her undergraduate genetics course personally invited her to take his graduate level genetics course. By the time she graduated with a bachelor of science degree in 1923, her future had been decided.

BECOMING A CYTOGENETICIST

After graduation, McClintock wanted to enroll in graduate school to study genetics, but the genetics program was part of the plant-breeding department, which did not admit women. Instead she enrolled in the botany department with plans to focus on cytology, the study of cells, but she also attended genetics classes taught by the plant-breeding department. To earn money, McClintock worked in the lab of a cytologist who studied maize (corn). Each maize cell had 10 chromosomes, the structures that carried the "heritable factors" (genes), though at the time the nature of the genes was unknown. Chromosomes are linear structures made mostly of protein and deoxyribonucleic acid (DNA), now known to be the molecular carrier of genetic information. Work out of the laboratory of Thomas Hunt Morgan, who was then at Columbia University, had demonstrated that genes existed in a linear arrangement on chromosomes. While genes have different molecular structures, at the cellular level, these differences are not apparent in the chromosomes by using standard staining techniques and viewing through the microscope. McClintock recognized this and developed her own methodology. As a result she was able to observe differences in lengths and shapes of chromosomes. Her technique enabled cytogeneticists to distinguish and identify each of the 10 maize chromosomes in order to further analyze their roles. McClintock's efforts advanced the new field of cytogenetics by helping scientists relate chromosome functions to genetic properties.

Maize is suitable for genetics research because the kernels of each ear can be one of multiple colors or textures, characteristics controlled by the maize genes. To study how traits such as these are inherited, one can control pollinations between the maize plants in the field. The pollen grains mature in anthers on the tassels, the male flowers, at the top of the cornstalk, and the embryo sacs are found within the kernels on the ears. Each kernel starts as an ovule and has its own silk that grows out of the husk at the top of the ear. Covering the ears of corn with a paper bag prior to silking prevents pollination from occurring naturally, as by wind carrying pollen grains from one plant to another. One can then collect the pollen by shaking the tassels into a clean bag. Fertilization occurs by physical transfer of the pollen to the cornsilks of an ear of a specifically chosen plant. The pollen sticks to tiny hairs on the silks, and within a few minutes, the pollen grain germinates and develops a pollen tube that grows down the silk to fertilize an ovule, which then develops into a kernel. In this manner a plant breeder can control which plants breed with which other plants to study the manner of inheritance for specific traits. McClintock performed similar experiments on maize during her graduate career. She made observations on the chromosome structures for plants with different characteristics before pollinating and in the offspring resulting from the pollinations. She studied the maize linkage groups, groups of genes that are inherited together on a single chromosome. In 1927 she received her doctorate from Cornell, but she continued working there as an instructor and a maize genetics researcher.

McClintock continued studying the individual maize chromosomes with the goal of figuring out which genes each one carried. One major finding made by McClintock, in collaboration with a graduate student and friend named Harriet Creighton, was that chromosomes exchange genetic information during cell division. Genetic recombination correlated with physical crossing of the chromosomes. Though scientists had suspected as much, McClintock and Creighton produced the first evidence demonstrating this to be true. When two genes occur along the same chromosome, a phenomenon called crossing over or recombination can separate the two so-called linked genes. If the two genes of interest each give two different distinguishing characteristics, then controlled breeding experiments allow a researcher to track crossing over events by simply observing the combinations of the characteristics in the parents and the offspring. McClintock and Creighton followed this exchange by observing the presence of unique chromosomal structural characteristics under the microscope. With the support and encouragement of Morgan, in 1931 they published "A Correlation of Cytological and Genetical Crossing-Over in *Zea mays*" in the *Proceedings of the National Academy of Sciences*. This discovery was fundamental in understanding how chromosomes carry genes and pass them on to the next generation of cells.

Despite her successes, universities were hesitant to hire a woman as a research scientist, and she was not interested in teaching at a women's college. From 1931 to 1933, with a fellowship from the National Research Council, McClintock undertook part-time research at Cornell, in addition to working at the California Institute of Technology and the University of Missouri. When her National Research Council money ran out in 1933, she obtained a Guggenheim fellowship to study at the Kaiser Wilhelm Institute in Berlin, Germany. She was appalled at the treatment of Jews in Germany, however, and was anxious to return home in 1934. With support from the Rockefeller Foundation, she was appointed a research associate at Cornell from 1934 to 1936.

As recorded in a memoir written by Nina Fedoroff, McClintock's work at Cornell led to an explosion of cytogenetic discoveries. In addition to those already mentioned, the maize group determined the physical location of many genes along the length of the chromosomes, identified the genetic consequences of improper pairing during mitosis, established that unstable ring chromosomes caused phenotypic variegation, and found that the centromere (the region where sister chromatids remain joined until the later stages of mitotic division) is divisible.

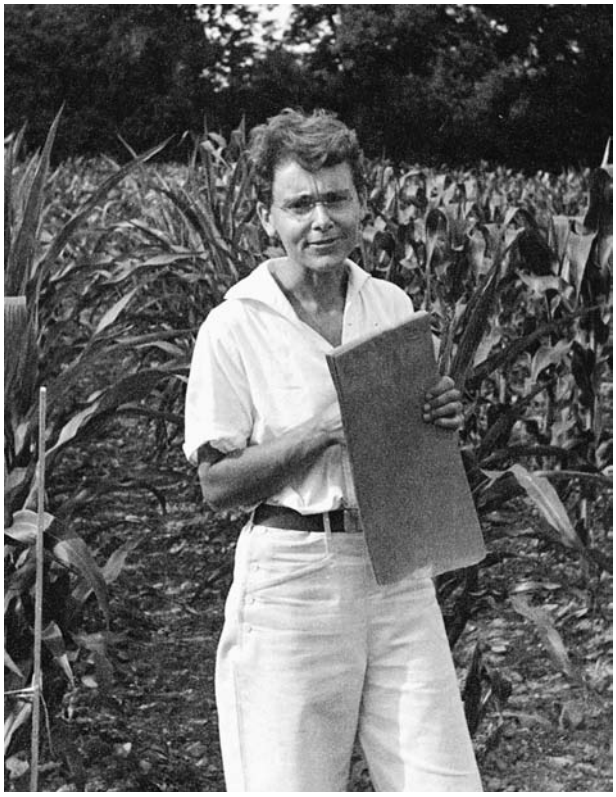
During the nine years since she had obtained her doctorate degree, McClintock examined chromosomal structural abnormalities induced by X-rays, which physically broke apart the chromosomes and led to different colors and textures in the kernels of the future generations. She also identified a region at the end of the sixth chromosome as the nucleolar organizer region based on her observation that, when broken in two, each part could form an independent nucleolus of the nucleus.

Finally, in 1936 the University of Missouri offered her an assistant professorship, though she was excluded from regular academic activities such as faculty meetings. Continuing her studies on X-ray treated plants, she noticed that the ends of broken chromosomes sometimes fused with one another, temporarily stabilizing them. They broke apart once again when the cell divided, but sometimes they appeared to heal. Her data revealed a mutant in which a broken end that normally would heal did not, suggesting a mutation inactivated an enzyme responsible for this process. McClintock hypothesized the existence of unique structures at the end of chromosomes, called telomeres, that functioned to maintain stability. Cell biologists have since intensively examined the structure and function of telomeres and linked them to cell senescence, cancer, aging, and other biological phenomena. Though McClintock was unquestionably productive, her colleagues at the University of Missouri viewed her as difficult and odd. With little chance for attaining tenure, she left in 1941.

Knowing the prospect of obtaining a full-time academic appointment at a primarily research university was unlikely, that summer she got a job working at Cold Spring Harbor Laboratory on Long Island, New York. The Carnegie Institution of Washington, which owned and operated the facility, gave her a full-time, permanent appointment as a staff member in 1942, and made her a distinguished service member in 1967. Despite McClintock's early career struggles, her publication record was outstanding and other scientists respected her. In 1944 the prestigious scientific organization the National Academy of Sciences elected her as only the third woman member, and in 1945 the Genetics Society of America elected her president (she had served as vice president in 1939).

JUMPING GENES

At Cold Spring Harbor, McClintock took up the genetic analysis of maize progeny resulting from the self-fertilization of plants that had at least one broken chromosome number nine. Such self-fertilizations often result in progeny that exhibit varied phenotypes, and the seedlings from these plants also exhibited a variety of colors ranging from the normal green to white, light green, or pale yellow. McClintock typically worked alone and performed all the tasks related to her research at Cold Spring Harbor, even the jobs normally completed by lab assistants or field workers. By spending so much time with the plants, she noticed unusual patterns in the kernel colors and in the leaf and corn stalk colors of the progeny from the parents with broken number nine chromosomes. The mutations appeared unstable within the life span of a single plant. The plants had areas of color that did not make sense, including some that caused variegation from the recessive to the dominant phenotype. Patches of color all resulted from cells that were derived from a single mutated cell. McClintock could estimate at what point during development a mutation had occurred based on the size of the patch. By close examination of the patches, which were clear evidence of mutation, she recognized a regularity, a constant rate of mutation. Constancy or regularity implied control, and until her discovery, the concept of controlling genes, such as during development, was new. The timing of a dissociation event could affect large numbers of cells if it occurred early on in the organism's development, or it could produce more localized changes if it occurred later in development. Realizing the importance of her discovery, she dropped all of her other projects and focused on elucidating the mechanism of this genetic control of



Using maize as a model organism for examining the role of chromosomes in heredity, Barbara McClintock discovered transposable elements, commonly known as jumping genes. *(Courtesy of the Cold Spring Harbor Laboratory Archives)*

development. She reported her findings in the annual report to the Carnegie Institution in 1946.

Unlike the variegation she observed as a result of X-ray treatment, these patterns seemed more orderly and occurred in pairs. For example, if one portion of a leaf seemed to have more green streaks, another portion seemed to have fewer. McClintock suspected the changes resulted from sister cells, cells formed by the division of one parent cell, and she tried to relate the patterns of variegation to changes in the chromosomes. The notion that "one cell gained what another had lost" consumed her thoughts for the next two years.

Her research efforts and perceptive analysis revealed a unique mutation that always resulted in the breakage of chromosome nine at a specific site. She called this chromosome-breaking locus *Dissociation* (*Ds*). This locus underwent dissociation mutations only when another dominant factor that she called *Activator* (*Ac*) was also present. By 1948 she had gathered enough evidence to conclude that both the controlling element (the *Ac* loci) and the dissociated element (the *Ds* loci) moved from one place to another; they physically relocated within the genome. Biologists have named this phenomenon transposition and the mobile segment a transposable genetic element. In the late 1940s McClintock had identified several mutable loci. She figured out that insertion of the mobile segments at other positions caused unstable mutations and concluded that genes were not the static entities that scientists believed they were. On the contrary, she believed pieces of the chromosomes were moving around, jumping from place to place. This apparent self-rearrangement contradicted Mendelian inheritance patterns, but her careful and detailed observations convinced her that what she saw was a real phenomenon. She thought these jumping genes explained some of the unexpected occurrences that scientists observed.

Since 1946 McClintock had been recording her observations in the *Carnegie Institution of Washington Yearbook,* but few cytogeneticists read the institution's annual reports. After conducting additional experiments to confirm her observations, McClintock wrote a paper, "The Origin and Behavior of Mutable Loci in Maize," published in the *Proceedings of the National Academy of Sciences* in 1950. Though *Proceedings* is a highly prestigious journal, the paper went largely unnoticed. In the summer of 1951 she presented her findings in a talk titled "Chromosome Organization and Genic Expression" at the Cold Spring Harbor Symposium on Quantitative Biology, expecting a favorable response. She was nervous, and rightly so, since she had to cram four years of her revolutionary research and the necessary background information into one lecture. When she was done explaining her elegant model for the genetic controlling elements, nobody in the audience asked a single question. Some admittedly did not understand what she was saying; she was a brilliant, accomplished cytogeneticist, and her experiments were complex. Others felt that the idea that genes jumped around was simply incredulous; they chose not to listen. Despite the indifference of her colleagues, she persisted in her studies on transposons.

Continued examination of genetic control in maize led to her discovery of a new element, *Suppressor-mutator* (*Spm*), which was also transposable, a characteristic that made its recognition possible. In addition, *Spm* produced some kind of substance that helped control the expression of other mutant genes. This was the first example of a trans-acting regulatory factor, something produced at one site but that acts at another. She presented this new system of regulation and control at the Cold Spring Harbor Symposium in 1956, but again, the scientific community was not ready to hear it.

Other scientists were aware that cells and organisms somehow regulated the synthesis of certain proteins, but McClintock's reports were the only pub-

lished evidence of mechanisms for achieving regulation at the genetic level until 1960, when two French scientists, Jacques Monod and François Jacob, published the first report of a molecular mechanism for regulation. A more complete English version titled "Genetic Regulatory Mechanisms in the Synthesis of Proteins" appeared in the *Journal of Molecular Biology* in 1961. McClintock was thrilled to read their account. They presented an elegant analysis of a coordinated system for gene expression. They described how one gene controlled another set of genes, called the *lac* operon, that encoded proteins involved in catabolism of the sugar lactose. Like the *Spm* system, Jacob and Monod's system consisted of two controlling elements—one positioned adjacent to a structural gene and in control of its action, and the other indirectly controlling the gene. Her controlling elements were transposable, whereas theirs were not, but to McClintock, that fact was irrelevant in the operation of the system—it was simply a characteristic that facilitated their discovery.

The French scientists participated in the Cold Spring Harbor Laboratory Symposium in 1961, shortly after their paper appeared in English. They mentioned McClintock's discovery during their own presentation. McClintock also spoke at the meeting, carefully pointing out how her own work in maize related to their system. This time scientists became excited, but about the Frenchmen's discovery, not McClintock's.

Discouraged, McClintock stopped trying to present her transposon research to other scientists. She did continue her experiments and documenting her findings, however. Her data suggested that not only did genes relocate, but also that they could act differently or serve different roles based on their relative position. McClintock thought that stress from poor environmental conditions could induce such changes. Molecular biologists are still trying to figure out what mechanisms trigger the movement of mobile genetic elements.

Meanwhile, since McClintock's initial publication regarding transposons, biologists had clearly demonstrated that DNA was the carrier of heritable traits and solved its molecule structure. An understanding of how DNA replicated and encoded for traits was emerging. Researchers were figuring out how to isolate and study individual genes. A mutation in bacteria was found to be the result of the insertion of foreign DNA, and scientists were able to visualize looped structures containing the insertion. These and other studies opened the minds of scientists to the concept of mobile genetic elements.

By the end of the 1970s, other geneticists started reporting the presence of jumping genes in bacteria, yeast, and fruit flies. Evidence demonstrated that mobile genetic elements played a role in antibiotic resistance in bacteria. Whereas they had thought McClintock's findings in maize were unbelievable, or if real, then rare and therefore insignificant, the fact that the phenomenon appeared to be widespread brought long-deserved attention to McClintock's findings from decades prior. By the 1980s, scientists began to understand how transposons might explain unusual types of mutations or changes to the DNA. As time passed, the importance of transposable elements in evolution and in the development of cancer became apparent.

Today the widespread existence of mobile genetic elements is accepted as fact. Such pieces of DNA can move to an alternate site within a chromosome or onto another chromosome. The mobile elements range in size from a few hundred to tens of thousands of nucleotide base pairs in length. Some are derived from viruses that have inserted their genomes into the host's genome. These can move into or out of chromosomes in a site-specific manner, and relics left behind during this process make up a significant portion of the genomes of many organisms. Other transposable elements, called DNA-only transposons, can insert into many different sites; they are not as selective as other mobile genetic elements. Researchers have found both viral-like and DNA-only transposons in maize. The movement of transposable elements can lead to the deletion or addition of nucleotides in the region, as well as rearrange neighboring sequences. These mutations play an important role in the course of evolution.

McClintock finally began to receive accolades for the discovery she first reported in 1950. The National Academy of Sciences gave her its Kimber Genetics Award in 1967. President Nixon awarded McClintock a National Medal of Science in 1970 for "establishing the relations between inherited characters in plants and the detailed shapes of their chromosomes, and for showing that some genes are controlled by other genes within chromosomes." Harvard University, Yale University, and the University of Cambridge presented her with honorary doctor of science degrees. Between 1947 and 1981 she was presented with a total of 13 honorary degrees. In 1981 she received the first MacArthur Laureate award and the Albert Lasker Award for Basic Medical Research. McClintock did not have a telephone at home because she thought they were an annoyance. On October 10, 1983, she first heard by radio the announcement that she won the Nobel Prize in physiology or medicine. She was the first woman to win an unshared Nobel Prize in physiology or medicine and only the third woman to win an unshared Nobel in any science category.

In honor of McClintock's 90th birthday, her colleagues wrote and compiled a collection of essays

describing the influence of McClintock's work on genetics research. The result was *The Dynamic Genome*. Her friends presented this book to her at a birthday celebration held at the home of James D. Watson, one of the codiscoverers of the structure of DNA and the director of Cold Spring Harbor Laboratory. Thus, at last she received respect and appreciation for her labors and contributions to science. A few months later, on September 2, 1992, McClintock died.

The story of McClintock's discovery of jumping genes and the scientific community's slow realization of the importance of her discovery illustrates that science, even Nobel Prize–winning science, is a human endeavor, and therefore fallible. Personal characteristics of scientists play a role in how discoveries come about and how they mature from a spark of imagination to become scientific fact. Stubborn scientists, those who do not open their minds to new ideas, sometimes limit or prevent the progress in their fields. McClintock's personal confidence and faith in her abilities helped her withstand the years of ridicule and neglect. She reportedly admitted that she did not worry about how her colleagues viewed her and took solace in knowing that she was right and that others would eventually come around. Today all genetics references discuss the transposable elements McClintock discovered more than 60 years ago.

See also CHROMOSOMES; INHERITANCE; MENDEL, GREGOR; MORGAN, THOMAS HUNT.

FURTHER READING

Fedoroff, Nina V. "Barbara McClintock (June 16, 1902–September 2, 1992)." In *Biographical Memoirs. National Academy of Sciences*. Vol. 68. Washington, D.C.: National Academy Press, 1995.

Fedoroff, Nina, and David Botstein, eds. *The Dynamic Genome: Barbara McClintock's Ideas in the Century of Genetics*. New York: Cold Spring Harbor Laboratory Press, 1992.

Keller, Evelyn Fox. *A Feeling for the Organism: The Life and Work of Barbara McClintock*. San Francisco: W. H. Freeman, 1983.

Lewin, Roger. "Naturalist of the Genome." *Science* 222, no. 4622 (October 28, 1983): 402–405.

McClintock, Barbara. *The Discovery and Character of Transposable Elements: The Collected Papers (1938–1984) of Barbara McClintock*. New York: Garland, 1987.

———. "The Origin and Behavior of Mutable Loci in Maize." *Proceedings of the National Academy of Sciences* 36 (1950): 344–355.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1983." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1983/index.html. Accessed January 25, 2008.

Spangenburg, Ray, and Diane Kit Moser. *Barbara McClintock: Pioneering Geneticist*. New York: Chelsea House, 2008.

Mendel, Gregor (1822–1884) Austrian *Geneticist*

Gregor Mendel was the first to investigate systematically the transmission of traits from parents to offspring and to mathematically interpret the results. His research on the transmission of heritable traits using garden peas led to the establishment of the basic laws of heredity. He made his discoveries less than a decade after Charles Darwin published his theory of evolution by means of natural selection. Despite the fact that Mendel's work offered explanations for many unanswered questions concerning Darwin's revolutionary theory, his work went unnoticed for 35 years. When it was rediscovered, it induced a flurry of biological research examining the principles of heredity.

STUDIES NATURAL SCIENCES

Johann Mendel was born on July 22, 1822, in Heinzendorf, which then was part of the Hapsburg Empire (now Hynčice, Czech Republic). His father supported his family as a peasant farmer, and his mother instilled in her only son a love for plants. Johann, who had an older and a younger sister, showed intellectual promise at an early age. In 1833 his schoolmaster recommended he attend the more advanced Piarist secondary school at nearby Leipnik (now Lipnik), 16 miles (26 km) from home. Johann continued to excel academically, and in 1834 he enrolled at the Gymnasium in Troppau (Opava), about 22 miles (35 km) from home.

Mendel planned to become a teacher. After completing six years at the Gymnasium, he still needed to complete two years of philosophical studies before he could enroll in a university, but his parents could not afford further schooling. His younger sister offered part of her promised dowry so Johann could continue his studies. He also tutored pupils privately to earn money and entered the Philosophical Institute at Olmütz (now Olomouc) in 1841. He was particularly interested in the natural sciences. After two years, he could have enrolled at a university, but he was tired of the constant struggle to make ends meet, so he entered the Augustinian monastery of St. Thomas at Brünn (now Brno), then the capital of the province of Moravia. The recommendation from his professor was so positive that Mendel was admitted without an interview.

Johann Mendel began his novitiate in October 1843, taking the name Gregor. During the normal probationary period Mendel studied mostly classical subjects, but he pursued his studies of plants and min-

erals in his free time. He also studied agricultural sciences, as sheep breeding and fruit and wine cultivation formed the basis of the Brno economy. The abbot of the monastery, C. F. Napp, encouraged independent studies and arranged the construction of an experimental garden and a herbarium in the monastery.

In 1847 Mendel was ordained a priest. He continued to study theology until 1848, at which time he was made a chaplain of the monastery parish. Mendel, however, was too shy and sensitive to effectively minister to the sick and suffering parishioners, and he became physically ill from nerves. Napp took pity on him and appointed him to teach the classics and mathematics to seventh graders in the town of Znojmo (Znaim), in southern Moravia. Mendel performed his duties well and accepted a position as a substitute teacher at the Znojmo Gymnasium in 1849. He was a patient teacher and impressed the faculty with his efforts. In order to be appointed a regular teacher, however, Mendel had to pass a competence exam.

Obtaining a teaching certificate turned out to be Mendel's albatross. He did well on his physics and meteorology exams but failed the zoology and geology portions. Explanations for his failure included that he did not have the advantage of a university education and that he had attempted to prepare on his own while teaching a full load of courses. Despite this letdown, he had a good reputation as a teacher. The following year he substituted for an ill natural history teacher at the Brno Technical School. During his time there, Mendel became an extraordinary member for the Natural Science Section of the Agricultural Society.

When Mendel was 29 years old, Napp sent him to Vienna University to prepare further for his teacher's qualifying examination. During the period 1851–53, Mendel mostly studied physics, but also took mathematics, chemistry, zoology, botany, plant physiology, and paleontology. He was particularly interested in botany and plant hybridization, but his studies in physics and mathematics also greatly influenced his later work. In physics he discovered the simplicity of natural laws, and in mathematics he learned to use probability theory and statistical analysis.

After completing his studies at Vienna, he returned to the monastery in July 1853. It remains unknown why he did not immediately retake his teacher's qualifying exam. A post opened up at the Realschule, a nontraditional technical school, and he taught physics and natural history there for the next 14 years. Mendel also took charge of the natural science collections. His expertise of specialized flora was admired, and he often took students on botanical excursions. Mendel did attempt to retake the qualifying exam in 1855, but he failed a second time, most likely due to nerves. He continued teaching, but, without a full appointment, he earned only half-pay.

INHERITANCE IN *PISUM*

Mendel devoted his free time to conducting a series of independent studies at the monastery. In the early 1850s, he started performing artificial pollinations with *Pisum,* the ordinary garden pea, to determine how traits were passed from generation to generation. In 1854 he became a full member of the Natural Science Section of the Agricultural Society in Brno, which later evolved into the Natural Science Society. The economic aspects of plant hybridization thrust the topic into the spotlight at the society's meetings. Mendel began a series of experiments for which he sought to examine the transfer of traits between generations. The results of these experiments led to the formulation of the laws of heredity, which form the basis of the field of genetics. Mendel presented his findings during two lectures before the Natural Science Society in February and March of 1865.

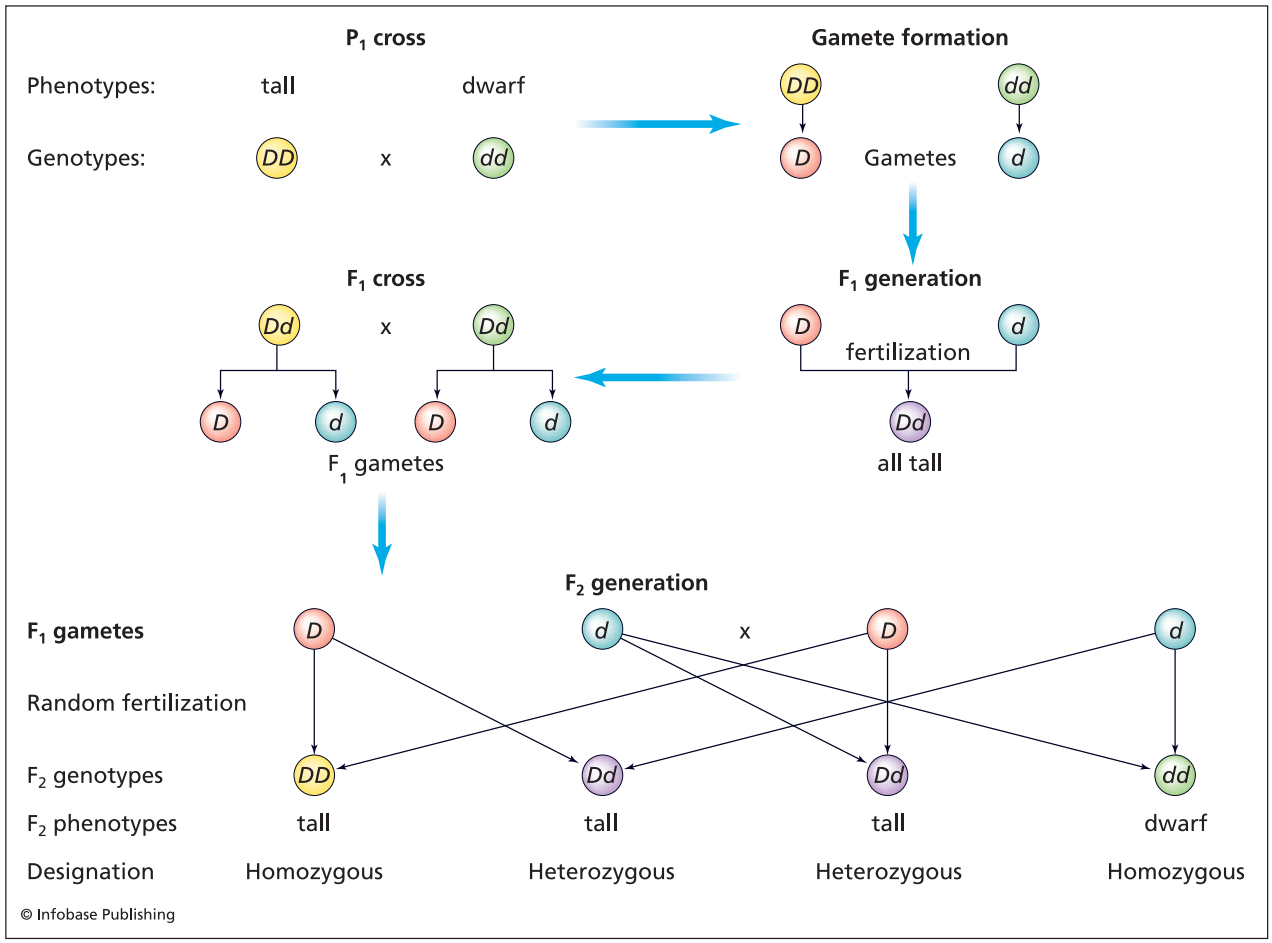
With great care, Mendel selected *Pisum* to utilize in his breeding experiments. The plants were simple

Gregor Mendel is considered the father of classical genetics. *(National Library of Medicine)*

to grow, he could easily control pollinations, they had several easily distinguishable characteristics to examine, and the hybrids were fertile. Hybrids are new varieties of plants created by crossing two distinct varieties or species. Mendel spent two years ensuring that the 34 varieties of peas he planned to use were true breeding, meaning self-pollination resulted in the constancy of specific traits generation after generation. The seven characteristics chosen for analysis were seed shape, seed color, flower color, pod shape, pod color, flower position, and stem length. Mendel also made sure to collect large amounts of data to eliminate any misleading effects due to chance. Over a seven-year period, he cultivated and studied almost 30,000 plants.

Starting with a single pair of traits, Mendel began his methodical pollinations. First, he pollinated round-seeded pea plants with pollen from wrinkle-seeded plants. All of the seeds produced were round. When he performed the reciprocal cross, that is, when he pollinated plants of wrinkled-seeded peas with pollen from round-seeded plants, the same results were observed. (The hybrid offspring from these initial crosses are often referred to as the F_1 generation.) Next, Mendel planted the round hybrid seeds and allowed them to self-fertilize. (The offspring of the hybrids are often referred to as the F_2 generation.) The self-fertilizations resulted in 5,474 round and 1,850 wrinkled seeds. Though the wrinkled seeds had seemed to vanish in the previous generation, now they reappeared. Mendel called the trait that prevailed after the first set of crosses "dominating" (now called dominant) and the trait that disappeared for a generation he called "recessive."

Mendel performed similar experiments examining the transmittance of the other six traits and obtained similar results. In all cases, one of the traits was dominant and the other recessive. More remarkably, all hybrid offspring exhibited a 3:1 ratio of the dominant to the recessive trait. These findings were important because, at the time, many scientists believed that inherited characteristics constituted an intermediate form of expression of the parental traits. This data clearly showed that the parent's traits were not blended in the hybrids.



Mendel used true-breeding *Pisum* parents to obtain hybrid F_1 offspring and then allowed the F_1 to self-fertilize in order to obtain the F_2 generation. Further controlled self-fertilizations revealed the original parental genotypes.

MENDEL'S RESULTS

Trait	Dominant Total	Recessive Total	Overall Total	Ratio Dominant : Recessive
seed shape	5,474	1,850	7,324	2.96 : 1
seed color	6,022	2,001	8,023	3.01 : 1
seed coat color	705	224	929	3.15 : 1
form of pod	882	299	1,181	2.95 : 1
pod color	428	152	580	2.82 : 1
flower position	651	207	858	3.14 : 1
stem length	787	277	1,064	2.84 : 1

Mendel continued his investigations, allowing the F_2 plants to self-fertilize. Keeping very meticulous records, he examined all the offspring of the resultant F_3 generation. In the data, he recognized that the 3:1 ratio of observed characteristics, or phenotypes, was really a disguised 1:2:1 ratio. He concluded that each trait in an individual resulted from the combination of a pair of discrete units (today called alleles). Each parent contributed one unit for each trait to the offspring. These were transmitted to the offspring by the gametes, the sex cells which fuse during fertilization. Because each parent possessed two alleles for each particular trait, there was a 50 percent chance that the offspring would inherit either specific allele from that parent.

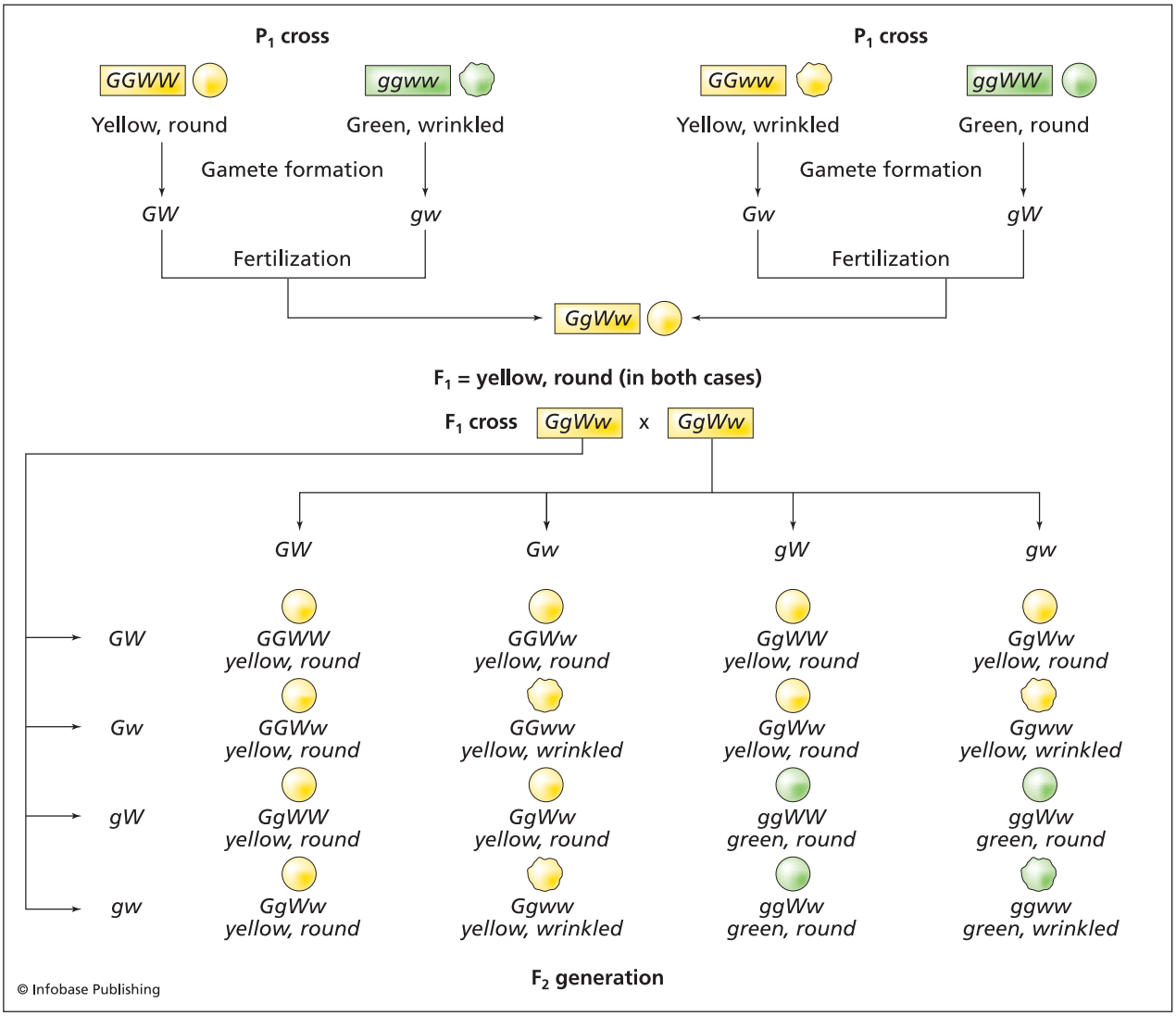
Mendel devised a method of alphabetical notation that is still used today. Each unit or allele was represented by a letter. For example, he used "*A*" for the trait of round or wrinkled seeds. The uppercase "*A*" signified the dominant allele, in this case, the allele that encoded the round phenotype. The lowercase form of the letter "*a*" symbolized the recessive form of the trait. Thus, each individual organism contained a pair of alleles that together determined whether the seeds would be round or wrinkled. An individual might possess one of three possible genotypes, or combinations of alleles: *AA, Aa,* or *aa*. The two combinations that have two of the same alleles (*AA* and *aa*) are called homozygous genotypes, whereas the combination of the two different alleles (*Aa*) is termed heterozygous. The heterozygous genotype leads to the dominant phenotype. In other words, plants with the *Aa* genotype will have round seeds.

Using this notation and terminology, it is easier to describe what happened in Mendel's pea crosses. He started with true breeding plants, meaning the genotypes were homozygous. When he crossed the true breeding parents with each other (*AA* × *aa*), all the offspring inherited one dominant and one recessive allele. Thus, all the hybrid offspring in the F_1 generation were heterozygous, *Aa,* and had the round phenotype.

In the second set of crosses, Mendel used the heterozygous F_1s as the parents (*Aa* × *Aa*). Since these individuals possessed both alleles, they were capable of contributing either the dominant or the recessive allele to their offspring. This led to three possible genotypes in the F_2 generation: *AA, Aa,* or *aa*. Both the *AA* and *Aa* genotypes encoded the round phenotype, but the combination of two recessive alleles allowed the recessive phenotype of wrinkled seeds to reappear after skipping one generation. The phenotypic ratio of offspring in the F_2 was 3 round: 1 wrinkled. Mendel had the remarkable insight to recognize a 1 *AA*: 2 *Aa*: 1 *aa* genotypic ratio hidden within this 3:1 phenotypic ratio.

To verify the genotypes, Mendel performed backcrosses of the hybrids with the recessive true-breeding parent. A backcross is a cross of an individual with one of its parents or another organism with the same genotype as one of its parents. Since the recessive true-breeding parent could contribute only the recessive allele to its offspring, the appearance of any offspring with the recessive phenotype necessitated a heterozygous parent. He also performed reciprocal crosses, meaning the genotype of the female in the first cross was presented as the genotype of the male parent in the second cross. It did not matter which phenotype (or genotype) was used to pollinate, the results were always the same. From this series of experiments, he formulated the law of segregation, which states that the allele pairs separate during gamete formation and then randomly reform pairs during the fusion of gametes at fertilization.

Mendel next wondered what the result would be if he examined two traits at the same time. He started



The 9:3:3:1 phenotypic ratio of a typical dihybrid cross demonstrates that alleles for one gene segregate independently of alleles for another gene.

by crossing plants that bred true for both seed shape and color. Round and yellow (*AABB*) were crossed with wrinkled and green (*aabb*). As expected, considering the F_1 genotypes must have been *AaBb*, all of the offspring exhibited the dominant phenotypes of being round and yellow. These offspring, which were heterozygous for both traits, were allowed to self-fertilize. This sort of cross is called a dihybrid cross. When Mendel performed this experiment, he obtained four different phenotypes in the F_2 generation occurring in the ratio of 9 round and yellow: 3 round and green: 3 wrinkled and yellow: 1 wrinkled and green. When looked at separately, the two traits still exhibited a 3:1 phenotypic ratio.

In order to gain information concerning the genotypes of the F_2 generation, he allowed them to self-fertilize. From this experiment, he determined that there were nine different genotypes in the F_2 occurring in the ratio of 1 *AABB*: 2 *AABb* : 1 *Aabb*: 2 *AaBB*: 4 *AaBb*: 2 *Aabb*: 1 *aaBB*: 2 *aaBb*: 1 *aabb*. Each trait independently exhibited the same behavior as when monohybrid crosses were performed. The transmittance of the alleles for one trait had no effect on the transmittance of the alleles for a second trait. He performed similar experiments pairing all of the different traits with one another. Each time the traits segregated into a 9:3:3:1 phenotypic ratio and a 1:2:1:2:4:2:1:2:1 genotypic ratio in the F_2. From these observations, Mendel formulated what is now known as the law of independent assortment. The law of independent assortment states that each allele pair segregates independently during gamete formation. Today we know this law applies only when the two traits being examined are located on different

chromosomes or are sufficiently distant from one another on the same chromosome.

The lectures Mendel presented in 1865 were published the following year in the Natural Science Society's *Proceedings*. Neither the lectures nor his paper aroused much interest. The fact that this was a rather obscure journal contributed to the fact that it went largely unnoticed following its publication. In addition, neither the members of the society nor the rest of the world were intellectually prepared for the mathematical methodology or far-reaching implications of Mendel's research. Historians know that the evolutionary theorist Charles Darwin received a copy of the paper, but he probably never read it.

The powerful deductions contained in the paper, "Experiments with Plant Hybrids," are summarized as follows. Hereditary determinants are individually distinct in nature. Today these determinants are known as genes. Genes exist in pairs. Each adult plant carries two copies, or alleles, of each gene for a specific trait. The alleles may be two types, dominant or recessive. An individual may have two alleles of the same type or one copy of each. The members of a gene pair separate equally during gamete formation. Consequently, each gamete carries only one member of a gene pair. The union of gametes is random. These concepts form the basic principles of genetics. They are the simplest guidelines to which many exceptions have since been discovered.

METEOROLOGY AND BEES

Though he is most famous for his experiments in plant breeding, Mendel also was considered an authority on meteorology. He was a member of the Vienna Meteorological Society and took daily observations at the monastery. Beginning in 1857, he meticulously recorded data including temperature, rainfall, air pressure, and ozone levels, but he also noted wind direction as indicated by a flag posted on a nearby tower and wind force as determined by the smoke coming from chimneys across the horizon. He generated a tabular overview of this data, which he presented to the Natural Science Society in 1862. This graphical summary was quite popular among other amateur meteorologists. Mendel also enjoyed using statistical analysis to forecast the weather for farmers. A paper from 1870 describing the causes of tornadoes demonstrated his scientific approach to meteorology.

In 1863 Mendel became a member of the Brno Agricultural Society. In 1868 Mendel was elected abbot of the monastery to replace Napp, who had passed away. The duties of his new position were time-consuming, leaving him little time to devote to his research. His views on many political matters conflicted with the government's opinions; thus, he was not a very popular public figure. He joined the Society of Apiculturists in 1870 and studied heredity in bees. He hoped to find that the principles he developed while studying plants also applied to animals. This endeavor was not successful due to the complex mating behavior of bees.

Though he never married, family was important to Mendel. He enjoyed playing chess with his nephews and regularly corresponded with all his family members. He repaid the debt to his younger sister, who shared her dowry to support his education, by financing the college educations of her three sons. Mendel's health declined near the end of 1883. His kidneys and his heart failed. On January 6, 1884, Mendel died without ever having received recognition for his remarkable insight into the laws of heredity. His remains were interred in the monastery tomb in the Central Cemetery.

REDISCOVERY OF MENDEL'S WORK

Mendel made one of the most significant discoveries in biology in 1865, and 35 years later its historical significance was finally brought to light. At the turn of the 20th century, three separate researchers were independently researching similar phenomena when they stumbled upon Mendel's paper. Dutch botanist Hugo de Vries, German botanist Carl Correns, and Austrian botanist Erich von Tschermak all thought they had attained novel results when they read Mendel's findings. English biologist William Bateson also deserves some credit for exposing Mendel's original research. In 1902 he published *Mendel's Principles of Heredity, a Defense* and coined the term genetics, defined as the study of the mechanisms of inheritance. Though all of these men recognized the significance of Mendel's work, whereas his 19th-century counterparts did not, none of them had devised the idea of pairs of discrete units to represent each trait.

Following his rediscovery, a statue of Mendel was erected outside the monastery in Brno in 1910. In 1922 a sandstone monument was placed in Mendel's experimental garden. To celebrate the centennial, in 1965 Brno hosted a Mendel Memorial Symposium. The Mendelianum, part of the Moravian Museum in Brno, opened. In 2002 a new international exhibition, the Mendel Museum of Genetics, was established in the Abbey of St. Thomas in Brno, where Mendel lived and worked.

Beginning around 1911, Mendel's data began to stir up controversy. Throughout the 1900s, there were several attempts to show that his data was not realistic. The data was too good to be true! Critics suggested that Mendel either failed to report some of his data or fabricated his numbers. His data fit the expected ratios better than chance alone predicted. Numerical analysis rarely would yield results so close

to the expected values. For example, if someone flipped a coin 200 times, what are the chances that 100 of those events would reveal heads, and 100 times tails? Using principles of probability, one could calculate the likelihood of getting exactly 100 heads and 100 tails on 200 coin tosses to be approximately 5.63 percent. Perhaps the coin would land on heads 95 times and tails 105 times, or 111 and 89. Though a preponderance of evidence collected over time has supported the expectation of achieving a 3:1 ratio in Mendel's pea crosses, statistical analysis reveals that the probability of the actual outcome being so close to the expected is quite low. A detailed statistical analysis performed by the British scientist Ronald Fisher purported the probability that Mendel would have achieved his published results by chance alone was only 1 in 30,000.

Some scientists believed that Mendel adjusted his data in order to fit his hypothesis. They thought his numbers were too close to the predicted 3:1 ratio. Mendel may have subconsciously sorted plants with questionable phenotypes into categories that favored his hypothesis. Whatever the truth, Mendel was an experienced teacher and probably left out what he considered extraneous information from his landmark paper. He did not feel obligated to report all the details and deleted those that did not contribute to his main points. Everything known about Mendel personally indicates he did not manipulate the numbers dishonestly. Whether or not he presented skewed results to emphasize his point, Mendel is admired for his brilliant insight. No other scientists had performed such meticulous experiments and predicted or explained the outcome. No one else before him or for 35 years afterward had perceived the 3:1 ratio as a 1:2:1 ratio. No one else deserves the credit for transforming a seemingly random set of phenomena into a logical set of laws of heredity.

See also CHROMOSOMES; DARWIN, CHARLES; GENETICS; GENOMES; INHERITANCE; REPRODUCTION.

FURTHER READING

Aaseng, Nathan. *Genetics: Unlocking the Secrets of Life*. Minneapolis, Minn.: Oliver Press, 1996.

Blumberg, Roger B. Mendelweb home page. Available online. URL: http://www.mendelweb.org/. Accessed January 22, 2008.

Carlson, Elof Axel. *Mendel's Legacy: The Origin of Classical Genetics*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

Henig, Robin Marantz. *The Monk in the Garden: The Lost and Found Genius of Gregor Mendel, the Father of Genetics*. Boston: Houghton Mifflin, 2000.

Orel, Vítězslav. *Gregor Mendel: The First Geneticist*. Oxford: Oxford University Press, 1996.

metric system The metric system, formally called the International System of Units (SI), is a system of units used by scientists worldwide to indicate measurable quantities such as length, time, and mass. By the late 18th century, different countries had developed a variety of means for measuring and reporting quantities of length, area, volume, and mass, complicating the communication of important information. The metric system originated in France, when in 1791 a committee of the French Academy of Sciences recommended using the meter as a standard unit of length. The original definition of the meter was one-ten millionth of the Earth's meridian quadrant at sea level. The Metric Convention of 1875 established the International Bureau of Weights and Measure (BIPM). The original treaty was signed by 17 nations, including the United States, and since then nations all over the world have adopted the metric system for reporting scientific information, though the United States and

COMMONLY USED METRIC UNITS

Quantity	Base Unit	Symbol
length, width, distance	meter	m
mass	gram	g
time	second	s
temperature	kelvin	K
	degree Celsius (K - 273.15)	°C
area	square meter	m^2
	hectare (10,000 m^2)	ha
volume	liter	L
	cubic meter (equals 1 L)	m^3
speed, velocity	meters per second	m/s
	kilometers per hour	km/h
density	kilogram per cubic meter	kg/m^3
force	Newton	N
pressure, stress	kilopascal	kPa
power	watt	W
energy	kilojoule	kJ
	kilowatt hour	kW·h
electric current	ampere	A

PREFIXES FOR MULTIPLES OF COMMON METRIC UNIT MEASUREMENTS

Prefix	Quantity	Abbreviation
yotta	10^{24}	Y
zeta	10^{21}	Z
exa-	10^{18}	E
peta-	10^{15}	P
tera-	10^{12}	T
giga-	10^{9}	G
mega-	10^{6}	M
kilo-	10^{3}	k
hecto-	10^{2}	h
deka-	10^{1}	da
base	1	
deci-	10^{-1}	d
centi-	10^{-2}	c
milli-	10^{-3}	m
micro-	10^{-6}	μ
nano-	10^{-9}	n
pico-	10^{-12}	p
femto-	10^{-15}	f
atto-	10^{-18}	a
zepto-	10^{-21}	z
yocto-	10^{-24}	y

Great Britain also use other methods for reporting measurements.

The metric system provides a common standard for measurement, allowing for the precise communication of information despite other potential barriers such as language. International agreement has defined seven base units for measuring the fundamental physical quantities of length (meter), mass (kilogram), time (second), electric current (ampere), thermodynamic temperature (Kelvin), amount of a substance (mole), and luminous intensity (candela). Other units, called derived units, can be formed by combining base units according to algebraic equations relating the quantities. The table Commonly Used Metric Units lists some of the most common metric units and their abbreviations.

Each type of measurement utilizes only a single base unit quantity. The addition of particular prefixes indicates differences in quantity by multiples of 10, eliminating the need for memorization of conversion factors and facilitating calculations. For example, the meter is the fundamental unit of length, and the prefix milli- indicates 1,000-fold less, so a millimeter is one-thousandth of a meter. Using U.S. customary units, for length calculations, one might have to convert from inches to feet (12 inches equals one foot), from feet to yards (three feet equals one yard), or from feet or yards to miles (5,280 feet or 1,760 yards equals one mile).

In the metric system, the international meter is the fundamental unit of length, a one-dimensional extension in space. In 1983 the General Conference of Points and Measures, which oversees the BIPM, defined the meter as the distance light travels in a vacuum during a time interval of 1/299,792,458 of a second.

A second fundamental physical quantity, mass, is indicated in grams. The standard mass to which all other masses are compared is the international prototype kilogram, a cylinder of platinum-iridium alloy weighing exactly one kilogram. The BIPM near Paris, France, keeps this standard, but numerous replicas are kept in various locations worldwide.

The metric system uses seconds to report quantities of time, the dimension of the physical universe that orders the sequence of events at a given location. Traditionally, units of time depended on astronomical events such as solar days, the average length of time between passages of the Sun across the meridian, but the fact that the Earth has a nonuniform rate of rotation led to a revised definition based on the immutable properties of an atom. The current definition states that one second equals 9,192,631,770 periods of the radiation corresponding to the transition between two hyperfine levels of the ground state of the cesium 133 atom. The BIPM maintains international atomic time.

The United States commonly uses a nonmetric system, called the customary system, customary units, or inch-pound units, for weights and measures. Lengths are reported in inches, feet, yards, or miles, and mass is measured in ounces or pounds. Conversions are complicated and require knowing the conversion factors. For example, to convert someone's weight from the customary unit of pounds to the metric unit of kilograms, one would multiply the number of pounds by 0.45359237 to obtain the equivalent number of kilograms. The Metric Act of 1866 authorized the use of the metric system in trade and commerce, but the U.S. Metric Conversion Act of 1975 designated the metric system as the preferred system for weights and measures for trade

and commerce and directed the federal government to convert to the metric system. Labels on food products use metric units, as do blood chemistry tests and drug dosages, and metric units are making their way into other uses as well.

FURTHER READING

The United States Metric Association home page. Available online. URL: http//www.metric.org. Updated January 10, 2008.

microbiology Microbiology is the branch of biology that deals with microorganisms, living things that are generally too small to be seen without assistance. Certain microorganisms can be seen with the naked eye, and, though most are unicellular, some are multicellular or acellular. Microorganisms, or microbes, are the most abundant and diverse organisms on Earth. Each of the three domains of life (Archaea, Eubacteria, and Eukarya) contains microbial representatives. Prokaryotes, classified as members of the kingdom Monera, include both archaeans and bacteria, though archaeans actually resemble eukaryotes more than they resemble bacteria. All of the eukaryotic kingdoms (of the five kingdom classification system: Plantae, Animalia, Fungi, and Protista) include microorganisms. Though they are not considered living organisms, viruses, viroids, and prions also fall within the realm of microbiology. The major groups of microorganisms include bacteria, archaea, algae, protozoa, fungi, parasitic worms, and viruses.

Microbiology as an independent branch of the biological sciences originated when the Dutch draper Antoni van Leeuwenhoek first observed microorganisms in pond water using hand-crafted lenses in the 1670s. Though one hundred years earlier some physicians believed that invisible organisms were involved in disease, it was not until scientists could see them or had a means to study them that microorganisms were recognized as living entities. As a field microbiology involves the study of all aspects of microorganisms—their origin and history, evolution, genetics, cell structure, physiology, metabolism, reproduction, pathogenicity, ecology, and industrial applications, to name a few.

MICROORGANISMS AND DISEASE

Many people think of germs and disease when they hear the term *microbiology*. While it is true that microorganisms cause infectious diseases, it is also important to remember that microbes are beneficial to humans in many more ways than they can cause harm. Numerous observations made throughout the 17th and 18th centuries led up to and suggested a causative link between microorganisms and disease. In 1867 the physician Joseph Lister instituted the use of aseptic techniques during surgeries, greatly reducing the incidence of postoperative infections and deaths. Credit for definitively establishing the germ theory of disease goes to the German physician Robert Koch and the French chemist Louis Pasteur, both considered founders of microbiology. Pasteur's other contributions to microbiology include recognizing that fermentation was a biological process, solving the problem of souring during wine- and beer-making, debunking the notion of spontaneous generation, development of the first vaccines, and identifying the causative organisms for cholera, anthrax, rabies, and silkworm diseases. Koch and his colleagues invented most of the techniques used for growing microbes in the laboratory and maintaining sterile conditions. He also established a set of proofs designed to determine if a particular organism caused a specific disease, called Koch's postulates. Near the end of the 19th century, the improvement in methods for growing, handling, and studying microorganisms ushered in what is called the golden age of microbiology, a period during which the etiological agents of numerous diseases were identified and several vaccines developed.

While society tries to avoid microorganisms that cause harmful diseases, other microorganisms keep humans healthy. Normal microbiota, also called normal flora, live on or in the human body. Their presence helps fight infection by potential pathogens by competing for space and nutrients or by secreting substances that render the environment unfavorable for other microorganisms. Bacteria that inhabit the intestinal tract even produce necessary vitamins, maintain an optimal pH, and aid in digestion by breaking down lactose.

Society's comprehension of the role of microorganisms in health and disease has greatly improved since the golden age of microbiology. Microbiologists now have identified and characterized the different classes of microorganisms, have a better understanding of the immune system, improved the techniques for controlling microbial growth, gained greater knowledge of how to prevent the spread of infections, and developed many types of antimicrobial drugs for treating infections. Microbiology is a required course in preparation for most health care careers, including medicine, dentistry, and nursing. Governments regulate health care practices designed to prevent the spread of communicable diseases and track epidemiological reports for certain diseases in order to prepare for outbreaks of particularly dangerous infections or of new, unidentified illnesses. With the widespread marketing of antimicrobial cleansers and hand sanitizers, even the average consumer is better

educated concerning hygienic practices that reduce the chance of contracting an infectious disease.

HABITATS AND ECOLOGY

Despite the bad reputation microorganisms have obtained due to the pathogenic nature of a small percentage of all microbes, the Earth would be uninhabitable without them. Prokaryotic organisms first appeared on Earth about 3.5 billion years ago and were the only inhabitants until about 1.8 billion years ago, when single-celled eukaryotic organisms emerged. Photosynthetic microorganisms changed the composition of the ancient atmosphere by releasing oxygen into it. Today photosynthetic microorganisms produce the majority of oxygen released into the atmosphere, maintaining a suitable environment for aerobic life-forms.

Microorganisms are essential to maintaining the health of Earth's ecosystems. They play important roles in both energy flow and nutrient cycling. In addition to generating oxygen required by many life-forms for respiration, photosynthetic microorganisms form the foundation of many food chains as primary producers. Many species of bacteria, algae, and plants serve as primary producers, supporting all other trophic levels of the food chain by converting carbon dioxide into organic compounds that can serve as food for other organisms. Some bacteria that act as primary producers are chemotrophs, organisms that are also autotrophic but obtain their energy from reduced inorganic compounds rather than sunlight. Consumers, all the organisms other than the primary producers in a food web, that is, the network of interconnected relationships in an ecosystem, are heterotrophs that directly or indirectly depend on the biomass made by primary producers.

The organic matter produced by living organisms in an ecosystem must eventually be recycled into the environment. Decomposition is the process by which nonliving organic material such as dead organisms, fallen leaves, and waste materials are converted back to their inorganic forms. Prokaryotic organisms and fungi are the major decomposers of organic materials from all trophic levels. The inorganic substances they release back into the environment can be absorbed and used by autotrophs to create new organic materials. This process of chemical recycling is essential to continued life on Earth, since nutrients such as carbon, nitrogen, oxygen, and phosphorus are available in limited quantities due to the law of conservation of matter. Microorganisms play a major part in chemical cycling, but so do geological processes such as weathering, fossilization, and erosion.

Their diverse metabolisms allow microorganisms to thrive in a variety of conditions. Microbial life has been found in all imaginable environments: in a sample of ice taken from a 120,000-year-old Greenland glacier, in boiling hot springs of Yellowstone National Park, more than two miles (3.5 km) below the Earth's surface, and even inside other living organisms. Such close associations between organisms are called symbioses, and the relationships can be beneficial to one or both organisms or cause one of the organisms harm. For example, adult humans contain 10 times more microorganisms living in or on their bodies than the number of cells that compose their bodies. Most of these are harmless, though some are potential pathogens. The ubiquity of microorganisms emphasizes their importance in the functioning of the biosphere.

APPLIED MICROBIOLOGY

In addition to keeping nutrients and energy flowing through the Earth's ecosystems, humans have figured out how to manipulate microbes in other ways that benefit society. The production of various foods and beverages exploits the natural metabolic activities of many bacteria and fungi. Yeast, a type of unicellular fungi, ferment sugar into alcohol during the synthesis of beer and wine. Baker's yeast, *Saccharomyces cerevisiae,* produces carbon dioxide as an end product in fermentation in bread-making. The release of gas makes the bread porous, and yeast enzymes help break down the flour to make the dough workable. The different fermentation pathways characteristic of different microbes give a variety of odors and flavors to different types of bread. The same is true for the production of cheese. The addition to milk of bacteria that generate lactic acid as a product of fermentation results in the formation of curds. The subsequent addition of different varieties of bacteria or fungi to the curds imparts the different flavors, textures, and aromas characteristic of particular varieties to the resulting cheese. For example, the mold *Penicillium roqueforti* gives Roquefort, bleu, and gorgonzola cheese their distinctive, strong flavors. The production of other dairy products such as sour cream, yogurt, and buttermilk also depends on microbial fermentation, as do other food products such as pickles, pepperoni, sauerkraut, and soy sauce.

Sewage treatment facilities depend heavily on microorganisms to remove organic matter from wastewater before releasing it into the environment. During the primary phase of treatment, skimmers remove bulky suspended materials and heavier particles sediment to the bottom of large tanks. A mixture of bacteria, algae, and protozoa aerobically digest much of the organic matter left in the remaining aqueous suspension in the secondary phase of treatment. The last phase involves additional physical and chemical treatments before discharging the water into the environment. The solid matter left behind

contains many inorganic fertilizers such as nitrogen, phosphorus, and potassium. In some places, further digestion by anaerobic microorganisms of the sludge remaining after biodegradation of the secondary phase of treatment produces gases such as methane that can be harvested as an energy source.

Genetic engineering is the deliberate alteration or recombination of the genome of an organism. Bacteria and viruses are important laboratory tools for manipulating genomes. Bacteria harbor plasmids, small, circular extrachromosomal pieces of deoxyribonucleic acid (DNA). Researchers can remove plasmids from bacterial cells, insert specific genes into them, and put them back into other cells. In this manner, genes that confer specific traits or characteristics can be moved around between organisms. For example, engineered bacterial cells can synthesize the protein human growth hormone because a researcher inserted the gene encoding it into a plasmid, and then introduced the plasmid into a bacterial strain. Biotech companies use microorganisms to produce large quantities of antibiotics, hormones, vitamins, and vaccines. Like plasmids, viruses can also carry genes from one cell to another.

Other microorganisms naturally produce substances that are valuable to humans. Many industries benefit from the mass production of enzymes, solvents, amino acids, or organic acids by microorganisms. Factories grow large batches of the microorganisms, and then extract and purify the desired product from the culture.

The mining industry also uses microbes. Mining is the extraction of valuable minerals from the Earth. Biomining uses microorganisms such as *Thiobacillus ferooxidans* to leach out minerals such as copper and gold from ores, and bacteria can extract minerals more efficiently from low-grade ores, an important consideration economically and as the availability of higher grades decreases. Bioprocessing also causes fewer environmental and health hazards.

Bioremediation involves the use of microorganisms to remove or break down undesirable substances from the environment. Examples of substances broken down naturally by microorganisms include crude oil, sewage effluent, chlorinated compounds, pesticides and herbicides, gasoline contaminants, and wood preservatives. Landfills are filling at an enormous rate with substances that are not biodegradable, such as glass and plastics, and the environment of most landfills, dry and anaerobic, does not encourage growth of the microorganisms that most often biodegrade organic waste. Because of this, solid waste management is an area of top priority in microbiology research.

Some microbes serve as model organisms for basic biological research, research performed for the sake of understanding how organisms live, grow, and reproduce. Because it is inexpensive and easy to grow and manipulate, researchers frequently use the bacterium *Escherichia coli* or yeast cells to study process and phenomena that differ only slightly between kingdoms. For example, the genetic code is universal, meaning that the triplet codon AUG encodes for the same amino acid, methionine, in bacterial cells, sunflowers, and cougars. Biochemical pathways such as glycolysis are basically the same whether in fungi or human muscle cells. Molecular processes are much easier to study using microorganisms such as bacteria and yeast, but biologists gain valuable information that is relevant to other living systems.

The applications of microbiological knowledge spread far and wide, to the benefit of human society and Earth's other life-forms. As microbiologists continue to learn about the creatures that inhabit the microbial world, they learn more about themselves, the Earth and its other inhabitants, and the sometimes harmful/sometimes beneficial relationship in which microorganisms play a central role.

See also ALGAE; ARCHAEA; BACTERIA (EUBACTERIA); BIOGEOCHEMICAL CYCLES; BIOLOGICAL CLASSIFICATION; BROCK, THOMAS; ECOLOGY; ECOSYSTEMS; EUKARYA; EUKARYOTIC CELLS; FLEMING, SIR ALEXANDER; FUNGI; HISTORY OF LIFE; HOOKE, ROBERT; INFECTIOUS DISEASES; KOCH, ROBERT; LEEUWENHOEK, ANTONI VAN; ORIGIN OF LIFE; PASTEUR, LOUIS; PROKARYOTIC CELLS; PROTOZOA; SLIME MOLDS; VIRUSES AND OTHER INFECTIOUS PARTICLES.

FURTHER READING

American Society for Microbiology. "MicrobeWorld." Available online. URL: http://www.microbeworld.org/. Accessed November 20, 2008.

Chan, Al. "Microbes.info: The Microbiology Information Portal." Available online: URL: http://www.microbes.info/. Last modified February 1, 2008.

Cowan, Marjorie Kelly, and Kathleen Park Talaro. *Microbiology: A Systems Approach*. New York: McGraw Hill, 2006.

Digital Learning Center for Microbial Ecology, Michigan State University. "The Microbe Zoo." Available online. URL: http://commtechlab.msu.edu/sites/dlc-me/zoo/index.html. Accessed November 20, 2008.

Hogg, Stuart. *Essential Microbiology*. Hoboken, N.J.: John Wiley & Sons, 2005.

microscopy Microscopy is an invaluable tool for studying the life sciences. Different types of microscopy allow one to visualize microorganisms, anatomical structures, and tissue samples at the subcellular and even molecular levels. In 1590 Zacharias Janssen constructed the first microscope by putting

two lenses in a tube. In the late 1600s, Robert Hooke and Antoni van Leeuwenhoek observed individual cells and microorganisms using simple microscopes, namely, microscopes with a single lens, demonstrating the utility of such an instrument. Not more than ground lenses attached to a handle, primitive microscopes resembled magnifying glasses, but with them one could observe specimens magnified up to 200 times. Tremendous advancements led to the development of modern microscopy. Today two basic kinds of microscopes meet the different needs of life scientists: light microscopes and electron microscopes.

LIGHT MICROSCOPY

Light microscopes use light as the source of illumination. Most light microscopes are compound microscopes, meaning they contain more than one lens between the eye and the object being viewed. The condenser lens system focuses the light necessary for adequate viewing onto the specimen, and the objective and ocular lenses magnify the specimen. Five types of light microscopy include

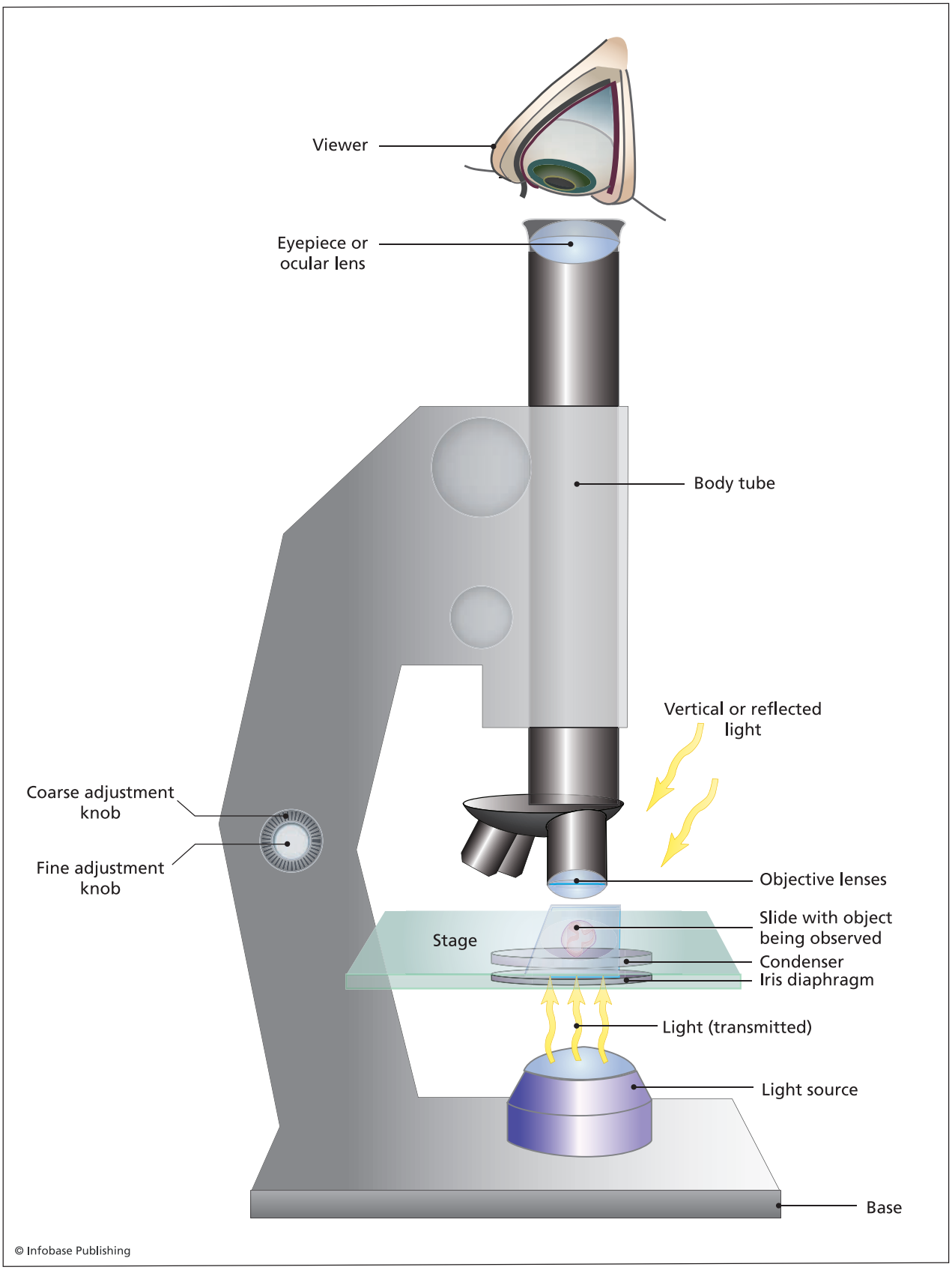
- brightfield,
- darkfield,
- phase contrast,
- differential interference,
- and fluorescence microscopy.

Objects that interfere with the transmission of light will appear dark against a light background in brightfield microscopy. A standard piece of laboratory equipment, the compound brightfield light microscope consists of a base, a stage that holds the microscope slide, an arm for carrying the instrument, and a body tube for transmitting the image. A lamp situated at the base of the microscope emits light that travels upward and passes through the condenser, which uses a set of lenses to gather light from the source and focus it to evenly illuminate the specimen. An iris diaphragm controls the diameter of the light beam that enters the condenser. The specimen lying on the stage refracts the light, which then travels up toward an objective lens that magnifies the image. The light then passes through the ocular lens where the image is further magnified to the eye of the observer. The microscopist focuses the instrument by turning the coarse or fine adjustment knobs, which in turn move the stage holding the slide up or down, until the light from the condenser hits it just where the beam converges and the image is sharp.

Magnification is the apparent enlargement of an object when viewed through the microscope. The total magnification is the product of the magnification of the objective lens and the magnification of the ocular lens. Many microscopes have a rotating nosepiece that can hold up to five objective lenses with different magnifications. The practical limit of magnification using light microscopy is approximately 1,300 times because as magnification increases, resolution decreases. This practical maximum magnification allows for the viewing of objects as small as one-millionth of an inch.

Resolution or resolving power is a measurement of how far apart two objects need to be in order for the viewer to distinguish them as separate entities. Without good resolution, objects appear to merge together into one blurry mass. The maximum resolution possible with light microscopy is approximately 7.87×10^{-6} inches (0.2 μm), too great to clearly resolve viral particles or very small bacteria. Resolving power is largely a function of the condenser's capabilities and the numerical aperture of the objective lens. The condenser improves resolution by eliminating stray light rays that could cause a glare when looking at the specimen. The numerical aperture is a measure of how efficiently a lens bends light. One can maximize the resolving potential by properly illuminating the specimen. The first step is to adjust the light intensity. Most light microscopes have a rheostat that allows one to change the amount of current, and therefore to maximize or dim the light generated at the source. The optimal amount of light depends on the type of specimen being viewed. More light is usually better for specimens that have considerable pigment or that are stained, whereas unstained samples with low contrast are easier to observe when less light is used. Another way to control the amount of light that illuminates the specimen is to adjust the diameter of the opening of the iris diaphragm that controls the amount of light that enters the condenser. A larger opening increases the cone of light, letting more through. A general rule for achieving optimal illumination is to maximize the light intensity and minimize the opening of the iris diaphragm. Objective lenses with very high magnifications require the use of immersion oil to maintain resolution. As the light passes from the glass slide containing the specimen to the air, peripheral rays are refracted, thus less light reaches the objective lens, which at high magnifications has a tiny diameter. The addition of oil prevents light rays from going astray as they change mediums (from glass to air) because oil and glass refract light in the same way, whereas air and glass do not.

Many biological specimens are transparent, and viewing something clear against a white background is difficult. Staining specimens helps increase the contrast between a specimen and the background for viewing with brightfield microscopy. Basic stains are positively charged, and the surfaces of cells are usually negatively charged due to the presence of acidic

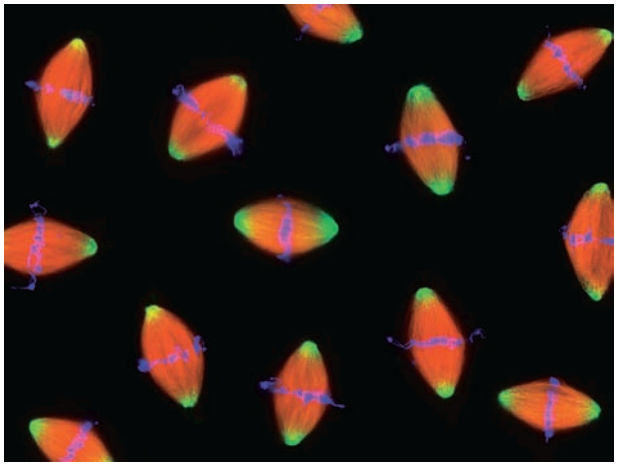


The compound light microscope is a standard piece of equipment in most biology laboratories.

proteins and of the phosphate groups of the phospholipids. Electrical attraction draws the two together, giving the cell color and increasing its contrast with the background. Cells repel acidic stains, which are used in a technique called negative staining. In this type of staining, the cells appear as bright spots on a dark background. Differential stains use several reagents and different types of cells respond differently to the reagents, allowing the observer to distinguish between cell types. For example, the Gram stain allows one to identify gram-positive and gram-negative cells based on structural differences in the cell envelopes. Unique structures such as flagella or capsules can be visualized using special staining techniques.

While staining is useful in brightfield microscopy, the process kills the specimens and can distort the shape. Viewing living organisms is necessary to detect motility and ensure the true shape is being observed. Darkfield and phase contrast microscopy provide better contrast by using special condensers, making it easier to see unstained, living organisms. In darkfield microscopy, the background is dark while the specimen is light, similar to negative staining. A darkfield condenser allows only light rays that pass through the object being viewed to reach the objective lens. This makes the objects appear brighter than the background. Phase contrast microscopy exploits the different refractive, or light-bending, properties of objects and converts these refractive differences into variations in light intensity. Light rays in the shape of a ring are sent toward the object, and light rays that pass through the object emerge out of phase from the light rays that do not pass through the object. A part called a phase plate, positioned in between the specimen and the eyepiece, increases the phase difference, making objects appear various shades of gray. Phase contrast microscopy is especially useful for observing live specimens and detailed intracellular structures that cannot be seen using ordinary light microscopy.

The dissecting microscope is a unique type of light microscope that allows the viewer to observe images in three dimensions. The light source can be built in or supplied from an auxillary lamp separate from the microscope that illuminates the specimen without the light first passing through a condenser lens. A mirror located below a transparent stage reflects the light through the specimen. The stage of a dissecting scope is low, and space between the stage and the objective lens is sufficient for the investigator to handle or manipulate the specimen while viewing it. Dissecting microscopes are commonly used for performing dissections of anatomical structures that are too small to see detail clearly with the naked eye. The ocular lenses usually magnify the object tenfold, whereas the objective lens usually magnifies only twofold or not at all. Some dissecting scopes have a zoom lens that increases the objective magnification up to sevenfold.



Fluorescent labeling allows one to distinguish the DNA (pink), microtubules (red), and poles of the spindle apparatus (green) in these dividing cells. *(Dr. Torsten Wittmann/Photo Researchers, Inc.)*

Differential interference microscopy also allows one to view details of live specimens. A set of prisms adds colors to the image. The colors are not natural, but they add contrast and make the image appear more three-dimensional.

Fluorescence is the absorption of ultraviolet or near-ultraviolet light and the subsequent emission of visible light from an object. Fluorescence microscopy initially was used to detect plant and animal cell structures that naturally fluoresced, but the development of new techniques allows for the observation by this method of anything for which antibodies can be made. Fluorescence microscopes have an ultraviolet light source and a filter that protects the observer's eyes from this dangerous form of radiation. Antibodies that specifically recognize a molecule or structure of interest are chemically linked to fluorescent dyes. These dye-conjugated antibodies are added to a slide that contains a specimen. The antibodies recognize and bind their antigens. When viewed under a fluorescence microscope, the fluorescence-tagged structure or molecules emit brightly colored visible light. This technique is ideal for pinpointing intracellular locations of certain molecules or for diagnosing specific diseases.

ELECTRON MICROSCOPY

Electron microscopes are much larger and more complicated than light microscopes but are invaluable for studies in biological, medical, and materials science.

They use beams of electrons instead of light to illuminate the samples. Because electrons have wavelengths about 100,000 times shorter than wavelengths of visible light, they can penetrate into much smaller crevices and therefore have much better resolution at high magnifications. Electron microscopes can achieve magnifications of biological specimens of up to 1,000,000 times with a resolution approaching 3.94×10^{-8} inches (0.001 µm). There are two main types of electron microscopy: transmission electron microscopy (TEM), which was invented in 1931, and scanning electron microscopy (SEM), invented in 1942.

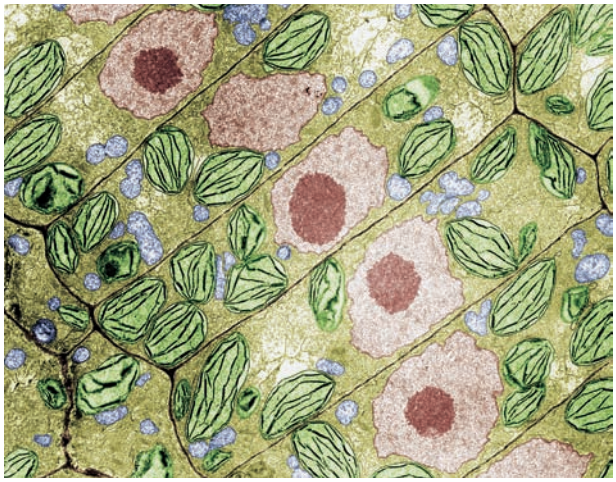
TEM is best for viewing viruses or the detailed structures of cells. In TEM, a specimen is placed on a small copper mesh grid and inserted into the chamber of an electron microscope. At the top of the chamber, a heated wire filament emits a stream of electrons into the chamber. Since any matter could deflect the pathway of the electrons, all matter, including air, must be absent from the chamber, a state called a vacuum. The electrons travel toward the copper grid, which is a conductor of electricity. Whereas a glass condenser lens focuses light onto the specimen in light microscopy, a ring-shaped electromagnetic lens focuses the stream of electrons onto the specimen in electron microscopy. As they hit the specimen, the electrons scatter to varying degrees based on the density of the object they encounter. Scattering is greater when electrons bounce into very dense objects. Only parts of the beam are transmitted through the specimen and hit a fluorescent screen. The scattering patterns of the electrons on the screen are recorded onto photographic film. Darker areas on the screen represent denser areas through which fewer electrons were transmitted. Less dense areas appear lighter. The two-dimensional image on the fluorescent screen can also be coupled to a digital camera for playback at a later time. The resulting images are black and white, though computers can add color to enhance the pictures.

The simplest method for preparing biological samples for TEM is negative staining, a process in which the specimen is coated with a heavy metal to provide greater contrast in the scattering of electrons. The specimen is placed in a solution of a heavy metal salt on a copper grid that has been coated with a thin layer of carbon. The excess liquid is blotted off, and the sample is ready for viewing. Negative staining works well for viruses or other specimens that can be isolated. To look at cells or tissues, the specimen first must be fixed by treatment with an organic solution that quickly kills the cells while preserving their structure as much as possible. Metallic osmium is added to increase the contrast, and then the sample is dehydrated with alcohol. Because electrons must penetrate the specimen to produce the image, the prepared samples must be extremely thin—7.87×10^{-7} to 3.94×10^{-6} inches (20 to 100 nm). To accomplish this, the specimens are embedded in a hard plastic resin, and then sliced into sections using an instrument called a microtome.

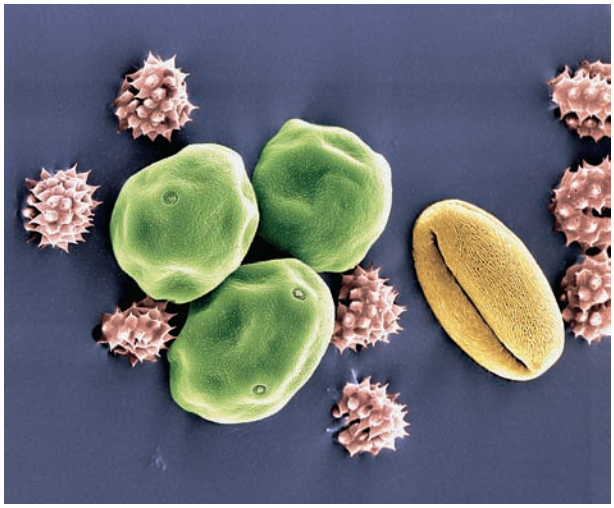
Both negative staining and sectioning can distort the specimen. Freeze-etching is a process that attempts to preserve the natural structure of cellular membranes and organelles. The cells are rapidly frozen and then split open to expose the interior. A replica of the exposed surface is created by coating it with carbon and a metal. The replica can then be examined in the electron microscope.

The goal of SEM is to obtain a greater depth of focus in order to create a three-dimensional image by scanning the surface of a whole specimen. In SEM, electrons are generated from an electron gun at lower voltages since the electrons are not transmitted through the sample. Instead, the electrons bounce off the surface of a specimen that has been coated with a metal like gold or platinum, and a sensitive detector picks up the deflected electrons and displays the pattern onto a computer screen.

Because the drying and fixation that is necessary for preparing TEM samples can cause shrinking, tearing, and wrinkles on the surface of the specimen, more delicate methods of preparation are necessary for SEM. In critical-point drying, the specimen is dehydrated in ethanol or acetone, and then placed in a special drying chamber with a fluid such as carbon dioxide or Freon. The temperature of the chamber is raised to the critical point at which the liquid and gaseous states are in equilibrium. Any remaining liquid converts directly to gas without distorting the surface of the specimen. The dried specimen is then



This TEM micrograph of duckweed cells shows cell walls, nuclei, and chloroplasts. *(Biophoto Associates/ Photo Researchers, Inc.)*



SEM reveals the fine details of surface structures, as observed in these pollen grains from different plant species. *(Eye of Science/Photo Researchers, Inc.)*

layered with carbon and a metal and then can be viewed.

The requirement for an electron microscopy specimen to be placed in a vacuum prevents the observation of living organisms. Newer microscopes called probe microscopes, including the scanning tunneling microscope (STM), the atomic force microscope (AFM), and the near-field scanning optical microscope, allow observation of living organisms. These microscopes use microscopic probes to create images. The probe of an STM skims over the specimen without ever touching it, all the while collecting information from the charged electrons on the surface of the object. STMs can reach magnifications of 500 million, allowing scientists to view objects at the atomic level. AFMs use probes made of silicon, and near-field scanning optical microscopes use tiny lasers. Another innovation is acoustic microscopy, in which sound waves are bounced off the surface of a specimen to obtain information about its structure.

See also CELL BIOLOGY; HOOKE, ROBERT; LEEUWENHOEK, ANTONI VAN; MICROBIOLOGY.

FURTHER READING

Egerton, Ray F. *Physical Principles of Electron Microscopy: An Introduction to TEM, SEM, and AEM*. New York: Springer Science and Business Media, 2005.

Hayat, M. A. *Principles and Techniques of Electron Microscopy: Biological Applications*. 4th ed. New York: Cambridge University Press, 2000.

The Nobel Foundation. "Microscopes." Available online. URL: http://nobelprize.org/educational_games/physics/microscopes/index.html. Accessed January 15, 2008.

Sullivan, James A. Cells Alive! Available online. URL: http://www.cellsalive.com/. Accessed January 15, 2008.

Miller, Stanley (1930–2007) American *Chemist* Stanley Miller's name is familiar among biologists for his experiment that demonstrated inorganic compounds could assemble into small organic molecules under conditions presumed to simulate the early Earth's atmosphere. With the catalyst of an electrical spark, amino acids—the building blocks of proteins—formed from ammonia, methane, hydrogen, and water.

PREBIOTIC SYNTHESIS OF ORGANIC COMPOUNDS

Stanley Lloyd Miller was born in Oakland, California, on March 7, 1930, to Nathan Harry Miller, an assistant district attorney, and Edith Levy Miller. For undergraduate studies, Stanley enrolled at the University of California at Berkeley. After earning a bachelor of science degree in chemistry in 1951, he entered the graduate program at the University of Chicago. While there, he attended a seminar given by the Nobel Prize–winning chemist (1934) Harold Urey, who had discovered the heavy form of hydrogen, known as deuterium. Urey is also known for his contributions to the theory of the origin of Earth and other planets. During the seminar, Urey proposed that the atmosphere of the early Earth was reducing, meaning an abundance of molecular hydrogen (H_2) was present. Today's atmosphere is oxidizing, meaning an abundance of free oxygen is present. Oxygen oxidizes, or breaks down, the covalent linkages in organic molecules, thus today's atmosphere would not support their synthesis. All biomolecules are organic molecules, but in living organisms they are normally synthesized within the protective confines of a cell or surrounded by tissues in multicellular organisms.

So how did the first organic molecules form? In the 1920s Aleksandr Oparin and J. B. S. Haldane independently proposed that life originated in a primordial soup, and that the first life forms were heterotrophic. Organisms that are heterotrophic cannot synthesize organic molecules from an inorganic carbon source, such as carbon dioxide. In contrast, autotrophs can synthesize organic molecules. Photoautotrophs, such as plants and green algae, use energy in the form of visible light to accomplish this via a process called photosynthesis. Many scientists did not believe Oparin's heterotrophic origin of life was correct; rather, they thought that the first life forms were photosynthetic. Oparin had gone so far as to suggest that someone attempt to synthesize organic compounds using the reducing conditions believed

to characterize the early atmosphere, but no one had done so, or at least not successfully. Melvin Calvin, a chemist who elucidated the biochemical pathways responsible for the fixation of inorganic carbon from carbon dioxide into organic compounds during photosynthesis, had attempted prebiotic (meaning before life existed) synthesis of organic molecules from carbon dioxide, water, and helium ions via irradiation, but he only achieved a minimal amount of formaldehyde (CH_2O). Urey was not impressed with the results. Calvin later won the 1961 Nobel Prize in chemistry for his work on photosynthesis.

While Urey's seminar interested Miller, he preferred theoretical research rather than laboratory research. Miller began work toward his dissertation under the supervision of the nuclear physicist Edward Teller, but after one year Teller left for California. So Miller asked Urey if he could attempt prebiotic synthesis experiments. Urey was not convinced this was an appropriate investigation for a young graduate student, as he felt the experiment did not have a high probability of succeeding and would take too long.

Miller persisted, and together Miller and Urey designed an experiment to examine the possibility of prebiotic synthesis under these conditions. Two glass flasks were connected by two glass tubes. One flask contained water, which was heated to simulate the hot oceans and create water vapor through evaporation. All of the air was pumped out of the second flask, which was then filled with the energy-rich gases ammonia, methane, and hydrogen. Miller and Urey thought that either ultraviolet radiation or an electrical discharge would be an appropriate source of energy to catalyze the reaction, as these energy sources would have been available from the Sun or lightning during the early Earth. The gas-filled flask contained two electrodes though which an electrical current could be sent. A condenser connecting the flasks was set up to collect any newly synthesized compounds.

After pumping out the air, filling the apparatus with the appropriate gases, heating the water, and turning on the electrical spark, Miller waited. After two days, he noticed the water had yellowed and a residue collected around the electrodes of the gas-containing flask. Unable to restrain from waiting any longer, he stopped the experiment to assay the water for the presence of amino acids. Using paper chromatography, Miller discovered that the amino acid glycine was present.

He set up the experiment once again, but this time waited for a week, at which time the water was yellowish brown and the gas flask contained a coating of scum. Analysis showed that not only was glycine present and more abundant, but that other amino acids were also present. Miller had demonstrated that inorganic compounds could spontaneously form into amino acids in a reducing atmosphere, plausible conditions of primitive Earth.

Miller first presented his results at a department of chemistry seminar in the same auditorium where he had heard Urey's seminar 18 months earlier. Distinguished scientists such as previous or future Nobel Prize winners and participants in the Manhattan Project typically gave these seminars, but that day they composed the audience. Though many assumed they would be able to find flaws in Miller's work, they left amazed that a 23-year-old graduate student may have determined how organic compounds were originally made billions of years ago.

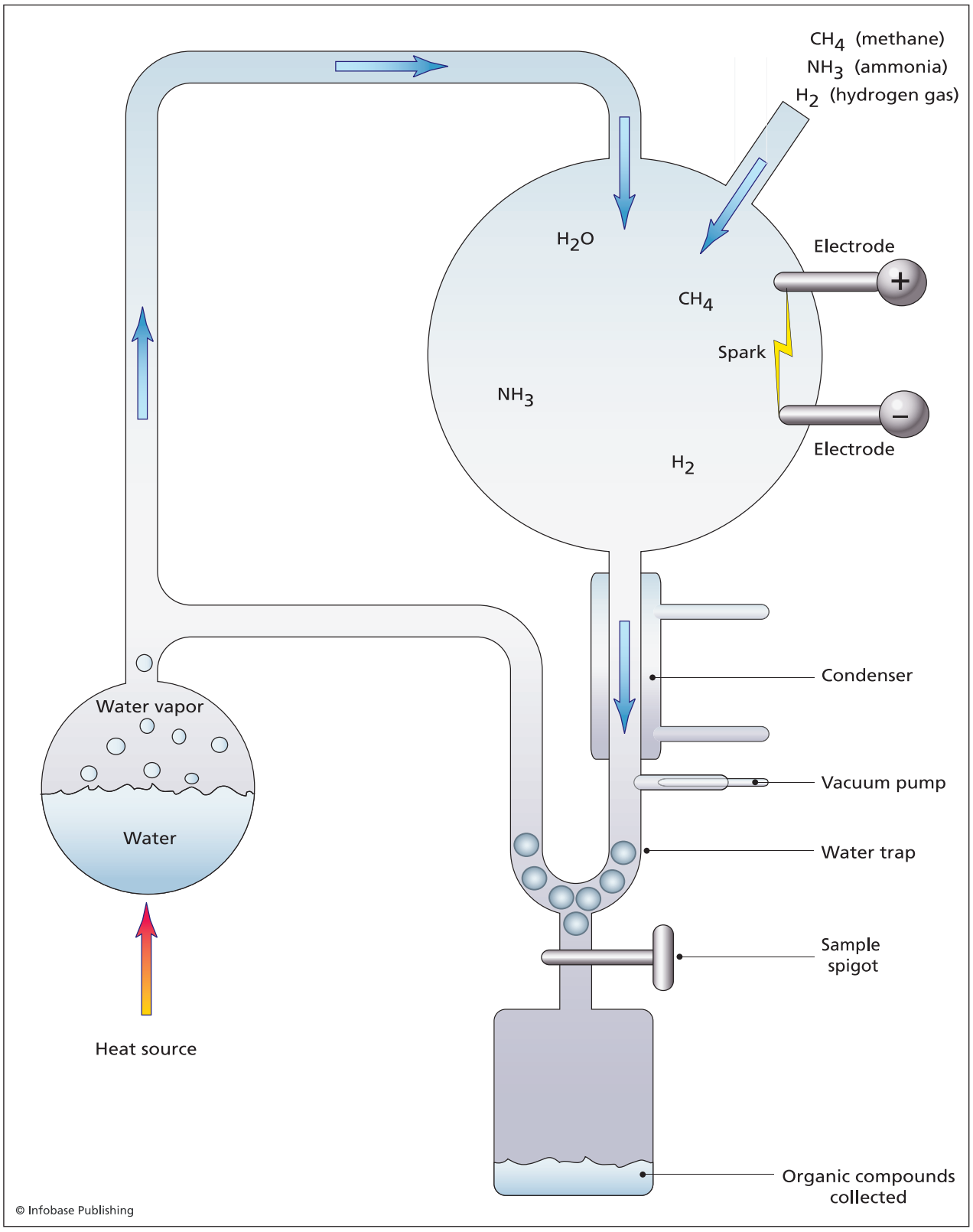
Miller published his results in the journal *Science*. According to Miller's brother Donald, also a chemist, Stanley had included his adviser's name on the manuscript, but Urey asked him to remove it, saying that he already had a Nobel Prize.

CAREER AFTER THE FAMOUS EXPERIMENT

After earning the doctorate degree, Miller performed postdoctoral research in the department of biochemistry at the College of Physicians and Surgeons at Columbia University. In 1960 the University of California recruited him as an assistant professor for the newly created San Diego campus, then made him associate professor two years later. He became a full professor of chemistry in 1968 and later an emeritus professor of chemistry and biochemistry. He continued his research on the chemical origins of life for four decades.

In the last decade, Günter Wächtershäuser, a chemist-turned patent lawyer from Munich, has propelled a new theory regarding the origin of life into the limelight—the iron-sulfur world theory. He suggested that life first arose on mineral surfaces near submarine volcanic vents, catalyzed by metal catalysts, as opposed to the conditions Miller used in his landmark experiment. Others have called into question the composition of gases that Miller used, saying that it did not accurately reflect the early atmosphere. Even if Miller did not use the exact composition of gases present at the time that life was emerging on Earth, and even if his assumption that electrical discharges stimulated the biotic formation of the organic compounds was incorrect, Miller's contribution is still impressive and significant in two respects. He was the first to demonstrate experimentally that organic compounds could form spontaneously from inorganic compounds, and his experiment generated excitement that led to an explosion of experimental research into the origin of life.

The National Academy of Sciences elected Miller to membership in 1973. In 1983 the International



The apparatus designed by Miller and Urey consisted of a flask of water to represent the oceans, connected to a flask containing energy-rich gases presumed to be present in the early atmosphere. Electrical discharges passed through the gas flask to simulate lightning, which he thought provided the energy to assist in the abiotic formation of organic compounds.

Society for the Study of the Origin of Life awarded Miller the Oparin Medal. He served as president of this society from 1986 to 1989. He also belonged to the American Chemical Society, the American Association for the Advancement of Science, and the American Society of Biological Chemists. Miller was made an honorary counselor of the Higher Council for Scientific Research of Spain in 1973.

After suffering a series of strokes beginning in 1999, Stanley Miller died of heart failure near his home in National City, California, on May 20, 2007. His classic experiment demonstrated that organic molecules could have spontaneously formed from inorganic compounds in a reducing atmosphere. Considered the father of origin-of-life chemistry, he gave scientists hope that, someday, the reconstruction of the creation of the first macromolecules or living cells from a primordial chemical soup might be possible. To date, scientists have not been successful in this endeavor, but many are trying.

See also BIOMOLECULES; CALVIN, MELVIN; CHEMICAL BASIS OF LIFE; ORGANIC CHEMISTRY; ORIGIN OF LIFE.

FURTHER READING

Miller, Stanley. "A Production of Amino Acids Under Possible Primitive Earth Conditions." *Science* 117 (1953): 528–529.

Miller, Stanley, and Harold Urey. "Organic Compound Synthesis on the Primitive Earth." *Science* 130 (1959): 245–251.

molecular biology The branch of life sciences concerned with the study of life at the molecular level is called molecular biology. The field of molecular biology significantly overlaps with biochemistry, genetics, and cell biology. Though by definition molecular biology encompasses the study of the structure and function of all biomolecules (including proteins, carbohydrates, lipids, and nucleic acids) molecular biology has come to imply molecular genetics, the study of gene structure and function at the molecular level. Organisms from across kingdoms are remarkably similar at the molecular level, thus knowledge gained from studying bacteria or yeast provides insight into the molecular processes in reptiles and beetles.

HISTORY OF MOLECULAR BIOLOGY

Molecular biology emerged as a recognized field of life science in 1953, when James Watson and Francis Crick announced their discovery of the structure of deoxyribonucleic acid (DNA) to be a double helix bound together by hydrogen bonds between specifically paired nucleotide bases on opposite strands. Several other discoveries leading up to that landmark discovery, however, make up a period that could be called the gestational period of molecular biology.

In 1902 the English physician Archibald Garrod combined his genetic observation that a metabolic disease known as alcaptonuria behaved as a Mendelian recessive trait with his belief that the biochemical cause of the disease was a defective enzyme. Based on this merger, he proposed that defective genes gave rise to defective enzymes. In 1941 the American geneticists George Beadle and Edward Tatum carried out experiments with the mold *Neurospora* that provided experimental evidence that genes encoded enzymes. They created single gene mutations that resulted in the lack of certain enzyme activities and summarized their conclusions into their famous "one gene–one enzyme" hypothesis, stating that each gene encodes the synthesis of a single, specific enzyme. A more accurate phrase based on knowledge gained since then is "one gene–one polypeptide."

In 1944 researchers from Rockefeller Institute in New York—Oswald Avery, Colin MacLeod, and Maclyn McCarty—published evidence demonstrating that DNA was the molecular carrier of genetic information in bacteria, but many scientists still doubted that such a seemingly simple molecule could perform such a crucial task for life. Biochemists had determined the composition of DNA; it consisted of four different nucleotides. Chromosomes were known to carry the hereditary material, but they contained both DNA and protein, and proteins were made up of 20 different amino acids. Thus many believed protein was a much more likely candidate for the carrier of all the complex and varied genes within an organism. In 1952 Alfred Hershey and Martha Chase published their results from experiments on bacteriophage (a virus that infects bacteria), supporting the belief that DNA was the hereditary material. These experiments led to an increased interest among biologists in the structure of DNA, the molecule of life.

When Watson and Crick published their landmark paper, "A Structure for Deoxyribose Nucleic Acid," describing the structure of DNA, they ended it with the statement "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Indeed, once the structure of DNA was uncovered, an explosion of research into the mechanisms of DNA replication and of gene expression followed. Until this time, geneticists believed that genes were the smallest units of genetic function, mutation, and recombination, but Watson and Crick's model for DNA suggested that individual base pairs may be mutated. An American whose training was in physics, Seymour Benzer, demonstrated that genes had finer structures that could be

mutated and recombine. This meant that different alleles could occur within the same gene.

The next major advancement in molecular biology was the deciphering of the genetic code, a task carried out by Crick, Leslie Barnett, Sidney Brenner, and R. J. Watts-Tobin. Through an elegant series of genetic experiments, they determined that

- a group of three nucleotide bases (now called a codon) encoded one amino acid;
- the code was not overlapping;
- the sequence began at a specific starting point; and
- some amino acids were encoded for by more than one group of three bases.

Several pioneering molecular biologists helped define the amino acid encoded by each triplet codon, and Brenner went on to show that specific codons acted as chain terminators (stop codons). In the 1960s Charles Yanofsky and Brenner independently provided evidence that gene structure and protein structure were colinear, in other words the linear sequence of nucleotide bases along the length of a strand of DNA determined the linear sequence of amino acids in a polypeptide. These studies paved the way for investigations into the molecular nature of mutations—how a change in DNA sequence could lead to an altered amino acid sequence.

Meanwhile, two French scientists were researching the mechanisms by which the bacterium *Escherichia coli* regulated the expression of its genes that controlled the catabolism of the carbohydrate lactose. François Jacob and Jacques Monod published their model for what they called enzyme induction, or genetic regulation of protein synthesis. Today the regulation of gene expression remains an important focus of research in many molecular biology labs. The regulation of gene expression is intimately related to biological processes and phenomena, including cellular reproduction, development, communication between cells, and metabolism. Improper genetic regulation can lead to a variety of disorders and diseases, including cancer.

The work of the Austrian organic chemist Max Perutz brought proteins into the realm of molecular biology. His research on the oxygen-carrying proteins hemoglobin and myoglobin demonstrated how one could use molecular biological knowledge and techniques to study the structure and function of proteins. By comparing the amino acid sequences of these proteins between different vertebrate species, Perutz and his colleagues determined that the interior portion of all the globins was nonpolar. He went further to show how the function of mutant globins was altered by not preserving this folding of polypeptide chains in a manner that protected the interior hydrophobic nature of proteins, and that these mutations could cause the clinical symptoms of diseases such as anemia.

Due to a better working knowledge of the molecule of heredity and the process of protein synthesis, around 1970 molecular biology gave birth to genetic engineering and the biotechnological revolution, making possible the manipulation of genes within and between organisms and even different species. Molecular biological techniques have led to revolutionary discoveries and inventions that have changed the way biological research is carried out, the approach to medicine, forensic investigations, the industrial manufacture of many chemicals, drug design and production, and more.

SCOPE OF MOLECULAR BIOLOGY

The major goal of molecular biology is to understand the structure and function of genes. The structure of DNA elucidated the basic mechanism by which it could self-replicate—by separation of the two strands and duplication of the specific base pairings. Many of the enzymes, other factors, and the basic process have been unraveled, but DNA replication is still a main topic of molecular biological research. The regulation of DNA replication and its coordination with the cell cycle is another major focus, as improper control can lead to permanent mutations in an organism's DNA and potentially to cancer. Mutations do occur, however, and the cell has mechanisms for recognizing and repairing many types of mutations. Because genetic mutations form the basis of many diseases or disorders, medical researchers have focused much molecular biological research into the causes, effects, and prevention of genetic mutations as well as natural and assisted DNA repair mechanisms.

Genes direct the synthesis of polypeptides in a multistep process known as gene expression, another leading area of molecular biological research. First, the process of transcription occurs, during which the DNA opens up and enzymes synthesize a molecule of ribonucleic acid (RNA) that contains the coded information from the DNA. Depending on whether the cell is prokaryotic or eukaryotic, the messenger RNA (mRNA) transcript may undergo processing, during which enzymes modify the mRNA by adding or removing chemical groups or stretches of base pairs. In eukaryotic cells, the cell then transports the mRNA from the nucleus, where transcription occurs, into the cytoplasm for the next step in gene expression, translation. Ribosomes carry out the process of translation; they scan the mRNA and, based on the linear sequence of nucleotides, incorporate the encoded amino acids to create a chain called a polypeptide. Several polypeptides may combine to form

the complete protein, which assumes a specific three-dimensional configuration that is necessary for the protein to perform its cellular function. Sometimes additional processing is necessary to activate the protein, such as the removal of a few amino acids, the addition of phosphate groups at certain positions, or the addition of different carbohydrates. Molecular biologists study all of these processes in addition to the regulation of each step.

As is true for all life scientists, the educational requirements for molecular biologists depend on the type of position one desires. A bachelor's degree is sufficient for employment as a laboratory technician or assistant in either an academic research institution or a private corporation. One must obtain a graduate degree to hold a supervisory position, and a doctorate degree is usually necessary to serve as the head of a laboratory.

MOLECULAR BIOLOGICAL RESEARCH

Because molecular biology overlaps many other fields of biology, a molecular biologist may also be considered a specialist in another subdiscipline, such as biochemistry, developmental biology, or microbiology, depending on the type of research performed. A molecular biologist might investigate the role of p53 (a tumor suppressor gene) in controlling changes in aerobic respiratory activity in tumor cells. Another might research how histone protein modifications recruit other proteins involved in the progression of the cell through the cell cycle when breaks are present in the DNA. A scientist interested in intercellular communication might study the molecular components of a system that coordinates a physiological activity, such as the successive muscle contractions in the digestive tract of the nematode *Caenorhabditis elegans*.

Techniques based on knowledge of molecular biology or that exploit molecular processes have advanced other fields of life science research as well. For example, an evolutionary biologist might examine the number of differences in the sequence of a conserved gene to estimate when two species diverged from a common ancestor. An ecologist might use molecular techniques to follow the transmission and spread of protozoan and viral pathogens by arthropod vectors through a population of birds. A plant breeder might use molecular markers to assay for the inheritance of desirable traits such as drought-resistance in seedlings from a cross rather than waiting for the plants to mature and produce their own offspring.

See also AVERY, OSWALD; BIOMOLECULES; CRICK, FRANCIS; DEOXYRIBONUCLEIC ACID (DNA); FRANKLIN, ROSALIND; GENETICS; MACLEOD, COLIN MUNRO; MCCARTY, MACLYN; RECOMBINANT DNA TECHNOLOGY; WATSON, JAMES D.; WILKINS, MAURICE H. F.

FURTHER READING

Ausubel, Frederick M., Roger Brent, Robert E. Kingston, David D. Moore, J. G. Seidman, John A. Smith, and Kevin Struhl. *Current Protocols in Molecular Biology*. 5 vols. Hoboken, N.J.: John Wiley & Sons, 2004.

Lewin, Benjamin. *Genes IX*. Sudbury, Mass.: Jones & Bartlett, 2008.

Sambrook, Joseph, and David W. Russell. *Molecular Cloning: A Laboratory Manual*. 3rd ed. 3 vols. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2001.

Watson, James D., Tania A. Baker, Stephen P. Bell, Alexander Gann, Michael Levine, and Richard Losick. *Molecular Biology of the Gene*. 6th ed. San Francisco: Benjamin Cummings, 2007.

Weaver, Robert F. *Molecular Biology*. 4th ed. New York: McGraw Hill, 2008.

Morgan, Thomas Hunt (1866–1945) American *Geneticist*

Thomas Hunt Morgan headed a research team that essentially created the subfield of classical genetics. He is responsible for providing overwhelming evidence in support of Mendelian theory and the chromosome theory. His research on fruit flies firmly established the basic laws of inheritance. He also proved that genes were physical entities that existed on chromosomes in a linear arrangement with measurable distances between them, and he discovered sex-linked inheritance.

EDUCATION

Thomas Hunt Morgan was born on September 25, 1866, in Lexington, Kentucky. He was the oldest son of Charlton Hunt Morgan and Ellen Key Howard. His family was well known, as his uncle was a famous Confederate army general and his great-grandfather was Francis Scott Key, composer of *The Star Spangled Banner*. As a child, Tom enjoyed the outdoors and especially collecting natural treasures such as fossils and butterflies near a relative's summer home in Oakland, Maryland.

During his teenage years, Tom spent two summers working for the U.S. Geological Survey in the Kentucky mountains. After two preparatory years at the State College of Kentucky, he began coursework toward a bachelor of science degree in zoology. The members of the all-male student body were military cadets. The routine and expectations were strict, and Tom received his share of demerits. The science program was limited, but Tom excelled. He earned his degree summa cum laude in 1886 and was voted

valedictorian for his graduating class of three total members.

Before starting graduate school, Morgan spent the summer studying marine biology at the Boston Society of Natural History's marine biological station in Annisquam, Massachusetts. This station was the precursor to the Marine Biological Laboratory at Woods Hole. There he developed an interest in marine life that persisted throughout his scientific career. He entered Johns Hopkins University that fall. Though only a decade old, Johns Hopkins had an excellent academic reputation and was one of very few American colleges that concentrated on biology as a science. The faculty emphasized the importance of inquiry and laboratory research. Biology was in the process of transforming from a descriptive discipline to an experimental science.

As a doctoral candidate, Morgan studied embryology, that is, the study of embryos, or animals during early development. The goal of his research was to classify sea spiders, *Pycnogonida*. A morphological, or structural, approach suggested they were crustaceans, a class that includes aquatic animals such as crabs, lobsters, and crayfish. Morgan took a comparative embryological approach. By comparing their anatomies during different stages of development, he determined that sea spiders more closely resembled arachnids, a class including true spiders and scorpions.

After completing two years of graduate study at Johns Hopkins, Morgan was eligible for a master of science degree from the State College of Kentucky. The only requirements were two years of study at another institution and a satisfactory examination by the college faculty. The faculty was so impressed with Morgan that they offered him an immediate position as a professor, but Morgan chose to pursue his schooling.

Morgan earned a Ph.D. in 1890 for work on the development of sea spiders and was awarded a one-year postdoctoral research fellowship. He spent the following year conducting research and traveling to the Bahamas, Jamaica, and the zoological station in Naples, Italy. Morgan's training from Johns Hopkins taught him to be mentally flexible, to only accept as true that which had been thoroughly examined, and to reject that which was false. Science left no room for subjectivity or emotional attachments to merely fashionable theories.

EARLY CAREER

In 1891 Morgan took a position as an associate professor of biology at Bryn Mawr College, an intellectually rigorous college for women in Pennsylvania. As a teacher, he was well liked, but he was not a very organized lecturer. He lectured as if he were thinking aloud. The students either loved or hated his classes, and he always welcomed those seeking extra help.

Thomas Hunt Morgan received the Nobel Prize in physiology or medicine in 1933 for his numerous advances in the field of classical genetics. *(National Library of Medicine)*

Embryological studies of marine animals remained the focus of his research at Bryn Mawr. He studied sea acorns, frogs, and ascidian worms. His research convinced him that biology needed to advance to more experimental analysis rather than remain simply descriptive. Morgan also examined the processes by which sea urchin eggs duplicated and differentiated into multicellular, multifunctional adults. He found that developmental cues were mostly intrinsic to the organism. Gravity did not play an important role in the early development, as some scientists thought. The role of most environmental influences was comparatively small.

In 1894 Morgan returned to Naples for a year. After this sabbatical, he was promoted to full professor. He had published his first book in 1897, *The Development of the Frog's Egg: An Introduction to Experimental Embryology*. That same year he was elected to the board of trustees for the Marine Biological Laboratory at Woods Hole, which was rapidly becoming a mecca for marine biological research.

He remained an active board member until 1937. In 1901 Morgan published *Regeneration,* a book that summarized the current state of knowledge on the subject of regeneration, the process by which body parts are replaced by new tissue growth. For example, if a sea star loses one of its arms due to injury, it will grow another one in its place. Morgan likened the process of regeneration to embryological development and emphasized the importance of experimental analysis in discovering natural laws that governed both processes. He also published *Evolution and Adaptation* in 1903. In this book, Morgan attacked the theory of evolution by means of natural selection as proposed by English naturalist Charles Darwin, claiming there were too many loopholes. Later in life, he came to accept the theory, and even published other books on the topic, which tied the concepts of heredity to evolution by natural selection, *A Critique of the Theory of Evolution* (1916) and *The Scientific Basis of Evolution* (1932).

When he was offered a position as chair of experimental zoology at Columbia University in New York in 1904, Morgan accepted it. Before moving, he married an 1891 Bryn Mawr alumna named Lilian Vaughan Sampson, a cell biologist. They had four children together.

At Columbia, Morgan continued to study sea urchins in order to examine the question of whether environment or heredity played a larger role in embryonic development. His research showed inheritance proved more important. This sparked his interest in heredity. Another hot research topic at the time was the mechanism of sex determination. Some scientists believed that environment controlled the outcome of an organism's gender, while others believed it was heredity. Morgan followed this scientific debate with interest.

Heredity constituted an exciting new field of research. The work of the Austrian monk Gregor Mendel had been rediscovered in 1900. Mendel had proposed that specific factors (today called genes) were responsible for inherited characteristics. He said that individuals had two copies of each gene, and that the members of each gene pair separated during gamete (sex cell) production so that offspring received one member from each parent. The members of the gene pair, or alleles, could be dominant or recessive. Dominant characteristics were expressed even if only one dominant allele were inherited, while recessive alleles were outwardly expressed only if both inherited alleles were recessive. Morgan did not immediately accept Mendelian theory because he felt that definite experimental evidence was lacking.

One of Mendel's rediscoverers, the German botanist Carl Correns, hypothesized that chromosomes, prominent structures located within the nucleus of cells, might contain genes. In 1903 American cytologist Walter Sutton published a paper firmly stating that the behavior of chromosomes during the cellular process of meiosis exemplified the behavior of Mendel's factors of inheritance. (Meiosis is the process of making gametes, or eggs and sperm cells.) The following year, the German biologist Theodor Boveri corroborated these conclusions. Most of the world immediately accepted that genes were located on chromosomes, ushering in the era of the chromosome theory of inheritance; however, Morgan remained cautious.

A cytogeneticist working at Bryn Mawr named Nettie Maria Stevens hypothesized that the sex of an organism was determined by the inheritance of a specific sex chromosome. The sex chromosomes are termed the X and the Y chromosomes. She performed experiments to confirm her hypothesis using the yellow meal worm beetle, *Tenebrio molitor,* as a model organism. She determined that sperm carried either an X or a Y chromosome, while eggs all carried X chromosomes, and she showed that when an egg was fertilized by a sperm carrying the Y chromosome, a male organism resulted. When an egg was fertilized by a sperm carrying an X chromosome, a female resulted. She then expanded her studies to include different species. Working independently, a colleague and friend of Morgan at Columbia, Edmund Beecher Wilson, also demonstrated that the sex of an organism was determined chromosomally using the seed bug *Lygaeus furcicus* as a model organism.

SEX-LINKED INHERITANCE IN *DROSOPHILA*

Morgan started using fruit flies, *Drosophila,* as a model system around 1908. These flies were optimal research material since their generation time was only two weeks long, they were easy to breed, they were cheap to maintain in the lab, they did not require much laboratory space, and, importantly, they had only four pairs of chromosomes. One of Morgan's graduate students, Fernandus Payne, was trying to produce blind mutants by culturing flies in the dark. After breeding 69 generations in the dark without success, the student tried inducing blind mutations by exposing the flies to X-rays, radium, and various environmental conditions.

Though Payne had no luck producing blind mutants, in May 1910, a white-eyed male fly was born in Morgan's laboratory. (The source of this fly has been disputed.) Morgan mated this male fly with a normal red-eyed female. All 1,240 of the offspring had red eyes. (Actually, three did have white eyes but are believed to have been the result of spontaneous mutation.) In Mendelian terms, this meant that the red-eyed phenotype (observed characteristic) was dominant to white eyes. Next, he mated the red-eyed

Drosophila melanogaster is a fruit fly commonly used in breeding and genetics research. The male (shown on the left) is slightly larger than the female (shown on the right). *(Biology Media/Photo Researchers, Inc.)*

offspring (F_1) with each other and found the white-eyed phenotype reappeared in the next generation (F_2). According to Mendel's research, the recessive phenotype was expected to reappear in one-fourth of the offspring. This was what Morgan observed, but interestingly, though one-fourth of all the offspring had white eyes, all of them were males. None of the female flies had white eyes. Though in the past he had argued against Mendel's conclusions, Morgan's work actually provided overwhelming evidence in support of Mendel's laws and the chromosomal theory of inheritance.

Morgan correctly concluded that the gene controlling eye color was located on the X chromosome. Today, genes located on either the X or the Y chromosome are referred to as sex-linked. Since male flies possessed only one X chromosome, a recessive allele for white eyes would not be masked. Females have two X chromosomes, thus they are likely to have a dominant red-eyed allele, which would prevent the outward expression of the recessive white-eyed phenotype. The females in the F_1 generation must have possessed one X chromosome containing a dominant allele and one X chromosome with a recessive allele. Organisms that possess both a recessive allele and a dominant allele and have the dominant phenotype are called carriers. These experiments clearly demonstrated that specific genes are associated with specific chromosomes and that certain genes are sex-linked. This work also contributed to the understanding of genetic sex-determining mechanisms.

GENE MAPPING

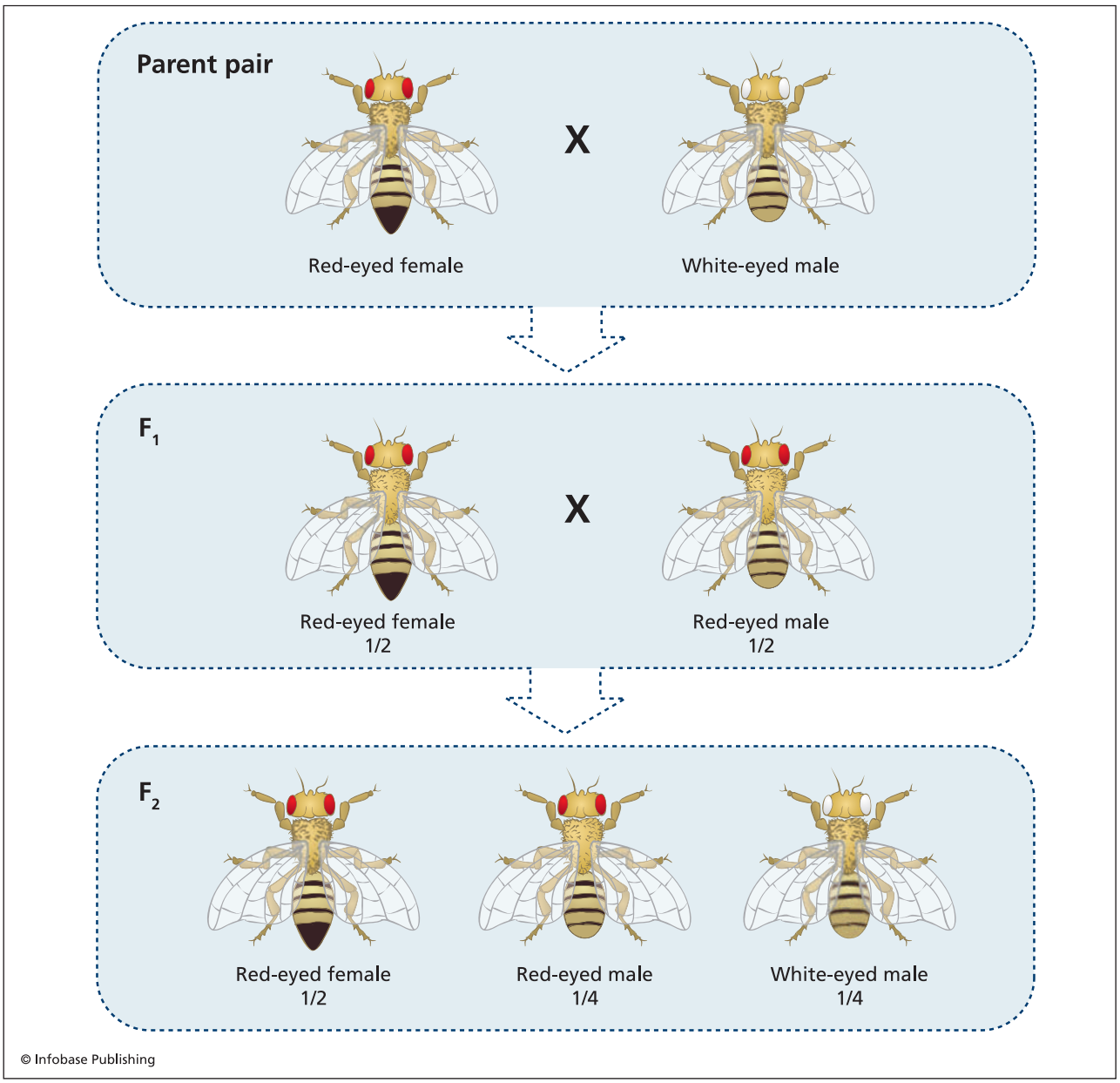
Following the success of these experiments, the members of Morgan's laboratory excitedly performed more breeding experiments with *Drosophila*. Two undergraduate students, Alfred H. Sturtevant and Calvin B. Bridges, and a graduate student named Hermann Joseph Muller performed the bulk of the labor. They spent winters at Columbia and summers at Woods Hole. The two labs were affectionately referred to as the "fly room." Together, Morgan's team began finding a few new mutations each month. By 1912 they had discovered 40 types of flies with mutations such as crooked or tiny wings and yellow bodies.

Each new mutant type was methodically mated, and the progeny were mated with its siblings, its parents, and with other mutants. It soon became apparent that these mutations were inherited in groups. Since the fruit fly has four different chromosomes, there were four groups, each corresponding to a particular chromosome. All the mutants were carefully classed. The data supported the hypothesis that traits were linked with other traits. Furthermore, the number of genes in a linkage group correlated with the length of the associated chromosome. Morgan imagined the genes were arranged in a linear fashion along the length of a chromosome, like beads on a string.

As their research progressed, the team began to notice that traits that were thought to be linked, that is, grouped together on the same chromosome, sometimes were inherited independently. Furthermore, some genes appeared more tightly linked than others. How could this be? An astute observer, Morgan also noticed that chromosomes occasionally appeared visibly tangled during the process of meiosis. In 1909 the Belgian cytologist F. A. Janssens had described this intertwining of chromosomes during meiosis and claimed that parts of the chromosomes physically exchanged fragments during the process. Morgan called this event crossing over or recombination.

Sturtevant brilliantly related the frequency of these events to the distance between genes. Genes that were distant to each other along the length of a chromosome had a greater chance of becoming "unlinked" during meiosis than genes that were near to one another. In other words, the greater the distance between two genes, the more space in which a break could occur. Morgan's lab members performed numerous crosses of flies with mutations of linked genes, calculated the frequency of the linked traits segregating, and, from this, estimated the genetic distance between the genes. The unit for such chromosome mapping measurements is called the centimorgan, in Morgan's honor. The mapping work clearly demonstrated that genes exist in a linear arrangement on chromosomes.

Exceptions to Mendelian theory arose and complicated interpretation of the fly room's data. Lethal genes caused the embryos to die before birth. Some



Morgan performed a series of controlled crosses involving white-eyed male mutants that demonstrated eye color was a sex-linked characteristic in *Drosophila.*

genes were found to have multiple alleles, not simply one dominant and the other recessive. Some traits were affected by more than one gene. The concept of crossover interference was proposed to explain why some crossover frequencies did not turn out as expected based on genetic distances. All of these situations have been studied thoroughly and now are accepted as common modifications to Mendelian inheritance. Sturtevant also thought that the position of a gene on a chromosome affected its expression. This suggestion was furiously refuted by staunch Mendel supporters though it later proved to be a real effect.

LATER CAREER

In addition to his numerous peer-reviewed papers in renowned scientific journals, Morgan published several textbooks, which shaped the field of genetics. A joint effort of Morgan, Bridges, Sturtevant, and Muller, *Mechanism of Mendelian Heredity* was published in 1915. His best-known work, this book clearly described the relationship between genes and chromosomes, outlined the role of chromosomes in heredity, and helped to establish genetics as an experimental rather than a descriptive science. In 1926 Morgan published *The Theory of the Gene,* which clearly summarized all that had been discov-

ered regarding the transmission of traits to offspring. The following year he wrote a text titled *Experimental Embryology*.

In 1927 the California Institute of Technology invited Morgan to Pasadena to found a new division of biology. He could not refuse this opportunity to establish an entire department based on his own philosophies about biology and the importance of experimental analysis. Much of his lab followed him, and he remained there for the rest of his career. His research after moving to California focused on embryological issues, such as egg cleavage and factors affecting development. Thus, he began and ended his scientific career by studying embryology.

Morgan was awarded the Nobel Prize in physiology or medicine in 1933 "for his discoveries concerning the role played by the chromosome in heredity." He generously shared the prize money with his former students and lifelong coworkers, Bridges and Sturtevant, to support their children's college education. Though his genetic discoveries leading to the Nobel Prize are his most famous, Morgan considered himself a broadly trained experimental zoologist and made significant contributions to the fields of embryology and developmental biology as well. He was a remarkable, multitalented scientist who was respected and admired by his colleagues. Because of this, he was elected a member of numerous scientific organizations and served as president of several, including the prestigious National Academy of Sciences (1927–28) and the Association for the Advancement of Sciences (1929). He was awarded both the Darwin Medal (1924) and the Copley Medal (1939) from the Royal Society of London.

Though he officially retired from Cal Tech in 1942, Morgan continued with his administrative responsibilities until his death in 1945 from a ruptured artery following a severe attack from a chronic ulcer.

Thomas Hunt Morgan and his students performed a remarkable number of experiments that lent unquestionable support to Mendel's laws of inheritance and generated large amounts of data which greatly advanced the science of genetics. Because of Morgan's research, geneticists have a better understanding of the physical entity of the gene and how genes are transmitted from generation to generation.

See also CHROMOSOMES; DARWIN, CHARLES; EVOLUTION, THEORY OF; GENETIC DISORDERS; GENETICS; INHERITANCE; MENDEL, GREGOR; POINT MUTATIONS.

FURTHER READING

Allen, Garland E. *Thomas Hunt Morgan: The Man and His Science*. Princeton, N.J.: Princeton University Press, 1978.

Morgan, Thomas H. *The Theory of the Gene*. Revised and enlarged edition. New Haven, Conn.: Yale University Press, 1928. Electronic edition published by Electronic Scholarly Publishing. Available online. URL: http://www.esp.org/books/morgan/theory/facsimile/. Accessed January 26, 2008.

Morgan, Thomas H., Alfred H. Sturtevant, Hermann J. Muller, and Calvin B. Bridges. *The Mechanism of Mendelian Heredity*. New York: Henry Holt, 1915. Electronic edition published by Electronic Scholarly Publishing. Available online. URL: http://www.esp.org/books/morgan/mechanism/facsimile/. Accessed January 26, 2008.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1933." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1933/. Accessed January 22, 2008.

Shine, Ian, and Sylvia Wrobel. *Thomas Hunt Morgan: Pioneer of Genetics*. Lexington: University Press of Kentucky, 1976.

Mullis, Kary (1944–) American *Biochemist*

In 1983 Kary Mullis conceived the idea of the polymerase chain reaction, a technique for exponentially and rapidly amplifying specific fragments of deoxyribonucleic acid. The utility of the method he invented for fields as far ranging as genetic engineering, forensics, and evolutionary biology earned him the Nobel Prize in chemistry in 1993.

CHILDHOOD AND EDUCATION

Kary B. Mullis was born on December 28, 1944, in Lenoir, North Carolina, to Bernice Alberta Barker Mullis and her husband, Cecil Banks Mullis. Kary spent his early childhood at his grandparents' farm in the rural foothills of the Blue Ridge Mountains. Kary's first experience with science involved a chemistry set that he received for Christmas and supplemented with chemicals purchased from the local drugstore. The enthusiasm he demonstrated delighted the chemistry teacher at Dreher High School in Columbia, South Carolina, where the Mullis family had lived since Kary was five years old, and she allowed him free reign in the laboratory after school hours. He launched homemade rockets by heating concoctions of potassium nitrate and table sugar, and once sent a live frog a mile into the sky. Kary, who was president of the Junior Engineering Technical Society, and a friend once put on a science demonstration for elementary students during which a crucible exploded in the opening moments during a pyrotechnic display.

Mullis received a bachelor of science degree in chemistry from the Georgia Institute of Technology in 1966. During the summers, he and a friend

synthesized and sold organic chemicals to a supply company. They set up a lab in his friend's garage and specialized in making chemicals that other companies discontinued, sometimes because they were too dangerous to make for the limited demand. They moved their lab into an old chicken coop after the friend's grandmother accidentally was tear-gassed upon entering the garage to do some laundry.

As an undergraduate, Mullis married a woman named Richards, who gave birth to a daughter, Louise. The University of California at Berkeley (UCB) accepted Mullis's application for graduate study in biochemistry, and the family moved west. His dissertation adviser, Joe Neilands, specialized in iron transport of microorganisms, but he encouraged his graduate students to explore their own interests. Mullis completed a dissertation titled, "Structure and Organic Synthesis of Microbial Iron Transport Agents" in 1972. He had published a paper unrelated to his doctoral dissertation in the prestigious journal *Nature* in 1968. "The Cosmological Significance of Time Reversal" suggested that half of the matter in the universe was going backward in time. After obtaining his doctorate, Mullis, by then divorced, remained at UCB for one year as a lecturer in biochemistry.

DEVELOPS AN INTEREST IN DNA

In 1973 Mullis moved to Kansas City, where his second wife entered medical school at the University of Kansas (UK). She left him, and the following year he met and then married his third wife, Cynthia Gibson, a nurse with whom he had two sons, Christopher and Jeremy. In Kansas, Mullis researched the biochemistry of a chronic lung disorder in pediatric cardiology at the UK Medical Center. Mullis moved back to Berkeley, where he managed a restaurant and coffee shop for two years, then in 1977 he got a job at the University of California in San Francisco working on endorphins, a group of morphinelike chemicals in the brain that suppress pain and promote a feeling of well-being. After attending a seminar about the cloning of the somatostatin gene, the gene encoding a polypeptide hormone that regulates the secretion of other hormones such as growth hormone and insulin, Mullis became inspired to learn more about DNA and its synthesis.

In 1979 Mullis took a job as a DNA chemist at Cetus Corporation, in Emeryville, California, where he quickly learned about DNA replication and improved the efficiency of production of oligonucleotides, short stretches of single-stranded DNA. One of the groups at Cetus was trying to come up with a method for detecting point mutations, alterations to a single base pair on a molecule of DNA that can have devastating effects and are responsible for some inherited diseases. The oligomer restriction assay consisted of a series of simple steps to detect changes in a nucleotide sequence, but it was not very reliable or efficient. The first step was to heat the DNA sample in order to denature, or separate, the two complementary strands. The addition of a radioactively labeled oligonucleotide that was complementary to the denatured target DNA resulted in hybridization between the oligonucleotide and its complementary sequence on the "template" strand, a process resembling a zipping together of the two strands. If the DNA did not have a mutation, then subsequent treatment with an enzyme called a restriction enzyme would cut the DNA at a specific site. If the target DNA did have a point mutation, then the restriction enzyme would not cut it. Analysis of the DNA following this treatment revealed whether or not the sequence contained the mutation that caused a particular disease.

Mullis thought about improving the oligomer restriction assay by adding DNA polymerase to the tube in order to extend the oligonucleotide. The DNA polymerase would "read" the next unpaired nucleotide from the template strand and add the appropriate matching nucleotide. If he used a special type of nucleotides called dideoxynucleotides, the polymerase could add only one single nucleotide to the oligonucleotide. By setting up four separate reactions, each containing a different radioactively labeled dideoxynucleotide, one could determine which nucleotide was present at the site of the putative mutation on the template strand. Mullis's technique for identifying point mutations worked on purified DNA samples, but the sensitivity of the technique was not sufficient if the region containing the sequence of interest was rare. While others pondered means to increase the final signal strength, Mullis wondered how he could increase the relative concentration of that one particular stretch of DNA.

CONCEIVES OF PCR

Mullis was concentrating on this challenge as he was driving to Mendocino on what has become a legendary Friday night in May 1983. He thought that since oligonucleotides were cheap and easy to make, why not put two into the reaction, with one binding to each strand of the double-stranded DNA molecule? Due to the unique directionality of the two complementary DNA strands, each oligonucleotide would direct synthesis toward the other. If the oligonucleotides were different initial lengths, Mullis could separate them later, and one could act as a control for the other. Though this procedure sounds rather complex, it is based on sound principles of known processes and is technically simple to perform in the laboratory.

Mullis had not yet solved the low concentration problem but had conceived a cheap and useful control mechanism for his experiments. While pondering potential complications, he considered the possibility of contaminating nucleotides in the mixture. If nucleotides other than the special dideoxynucleotides were present, then results would be difficult to analyze. To remove the contaminating building blocks, he could incubate the sample with DNA polymerase to use them all up, and then heat the reaction mixture to separate the DNA strands. Cooling the mixture would allow fresh, unextended oligonucleotides to hybridize to the target sequence, and the subsequent addition of the dideoxynucleotides and fresh DNA polymerase should overcome the problem. The thought then occurred to him that if the newly extended oligonucleotides were long enough, they also might hybridize to the unextended oligonucleotides added in the second round. He began to wonder if his idea was hopeless. Mullis suddenly realized the outcome of this contrived scenario would be the same—the sequence of the DNA in the target and in the extended oligonucleotides would be identical, but the concentration of the DNA of interest would be doubled. By purposefully adding what he had previously considered annoying "contaminating" nucleotides, he could ensure this happened, and the process could be repeated over and over. The first round of DNA polymerase action would double the amount of target DNA, two rounds would quadruple it, three rounds would increase it by a factor of eight, and so on. After 10 cycles, one million times the original amount of DNA would be present. Mullis not only solved the concentration problem, but in a breakthrough moment, he invented the polymerase chain reaction (PCR).

To summarize, the polymerase chain reaction involves a series of stepwise reactions that create multiple copies of a specific sequence of DNA. The enzyme that synthesizes DNA, DNA polymerase, requires the presence of oligonucleotides primers. The researcher designs primers that have sequences complementary to a specific region of DNA. Two different primers must be used, one must bind each of the two strands of a double-stranded template, and they each must prime DNA replication to proceed in the direction toward the other spanning the segment of DNA to be amplified. One round of PCR includes a denaturation step to break the hydrogen bonds between the complementary strands of DNA, an annealing step that allows the primers to bind to their complementary sites on the templates, and an elongation step, during which DNA polymerase adds nucleotides to the growing chain. At the completion of one round of PCR, the amount of DNA has doubled (2^1) theoretically. After two rounds, the amount has quadrupled (2^2). After three rounds the amount has increased by eightfold (2^3), and so on.

Mullis had come up with a way to provide an experimenter with an unlimited supply of any specific DNA, but his idea met with an indifferent reception. Believing the concept of PCR was novel and sound, he continued to talk about it with his friends and colleagues, but Mullis alone foresaw the future success and potential utility of this procedure.

Mullis did not perform his first experimental attempt at PCR until September 1983. For his target DNA, he chose a 400-base pair fragment from the human nerve growth factor gene, a single copy gene within the human genome, and designed the appropriate primers. He combined human DNA with the nerve growth factor primers in a small tube, boiled the mixture to denature the DNA, cooled it, added DNA polymerase, and left it sitting at 98.6°F (37°C) overnight. The next day, he anxiously looked for a 400-base pair fragment using a procedure called gel electrophoresis and a stain that causes DNA to fluoresce when exposed to ultraviolet radiation. He saw nothing, but he was not surprised. The rate for dissociation of the two strands of DNA was too slow, meaning he would have to heat the tube after each cycle to denature the DNA. Because high temperatures destroy DNA polymerase, fresh enzyme would have to be added after every denaturation event. The procedure was going to be much more time-consuming than Mullis originally anticipated. He spent three months making several modifications to the reagent concentrations and the temperatures and lengths of incubations, and he even changed the target DNA to a region of a plasmid. (Plasmids are closed, circular DNA molecules found in some bacteria.) Persistence paid off, and, on December 16, 1983, Mullis amplified a short fragment of DNA by PCR. He continued to work on improving his technique.

By June 1984, Mullis was at risk of losing his job at Cetus due to his perceived inability to work as part of a multidisciplinary project team. The personnel department gave Mullis one probationary year to prove the merits of PCR. Though Mullis believed he already had demonstrated its success, his peers demanded further proof, including better controls and more complete experiments. By that November, experiments clearly showed PCR worked, and skilled technicians refined the conditions until they obtained reliable, quantitative data showing amplification of DNA hundreds of thousands of times. Cetus filed the first PCR patent on March 28, 1985.

Though this accomplishment soon would revolutionize biotechnological research, remarkably, the prestigious scientific journals *Nature* and *Science* both rejected its publication, calling the paper too technical and unoriginal. Mullis did publish his

description of PCR in *Methods in Enzymology* in 1987 under the title "Specific Synthesis of DNA in Vitro via a Polymerase-Catalyzed Chain Reaction." In December 1985, *Science* had published the first application paper, "Enzymatic Amplification of Beta-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia," with Mullis listed as the third of seven coauthors. Mullis presented PCR at Cold Spring Harbor in May of 1986, and his talk, "Specific Enzymatic Amplification of DNA in Vitro: The Polymerase Chain Reaction," was published in the symposium's proceedings.

After inventing PCR technology in 1983, Mullis anticipated the need for a thermostable DNA polymerase to automate and popularize the method. The use of a heat-stable polymerase would relieve researchers from having to add fresh enzyme after the denaturation step of every cycle. In 1986 David Gelfand and Susanne Stoffel of Cetus purified *Taq* polymerase, the DNA polymerase from *Thermus aquaticus*. The bacteriologist Thomas D. Brock first isolated *T. aquaticus,* a thermophilic (heat-loving), rod-shaped, prokaryotic organism, from hot springs in the Great Fountain area of the Lower Geyser Basin of Yellowstone National Park in the late 1960s. The organism grows at temperatures between 122–176°F (50–80°C), and its enzymes function optimally around 158°F (70°C) but can withstand heating up to 203°F (95°C), the temperature at which denaturation usually is performed during PCR. The discovery of organisms that lived in such extreme environments was remarkable because high temperatures destroy typical biological molecules such as proteins and DNA.

Gelfand, Stoffel, and others received the patent for purified *Taq* polymerase in 1989, the same year that the enzyme was named "Molecule of the Year" by *Science,* one of the journals that had rejected Mullis's original paper describing PCR only three years earlier. Today, most PCR is performed using cheaply produced, cloned, recombinant *Taq* polymerase. Using an automated thermocycling machine, the process takes only a few hours to complete a few dozen cycles, depending on the specific parameters of the program.

RECOGNITION FOR INVENTING PCR

The Royal Swedish Academy of Sciences awarded Mullis the Nobel Prize in chemistry for 1993, shared with Michael Smith (1932–2000), for their contributions to the developments of methods within DNA-based chemistry. Smith was a Canadian molecular biologist from the University of British Columbia who developed the technique of oligonucleotide-based, site-directed mutagenesis of DNA. The rapid awarding of the Nobel Prize for something discovered less than a decade before was testament to the profound impact PCR had on scientific research. That same year, Mullis received the prestigious Japan Prize for his invention of PCR.

Cetus became involved in a battle with E. I. Dupont de Nemours and Company over rights to the patent for PCR. Cetus eventually won, but later sold the patent to Hoffmann-La Roche for $300 million. Mullis was rewarded for his contributions with a check for $10,000, the largest bonus Cetus ever had paid to a scientist. Mullis left Cetus in 1986, and Xytronyx, in San Diego, hired him as director of molecular biology. He worked on DNA technology and photochemistry for a while, and, in 1987, he began consulting privately on nucleic acid chemistry for numerous corporations, making it his full-time occupation in 1988.

Modifications to the technique of PCR improved its efficiency, reliability, and versatility, and, as Mullis predicted, its use spread like wildfire. As a result, the discoverer was inundated with numerous awards in the early 1990s, including the National Biotechnology Award (1991), the R&D Scientist of the Year Award (1991), the California Scientist of the Year Award (1992), the Thomas A. Edison Award (1993), and many more. He also received an honorary doctorate degree from the University of South Carolina in 1994 and was inducted into the National Inventors Hall of Fame in 1998.

Not everyone agreed that Mullis deserved so much recognition; in fact, several of his scientific colleagues resented that fact that he received so much publicity, much less the coveted Nobel Prize. As a confessed psychedelic drug-using surfer, Mullis was not an ideal poster boy for science, and he had a reputation for creating unwanted controversy. Mullis declared that the human immunodeficiency virus (HIV) did not cause acquired immune deficiency syndrome (AIDS), despite the fact that this theory was widely accepted by the medical community. He also argued that the ozone layer was intact, opposing the findings of reputed atmospheric scientists. He even declared that he had an experience with extraterrestrials, asserted that his dead grandfather once spent several days visiting him, and affirmed astrological claims.

Even if many of Mullis's outlandish beliefs seem silly, the benefits of the procedure he imagined and first performed cannot be denied. Today, the versatile procedure of PCR is used to diagnose genetic disorders, identify infectious disease agents, determine paternity, perform forensic analyses, and relate extinct organisms with extant life-forms. All molecular laboratories employ PCR as a standard procedure within their modern research programs.

Since winning the Nobel Prize, Mullis published *Dancing Naked in the Mind Field* (1998), an autobiographical exploration of his thoughts and opinions on a variety of subjects, including modern science, the use of hallucinogenic drugs, the workings of large companies, and romantic relationships. That same year, Mullis married his fourth wife, Nancy Lier Cosgrove, with whom he lives in Newport Beach, California, and in Anderson Valley, California. He then worked at Burstein Technologies in Irvine, where he was vice president and director of molecular biology. More recently, he founded Altermune, LLC, a venture resulting from his newest patent on chemically programmable immunity. His approach is to stimulate a specific immune response by administering a chemical linker that joins an individual's preexisting antibodies to a moiety that specifically recognizes and binds a pathogen that has invaded that person's body. This novel concept is currently being tested in rodents, and Mullis hopes it will work in humans.

See also BIOMOLECULES; DEOXYRIBONUCLEIC ACID (DNA); MOLECULAR BIOLOGY; POLYMERASE CHAIN REACTION; RECOMBINANT DNA TECHNOLOGY.

FURTHER READING

Mullis, Kary B. *Dancing Naked in the Mind Field*. New York: Pantheon Books, 1998.

———. Kary Mullis: Chemist, Author, Nobel Laureate. Available online. URL: http://www.karymullis.com/. Accessed January 15, 2008.

Mullis, Kary, and Fred A. Faloona. "Specific Synthesis of DNA in Vitro via a Polymerase Catalyzed Chain Reaction." *Methods in Enzymology* 255 (1987): 335–350.

Mullis, Kary, Fred Faloona, Stephen Scharf, Randall K. Saiki, Glenn T. Horn, and Henry A. Erlich. "Specific Enzymatic Amplification of DNA in Vitro: The Polymerase Chain Reaction." *Cold Spring Harbor Symposia on Quantitative Biology* 51 (1986): 263–272.

The Nobel Foundation. "The Nobel Prize in Chemistry 1993." Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1993/. Accessed January 15, 2008.

Rabinow, Paul. *Making PCR: A Story of Biotechnology*. Chicago: University of Chicago Press, 1996.

musculoskeletal system The musculoskeletal system comprises the muscular and the skeletal systems. The purpose of the muscular system is locomotion or movement. The functions of the skeletal system are to provide structural support, protect the body's internal organs, and aid in movement by providing a simple mechanical lever system against which the muscles work.

TYPES OF SKELETAL SYSTEMS

Different types of skeletal systems include hydrostatic skeletons, exoskeletons, and endoskeletons. Many soft-bodied invertebrates such as hydra, flatworms, roundworms, and earthworms have a hydrostatic skeleton that provides support for the organism. The gastrovascular cavity or coelom is filled with fluid, and the pressure against the closed compartment gives shape to the animal. Hydrostatic skeletons work on the same principles as a water balloon. When the balloon contains only a small amount of water, it is floppy and applying pressure to it causes its shape to change. As the water content inside increases and fills the balloon, the pressure the water exerts on the balloon gives it a more defined shape that resists changes by the application of gentle pressure. Hydrostatic skeletons provide enough rigidity to allow crawling or burrowing, but cannot support the body upright off the ground against the pull of gravity for locomotion such as walking. Peristalsis, a rhythmic contraction of muscles along the length of an organism or structure, is supported by hydrostatic skeletons. In an earthworm, for example, the organism contracts circular muscles, stretching the animal lengthwise and forcing fluid toward the ends. When longitudinal muscles that extend along the length of the animal contract, the animal shortens and fluid pushes outward, making the worm plump. In coordination with bristles that grab the ground as it alternates contraction of its circular and longitudinal muscles, the worm moves forward. Hydrostatic skeletons also offer some cushion and protection for the internal organs.

Exoskeletons are hard, protective coverings that exist on the outside of an animal and provide points of attachment for muscles. Invertebrates such as mollusks and arthropods have exoskeletons. Mollusks like clams and oysters secrete calcium carbonate from their mantle, and it hardens to form a shell. As the animal grows, the shell's diameter increases by the addition of more calcium carbonate to the outer edge. The muscles of bivalves are attached to the two hinged shell halves and contract to close them. The exoskeletons of arthropods, including insects, spiders, and crustaceans, are called cuticles. They form from secretions of the epidermis and consist of fibrils of the polysaccharide chitin embedded in a protein matrix. The rigidity of the cuticle varies depending on its location within the body. Portions of the cuticle whose main job is protection are hardened by the addition of extra calcium salts, whereas areas surrounding joints contain less inorganic salts and less cross-linking between the proteins, and are therefore more flexible, allowing movement. Because the cuticle is nonliving, it does not grow with the animal and must be shed periodically by molting.

Endoskeletons are located inside the body and are composed of a hard material. In sponges, spicules made of inorganic substances and protein fibers provide internal support. Because sponges are sessile, meaning they remain attached to a surface such as a rock, the skeletal components do not have a locomotor function. Hard, calcareous plates called ossicles lie just beneath the skin of echinoderms to provide protection and support. They are bound together by proteins to various degrees depending on the amount of movement that is required. Sea stars need flexibility to move their arms, so their ossicles are loosely bound, whereas the ossicles of sea urchins are more tightly bound.

The endoskeletons of chordates are made of bone and cartilage connected at joints that allow movement. Mammalian skeletons, including those of humans, are divided into axial and appendicular components. The axial skeleton consists of the skull, the backbone, and the rib cage. In humans, 29 skull bones include those that make up the cranium, which protects the brain against injury, facial bones, and bones in the middle ear that play a role in hearing. The skull is attached to the top of the vertebral column, which is made up of 26 vertebrae that hold the body upright. The rib cage contains 12 pairs of ribs that surround the chest, protecting the body's most vital organs, and attach to the sternum or breastbone at the front of the chest.

The appendicular skeleton of humans includes 126 bones that make up the pectoral and pelvic girdles and the limb bones. The pectoral girdle consists of two shoulder blades, called scapulas, and two long curved collar bones called clavicles that attach to the top of the sternum. The pelvic girdle contains two wide pelvic bones that help distribute the weight of the upper body over the legs. The appendages, or limbs, are attached to the girdles, which connect the axial and appendicular skeletons to one another. The humerus is located in the upper arm, the radius and ulna in the lower arm, and the carpals and metacarpals make up the wrists and hands, and the phalanges make up the fingers. The femur, or the thigh bone, is the longest bone in the human body. The patella, also known as the kneecap, connects the femur to the lower leg bones, the tibia or shinbone, and the fibula. The tarsals, metatarsals, and phalanges make up the heels, feet, and toes, respectively.

JOINTS

Joints, also called articulations, are where two bones come together. At the junction, cartilage cushions the surfaces of the opposing bones against pressure and stress from weight bearing and force. A capsule of connective tissue covers the ends of the bones at the joint and contains synovial fluid, a substance that resembles egg white and functions to lubricate the joint during movement. Ligaments are the strong bands of tissue that hold bones together and prevent them from moving too far. Animals can perform a variety of movements due to five different types of joints: ball-and socket, pivot, hinge, gliding, and saddle joints. Some joints are immovable, such as the sutures in the skull. Others are slightly movable, such as where the bones of the rib cage meet the sternum or between the vertebrae of the spinal column. The rest are referred to as freely movable or synovial joints, and their structure determines the degree and type of movement allowed.

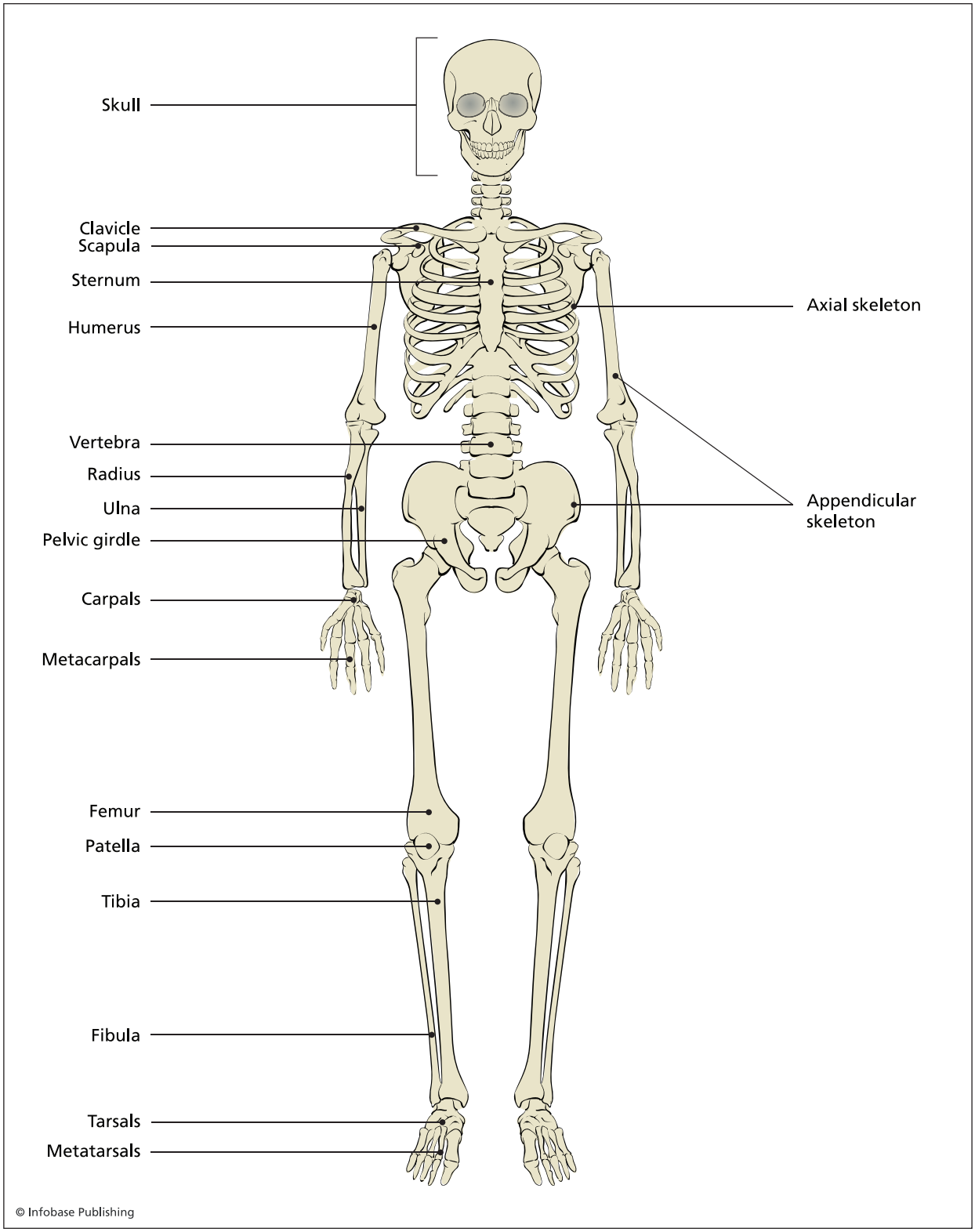
Ball-and-socket joints occur in the shoulders, where the humerus meets the shoulder girdle, and the hips, where the femur meets the pelvic girdle, and enable movement in all directions. The joint where the head meets the top of the spine is an example of a pivot joint that allows turning or rotation. Hinge joints restrict movement to a single plane, as in the knuckles of the fingers and toes. Gliding joints allow sliding motions and provide much flexibility when several occur together as in the wrists or ankles. Rotation, bending, and straightening are all permitted by saddle joints, like at the base of the thumb.

BONE TISSUE

During early development, the skeleton of most vertebrates consists of mostly cartilage that serves as a framework upon which bone is formed by the deposition of minerals. Bone is a living, dynamic tissue that contains living cells, requires a blood supply, and is innervated, meaning it is supplied with nerves. Osteoblasts, the cells that build bone, first deposit a matrix of the protein collagen and then secrete calcium, magnesium, and phosphate ions that harden into hydroxyapatite. Because bones are hard, they confer protection to the internal organs and provide a firm base against which muscles can pull to accomplish movement. A lesser known function of bone is storage of minerals such as calcium, magnesium, and phosphorus.

There are two types of bone tissue: compact and spongy. Approximately 80 percent of the human skeleton consists of the denser compact bone. At the microscopic level, compact bone consists of units called Haversian or osteonic canals that extend lengthwise. Surrounded by several layers of mineralized tissue that form concentric rings when viewed in cross section, the Haversian canals house the blood vessels and nerves that service the bones. Mature bone cells, osteocytes, are located in the lacunae, the spaces between the rings. Located inside the outer layer of hard compact bone, spongy bone tissue is

more loosely packed, resembling a honeycomb, and its spaces are filled with bone marrow that stores energy and is the site for blood cell synthesis. A tough membrane called the periosteum surrounds and protects the bones and contains many blood vessels that bring nutrients to the bone tissue.



An adult human skeleton comprises 206 bones that protect the body's soft organs and work with the muscular system to accomplish movement.

SKELETAL MUSCLE STRUCTURE

Muscle tissue consists of cells that can contract to produce movement. A muscle is considered an organ of the muscular system and consists of muscle tissue, connective tissue, nerve tissue, and vascular tissue. There are three main types of muscle:

- smooth,
- cardiac,
- and skeletal muscle.

Smooth muscle, which is under involuntary control, is found in the walls of blood vessels, the digestive tract, the bladder, and the uterus. Each smooth muscle cell has a single nucleus and is elongated with tapered ends. Cardiac muscle is found only in the walls of the heart. Like smooth muscle cells, each rectangular-shaped cardiac muscle cell has one nucleus and is under involuntary control, but cardiac muscle has a striated appearance like skeletal muscle. Skeletal muscles usually span a joint and are attached to the bones by tendons. Each skeletal muscle contains hundreds or thousands of long, cylindrical muscle fibers bundled together by connective tissue. Each fiber is an individual multinucleate cell formed from the fusion of many embryonic cells and contains numerous myofibrils. Two kinds of myofilaments make up the myofibrils: thin filaments and thick filaments. Thin filaments are made primarily of the protein actin, and thick filaments are made of the protein myosin. The two types of filaments alternate to form the smallest unit of muscular contraction, the sarcomere. Their longitudinal arrangement gives skeletal and cardiac muscles their striated appearance.

Within a sarcomere, the thin filaments are connected to Z lines that define the borders of the sarcomere. The thick filaments are located in between the thin filaments. In the relaxed state, the thick filaments do not extend all the way to the Z lines. The region that contains only thin filaments is called the I band, and it includes portions of two adjacent sarcomeres. The center region that contains only thick filaments in the relaxed state is called the H zone, and it is bisected by the M line. The A band is the portion that spans the thick filaments; its width equals the width of the sarcomere in the contracted state.

MUSCLE FUNCTION

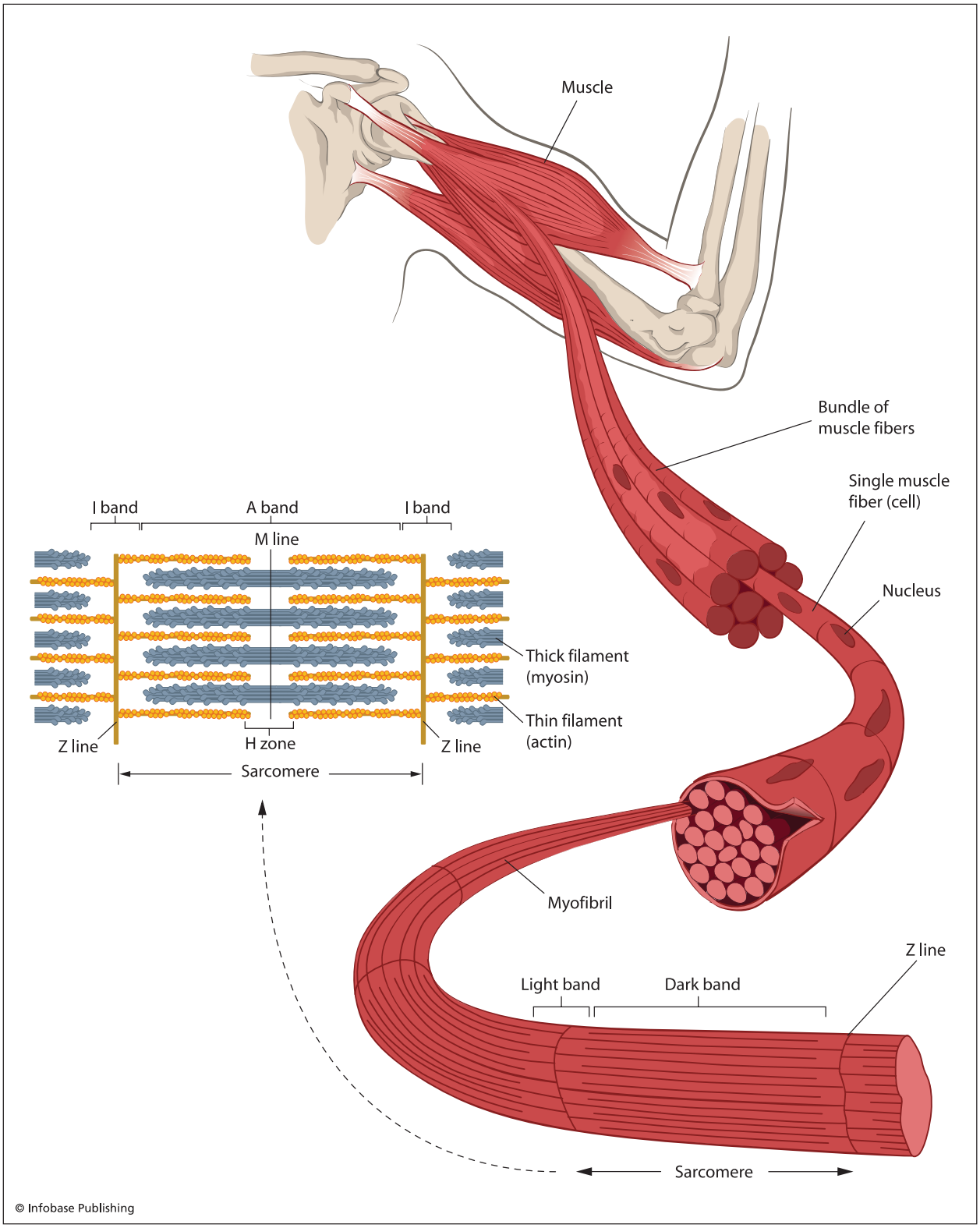
Muscle contracts actively, but elongation, or relaxation, is passive. In order for a muscle to contract, it first must receive a signal from the nervous system. A motor unit consists of a motor neuron and the muscle cells it controls. One neuron can branch out and contact several individual muscle fibers, allowing one signal to stimulate a whole group of fibers to contract simultaneously. The intensity of contraction increases as the number of stimulated fibers increases. Muscles that require fine coordination, such as the fingers, have motor units with only a few muscle fibers. Muscles that perform mostly heavy work, such as the thighs, have hundreds of muscle fibers belonging to a single motor unit.

The contraction of a muscle is due to the contraction of the sarcomeres within the myofibrils of the muscle fibers in the bundle. When relaxed, the myosin and actin filaments overlap slightly. During contraction, the actin and myosin slide along each other, increasing their overlap, pulling the Z lines together, and shortening the sarcomere. The width of the A band stays the same, while the H zone and I bands decrease in width. The lengths of the thick and thin filaments in the sarcomere remain constant, but the filaments slide past each other. The shape of myosin resembles a golf club, with a long, fibrous tail attached to a globular head. Myosin tails remain attached to other myosin tails to form the thick filament, while the head regions protrude and interact with the actin of the thin filaments. The myosin head turns, causing the actin to slide laterally, and the amount of overlap increases. One high energy molecule of adenosine triphosphate (ATP) is hydrolyzed, and the released energy is used as the myosin head detaches and then reattaches to the actin farther along the filament. As the myosin head continues to grab and pull the actin filament, the thick and thin filaments slide past one another, and the sarcomere shortens.

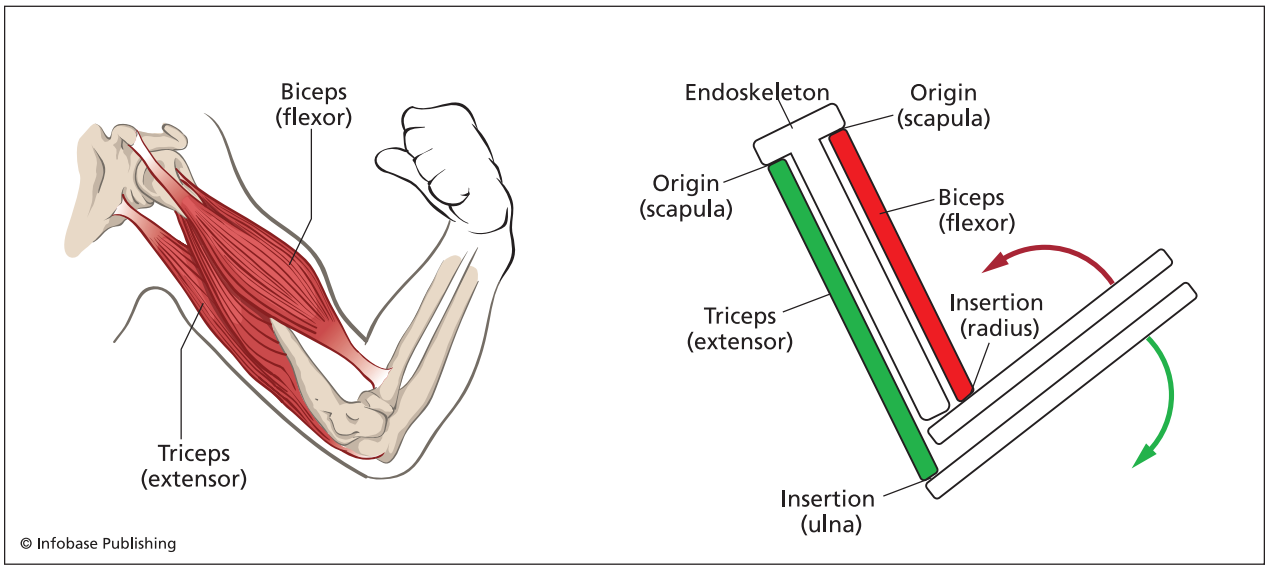
The bodily movements caused by the contraction of skeletal muscles occur because the two opposite ends of muscles are attached by tendons to two different bones. One of the bones moves and the other remains stationary. The point of attachment at the stationary bone is called the origin, and the attachment site at the bone that moves is called the insertion. Contraction of the muscle pulls the insertion toward the origin. Skeletal muscles usually occur in antagonistic pairs. A flexor muscle causes a joint to bend, whereas contraction of an extensor muscle causes the joint to straighten. For example, the biceps (flexor) and triceps (extensor) form an antagonistic pair of muscles. When the biceps contract, the arm bends at the elbow joint, and when the triceps contracts, the arm straightens out.

Because ATP is required to move the myosin head, muscle contraction requires a lot of energy. ATP also helps the muscle cells to regulate their concentration of calcium ions. This is important because the binding of the myosin heads to the actin filaments is regulated by calcium ions. Since aerobic respiration is the most efficient means for cells to make ATP, oxygen is consumed rapidly by the muscle cells during prolonged exercises such as walking. In contrast, brief, intense exercises such as weight lifting or sprinting use ATP

created mostly by anaerobic pathways like fermentation. When ATP is consumed at a faster rate than cells can produce it, muscle fatigue occurs, leading to soreness. Some muscles, such as those involved in maintaining posture, are always partially contracted. Since prolonged contraction leads to muscle fatigue,



In skeletal muscle, units called sarcomeres make up myofibrils, which assemble into individual muscle cells that bundle together to form a functional muscle.



Skeletal muscles usually work in antagonistic pairs. When a flexor muscle contracts, the joint is bent, and when the opposing extensor muscle contracts, the joint straightens.

the nervous system switches the motor units involved in maintaining the contracted state.

See also ANATOMY; ANIMAL FORM; CELLULAR METABOLISM; GENETIC DISORDERS; INVERTEBRATES; NERVOUS SYSTEM; PHYSIOLOGY; VERTEBRATES.

FURTHER READING

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton.* 2 vols. Detroit, Mich.: U*X*L, 2000.

Sayler, Mary Harwell. *The Encyclopedia of the Muscle and Skeletal Systems and Disorders.* New York: Facts On File, 2005.

Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology.* 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

White, Tim D., and Pieter A. Folkens. *The Human Bone Manual.* Burlington, Mass.: Elsevier Academic Press, 2005.

nervous system As the body's command control center, the nervous system displays complexity unparalleled by other physiological systems. Its main functions are to detect sensory input from inside the body and from the external environment, to integrate the information, and to respond accordingly. The nervous system is responsible for coordinating all body processes, movements, thoughts, and other activities. Communication is achieved via rapid-fire electrical signals that travel through a network of nerve fibers that branch throughout the body.

ORGANIZATION OF THE NERVOUS SYSTEM

Though one characteristic of life is the ability to respond to the environment, not all animals have a complex nervous system. The simplest nervous systems, as are found in cnidarians, do not have an area of central integration, though a nerve net allows them to respond to environmental stimuli. Slightly more complex, sea stars have a central nerve ring with nerves branching from it into each arm, allowing the animal to coordinate the movement of hundreds of tube feet along the arms during feeding. Bilaterally symmetric animals have nervous systems that are slightly more complex. Cephalization, the concentration of nerve tissue and sensory organs at the anterior end of an organism, often accompanies bilateral symmetry. In flatworms such as planaria and annelids such as earthworms, nerve cords—thick bundles of nerves that run the length of the animal body—extend from the primitive brain. Annelids also have larger brains and branched ganglia, clusters of nerve cell bodies that work together, in each body segment. The presence of ganglia in the segments allows annelids to carry out some simple reflexes, responses to stimuli that do not require processing by the brain. (The knee-jerk reflex is an example of simple reflex in humans. When tapped just below the knee, the spinal cord integrates the information and communicates a response directly to the lower leg, which immediately extends.) Arthropods (members of the phylum that includes insects, spiders, and crustaceans) also have ganglia arranged in segments and exhibit even more centralization at the brain. Mollusks exhibit a wide range of nervous system organizations dependent on their particular ecological niche. Mostly sessile mollusks such as clams have very little cephalization whereas keen predators like squids and octopuses have the most sophisticated nervous systems among the invertebrates. Octopuses have extremely large brains and are even capable of learning.

Though they are diverse, the highly centralized and cephalized nervous systems of vertebrate animals are partitioned into two divisions: the central and peripheral nervous systems (CNS and PNS). The central nervous system is derived from the dorsal hollow nerve cord of chordates and consists of the brain and spinal cord. The brain serves as the control center and processes information. The spinal cord relays information from sensory input to the brain, carries signals from the brain to the body, and responds to some simple stimuli. Layers of connective tissue called meninges encase the CNS. Blood does not penetrate the brain, but salty cerebrospinal fluid that contains nutrients, hormones, and white blood cells fills the center of the spinal cord and spaces called ventricles in the brain. Cerebrospinal fluid is also found in between the meninges in mammals, functions as a shock absorber for the brain, and provides a regulated extracellular environment for

"THE BIOLOGY OF ADHD"

by Peggy Shadduck Palombi, Ph.D.
Transylvania University

Attention-Deficit/Hyperactivity Disorder (ADHD) is a behavioral disease that is thought to be caused by an imbalance in specific chemical neurotransmitters in the brain. The disorder is classified as a mental or psychiatric illness and is characterized by an inability to concentrate and control impulses to carry out certain behaviors; in other words, the patient acts without thinking about the consequences. To be classified as attention deficit disorder, this lack of impulse control must be sufficient to interfere with the person's ability to carry out daily tasks such as following directions, completing work carefully and on time, and refraining from risky or disruptive behaviors. The person may not appear to be listening when given instructions and may have great difficulty in organizing tasks, seeming to be forgetful and easily distracted by things they see or hear. Not all diagnosed individuals exhibit the hyperactivity component, which includes nearly constant fidgeting and wiggling as well as talking, humming, and drumming or tapping on a surface in many individuals. Some people cannot manage to stay in a seat for more than a few minutes. They may also interrupt when others speak and have difficulty waiting for their turn.

ADHD is quite a complex disorder and may represent a whole group of brain disorders that affect inhibitory control, ability to consider the future when making decisions, ability to handle delay, and attentional ability. Experiments using rodents, primates, and other laboratory animals indicate a potential difference between errors of omission, in which the person's poor attentional abilities lead them to skip over parts of a task or to not complete the task, and errors of commission, in which the person has difficulty waiting for something or delaying a reward. In other words, ADHD can include both the inability to DO something because someone has been distracted by something else and the inability to NOT DO something because someone cannot wait.

Diagnosis of ADHD often occurs between the ages of six and 10, when children are first expected to follow a routine in a fairly structured school environment, but a child can exhibit symptoms as early as age two or three. Teachers and parents usually first observe the problem, but a physician must confirm the diagnosis. Since this is a disorder of the brain, no simple tests to determine if someone has ADHD exist. Instead, physicians rely on the observations of behavior by teachers and caregivers before and after the administration of medication. A significant improvement in a person's impulse control, attention, and/or hyperactivity following the administration of a stimulant medication confirms the preliminary diagnosis of ADHD based on questionnaires. Some people report that their symptoms subside during adolescence and adulthood, allowing them to stop taking medication to control the problem. Research does not clearly demonstrate whether ADHD really goes away as the brain develops further in those individuals, or whether they just mature in their ability to control their impulses such that the medication is no longer necessary. Many people still report symptoms of ADHD into adulthood, and sometimes diagnosis does not occur until then.

The first medication that was (and still is) widely used to treat ADHD is methylphenidate (brand name Ritalin). Classified as a stimulant, this medication triggers an increase in the amount of the neurotransmitters dopamine and norepinephrine in the brain. The effects are short-lived, lasting only a few hours in most people. Although at first glance giving a stimulant medication seems counterintuitive, scientists believe a lack of the inhibitory or suppressing chemicals in the brain causes this disorder. Methylphenidate causes an apparent increase in the activity of the brain circuits that

the neurons. Nerve tissue of the CNS consists of two distinguishable types of tissue. White matter consists mostly of bundled nerve fibers that are whitish in appearance due to the presence of myelin sheaths. Gray matter contains nerve cell bodies, dendrites, and axon terminals and is brownish-gray in color. In the CNS, collections of neurons are called nuclei, and collections of axons are called tracts.

Approximately 100 billion neurons and trillions of support cells called glia make up the brain, which consists of three main sections: the cerebrum, the cerebellum, and the brain stem. The large size of the cerebrum distinguishes humans from other vertebrates. A blood-brain barrier formed by capillaries that are less permeable than other capillaries in the body protects the brain from potentially dangerous substances in the blood. Necessary nutrients such as glucose can still pass through this barrier by specific membrane carriers. The cerebrum is divided by a deep groove into right and left hemispheres that communicate through a band of axons called the corpus callosum. Each hemisphere contains an extensively folded and furrowed cerebral cortex, which is less than one-fifth of an inch (5 mm) thick. Made of gray matter, the cerebral cortex surrounds white matter and the basal nuclei that control motor coordination. The surface of each side of the brain has four distinct lobes: the frontal lobe, the parietal lobe, the temporal

control or suppress excessive movement and unfocused thought. This allows the individual to keep the task at hand in the center of their attention rather than get distracted by all the other activity occurring in their brain. In people who do not have ADHD, however, methylphenidate can stimulate too much brain activity, resulting in feelings of excitement and hypervigilance or excessive focus; for this reason, people seeking these feelings often abuse methylphenidate and related substances. People who desire what are usually classified as unwanted side effects—appetite suppression and sleep suppression—also abuse these drugs. In the last decade, pharmaceutical companies have marketed a variety of new medications for the treatment of ADHD. These substances are designed to improve impulse control and focus without the abuse potential or side effects of methylphenidate. Another benefit of many of the new drugs is the longer duration of their effect on the body; rather than taking a pill every few hours, ADHD patients can now take only one or two pills a day.

The specific causes of ADHD are not known, although some evidence suggests a hereditary component. Children of people diagnosed with ADHD are themselves more likely to be diagnosed. The disorder affects more boys than girls, as do many childhood behavioral disorders. Recovery of function with maturation, as noted in some ADHD patients, indicates that the problem stems from delayed development in certain parts of the brain. However, deficits in function are still noted in teenagers and adults, more with attention difficulties than with hyperactivity, so the recovery may have more to do with the ability of some patients to learn how to compensate for the deficits than it does with improvement in their brain function. Children who exhibit the symptoms of ADHD also often have other learning problems (such as dyslexia) or other neuropsychological problems (such as depression or anxiety). This observation indicates how tightly attention and impulse control correlate with other behaviors and makes diagnosis and treatment difficult.

Evidence suggests that the areas of the brain most affected in ADHD patients are the lower areas of the frontal lobes and the basal ganglia, which are found in the lower front region of the mammalian brain. ADHD may also affect the cerebellum (located in the lower back part of the brain). Magnetic resonance imaging studies of children diagnosed with ADHD indicate that either the total number of brain cells (neurons) in these regions or the number of connections between the neurons may be slightly (3–4 percent) lower than normal. Dopamine and norepinephrine are two of several neurotransmitters that help the millions of neurons communicate with each other in the frontal and cerebellar regions of the brain, with serotonin also being heavily involved. The reduced number of neurons and/or connections may not be able to provide sufficient control of behavior through what researchers refer to as executive functions. In particular, evidence from human and animal research suggests people diagnosed with ADHD are not able connect with and use areas of the brain that help with planning or rehearsal of behaviors before they occur. Methylphenidate increases the levels of dopamine and norepinephrine, thereby enhancing the ability of the connections that do exist to work effectively. Serotonin may adjust or modulate the level or activity of dopamine and norepinephrine; so drugs that increase the serotonin level in these areas of the brain are also sometimes helpful in treating ADHD. Because dopamine, norepinephrine, and serotonin are involved in neuronal communication throughout the brain, not just in the frontal and cerebellar areas affected in ADHD patients, scientists are working on developing drugs that will be more selective for the frontal regions. The successful development of such drugs may eliminate the side effects of sleeplessness and appetite suppression, as well as the potential for abuse.

FURTHER READING

Barkley, Russell A. *Attention-Deficit Hyperactivity Disorder: A Handbook for Diagnosis and Treatment.* 3rd ed. New York: Guilford Press, 2006.

Nigg, Joel T. *What Causes ADHD? Understanding What Goes Wrong and Why.* New York: Guilford Press, 2006.

lobe, and the occipital lobe. A fifth lobe, the insula, is buried between the temporal and the parietal lobes. Functional areas within the lobes control different aspects of voluntary movement, thought, language, reasoning, and perception, qualities that are characteristically human. Without any external input, the human brain can generate independent thought and output information.

Located below the cerebrum and behind the brain stem, the cerebellum functions to coordinate skeletal movements and regulate posture and balance. The brain stem sits on top of the spinal cord and comprises several structures that maintain homeostasis, coordinate movements, and relay information to other parts of the brain: the medulla oblongata, the pons, and the midbrain. The medulla oblongata controls many autonomic, or involuntary, functions such as breathing, beating of the heart, blood pressure, swallowing, and vomiting. Tracts that communicate information between the cerebral cortex and the spinal cord pass through here, crossing the midline to the opposite side of the body en route. Because of this, the right side of the brain controls the left side of the body and vice versa. The pons also plays a role in regulating breathing and relays messages from sensory and motor pathways originating in other parts of the brain. The midbrain plays roles in eye movement and auditory and visual

reflexes. The thalamus, located in the upper brain stem, receives sensory input, processes it, and relays information to the cerebral cortex. Outgoing signals initiated by the cerebral cortex in response also pass through the thalamus. Located just underneath the thalamus, the hypothalamus regulates body temperature, is involved in emotions, and controls feelings of hunger and thirst. It also controls the release of certain hormones from the pituitary gland and regulates circadian rhythms, functions or bodily activities that correlate with a 24-hour period, such as sleep and wakefulness.

The spinal cord originates at the medulla oblongata and runs down the center of the vertebral column. Information communicated between the peripheral nervous system and the brain passes through the spinal cord. Some reflexes (automatic, involuntary responses to sensory input) are controlled by the spinal cord. The center of the spinal cord consists of gray matter, while the outer portion consists of heavily myelinated white matter. Tracts of axons make up columns of white matter. Ascending tracts carry sensory input to the brain, and descending tracts transmit responses to muscle cells or effector organs. The spinal cord branches into 31 pairs of nerves that, with 12 pairs of cranial nerves, carry information between the CNS and the entire body and head. Each nerve has a dorsal root made of sensory neurons that carry information to the CNS and a ventral root made of motor neurons that carry information from the CNS to muscles and other organs.

The PNS includes the cranial nerves that innervate the head and face and the spinal nerves that branch into the entire body. The nerves contain both sensory (afferent) and motor (efferent) neurons that carry information in the form of electrical signals to and from the CNS, respectively. The sensory division of the PNS receives stimuli from the internal and external environment and transmits the input to the CNS for processing. After processing, the CNS sends out information necessary for executing an appropriate response through the motor division of the PNS. For example, if someone is sitting in the bleachers at a baseball field and sees a baseball headed toward them, the sensory division transmits the visual input to the CNS. The brain processes the information and, in response, sends signals through the motor division instructing the person to duck. In the PNS, collections of neurons are called ganglia, and collections of axons are called nerves.

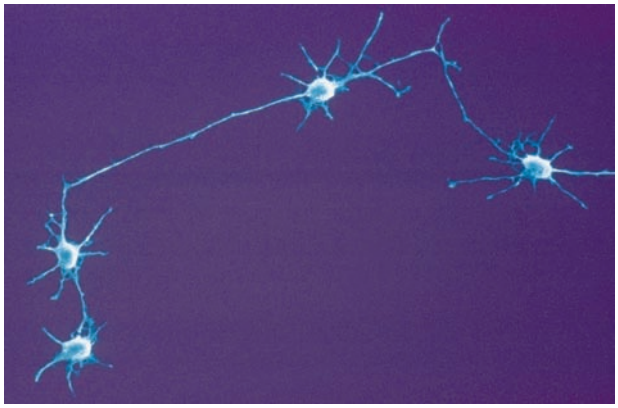
The motor division is further divided into the autonomic and somatic nervous systems. The autonomic nervous system controls involuntary muscles such as cardiac muscle responsible for the beating of the heart and the smooth muscles surrounding the stomach that contract to churn the food during digestion, processes that require no conscious input. Two divisions of the autonomic nervous system stimulate opposite physiological effects but function together to maintain balance or homeostasis. The sympathetic division conveys signals in response to stressful conditions that dilate the pupils, increase the heart rate, open the bronchioles, inhibit digestive activity, and dilate the blood vessels. This is called the fight-or-flight response because it prepares someone to take rapid action in dangerous situations, like being chased by a swarm of bees, but also dominates in times of emotional stress, such as during an important test. The parasympathetic division dominates during normal or resting conditions and acts to constrict the pupils of the eyes, slow the heart rate, constrict the bronchioles, and stimulate digestive processes. The vagus nerve is a major parasympathetic tract that innervates most of the body's internal organs. A lesser known third division, the enteric system, can work with the CNS or autonomously and is involved in coordinating digestive processes. Because the enteric system, which innervates the gastrointestinal tract, the pancreas, and the gall bladder, is capable of integrating information and carrying out a response without input from the CNS, its inclusion as part of the autonomic nervous system might change.

The somatic nervous system of the motor division of the PNS is considered voluntary because a person has some conscious control over the movements coordinated by this branch. This system consists of nerve fibers that carry sensory input to the CNS and nerve fibers that send signals from the CNS to effector cells (such as skeletal muscle cells) in response. Whereas pathways of the autonomic nervous system can be either excitatory, meaning the pathway stimulates an action) or inhibitory (meaning the pathway prevents an action from occurring), somatic pathways are always excitatory.

NEURON STRUCTURE

The nervous system consists of both neurons and supporting cells. Neurons are the functional units of the nervous system that transmit electrical signals, but they are outnumbered by supporting cells by more than tenfold. The supporting cells are called glia, meaning glue, and they perform a variety of functions including a role in the development of new neurons in embryos, providing structural and metabolic support for neurons, forming the blood-brain barrier, and producing myelin.

Neurons have a cell body that contains the nucleus and other typical organelles. Numerous dendrites and axons protrude from the cell body. The dendrites branch extensively and receive signals that they relay to the cell body. Axons, which may or



Neurons consist of a cell body, several dendrites that receive signals, and a long, thin axon that transmits neural impulses. *(Andrew Paul Leonard/Photo Researchers, Inc.)*

may not be branched at their ends, carry impulses from the hillock, the region where the axon joins the cell body, to another neuron or an effector cell. The longest axons can reach several feet (about one meter), or be hundreds of thousands of times shorter than that. Formed by Schwann cells in the PNS and oligodendrocytes in the CNS, a layer of insulation called a myelin sheath wraps around some axons and increases the efficiency with which they transmit electrical impulses by preventing the current from leaking as it passes.

NEURAL IMPULSE TRANSMISSION

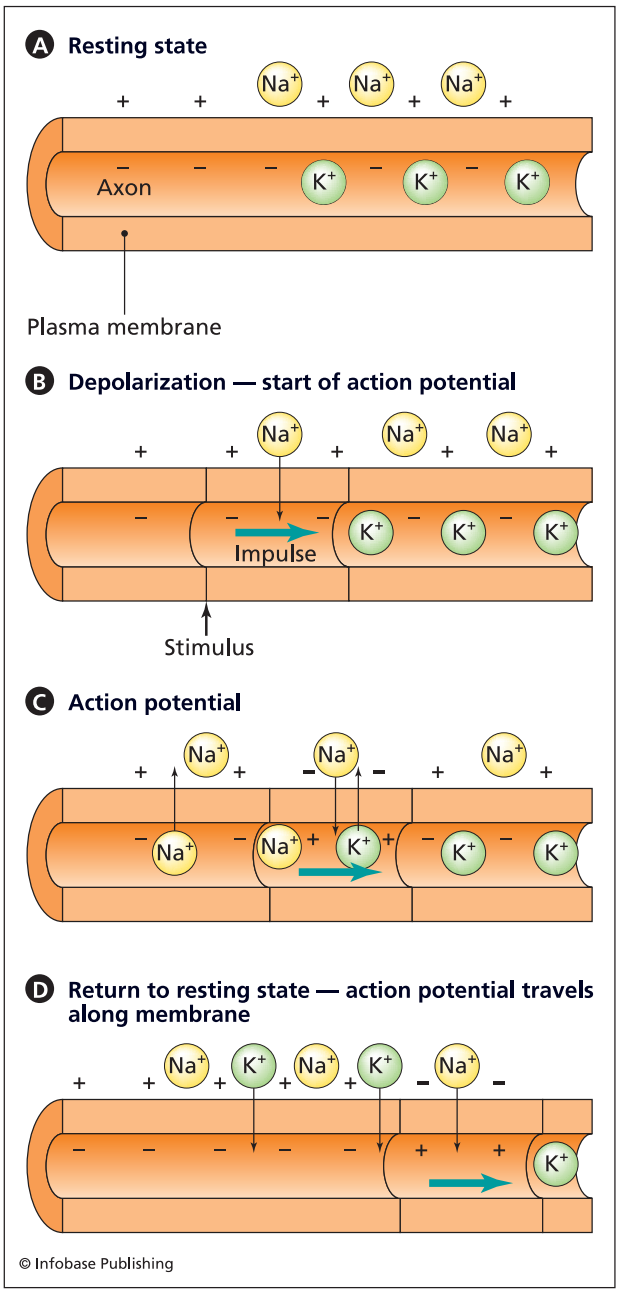
The function of neurons is the transmission of neural impulses, which are simply electrical signals that travel from a dendrite to a cell body or from a cell body along an axon. Living cells have a difference in charge across their cell membrane due to a difference in ion concentrations of a cell's cytoplasm and the extracellular fluids. Called the membrane potential, this difference can be expressed in millivolts (mV) with the voltage on the outside being zero. The charge inside the cell membrane is usually more negative, resulting in a typical membrane potential of -70 mV in a resting cell, a cell that is not transmitting an impulse. The major cations (positively charged ions) inside the cell are potassium (K^+) and, at a lower concentration, sodium ions (Na^+). The conditions of the extracellular fluid are reversed, with Na^+ existing in much higher concentrations than K^+. The main anion (negatively charged ion) outside of the cell is chloride (Cl^-), which is present inside the cell in much lower concentrations. The cell also contains numerous other anions such as phosphates, proteins, and amino acids. Because the lipid composition of the cell membrane does not permit the unrestricted passage of charged particles, K^+, Na^+, and Cl^- ions can pass only through selective openings called channels, but the charged organic molecules remain inside the cell. The cell membrane is practically impermeable to Na^+, but if it suddenly becomes permeable (by the opening of Na^+ specific channels), then both the concentration and the electrical gradients will cause Na^+ to rush into the cell. In contrast, the concentration of K^+ is much higher inside the cell, and it tends to diffuse in the opposite direction. Calcium ions (Ca^{2+}) and chloride ions (Cl^-) also tend to diffuse into the cell when the membrane becomes permeable to them.

Specialized cells like neurons have the ability to make changes in their membrane potentials; they are said to be excitable, meaning they are capable of responding to a stimulus. When a nervous impulse is not being transmitted, an excitable cell is said to be at rest, and the potential across its membrane is called the resting potential. The ion channels of neurons have gates that permit the passage of ions when opened, but they open only in response to a specific stimulus. In the case of sensory neurons, the gates open in response to a certain stimuli such as light or heat. Ion channels of interneurons, neurons that connect neurons to other neurons, open following excitation by another neuron. When the gates do open, the ions rush down their concentration gradient, changing the membrane potential. Specialized voltage-gated ion channels open in response to changes in membrane potential. Even a slight change can open the gates, increasing the membrane's permeability to that ion. Ions flow through and further alter the membrane potential. This is an example of positive feedback, when a response reinforces the stimulus, leading to an increased response until an external factor interrupts the cycle. Na^+ channels are unique in that they have two gates, an activation gate and an inactivation gate. At rest, the activation gate, which is closer to the cell's exterior, is closed and prevents Na^+ from entering the cell. The inactivation gate, which is closer to the cell's interior, is open at rest.

An action potential, or nerve impulse occurs when the local relative ion concentrations are reversed across a membrane. At first, a stimulus might simply reduce the electrical gradient by the influx of Na^+, making the interior of the cell less negative, a process called depolarization. If the depolarizing stimulus is strong enough to cause a large enough change in the membrane potential and a limit termed threshold potential is reached, then an action potential is triggered. An action potential is an all-or-none event; it happens or it does not happen. Threshold is normally -50 to -55 mV, compared to the typical resting potential of about -70 mV. Once triggered, the activation gates of the Na^+ channels in the vicinity open, allowing sodium to flow rapidly into the cell,

an action that reverses the membrane potential. The influx of Na^+ in one area triggers depolarization of the nearby membrane, causing it to reach threshold and open the activation gates of nearby Na^+ channels, and so on as the action potential self-propagates down the length of the axon. Meanwhile, at the initial trigger site, the Na^+ channel inactivation gates close, so no more Na^+ can pass through the membrane, and repolarization occurs by the opening gates of the K^+ channels. K^+ follows its concentration gradient and rushes out of the cell, restoring the negative membrane potential. At this time, the gates of the Na^+ channels return to their resting positions. During undershoot, the Na^+ channels are closed, so no more Na^+ passes, but the K^+ gates are slower to respond to the repolarization of the membrane, and K^+ keeps flowing out of the cell. The continued exit of K^+ causes the membrane potential to become more negative than its resting potential, a condition called hyperpolarization. During the refractory period a neuron cannot receive another action potential, ensuring that action potentials can travel only in one direction from the cell body to the axon terminal. It should be noted that while the change in membrane permeabilities to Na^+ and K^+ leads to significant alterations in the local electrical gradients, the change in concentrations of the ions is negligible. The Na^+-K^+ pump plays no direct role in the transmission of an action potential.

Because action potential is all-or-none, stronger stimuli are distinguished by their increased frequency rather than increased amplitude of the potential. As soon as the refractory period allows, a strong stimulus triggers another action potential. The speed that the impulse travels down the axon is determined by two major factors, diameter and myelination of the axon. Larger axon diameters increase the speed of conduction by lowering the resistance to current flow, just as a pipe with a larger diameter allows water to travel faster. Less of the fluid flowing through the pipe encounters resistance due to friction from the walls. In vertebrates, the interruptions in myelination between glial cells along the axons of some nerve fibers increase the rate of neural impulse transmission. These periodic gaps called nodes of Ranvier permit a process called saltatory conduction in which the action potential jumps from one node to the next. Extracellular fluid only contacts the axon at the nodes. After an action potential hits one node, the resulting depolarization spreads along the interior of the neuron all the way to the next node until reaching the end of the axon.



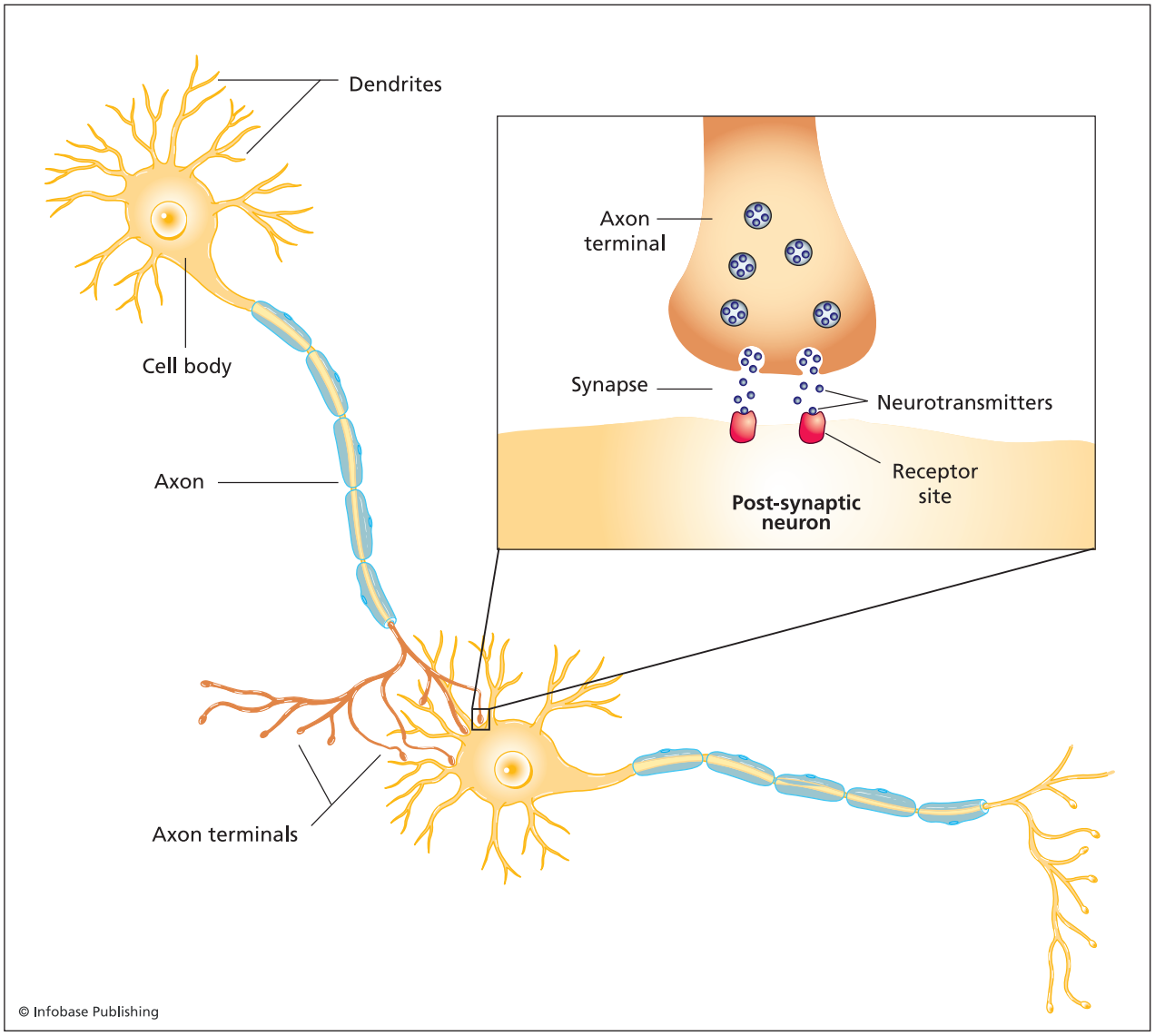
During the transmission of an action potential, a temporary reversal in the distribution of charge across the membrane moves in one direction along the length of a neuron.

SYNAPTIC TRANSMISSION

Synapses are the functional contacts between the end of a neuron and its target cell, either another neuron or an effector cell. There are three components to a synapse: the axon terminal of the presynaptic cell, the synaptic cleft (the region between the cells), and the membrane of the postsynaptic cell. There are two main classes of synapses, electrical and chemical synapses. The less common electrical synapses allow direct passage of a current from one cell to the next through channels that join to form pores between the two adjacent cells. Ions flow through the gap junction channels, altering the membrane potential of the

postsynaptic cell. Transmission through an electrical synapse is much faster than through a chemical synapse and can occur in either direction.

The majority of synapses are chemical synapses, through which neurotransmitter molecules carry the signal from the presynaptic to the postsynaptic cell. The synaptic cleft of a chemical synapse is much larger than in electrical synapses, and the mechanism for transmitting the impulse from one cell to the other is much more complex. Numerous vesicles filled with neurotransmitters are present inside the neuron of the presynaptic cells. Different types of neurotransmitters are found in different types of cells, but a neuron usually produces only one type. A postsynaptic cell, however, can respond to as many different neurotransmitters for which it has specific receptors. Examples of neurotransmitters include acetylcholine, glutamine, and dopamine. When an action potential reaches the end of an axon, the depolarization of the membrane stimulates the opening of voltage-gated Ca^{2+} channels. Ca^{2+} from the extracellular fluids rushes in and binds to regulatory proteins that initiate the fusion of the neurotransmitter-filled vesicles with the membrane at the axon terminus, a process termed exocytosis. As the membranes of the vesicle and the axon fuse together, the neurotransmitter stored inside the vesicle is released into the synaptic cleft and diffuses toward the postsynaptic cell. Receptors that are specific for that neurotransmitter recognize and bind it, an action that either



When an action potential reaches the end of an axon, synaptic vesicles fuse with the membrane, releasing neurotransmitter into the synaptic cleft. Specific receptors on the postsynaptic membrane recognize and bind the neurotransmitter molecules.

stimulates or inhibits the postsynaptic cell activity. If the neurotransmitter binds to a receptor linked to a chemical-gated ion channel, the gates will open and the ions will follow their concentration gradient. If it is a Na^+ channel, for example, the Na^+ will flow into the cell, reducing the negative charge of the cell's interior, and bringing the membrane potential of the postsynaptic cell toward threshold potential, an excitatory response. At an inhibitory synapse, binding of the neurotransmitter opens the gates of a channel for a positively charged ion, such as K^+, that is present at a higher concentration inside the cell than outside the cell. In this case, when the gates open, K^+ rushes out of the cell, increasing the negative charge inside and hyperpolarizing the membrane. A similar result at an inhibitory synapse would be achieved by opening Cl^- channels. Cl^- rushes into the cell because the concentration is higher in the extracellular fluid than the cytoplasm. This also increases the negative charge inside the cell and hyperpolarizes the membrane.

Whether the synapse is excitatory or inhibitory, the neurotransmitter is quickly removed from the synaptic cleft so the effect on the postsynaptic cell is not prolonged. One mechanism for accomplishing this is by digesting the neurotransmitter so it is not recognizable by the postsynaptic receptors. For example, the enzyme cholinesterase rapidly digests acetylcholine. Another mechanism for clearing neurotransmitter from the synaptic cleft is for the presynaptic cell to reabsorb it. Clearance of the neurotransmitter readies the synaptic cleft for transmitting the next action potential.

See also ANATOMY; ANIMAL FORM; BIOLOGICAL MEMBRANES; CELL COMMUNICATION; ENDOCRINE SYSTEM; EUKARYOTIC CELLS; INVERTEBRATES; PHYSIOLOGY; SENSATION; VERTEBRATES.

FURTHER READING

Greenspan, Ralph J. *An Introduction to Nervous Systems*. Woodbury, N.Y.: CSHL Press, 2007.

Kahle, Werner, and Michael Frotscher. *Color Atlas of Human Anatomy: Nervous System and Sensory Organs*. Vol. 3. 5th rev. ed. New York: Thieme, 2003.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 2. Detroit, Mich.: U*X*L, 2000.

Purves, Dale, George J. Augustine, David Fitzpatrick, William C. Hall, Anthony-Samuel LaMantia, James O. McNamara, and S. Mark Williams, eds. *Neuroscience*. 3rd ed. Sunderland, Mass.: Sinauer Associates, 2004.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

Turkington, Carol. *Encyclopedia of the Brain and Brain Disorders*. 2nd ed. New York: Facts On File, 2002.

Zimmerberg, Betty. "Synaptic Transmission: A Four Step Process." Multimedia Neuroscience Education Project. Available online. URL: http://www.williams.edu/imput/synapse/. Accessed January 15, 2008.

nutrition Animals must intake food to fulfill three needs: fuel in the form of chemical energy, raw materials for the synthesis of biomolecules, and essential nutrients that the body cannot produce on its own but that are necessary for good health. The subject of nutrition includes the sum of the processes by which an animal or plant takes in and utilizes food substances for nourishment. The body's metabolism determines the fate of the food a person ingests. Biochemical reactions break down the food substances, releasing the component parts and energy. The components can be used to build new biomolecules depending on the needs of the body's cells and tissues. The freed energy can be either used or stored for later usage.

ENERGY AND METABOLISM

Metabolism is the sum of all the body's chemical reactions that occur within the body and that provide energy for vital processes and activities and that build new materials. Catabolism includes all the biochemical reactions that break larger substances into smaller ones, and anabolism includes all the biochemical reactions that create larger substances from smaller components. Catabolic processes generally release energy, while anabolic processes require its input. Metabolic pathways involve a series of stepwise chemical changes, some of which are catabolic and some of which are anabolic, and some that are amphibolic, or serve both anabolic and catabolic functions. Classification of the pathway depends on the final result. Was something new built? Was energy released overall?

Life is an energy requiring process. In order to grow and develop, keep cells functioning properly, maintain homeostasis, move, reproduce, or perform many other activities associated with life, a person must obtain energy. As endotherms, humans maintain a constant internal temperature by generating heat from metabolism, thus even when relatively inactive, an adult human expends much energy each day. Basal metabolism refers to the use of energy in a fasting and resting organism solely to maintain vital cellular activity, respiration, and circulation as measured by the basal metabolic rate, the rate at which

heat is given off by an organism at complete rest. Because oxygen is consumed during metabolism, measurement of the amount of oxygen someone consumes during a particular activity or at rest can be used as an indicator of that person's metabolic activity. Age, gender, and genetic makeup influence the amount of energy a person involuntarily expends just by living. Other factors, such as a person's physical activity level, the amount of lean muscle in the person's body, the person's diet, and various hormones, also impact the amount of energy used by the body.

If a person brings in more energy than his or her body utilizes, then the excess energy is stored as glycogen or fat. All cells have a limited capacity for storing glycogen, whereas adipose cells have the ability to store enormous amounts of fat in droplets. While the body benefits from the presence of some fatty tissue as insulation and to protect or cushion some organs, obesity results when a person ingests more than he or she expends over an extended period of time and accumulates stored fat. In contrast, if someone expends more energy than he or she ingests, the body will utilize the fat stores, resulting in weight loss. If energy expenditures continue to exceed energy intake after depletion of the fat stores, the body will begin to break down muscle, the brain will become protein deficient, and death will eventually result.

The concept of calories provides a means for measuring the amount of energy available in a food source and for accounting for the amount of energy expended during certain activities. In physical chemistry, a calorie is defined as the amount of heat energy required to raise the temperature of one kilogram of water by one degree Celsius. When describing the energy content of food, the familiar term *Calorie* (with a capital C) is really a kilocalorie (kcal). Calories reported on the nutrition labels of food packages are really kilocalories. An average moderately active adult male burns about 2,400–2,800 kcal (or Cal) per day, whereas an average moderately active adult female needs roughly 2,000–2,200 kcal (Cal) per day.

Body mass index is an indicator of the amount of a person's body fat, and can be calculated by the following formula:

$$\text{BMI} = [\text{weight (lb)} / \text{height (in)} / \text{height (in)}] \times 703$$

or

$$\text{BMI} = [\text{weight (kg)} / \text{height (cm)} / \text{height (cm)}] \times 10{,}000$$

According to the Center for Disease Control and Prevention, people are underweight if their BMI is less than 18.5, normal if the BMI falls between 18.5 and 24.9, overweight if their BMI falls between 25 and 29.9, and are obese if the BMI is greater than 30. The criteria differ slightly for children and teens. The most recent data from the National Center for Health Statistics show that more than 30 percent of adults living in the United States are obese, and the trend is worsening. Since 1980 the number of overweight children and teens has tripled.

Because of the obesity epidemic, researchers have focused on identifying hereditary connections to obesity. They have found several genes that encode for proteins that regulate appetite. Adipose cells produce a protein hormone called leptin that suppresses appetite. As body fat decreases, the adipose cells produce less leptin, and appetite increases. Cells in the stomach wall secrete ghrelin, a hormone that stimulates feelings of hunger. After eating, the small intestine secretes a hormone called peripheral hormone peptide YY (PYY) that has the opposite effect of ghrelin, and the pancreas secretes insulin, which suppresses appetite. The mechanisms and complex interplay between these and other hormones are not yet understood, however, and no simple cure for obesity looms around the corner.

RAW MATERIALS OF BIOSYNTHESIS

Food supplies raw materials for the synthesis of new biomolecules in addition to providing energy. Biomolecules include substances such as proteins, carbohydrates, nucleic acids, lipids, and other nutrients. All biomolecules are considered organic molecules because they feature carbon skeletons and are made from living organisms. Autotrophs are organisms that use energy from the sun or from the oxidation of inorganic compounds to synthesize organic molecules from inorganic substances. Animals are heterotrophs, meaning they cannot synthesize organic compounds from inorganic substances, and therefore they must ingest organic compounds made by autotrophs or other heterotrophs.

The U.S. Department of Agriculture (USDA) makes recommendations for obtaining healthy amounts of nutrients including carbohydrates, lipids, proteins, vitamins, minerals, and fiber. Their most recent report, *Dietary Guidelines for Americans 2005,* encourages most Americans to consume fewer calories, exercise more, and make wiser food choices. In summary, the guidelines recommend eating more fruits and vegetables, eating a variety of fruits and vegetables, obtaining half of the grains from whole grain products, and consuming sufficient fat-free or low-fat milk products.

Carbohydrates include monosaccharides, disaccharides, and polysaccharides mostly from plant foods, but are also present in milk products as lactose.

Glucose and fructose are common forms of carbohydrates, and their main sources are vegetables, fruits, and high-fructose corn syrup. Sucrose, or table sugar, is a disaccharide consisting of one glucose molecule joined with one fructose molecule, and its sources include sugarcane, sugar beets, honey, and maple sugar. Starch, produced by plants, and glycogen, produced by animals, are both complex carbohydrates (carbohydrates consisting of hundreds or thousands of monosaccharide subunits) used for storing energy. Another complex carbohydrate, cellulose, is a plant cell wall component that humans cannot digest, but it is an important source of dietary fiber. The USDA recommends obtaining most of one's carbohydrates from fruits, vegetables, and whole grain products.

Most lipids consumed by humans are triglycerides, molecules consisting of a glycerol molecule linked to three fatty acids. Fats are triglycerides that are solid at room temperature, and oils are triglycerides that are liquid at room temperature. Saturated fatty acids have only single covalent bonds between carbon atoms of their fatty acids, meaning the adjacent carbon atoms share only one pair of electrons. Unsaturated fatty acids have one (monounsaturated) or more (polyunsaturated) double covalent bonds between the carbon atoms of their fatty acids. Higher levels of saturation make lipids harder at room temperature. For example, butter contains high levels of saturated fatty acids, whereas vegetable oils contain polyunsaturated fatty acids. The process of hydrogenation, adding hydrogen atoms to convert the double bonds of unsaturated oils into single bonds, results in the production of trans fats. Consumption of trans fats, common in margarine and many processed foods, raises levels of low-density lipoprotein (LDL) cholesterol and lowers levels of high-density lipoprotein cholesterol (discussed below), increasing the risk of cardiovascular disease. The USDA recommends that 20 to 35 percent of one's total caloric intake come from fats, with less than 10 percent coming from saturated fats, and limiting trans fats as much as possible.

Proteins are chains of 20 different amino acids combined in different arrangements to form tens of thousands of proteins. Many proteins in the body serve structural roles, providing form and strength for cellular structures or tissues. Enzymes are proteins that catalyze biochemical reactions. Other protein functions include acting as hormones, buffering the pH of the blood, transporting molecules across membranes, aiding in blood clotting, fighting infection, carrying molecular signals, or serving as an energy source. Milk, meat, grains, and beans all provide protein.

Many organic molecules can be synthesized from other organic precursors. For example, the catabolic pathway glycolysis breaks down glucose into pyruvate, an intermediate that an aminotransferase enzyme can convert into the amino acid alanine by one simple step. Animals have anabolic pathways that enable them to synthesize 11 of the 20 amino acids if they have a source of organic nitrogen in their diet. Nine amino acids are essential to adult humans, meaning they cannot synthesize them, and therefore must ingest them: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Some fatty acids are also essential. For example, humans cannot synthesize linoleic acid, an unsaturated fatty acid necessary for membrane synthesis.

Vitamins are organic substances that are essential in small quantities. They often serve as coenzymes and precursors of coenzymes in the regulation of metabolic processes but do not provide energy or serve as subunits for the synthesis of macromolecules. Some vitamins are fat-soluble (vitamins A, D, E, and K), and others are water soluble (vitamins B_1, B_2, niacin, B_6, pantothenic acid, folic acid, B_{12}, biotin, and C). A diet consisting of a wide variety of healthy foods will supply sufficient quantities of vitamins for most people, but many people also take vitamin supplements. Excess quantities of water soluble vitamins are excreted in the urine, while excesses of fat-soluble vitamins are stored in fat, thus overdoses can be dangerous.

Minerals are inorganic nutrients that are usually required in minute quantities. Humans require larger amounts of some minerals such as calcium and phosphorus for bone maintenance. Nerve and muscle function also require calcium, and phosphorus is a component of nucleic acids and adenosine triphosphate (ATP). Other minerals humans need include chloride, sodium, magnesium, potassium, zinc, copper, iron, iodine, and many others. Several minerals serve as cofactors necessary for enzyme function. Sodium, potassium, and chloride help regulate osmotic balance and are important in nerve cell function. Iron atoms bind to the heme groups of hemoglobin and aid in the transport of oxygen molecules in the bloodstream and also serve as a component of cytochromes that function in cellular respiration.

A healthy diet includes sources of fiber, also called roughage, an indigestible type of carbohydrate from plants. Two types of fiber, soluble and insoluble fiber, provide different benefits to the body. Soluble fiber helps reduce cholesterol and blood sugar levels. Insoluble fiber, consisting mostly of cellulose from plant cell walls, acts as a laxative, a substance that softens the stools and keep the contents of the intestines moving. This also reduces constipation and the strain of defecation. Fiber slows digestion and absorption, so glucose enters the bloodstream more

ESSENTIAL VITAMINS

Vitamin	Food Sources	Functions
vitamin B_1 (thiamine)	pork, peanuts, legumes, whole grains	coenzyme for several enzymes involved in carbohydrate metabolism, synthesis of neurotransmitters, synthesis on precursors of DNA
vitamin B_2 (riboflavin)	milk, eggs, enriched cereals, green vegetables, lean meats	coenzyme for redox enzymes, electron transfers
niacin (nicotinamide)	yeasts, meats, cereals, legumes, seeds, milk, green leafy vegetables, fish	component of the electron carriers NAP and NADP
vitamin B_6 (pyridoxine)	poultry, fish, pork, bananas, whole grains	coenzyme for amino acid and neurotransmitter synthesis and glycogen breakdown
pathothenic acid	meats, dairy products, whole grains, eggs, fish, poultry, avocado, sweet potatoes, soybeans	precursor for coenzyme A
folic acid	fortified cereals, citrus fruits, asparagus, brussels sprouts, spinach, baked beans, kidney beans, nuts	coenzyme in nucleic acid and amino acid metabolism
vitamin B_{12} (cobalamin)	meats, eggs, dairy products	coenzyme in nucleic acid metabolism, red blood cell maturation
biotin (vitamin H)	legumes, other vegetables, meats	coenzyme in fat, glycogen, and amino acid synthesis
vitamin C (ascorbic acid)	citrus fruits, berries, melons, tomatoes, potatoes, green peppers, leafy green vegetables	synthesis of collagen, antioxidant, neurotransmitter synthesis, iron absorption
vitamin A (retinol)	liver, eggs, orange and red fruits and vegetables, leafy green vegetables	vision, gene expression, antioxidant, prevents damage to cell membranes
vitamin D	dairy products, fatty fish (also made in the skin by sunlight exposure)	calcium absorption, mobilize calcium stores, promotes bone growth
vitamin E (tocopherol)	vegetable and seed oils, nuts, whole grains, wheat germ	antioxidant, preserves cell membranes
vitamin K (phylloquinone)	dark green vegetables, tea	blood clotting

slowly, helping to maintain relatively constant levels. As microorganisms in the large intestine partially break down the fiber, they produce organic acids that nourish the lining of the colon and act as fuel for the rest of the body. Sufficient fiber intake also reduces the risk of heart disease, obesity, certain types of cancer, diabetes, diverticular disease, gallstones, and kidney stones. Whole grains, nuts, legumes, fruits, and vegetables are all good sources of dietary fiber.

Cholesterol is a type of lipid that the body needs to maintain cell membranes, synthesize certain hormones, make vitamin D, and produce bile acids for digestion of fats. Too much cholesterol circulating in the bloodstream is unhealthy and results from eating excessive amounts of saturated fats from animal products such as high-fat meats and dairy products. High levels of one type of cholesterol, low-density lipoprotein (LDL) cholesterol, can lead to clogged arteries, increasing the risk for heart disease and stroke. High-density lipoprotein (HDL) cholesterol helps remove excess LDL from the bloodstream, reducing the risk of heart disease.

See also ANATOMY; ANIMAL FORM; BIOENERGETICS; BIOMOLECULES; CELLULAR METABOLISM; CIRCULATORY SYSTEM; DIABETES; DIGESTIVE SYSTEM; PHYSIOLOGY.

FURTHER READING

Caballera, Benjamin, ed. *Encyclopedia of Human Nutrition*. 4 vols. 2nd ed. With editors Lindsay Allen and Andrew Prentice. Burlington, Mass.: Academic Press, 2005.

Executive Office of the President and the Department of Health and Human Services. *Dietary Guidelines for Americans 2005*. Available online. URL: http://www.healthierus.gov/dietaryguidelines/. Accessed November 20, 2008.

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U.S. Department of Health and Human Services. *Your Digestive System and How It Works*. Bethesda, Md.: National Digestive Diseases Information Clearinghouse, May 2004. Also available online at http://digestive.niddk.nih.gov/ddiseases/pubs/yrdd/.

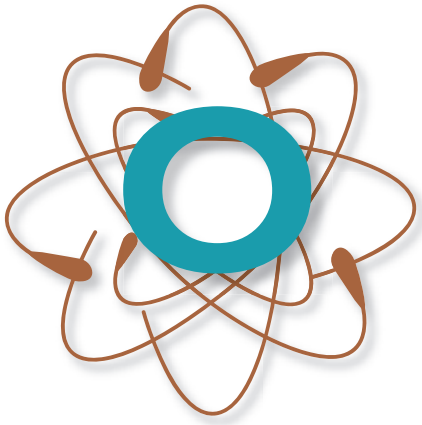
Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

U.S. Department of Agriculture. Center for Nutrition Policy and Promotion home page. Available online. URL: http://www.cnpp.usda.gov/. Last modified November 19, 2008.

———. Nutrition.gov home page. Available online. URL: http://www.nutrition.gov/. Accessed January 15, 2008.

Willett, Walter C., and Meir J. Stampfer. "Rebuilding the Food Pyramid." *Scientific American Special Edition* 16, no. 4 (December 2006): 12–21.



organic chemistry, its relevance to life science Organic chemistry is the study of chemistry as applied to organic molecules, those consisting mainly of carbon (C) and hydrogen (H). Organic chemists aim to study all aspects of organic compounds including their structure, composition, physical and chemical properties, and synthesis. The subject of organic chemistry is important to life science because the basic unit of life is the cell, and cells are composed of complex carbon-based compounds called biomolecules. The major groups of biomolecules are carbohydrates, proteins, nucleic acids, and lipids. Other biologically important molecules such as vitamins are also considered organic compounds, but most organic compounds are not biomolecules. For example, fossil fuels such as coal and crude oil are not considered biomolecules, but they are carbon-based, and are formed by millions of years of physical and chemical action on organic material made from or of former life-forms. Additional examples of compounds that fall within the realm of organic chemistry include synthetic rubber, polyvinyl chloride (PVC) as used in pipes and siding for houses, and solvents such as benzene and turpentine.

The element carbon is considerably important to living systems. The atomic number of carbon is six, meaning a carbon atom has six protons and six electrons. The most commonly occurring form of carbon also has six neutrons and therefore an atomic mass of 12. Carbon is unique in that it contains four valence electrons and is more likely to share them by forming covalent linkages than to give them away or accept electrons from other atoms in order to achieve a stable octet in its valence shell. The presence of four valence electrons allows carbon to participate in single, double, or triple covalent bonds with other carbon atoms or small atoms such as oxygen, nitrogen, hydrogen, and others. More than 10 million identified compounds contain carbon, but elemental carbon is not very reactive. Free carbon forms allotropes, including graphite, coal, diamond, and fullerenes, that differ only in their molecular arrangements.

Because covalent bonds between carbon atoms are very strong, organic molecules are stable and energy-rich. Chemical reactions involving organic molecules occur very slowly, and the products are typically more complex than inorganic reaction products. Most chemical reactions that occur in living organisms, called biochemical reactions, require a catalyst called an enzyme, which itself is a protein made from carbon-containing molecules. Biochemistry is the study of these types of reactions and of other chemical reactions caused by living organisms, and biochemists must study organic chemistry before they specialize in biochemistry.

Classification of organic compounds depends on the basic molecular structure of the molecule and on the presence and position of any functional groups. The flexibility of carbon allows it to form long unbranched chains, branched chains, circular or ringed compounds, and other structures. The specific functional groups and their site of attachment confer different physical characteristics and chemical reactivities to the molecule. For example, the addition of a particular side chain might make a molecule more soluble in water or make it more acidic. Three common functional groups in biomolecules include hydroxyl (-OH), carboxylic acid (-COOH), and amino ($-NH_2$) groups. Hydroxyl groups are very reactive and allow monomeric subunits of biological polymers to combine by dehydration synthesis, the joining of two molecules accompanied by the removal of an -OH

from one of them and a hydrogen atom (-H) from the other. For example, carbohydrates contain many hydroxyl groups, and monomers of glucose combine by dehydration synthesis to form carbohydrate chains as in glycogen or starch. Hydroxyl groups also play a role in solubilizing otherwise nonpolar molecules in aqueous solutions, an important consideration since the cytoplasm of cells is aqueous. Carboxylic acid groups are found in many biological molecules, including fatty acids and amino acids. At a neutral pH common to most living systems, they can readily give up the -H, leaving $-COO^-$ behind, making them acidic (hence the names fatty acid and amino acid). Amines, as found in amino acids, act in an opposite manner. At a neutral pH, water is a stronger acid, thus the amine becomes protonated; it accepts an H, becoming $-NH_3^+$. The acidic and basic properties conferred by amines and carboxyl groups give many biomolecules the properties that allow them to perform their specific functions.

Knowledge of organic chemistry in life science is also useful because many nonbiological organic chemicals affect living systems in a positive or negative manner. For example, medications such as acetylsalicylic acid (aspirin used to relieve aches and pains) and antibiotics are organic molecules. Antibiotics are chemicals that are either produced by or derived from microorganisms and that can kill or inhibit the growth of other organisms. They effectively treat many bacterial infections. Other organic chemicals harm living systems; for example, dichlorodiphenyltrichloroethane (DDT) was a common insecticide used during the 1940s. While DDT helped control mosquito populations, and therefore reduced outbreaks of insect-borne diseases such as malaria and typhus, scientists later found that the organic chemical caused profound environmental consequences. The chemical concentrates in biological systems, and exhibits increasingly toxic effects as one moves up a food chain. Many industries depend on the use of organic chemicals in manufacturing, and people utilize numerous organic chemicals while carrying out everyday activities—such as solvents in painting, as household cleaning agents, or as dyes to color fabrics. Because of the above-stated reasons and more, life scientists must have an understanding of organic chemistry not only to comprehend the molecular basis of life, but also to help preserve and protect living organisms.

See also BIOCHEMICAL REACTIONS; BIOCHEMISTRY; BIOMOLECULES; CHEMICAL BASIS OF LIFE.

FURTHER READING

Reusch, William. *Virtual Textbook of Organic Chemistry*. Available online. URL: http://www.cem.msu.edu/~reusch/VirtualText/intro1.htm. Updated July 16, 2007.

origin of life The oldest fossil evidence for life on Earth dates to 3.6 billion years ago, suggesting primitive life originated before then. The planet Earth itself originated at the same time as the rest of the solar system, about 4.55 billion years ago. Though not considered a geological time period, this event marked the beginning of Hadean time, which ended between 3.9 and 3.8 billion years ago, the age of the oldest known rocks. The intense heat characteristic of the early Earth persisted for billions of years due to the constant bombardment of matter from the solar system, and no ozone layer was present. As the Earth cooled, water vapor condensed, creating the oceans, and then rocks and continents formed. Microfossil evidence suggests that the first life-forms appeared within a few hundred million years after that. While scientists continue to debate the constitution of the Earth's first atmosphere, they agree that it was a reducing atmosphere; geological evidence suggests that the gases ammonia (NH_3), and methane (CH_4) were abundant, some hydrogen (H_2) was also present, and oxygen was present only as a component of water (H_2O). Over time, radiant energy from the Sun probably acted slowly to break apart those molecules and add carbon dioxide (CO_2) and nitrogen (N_2) to the mix. In contrast, the plentiful oxygen in today's atmosphere would oxidize any free organic molecules that would be necessary to create life. Just as the physical conditions on Earth differed greatly from today, so did early life-forms. As the geology of the Earth evolved, so did life, adapting in response to the changing conditions.

FORMATION OF SMALL ORGANIC MOLECULES

Scientists cannot know how exactly life first appeared, but by learning about the geological and chemical conditions that were present, they can attempt to simulate the environment in the laboratory to gain a better understanding. Most biologists agree that the first necessary step would be the formation of organic molecules from the available inorganic components. In the 1920s, a Russian biochemist named Aleksandr I. Oparin and others proposed that the reducing atmosphere may have created the necessary conditions to nurture the formation of organic compounds. Electrical discharges from lightning storms and ultraviolet radiation from the Sun could have supplied sufficient energy, and gases including NH_3, CH_4, H_2, and water vapor could have contributed the chemical elements necessary to build small organic molecules. In 1953 Stanley Miller and Harold C. Urey at the University of Chicago performed a famous experiment in which they demonstrated the creation of amino acids after circulating a mixture of gases meant to simulate the reducing early atmosphere—CH_4, NH_3, and H_2—past an electrical discharge to supply the energy

that lightning and ultraviolet radiation from the Sun would have provided. While some scientists do not think Miller's experiment represented the true conditions of the early Earth, Miller's work was the first to demonstrate the spontaneous production of organic chemicals from abiotic (nonliving) factors. Another explanation is that meteorites brought organic compounds to planet Earth. Geologists have shown that fragments from ancient meteorites contain amino acids in proportions similar to those generated by Miller and Urey's experiments. Hydrothermal vents may also have supplied the energy in the form of heat and chemicals necessary to synthesize amino acids. These openings on the deep ocean floor release mineral-laden hot fluids (up to 752°F or 400°C). Scientists have found not only amino acids, but also short chains of amino acids around these regions.

Some scientists doubt that the concentration of small organic molecules in the open oceans could have been high enough to allow the self-assembly of larger, more complex organic molecules, but those that were produced would have persisted in the absence of any life-forms to consume them or any free oxygen to oxidize them (break them down), and clays and inorganic crystals in shallow waters could have provided a surface on which these assemblies could occur. One researcher, James Ferris of the Rensselear Polytechnic Institute in New York, demonstrated that one type of clay, montmorillonite, catalyzed the polymerization of RNA molecules up to 50 nucleotides long. Furthermore, his results indicated the process generated the same sequences of the four possible ribonucleotides at frequencies greater than chance alone predicts, demonstrating selectivity, a characteristic necessary for this process to result in something biologically important.

GENETICS VERSUS METABOLISM

Two hallmarks of life are the ability to reproduce and simple metabolism. Before dividing, modern cells must first replicate their genetic material to pass on to the next generation. The deoxyribonucleic acid (DNA) contains all the information necessary to synthesize proteins that carry out all the cell's necessary functions, including the replication of the DNA. The process of protein synthesis is quite complicated, involves considerable specialized cellular machinery like enzymes and several types of ribonucleic acid (RNA), and requires a source of energy. Metabolism encompasses the extraction of energy from some molecules, transforming the energy, and building whatever macromolecules or cellular assemblages the cell needs, including DNA. Since replication and metabolism are so intimately linked, which developed first is a difficult question. Figuring out how the first cells developed self-replicating systems and metabolic processes will be a key step in understanding the origin of life.

Two major models describe possible mechanisms for the transition from organic molecules to "living" molecular systems, which then developed into protobionts, the name for aggregates of abiotically produced molecules enclosed by a membrane. The models differ in which came first, genetics or metabolism. The RNA world hypothesis incorporates the "genes first" model, which suggests that molecules of RNA formed spontaneously and had the ability to replicate themselves. Thomas Cech from the University of Colorado and Sidney Altman from Yale University independently demonstrated that RNA had catalytic properties, a discovery that earned them the Nobel Prize in chemistry in 1989. Scientists formerly believed only protein-based enzymes were capable of catalyzing biochemical reactions. Called ribozymes, catalytic RNAs have since been shown to synthesize RNA molecules using other RNA molecules as a template and to remove segments of RNA from different types of RNA molecules. This research supports an RNA world, in which RNA both catalyzed metabolic reactions and served as the genetic material. Other evidence includes the fact that RNA functions in both of these capacities (as a catalyst and in storing genetic information) in living cells in addition to serving an intermediary role between DNA and proteins during their synthesis. One problem with the RNA world hypothesis is that RNA is not stable, especially when exposed to ultraviolet light; however, it is more stable than DNA.

An alternative mechanism for explaining the jump from organic molecules to primitive living systems is the iron-sulfur world hypothesis. In 1990 the German patent attorney and hobby chemist Günter Wächtershäuser published his description for the hydrothermal origin of life, including the key idea that simple metabolism, in the form of chemical reactions that produced energy usable in other processes, preceded genetics. Proponents of the iron-sulfur world hypothesis believe that life originated in a setting similar to the environments created by hydrothermal vents in the ocean, characterized by near boiling temperatures, under high pressure, and with abundant carbon monoxide (CO), hydrogen sulfide (H_2S), and NH_3. On the surface of minerals such as iron pyrites (FeS_2) and nickel sulfides, CO became reduced, generating small organic compounds, which could concentrate on these surfaces and further catalyze their own formation. Another key step in the iron-sulfur hypothesis is the establishment of a self-sustaining, primitive metabolic cycle involving more complex molecules such as acetic acid and pyruvic acid, important compounds for the citric acid cycle and precursors for the formation of amino acids by

the addition of NH_3. Wächtershäuser began accumulating experimental evidence for his hypothesis in 1994, when he teamed with microbiologist Karl Setter to show that pyrite formation (FeS_2) could drive the formation of amide bonds, as found in amino acids. In 1997 Wächtershäuser and Claudia Huber successfully synthesized acetic acid in the laboratory by mixing CO, H_2S, nickel sulfide (NiS), and iron sulfide (FeS) at extreme temperatures, and in 1998 they re-created the synthesis of small peptides under similar conditions. In 2000 George Cody and his colleagues at the Carnegie Institute in Washington, D.C., showed that pyruvic acid could be synthesized from formic acid in the presence of H_2S under conditions similar to those used by Wächtershäuser, an important finding since experimental evidence for organic synthesis under high temperature and high pressure is limited due to the difficulty of creating the conditions in the laboratory.

In 2006 Trevor Dale of Cardiff University in Wales proposed a mechanism by which RNA and protein may have developed a prebiotic dependence on each other. In his model, crystallized protein fibers provided a support for the formation of RNA molecules, which in turn stimulated growth at the ends of the protein fibers. RNA growing on the surface of the protein fibers would be double-stranded, like DNA molecules. The mechanism by which DNA replicates is dependent on the property of double-strandedness. After the RNA and protein molecules reached a certain length, they would break off and the process would begin again with the shorter molecules. Though this model is still speculative, it is attractive because it also provides some explanation for the origin of ribosomes, the organelles made of RNA and protein that synthesize polypeptide chains.

PRIMITIVE CELLS

A key later step in the abiotic emergence of life would be the formation of primitive cells, the basic unit of life, from macromolecules. One defining factor of a cell is the presence of a cell membrane that encloses and protects the contents by preventing substances from leaking out of or into the cell. Phospholipids are molecules that have both hydrophilic and hydrophobic regions. Cell membranes are composed of a phospholipid bilayer, with the phosphate groups forming two sheets by orienting their polar heads toward the outside and the inside of the cell, and the nonpolar lipid portion sandwiched in between the two polar sheets. Lipids naturally come together in an aqueous solution to minimize the interaction of nonpolar and polar groups, excluding water. Micelles are spherical droplets formed when phospholipids are introduced into an aqueous environment. Phospholipids can also spontaneously form double layers and then sphere-shaped compartments when placed in aqueous solution. If such a double-layered formation happened to enclose a bit of solution that contained organic molecules, RNA and proteins, the results would structurally resemble a cell.

Several hypotheses exist for how membrane-bound spheres called protobionts formed and developed into primitive cells, or protocells. The bubble hypothesis purports that the bubbles formed when waves hit rocks or shorelines may have collected deposits of fatty molecules that accumulated in those areas, forming rudimentary membranes. Some of those microdroplets containing molecules such as amino acids may have been able to carry out metabolic reactions, transforming energy, and therefore able to "survive" and grow. Variations of the bubble hypothesis describe different ways these initial bubbles or spheres could have formed. Structures called coacervates form when a thin film of water molecules surrounds an aggregate of organic molecules held together by electrostatic interactions. The film of water allows some materials but not others to pass into the coacervate, resulting in a nonrandom arrangement of molecules. Coacervate droplets may have played an important role in the eventual establishment of cells. In the late 1950s Sydney Fox demonstrated that heating induced the polymerization of amino acids into proteinoids, which aggregated upon cooling to form structures called microspheres that were similar in size to bacteria. While not cells, microspheres can shrink and grow by dehydration and the absorption of water under different osmolarities. They can also absorb substances from the environment and form extensions that can pinch and break off, reminiscent of a simplified form of asexual reproduction called budding. Conditions on the early Earth may not have allowed for the polymerization of amino acids in an aqueous solution, but studies of microspheres have helped scientists consider the events and processes necessary for protocells to form from macromolecules.

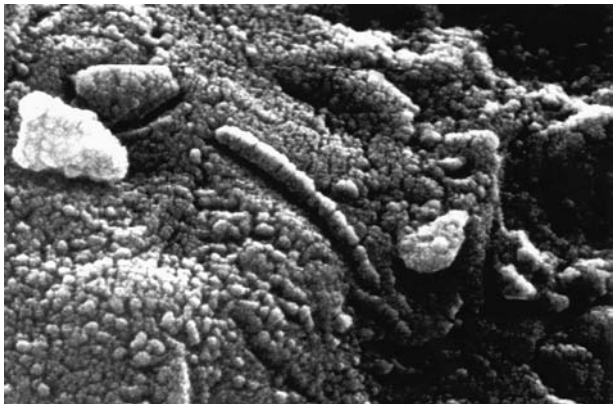
In 1970 Sol Spiegelman at the University of Illinois demonstrated that natural selection could act on nonliving systems. He introduced viral RNA 4,500 nucleotides in length to a salt solution containing RNA polymerase and free ribonucleotides, and replication ensued. After a while, he took a sample of the RNA and placed it in a tube with fresh components. The RNA polymerase replicated shorter molecules faster, so their relative proportion increased over time. After 74 generations the RNA was only 220 nucleotides long, demonstrating that replicating systems of macromolecules can evolve, or change, over time. In order to resemble modern life-forms, evolution would need to create an ancestral life-form that could store genetic information in the form of DNA,

replicate the DNA, synthesize proteins encoded by the DNA using RNA as an intermediate, and be contained within a membrane made of lipid molecules. Most biologists would agree that an ancestral lifeform with these properties would constitute a living cell.

Biologists continue to study the Earth's early conditions, probing possible mechanisms by which small organic molecules can form outside of preexisting organisms, examining the ability of these molecules to self-assemble and replicate, and postulating reasonable steps in the formation of primitive cells. The reconstruction of life in the laboratory would provide convincing evidence for chemical evolution and the abiotic emergence of cells, but attempts at reconstructing life from the bottom-up have not yet been successful.

PANSPERMIA AND EXOGENESIS

Alternative explanations for the appearance of life on Earth are panspermia, the notion that seeds for life on Earth came from outer space, and exogenesis, the hypothesis that life originated elsewhere and was transferred to Earth. Scientists have identified approximately 133 carbon-based molecules from space, including one identified in 2006 that contained a peptide bond, the type of chemical linkage that forms proteins. These molecules could have initiated the process of creating life on Earth, or they could be an indication that life already existed elsewhere in the universe and was brought here by way of a meteorite, comet, or interstellar dust particles. Thus, the hypothesis of panspermia does extend the framework of time to well beyond 4 billion years. If life appeared on Earth almost 4 billion years ago, it would have had to originate elsewhere prior to that time. Scientists have not found any remarkable evidence supporting the existence of life elsewhere in the universe. Exogenesis gained some credence in 1996, when the National Aeronautics and Space Administration (NASA) announced that a meteorite, known as ALH84001, had microscopic features that resembled microfossils, such as the ones used as evidence of life on Earth from more than 3.6 billion years ago. The world celebrated the finding, making the conjecture that life existed elsewhere in the universe. Since then, scientists have determined that the so called microfossils on ALH84001 could have been formed abiotically from organic molecules. No undisputed evidence supports the existence of extraterrestrial life, but evidence for the presence of water on Mars and on several moons in the solar system suggests that environments formerly considered inhospitable may in fact be habitable. Even if life on Earth did arise from outer space, exogenesis does not explain how life originated; it simply says it originated elsewhere.



Scientists originally thought microfossils found on a meteorite named ALH84001 (seen in this scanning electron micrograph) suggested the existence of life on Mars but later determined similar structures could form from organic molecules. *(AP Images)*

See also BIOLOGICAL MEMBRANES; BIOMOLECULES; EVOLUTION, THEORY OF; HISTORY OF LIFE; MILLER, STANLEY.

FURTHER READING

Berlinkski, David. "On the Origins of Life." *Commentary* 121 (2006): 22–33.

Brack, Andri, ed. *The Molecular Origins of Life: Assembling Pieces of the Puzzle*. New York: Cambridge University Press, 1999.

Cody, George D., Nabil Z. Boctor, Timothy R. Filley, Robert M. Hazen, James H. Scott, Anurag Sharma, and Hatten S. Yoder, Jr. "Primordial Carbonylated Iron-Sulfur Compounds and the Synthesis of Pyruvate." *Science* 289 (2000): 1,337–1,340.

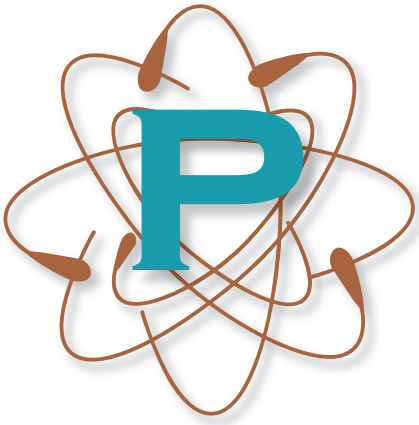
Dale, Trevor. "Protein and Nucleic Acid Together: A Mechanism for the Emergence of Biological Selection." *Journal of Theoretical Biology* 240 (2005): 337–342.

Futuyma, Douglas J. *Evolution*. Sunderland, Mass.: Sinauer, 2005.

Wächtershäuser, Günter. "Evolution of the First Metabolic Cycles." *Proceedings of the National Academy of Sciences* 87 (1990): 200–204.

———. "Life as We Don't Know It." *Science* 289 (2000): 1,307–1,308.

Warmflash, David, and Benjamin Weiss. "Did Life Come from Another World?" *Scientific American* 293 (2005): 64–71.



Pasteur, Louis (1822–1895) French *Chemist, Microbiologist* Louis Pasteur was an imaginative scientist of the 19th century whose research has profoundly affected society. His studies demonstrating that fermentation was a biological rather than a chemical process led to the development of pasteurization, a process that rejuvenated the French wine industry and is still used today to reduce possible contamination and to extend the shelf life of many beverages. Pasteur was among the first to determine that microorganisms cause infectious diseases in animals and humans, and he developed the first vaccines for many diseases, including rabies, anthrax, and fowl cholera. Modern hygienic practices stem from findings and recommendations from Pasteur's research. Because of his expansive work revealing the many relationships that humans have with microorganisms, he is considered a founder of microbiology.

CHILDHOOD AND EDUCATION

Louis Pasteur was born on December 27, 1822, in Dôle, France, a small town near the Swiss border. Louis began his education at age six in the local school at Arbois, where his family then lived. Louis was an average student who liked to fish with his friends in a nearby river and had an artistic talent for drawing portraits. In 1839 Louis entered the Royal College of Besançon, where he succeeded academically, but also still enjoyed art and creating portraits of friends and townsfolk. He earned his bachelor of letters degree in 1840 and began work on his bachelor of science degree. To offset expenses, he obtained a job as an assistant teacher. Two years later he passed his bachelor of science exam, and then took the entrance exam to the École Normale Supérieure. He ranked 15th out of the 22 students who were accepted, but this was not good enough for Louis, who turned down the offer of acceptance. Instead, he spent another year preparing by taking classes and giving lessons at a prep school, Lycée Saint-Louis. The following year he placed fourth on the entrance exam, and in 1843 he began his official training to become a professor.

At the École Normale Supérieure Pasteur developed a passion for chemistry and science. He completed his doctor of science degree in 1847, and his teachers strongly recommended him for a professorship, but Pasteur preferred to be in a laboratory doing research. He accepted a position as a laboratory assistant for Antoine-Jérôme Balard, the man who discovered bromine (used widely today to purify pool and spa water).

STEREOCHEMISTRY

While working on his doctorate, Pasteur's artistic nature attracted him to the beauty of crystals. Many substances such as table salt and sugar form unique crystals with sharp faces, regular angles, and beautiful colors. The structure of each crystal is dependent upon the arrangement of the atoms making up the substance. When light shines through solutions containing some dissolved crystals, the light beam is bent. These crystals are called optically active. Sometimes the path of the light bends to the right and sometimes to the left. Pasteur wondered why. To examine this, Pasteur concentrated on crystals that naturally formed in wine vats, namely, tartaric acid and racemic acid crystals. Tartaric acid and racemic acid were made of the same components and even in the same proportions, yet tartaric acid crystals rotated light, that is they were optically active, and racemic acid crystals did not.

Pasteur spent much time in the lab examining crystals with a magnifying glass, sketching his observations, making his own special equipment to measure the way crystals bent light, and pondering this apparent puzzle. Then one day, because of his acute attention to detail and his remarkable intuition, he noticed that the facets, or flat faces, in tartaric acid crystals all pointed in the same direction, but in racemic acid they pointed in both directions. He made an educated guess that the facets pointing in two different directions somehow cancelled each other out such that it appeared the light was not bent at all. To test this hypothesis Pasteur painstakingly examined crystals under the microscope and separated crystals with facets pointing in different directions. He made separate solutions of each and measured a light beam as it passed through each. His diligence was rewarded when he demonstrated that the light was bent to the right by one of the solutions and to the left by the other solution. The physical properties of molecules were dependent not only upon their composition, but also on their structures. By the age of 25, Pasteur had founded a new branch of chemistry called stereochemistry, which is concerned with the position and arrangement of atoms in molecules and how their arrangement affects the molecule's properties.

Shortly after this major discovery, Pasteur's mother died. He returned home briefly and then went to Dijon, where he worked as a physics professor. In January 1849 he was appointed professor of chemistry at the University of Strasbourg, where he met Marie Laurent, the 22-year-old daughter of the university's principal. They were wed in May 1849. Marie understood Pasteur's passion for science and supported him by handling home matters, assisting him in his writing, and urging him to explain his research clearly. They had five children together, but only two survived into adulthood because not much was known about the prevention or treatment of infectious diseases. Ironically, had his children been born into the next generation, which benefited from the knowledge of Pasteur's later discoveries, they may have all survived.

DEMONSTRATES FERMENTATION IS BIOLOGICAL

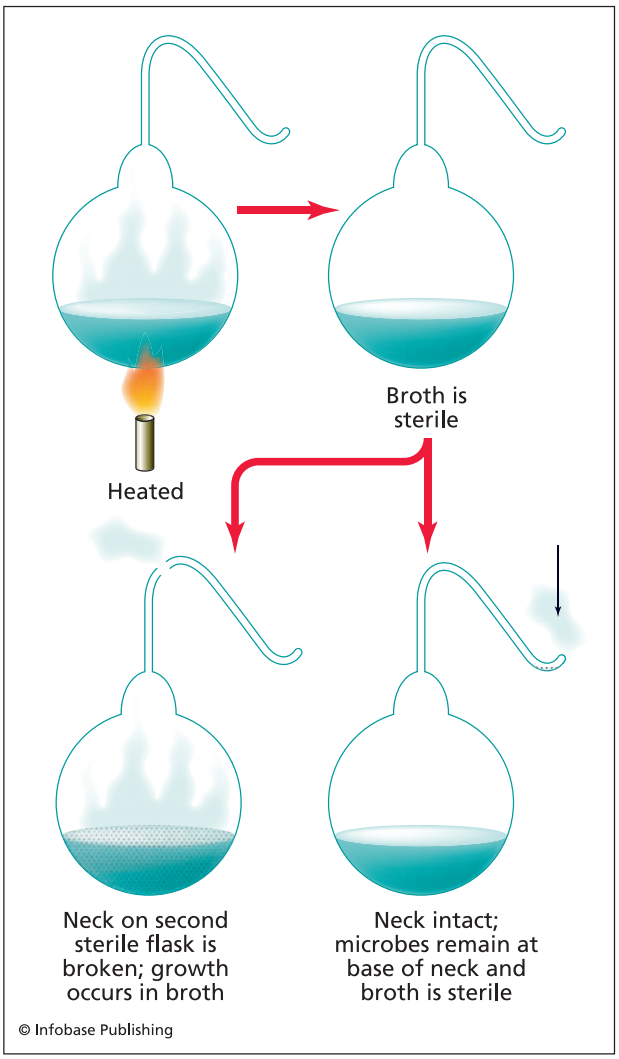
Pasteur took a new job as a professor of chemistry and dean of sciences at the University of Lille in 1854, when he was only 31 years old. His work began to center on the applications of science to benefit society. The first problem he attacked was the costly problem of spoilage at a local factory that produced alcohol from beet juice.

Fermentation is the biochemical conversion of sugar into alcohol and carbon dioxide gas. At the time, people thought that this was simply a chemical process, though they knew that yeast, a type of unicellular fungi, played a role. Pasteur studied drops of liquid from vats of fermenting beets under the microscope and noted the presence of yeast but also of many other tiny objects in the souring vats. He proposed that these other objects were the basis of the problem. He suspected that fermentation was a biological process, that the yeast were living organisms and that they converted the sugar into alcohol by their normal metabolic activities. From these studies, Pasteur developed his famous germ theory of fermentation, which stated that microorganisms were the cause of fermentation and that specific types of microorganisms caused specific types of fermentation. While the yeast made alcohol in the healthy vats, other contaminating microbes produced undesirable substances in the souring vats. In this case, bacteria had overrun the yeast and were producing lactic acid (the same substance that sours milk). Though other scientists laughed at this strange idea, through rigorous experimentation, Pasteur gathered much supporting evidence for his hypothesis. The remainder of Pasteur's scientific career revolved around the activities of these microscopic life-forms.

DISPROVES SPONTANEOUS GENERATION

In 1859 Pasteur became the director of scientific studies at the École Normale, where he himself had been a student. While the title was impressive, his work space and funding were limited. He undertook research in an attic lab with homemade equipment and incubated his microorganisms in a cupboard under a staircase. Nevertheless, during the next few years he took on one of his most famous challenges and moved from the study of chemistry into the field of biology to unravel the origins of microbial life. At the time, many people believed that living organisms could arise spontaneously from nonliving matter. He had learned to grow microorganisms in a broth that contained sugars and salts necessary for supporting life. If bottles or flasks containing this broth, or culture media, were uncapped briefly and then resealed, after a few days the broth would be teeming with tiny organisms. Pasteur believed the tiny life forms were carried in the air by dust particles that floated into the opened flasks. He hypothesized that cleaner air would have less microbes.

To test this hypothesis he filled 20 flasks with broth, heated them to kill any preexisting microorganisms, and exposed them to air from the Arbois countryside. He carried 20 others by mule to a mountain peak and exposed them to the icy mountain air from the top of Mont Blanc. All the flasks were resealed and brought back to the lab where growth was allowed to occur. Eight of the countryside flasks became cloudy, but only one of the mountain air flasks did. Many people remained skeptical



Pasteur used uniquely designed swan-necked flasks to dispel the theory of spontaneous generation.

of Pasteur's claims. Some argued that there must be a life-giving component to air, rather than life floating around in the air itself. They thought that heating the flasks destroyed the ability of the air in a sealed flask to support life. In response to this criticism, Pasteur fashioned swan-necked flasks to demonstrate conclusively that the microorganisms arose from the reproduction of preexisting microorganisms rather than spontaneously in the culture media. The unique shape of these flasks allowed air (including any magical life-giving components) to enter the flasks, yet trapped any dust particles and prevented them from entering the region of the flask that contained the broth. He boiled the liquid inside the flasks to kill any microbes that may already be present and then left them open to incubate. These flasks remained clear, no microbial growth occurred. When he tipped them to allow the sterile broth to contact the bend in the flask, growth soon appeared. This famous experiment ended the debates regarding spontaneous generation.

ASSISTS WINE, SILKWORM, AND BEER INDUSTRIES

Pasteur continued his studies on fermentation and he also taught subjects dealing with the chemistry of paints and the relationship between health and comfort, and architecture at École des Beaux-Arts in Paris. At this time, Emperor of the French Napoleon III pleaded for Pasteur's help in solving the country's wine-making problems. France was famous for its wines, but sometimes they became bitter or sour. Pasteur immediately suspected the problem stemmed from the presence of undesirable microorganisms in the fermentation mixture, as in beet juice. He set up a research lab to examine why wines became undrinkable in an old street café and found that most issues could be traced to a particular organism. When he boasted that he could predict the problem for certain wines by examining the microorganisms present, wine-tasting experts challenged him. Even though they tried to fool him by including perfectly good wines, Pasteur was able to distinguish successfully which wines were fine, bitter, or sour without even tasting them. He also determined that treating the wine at 131°F (55°C) for several minutes killed the harmful bacteria but did not affect the taste of the wine, and wine treated this way could be stored indefinitely. This process is now called pasteurization and, with slight modifications, it is also used for vinegar, beer, juice, milk, cheese, and eggs. Thus, Pasteur not only determined the cause of the problem, but also developed a practical application utilizing the results of his research to assist the industry.

After saving the wine industry, the French government asked Pasteur to help silkworm growers. Diseases of the silkworms, whose cocoons were used to manufacture fine silk, were ruining the French clothing industry. During the years from 1865 to 1869, Pasteur spent every summer in Alais studying diseases of silkworms, including pébrine, which caused small black spots to appear on their skin. Microscopic analysis of these worms revealed small, oval-shaped microbes. Pasteur predicted that pébrine could be controlled by picking out and destroying moths that showed signs of disease; however, when this was done, the newly hatched worms showed signs of a different disease that resulted in flabby bodies. Though he must have been discouraged not only by this, but by the death of his father and two of his daughters during this time, Pasteur persisted and was finally able to show that specific microbes caused specific diseases. He publicly demonstrated his ability to examine and successfully predict which diseases, if any, would affect the larva. Again, Pasteur's

impact lay in the methods of application of his new discoveries.

He returned in the fall of each year to the École Normale, where, in addition to teaching, he took charge of student residential life. Extremely disciplined himself, he was very strict, and the students complained, leading to his dismissal from the position. The school did not want to lose such a famous scientist, however, and gave him a new job as director of a lab. He was also made a professor at the Sorbonne at this time. Unfortunately, a stroke in 1868 left him partially paralyzed. Though he regained partial usage of his legs and speech, he required assistance in carrying out his research from this point on. Also, the war between France and Prussia forced him to leave Paris temporarily. France was defeated, which crushed Pasteur. He gave back an honorary doctorate degree that a German university had given him and turned down a job offer by an Italian university. Instead, he chose to do something that would help his mother country compete with Germany; he decided to study issues involved in beer-making. Problems France in making beer mirrored the fermentation problems Pasteur had solved in beet juice and wine. He was soon able to boost the economic success of the beer industry as well.

STUDIES CHOLERA, ANTHRAX, AND RABIES

Around 1877 Pasteur shifted his focus to the cause of disease. Perhaps this interest was fueled by the loss of so many loved ones. At the time, the notion that germs caused disease was a novel concept. Many believed that "bad air" or even sinful human nature was the cause of disease. Over the next decade Pasteur linked specific microbes to half a dozen human and animal diseases. Both he and Robert Koch are credited for discovering the germ theory of disease, which states that diseases are caused by specific microbes. Pasteur further suggested that the spread of disease could be prevented by killing the microorganisms that caused contagion and recommended that carcasses of animals that died from anthrax be burned rather than buried. He is also credited for revealing the underlying principles that led to aseptic technique—keeping a sterile environment—during surgeries. At the time, entering a hospital was practically a death sentence. The surgeon who first used carbolic acid as an antiseptic during surgery, Dr. Joseph Lister, publicly expressed his gratitude to Pasteur for his contributions to the medical field and for the many lives saved due to his recommendations. After implementing aseptic techniques, deaths following operations dropped from 50 percent to 3 percent.

Pasteur began by studying chicken cholera, a disease that had killed one-tenth of all the chickens in France. He figured out how to grow the causative organism in the lab and then injected it into healthy chickens, which promptly became ill. Once, perhaps by accident, his lab assistant Émile Roux left some cholera cultures in the lab for an extended period of time while Pasteur and his assistants took summer holiday. When these old cultures were injected into chickens the chickens become slightly ill, but surprisingly they recovered. When these same chickens were later inoculated with fresh culture, they remained healthy. Pasteur recognized that what happened with these chickens paralleled what happened when the English physician Edward Jenner injected persons with cowpox as a means to prevent possible future infection with smallpox. These chickens had been "vaccinated" against cholera. Somehow, the old culture had become weakened and had lost its ability to cause fatal disease but prevented the chickens from catching this same illness in the future. Immunologists have since learned that this is because the immune system builds antibodies when exposed to specific microorganisms and these antibodies remain in the body to prevent future infection by the same microorganism.

By 1881 Pasteur had developed an anthrax vaccine using similar techniques. Anthrax was killing hoards of cattle and other grazing farm animals. He publicly demonstrated this vaccine's usefulness in Pouilly-le-Fort. Pasteur's assistants inoculated 24 sheep, six cows, and one goat with his anti-anthrax vaccine. After 12 days, they inoculated these animals again. Then two weeks after the second vaccine injection he injected all the animals as well as 24 unvaccinated sheep, four unvaccinated cows, and one unvaccinated goat with fresh anthrax culture. Pasteur went home and returned two days later to a cheering crowd of not only farmers, but also veterinarians, scientists, reporters, and officials who had come to witness this historic event. All the vaccinated animals were healthy, but 21 of the unvaccinated sheep and the one unvaccinated goat were already dead. The four unvaccinated cows showed signs of fever and swelling, and eventually the other three sheep and the cows died. Even the people who had laughed at Pasteur and criticized him a month earlier could not deny the overwhelming life-saving success of this new vaccine.

Next he studied rabies. Rabies symptoms do not appear until a month or so after the virus is transmitted to a bite victim through the saliva of an infected animal, but by then there is little hope for recovery. The virus travels to the spinal cord and then throughout the body, causing paralysis and intense muscular spasms upon swallowing liquids. Death usually results from destruction of the portion of the brain that controls breathing. Because rabies is caused by a tiny virus, Pasteur was not able to find it using a

microscope, nor was he able to culture the microorganism in the laboratory using the conditions he had perfected for many types of bacteria. But he did not give up; instead, he grew the virus inside living animals. Pasteur removed spinal cords from infected animals and dried them for varying lengths of time. He gave a series of successive shots containing these ground-up spinal cords to 50 healthy dogs. Each shot contained tissue that had been dried for less time than the previous shot, thus each shot was stronger than the last one. Finally, he gave shots to the animals that were of a strength that should have caused the disease itself, but none of the animals became ill or died.

On July 6, 1885, a desperate mother brought her nine-year-old boy to Pasteur. Joseph Meister had been bitten more than a dozen times two days prior by a rabid dog. While Pasteur was confident of the success of his rabies vaccines in dogs, this was a human being, and he was very concerned with whether or not he should attempt to vaccinate the boy. After consulting two physicians who both stated that the boy would most certainly die if nothing were done, Pasteur agreed to attempt the vaccine. After administration of the 13-shot series, the boy went home and waited, but he never became ill.

Having heard of this success, three months later a 15-year-old shepherd named Jean-Baptiste Jupille, who had wrestled with and killed a mad dog while protecting his sheep and several younger shepherds, was brought to Pasteur with pleas for the vaccine. Though six days had already passed since the attack, the vaccine worked. Despite the obvious success, some of his colleagues still claimed the vaccine was useless and even dangerous. They questioned how it was known whether the bite victims would have even contracted the disease and worried the vaccine itself could cause rabies rather than prevent it. Nevertheless, over the next few years, thousands more came to Pasteur for his miracle rabies vaccine. The English Commission on Rabies studied the issue extensively and in 1887 declared that Pasteur's vaccine had indeed saved many lives.

ESTABLISHES PASTEUR INSTITUTE

Pasteur used the profits from the success of the rabies vaccine to set up a nonprofit biomedical research institute, which at the time specialized in the treatment of rabies and other microbiological problems. In 1888 the Pasteur Institute opened in Paris. After suffering two more strokes in 1887, Pasteur's health and memory were deteriorating, and he was unable to perform much research. He served as director of the institute until his death on September 28, 1895. A large public funeral was held in his honor at the palace in Versailles, and he was buried at the Pasteur Institute. Joseph Meister, who had received the first rabies vaccine, served as gatekeeper of the institute 55 years later during World War II when the Germans overtook Paris. According to rumor, commanded by German soldiers to open up the tomb of the man who had saved his life so many years before, he committed suicide rather than comply.

Pasteur's legacy has expanded globally to include 132 research units employing approximately 2,500 researchers. Eight Nobel laureates have been earned by staff of the Pasteur Institute. Scientists at the institute have produced many breakthroughs in biomedical research, including isolation of the AIDs virus, development of numerous vaccines and sulfa drugs, elucidation of the regulation of viruses, and identification of methods for cancer detection and treatment.

Pasteur was a brilliant researcher who had a knack for making logical and insightful predictions regarding his experiments and for knowing which lines of research to pursue vigorously. He did not limit himself to physics and chemistry, the fields in which he was formally trained, but instead considered himself simply a scientist. He felt that knowing how to form and test hypotheses from observations and previous data was more important than knowing all the content information in a given field. A remarkable experimentalist with a keen sense of intuition and a drive matched by few, he became a legend during his own lifetime.

See also BACTERIA (EUBACTERIA); CELLULAR METABOLISM; GERM THEORY OF DISEASE; KOCH, ROBERT; MICROBIOLOGY; SPONTANEOUS GENERATION; VACCINES.

FURTHER READING

De Kruif, Paul, with introduction written by F. Gonzalez-Crussi. *Microbe Hunters*. New York: Harcourt Brace, 1996.

Dubos, René. *Pasteur and Modern Science*. Madison, Wisc.: Science Tech Publishers, 1988.

Jacob, François. "The Pasteur Institute." The Nobel Foundation, April 6, 1998. Available online. URL: http://www.nobel.se/medicine/articles/jacob/. Accessed January 15, 2008.

Pauling, Linus (1901–1994) American *Chemist*

Modern scientists usually concentrate on a highly specialized topic for their doctoral dissertation research; unfortunately, breadth of knowledge sometimes is sacrificed to attain this focused expertise. Linus Pauling was an American scientist who embraced many varied interests ranging from mineralogy to quantum mechanics and immunology to evolution. His most significant scientific contribution centered on

his research related to the forces responsible for holding atoms together to create molecules, for which he received the Nobel Prize in chemistry in 1954. By understanding how different types of chemical bonds form, scientists can better understand the structure and therefore the function of molecules, including biomolecules of which living organisms are composed. Pauling was the first to describe a molecular basis for a disease, sickle cell anemia, and he was a vigorous proponent of vitamin C for use in treating the common cold. He became a controversial figure when he actively campaigned against nuclear testing, actions that earned for him the distrust of the U.S. government. He was accused of being a communist and refused permission to travel abroad. The rest of the world viewed him differently, and he was awarded the Nobel Peace Prize in 1962, becoming the only person to win two unshared Nobel Prizes. Many consider Pauling to be the greatest chemist of the 20th century.

EDUCATION AND TRAINING

Linus Carl Pauling was born on February 28, 1901, in Portland, Oregon. His father, Herman Henry William Pauling, was a pharmacist who died from a perforated ulcer when Linus was only nine, leaving his mother, Lucy Isabelle (Belle) Darling Pauling, to raise him and his two younger sisters. Linus was a voracious reader from an early age, and by the time his father passed away, he had already read Charles Darwin's *Origin of Species* and the Bible. As a young adult, he collected insects and minerals and explored chemistry with a childhood friend, performing simple experiments, setting off stink bombs, and making explosions. Linus entered Oregon Agricultural College (OAC, now Oregon State University) in Corvallis without obtaining his diploma because he lacked the required history courses. He studied chemical engineering, and, though he worked during the summers testing the composition of asphalt used to pave highways, financial difficulties forced him to leave school after only two years.

After hearing of Pauling's situation, the OAC faculty hired the 19-year-old as an instructor of quantitative analysis, a course he had just completed the year before. His pupils found his lectures entertaining and informative, and students vied to get into the classes taught by the "Boy Professor." The following year Pauling resumed his studies, graduating with a bachelor of science degree in chemical engineering in 1922. During college, independent reading of current chemistry journals brought to Pauling's attention ideas put forth by chemists Gilbert Newton Lewis and Irving Langmuir on the forces that held molecules together. Lewis and Langmuir proposed that elements were held together by the natural tendency of atoms to attain a stable structure with eight electrons in their outermost, or valence, shell. For example, if an atom had only seven electrons in its valence shell, it tended to share an electron pair with an atom that had only one, forming a covalent bond and providing each atom with a complete octet.

Wanting to learn more about chemical bonding, Pauling moved to the California Institute of Technology (Caltech) where he began studies toward a doctorate in physical chemistry. The offer of a generous stipend and the recent hiring of internationally known chemist Arthur Amos Noyes, who was in the process of transforming the chemistry department into a renowned research center, drew Pauling to Caltech. His dissertation research under Roscoe Dickinson centered on the use of X-ray diffraction to study the structures of inorganic crystals. Dickinson had earned his Ph.D. only two years before, and, as the resident expert in X-ray crystallography, he was qualified to teach Pauling the new and complicated technique. Pauling measured bond angles and distances in crystals of the mineral molybdenite, solving its structure for his thesis. He exhibited a natural ability to combine experimental and theoretical chemistry, and he intuitively recognized the relationship between the structure of molecules and their chemical behavior. By the time he earned his Ph.D. in chemistry in 1925, with minors in mathematics and physics, he had published 12 scientific papers on inorganic crystal structure. During graduate school he married Ava Helen Miller, one of his former students, to whom he remained married for almost 60 years and with whom he had four children.

After obtaining his doctorate, a Guggenheim fellowship took Pauling to the Institute of Theoretical Physics in Munich, Germany, where he mastered quantum mechanics, the burgeoning field of physics that attempted to explain the structure and behavior of matter at the subatomic level. Pauling met many influential physicists while in Europe, including Arnold Sommerfield, who directed the institute; Erwin Schrödinger; Max Born; Werner Heisenberg; J. Robert Oppenheimer; and Niels Bohr. Pauling established a name for himself by publishing an article describing atomic properties using wave mechanics in the prestigious journal *Proceedings of the Royal Society of London* in 1927. While writing the article, "The Theoretical Prediction of the Physical Properties of Many-Electron Atoms and Ions: Mole Refraction, Diamagnetic Susceptibility and Extension in Space," Pauling realized that quantum mechanics may reveal the answers to many questions regarding atomic behavior. He also recognized the significance of the work done by two German physicists working with Schrödinger, Walter Heitler and Fritz London, who used wave mechanics to model chemical bonding.

They demonstrated that as atoms approached one another, their electrons became attracted to each other's positively charged nucleus and rapidly jumped back and forth. At the same time, the positively charged nuclei repelled one another, resulting in a defined bond length between the two atoms.

PAULING'S RULES AND CHEMICAL BONDING THEORY

Upon returning to Caltech, the 26-year-old rising star became an assistant professor of theoretical chemistry and applied his newly adopted manner of looking at matter and energy to chemistry. He was promoted to associate professor in less than two years. Pauling quickly established himself as an expert in structural chemistry, publishing almost 50 papers on X-ray crystallography and quantum chemistry during his first five years on the faculty. Using both quantum mechanics and experimental data from inorganic crystals, he proposed that one could approximate the distances between atoms by simply adding the radii of the participating cations (positively charged particles) and anions (negatively charged particles). The values for ionic radii that he determined are still commonly used today, as are his values for covalent and van der Waals radii. He outlined a set of guidelines concerning the stability of crystal structures, making it easier for chemists to test the correctness of possible structures for complex ionic or covalent crystals. The rules described in "The Principles Determining the Structure of Complex Ionic Crystals," published in the *Journal of the American Chemical Society* in 1928, became known as Pauling's rules and further substantiated his international reputation.

As his interest in how atoms combined to form molecules increased, Pauling used quantum mechanics to explore exhaustively the formation and characteristics of chemical bonds. In 1931 he published his first paper of a series titled, "The Nature of the Chemical Bond," that led to his 1939 publication of a book by the similar name, *The Nature of the Chemical Bond and the Structure of Molecules and Crystals: An Introduction to Modern Structural Chemistry*. In this work he applied quantum mechanics to concepts including hybridization, resonance, and electronegativity, and the book became one of the most influential texts in scientific history. In recognition of his brilliant accomplishments, in 1931 Caltech promoted Pauling to full professor, and the American Chemical Society named him the best young chemist in the nation by awarding him their Langmuir Prize. In 1933 he became the youngest person to be elected to the National Academy of Sciences.

The valence bond theory (VB) and the molecular orbital theory (MO) constituted attempts to explain the sharing of electrons between atoms in a molecule. The VB approach considers individual atoms with their own electron orbitals coming together to form covalent bonds, whereas the MO approach collectively considers all of the atomic nuclei comprising the molecule encircled by sets of molecular orbitals. Pauling seemed to favor the VB approach, but it could not explain the quadrivalency of the atom carbon, its ability to provide four equal-energy orbitals to form four equivalent bonds with other atoms. To remedy this, he developed the concept of hybridization, the combination of the outer or valence orbitals of an atom to form hybrid orbitals. Mixing the orbitals changes their spatial arrangements and energies. He gave molecules three dimensions and imagined that atoms could change their shapes to form stronger bonds, a notion that was difficult for some chemists to accept.

To better understand the concept of hybridization, consider the element with the atomic number of six, carbon, the most important element in life science. Within a carbon atom, two electrons occupy the lowest energy level consisting of one *s* orbital. The four remaining valence electrons occupy the next energy level, consisting of one *s* orbital and three *p* orbitals. If each of the four valence electrons occupied a different orbital in the second energy level, the bond energies and lengths would not be equivalent. Combining the one low-energy *s* orbital with the three slightly higher-energy *p* orbitals gives four equivalent sp^3 orbitals with a weighted average energy. The number of hybrid orbitals must equal the number of combined original orbitals. When one carbon atom binds with four hydrogen atoms, methane (CH_4) is formed. Hybridization explains why all the carbon hydrogen bond lengths, strengths, and angles are equal, forming a perfect tetrahedron.

Pauling used the idea of resonance to explain the stability of chemical bonds, in particular, the carbon-carbon bonds of aromatic molecules such as benzene (C_6H_6). In 1857 German organic chemist Friedrich Kekulé proposed that carbon was tetravalent and described the structure of benzene as a ring with alternating single and double bonds that rapidly interconverted. This can be depicted as a hexagon with alternating single and double bonds followed by a double-sided arrow and another hexagon with single bonds replacing the first structure's double bonds and vice versa. Pauling used quantum mechanics to show that benzene was really an intermediate structure like a hybrid orbital. The resonant forms do not rapidly alternate in a dynamic equilibrium, but the true structure lies somewhere in between the two, making the distribution of electrons difficult to depict.

A third concept that Pauling explored in *The Nature of the Chemical Bond* was electronegativity, defined as the power of an atom within a molecule to attract electrons to itself. (Electron affinity is the power of a free atom to draw electrons to itself.) He

used this concept to estimate bond energies and to estimate dipole moments in polar covalent bonds of molecules in which one atom is slightly more positive than the other. Dipole moments are apparent in certain molecular geometric arrangements. Electronegativities may also be used to predict the character of chemical bonds. Ionic bonds result when electrons are donated by one atom and accepted by another, whereas covalent bonds form when two atoms each donate an electron to a shared pair. Most bonds are intermediate between the two extremes of ionic and covalent. The greater the difference in electronegativities of two elements, the more likely their atoms will form a characteristically ionic compound, and the lower the difference in electronegativities, the more likely the compound will be covalent.

STRUCTURE AND FUNCTION OF BIOLOGICAL MOLECULES

Around 1934 Pauling's interests shifted toward proteins, biomolecules consisting of long chains of 20 different amino acids. Proteins are the major structural building blocks of cells, and they perform most of the cellular work as enzymes that catalyze or speed up the rate of biological reactions. The first protein Pauling studied was hemoglobin, an iron-containing protein that carries oxygen (O_2) inside red blood cells throughout circulation. He determined that the oxygen molecule formed a covalent bond with the iron atom of hemoglobin using principles of magnetic susceptibility, a measurement of how easily sediments are magnetized when subjected to a magnetic field based on the iron content.

Pauling was the first to recognize and emphasize the structural, and therefore functional, significance of hydrogen bonding in proteins and other biomolecules. Hydrogen bonds are weak chemical attractions between a partially positive hydrogen atom and a partially negative atom of another molecule or a different portion of the same molecule. While the strength of an individual hydrogen bond is approximately one-twentieth of a covalent bond, the collective action of numerous hydrogen bonds may hold two molecules or different portions of the same molecule together tightly. After amino acids are joined together to create a long polypeptide chain during protein synthesis, the chain folds back on itself and sometimes combines with other polypeptides to form a unique conformation called the native form. Subjecting proteins to heat or acid causes them to denature, or unfold. If the treatment is mild, the unfolding may be reversible; if the conditions are harsh, the protein may be irreversibly damaged and will no longer function. Pauling examined this process and in 1936 concluded that mild denaturation and then renaturation involved the breaking and reformation of hydrogen bonds whereas irreversible denaturation was associated with the breakage of covalent linkages that resulted in inactive proteins.

Pauling also studied antibodies, proteins synthesized by the immune system that recognize antigens, usually other proteins. Antibodies are highly specific, meaning they recognize only one certain antigen and no others, a characteristic that intrigued Pauling. He formulated a theory based on complementarity, stating that the atoms of the antigen attracted complementary parts of the antibody, an idea that holds true today. He also incorrectly assumed that all antibodies were made of the polypeptide chains with the same sequence but folded differently to conform to their specific antigens.

In 1937 Caltech had named Pauling to the chair of the division of chemistry and chemical engineering, and the department enjoyed a renowned international reputation and a magnificent new bioorganic chemistry building outfitted with modern equipment. Pauling worked 12-hour days, seven days a week, and he loved his work. In 1941 he was diagnosed with Bright's disease, an ailment that prevents the kidneys from filtering the blood properly. He restored his health over several months by grossly modifying his diet—severely restricting his salt and protein intake and relying on vitamin and mineral supplements. After the attack on Pearl Harbor in December, Pauling's research for the next few years centered on war work, for which he received, in 1948, the Presidential Medal for Merit, the highest civilian honor in the United States. After the war and with the support of his wife, Pauling added his voice to the moral debate over the atomic bomb and to the campaign to place control of nuclear weaponry in civilian hands. His outspokenness and firm stance caused him problems later on.

PROTEIN STRUCTURE

In 1947 Pauling temporarily moved his family to England where he served as the Eastman visiting professor at Oxford. That same year he published an enormously successful college textbook, *General Chemistry*. Before returning home, while recovering from one of many serious colds he would suffer in the following decades, he started thinking about the structure of alpha-keratin, a protein found in hair and tissues with horns.

Pauling had spent time studying this protein a decade earlier, but new developments in the field convinced him he might make better progress solving the structure this time around. Believing the structure to be helical, he used paper models to construct the polypeptide chain and rotated all the single bonds, except the rigid peptide bond, in a stepwise manner until he achieved a helical structure that agreed with

Linus Pauling, shown here in his laboratory at Caltech, won the Nobel Prize in chemistry in 1954 for his research on chemical bonding and the Nobel Peace Prize in 1962. *(From the Ava Helen and Linus Pauling Papers, Special Collections, Oregon State University)*

the angles and interatomic distances calculated from X-ray crystallography performed by Robert B. Corey (also from Caltech) and colleagues. Referred to as an alpha-helix, this twisted arrangement involved hydrogen bonding between the -NH group of one amino acid and the carbonyl oxygen of another four amino acids positioned farther down the chain, with the carbonyl groups running parallel to the axis of the helix. He published a series of papers during 1950–51 describing this motif and others; a remarkable seven papers appeared in the same May 1951 issue of *Proceedings of the National Academy of Sciences*. Another structure he described that has withstood the test of time was the beta-pleated sheet, consisting of pairs of polypeptide chains lying side-by-side and stabilized by hydrogen bonds between the carbonyl oxygen on one chain and the -NH group on the other chain. Chains that run in the same direction are called parallel and chains that run in the reverse direction are called anti-parallel. Since Pauling's discovery, biochemists have identified these motifs in thousands of proteins.

Though Pauling was a gifted chemist, he was not correct about everything. One pride-damaging blunder was his proposal for the structure of DNA that consisted of a triple helix and protonated phosphate groups. James Watson, a codiscoverer of the correct double helical model, claimed that Pauling's obvious mistake was crucial in stimulating Watson to solve the structure within a matter of weeks. Pauling's error, however, did not diminish his reputation. In 1954 Pauling received the Nobel Prize in chemistry for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances. He was pleased to be recognized for such a broadly described accomplishment that seemed to encompass everything he had done during his career.

SICKLE CELL DISEASE

Sickle cell anemia is a hereditary disease in which the normally flat, disc-shaped red blood cells adopt a deformed, sickled configuration, causing them to become wedged in tiny vessels, blocking the flow of blood. The crescent-shaped blood cells also have a shorter lifespan, thus less oxygen is delivered to the tissues of afflicted individuals, causing a variety of symptoms, including fatigue, pain, an increased risk for infections, and even death. Pauling thought that the disease might be caused by an altered sequence of amino acids in the protein hemoglobin that may affect the protein's structure and, consequently, the structure of the red blood cells. Electrophoresis is a technique in which substances placed in a semi-solid matrix are subjected to an electric current, resulting in separation based on differences in their electrical charge. In 1949 Pauling demonstrated that normal and abnormal hemoglobin traveled at different rates in an electric field, suggesting a difference in the protein molecules. This was the first description of a disease caused by a change in protein structure. His astounding finding earned him honorary degrees from Cambridge, Oxford, and London Universities, nomination for the presidency of the National Academy of Sciences, and the presidency of the American Chemical Society. In 1956 Vernon Ingram and J. A. Hunt, working at the Medical Research Council Unit for Research on the Molecular Structure of Biological Systems, sequenced hemoglobin and found that the abnormal hemoglobin molecule had the amino acid valine instead of glutamic acid. A change in one single amino acid caused the disease.

The determination that sickle cell anemia was a molecular disease led Pauling together with Emile Zuckerlandl, to propose the concept of a molecular clock in 1962. Sequencing of proteins had become commonplace, and they observed a correlation between the number of amino acid residues altered in the same protein of two different species and the evolutionary distance between them. For example, horses and humans diverged long before gorillas and humans. Horse and human hemoglobin chains that were 150 amino acids long differed by 18 residues, whereas gorilla and human chains differed by only two amino acids. Pauling suggested that the rate of change of a protein was constant over time and protein sequencing data could be used to estimate when two species diverged. Modern evolutionary biologists compare nucleic acid sequence information to accomplish the same goal.

ANTIBOMB ACTIVISM

Since World War II, Pauling had used his scientific status to oppose the testing and use of nuclear weapons. He felt his unique perspective as a scientist on the effects of radioactive fallout obliged him to make a strong and public stance in opposition. He joined organizations such as the Emergency Committee of Atomic Scientists and circulated petitions protesting the development of nuclear weapons. In a national atmosphere dominated by fear of international communism led by the Soviet Union, against which nuclear weapons were the only defense, those who protested their use were considered communists themselves, or traitors. The U.S. government denied Pauling's application for a passport to lecture at a Royal Society meeting in 1952, an action that the scientific world viewed as an insult. Other requests also were denied, and colleagues and former friends began to withdraw from him.

On the announcement of Pauling's Nobel Prize in November 1954, the United States found itself in the embarrassing position of reluctantly having to grant Pauling, permission to travel to Sweden to accept the award or risk an international uproar. Pauling was instrumental in collecting thousands of signatures on a petition to end nuclear bomb testing presented to the secretary general of the United Nations in 1958. He also published the antibomb book *No More War!* that year and resigned his position as chair of the chemistry division under pressure from Caltech's president. The Soviet Union, the United Kingdom, and the United States did halt testing temporarily, and many among the U.S. public grew more sympathetic to Pauling's message about the dangers of nuclear weapons. For his efforts to end nuclear testing and for peace, the Nobel Committee awarded Pauling the Nobel Peace Prize for 1962.

VITAMIN C

The focus of Pauling's research shifted away from traditional chemistry toward the molecular basis of mental diseases, making his colleagues unhappy. Some expressed concern that his concentration on antiwar activism and the dangers of radioactive fallout left him out of touch with chemistry. Feeling misunderstood and unsupported, Pauling left Caltech after four decades and went to work at the Center for the Study of Democratic Institutions, a liberal think tank in Santa Barbara, California. In 1967 he became a research professor of chemistry at the University of California at San Diego, and then two years later he moved to Stanford University, from where he retired as a professor emeritus of chemistry in 1974. The lack of a stable work location interfered with his ability to do research.

Colds had always troubled Pauling, and in 1966 he began ingesting three grams of vitamin C, also called ascorbic acid, each day. To put this in perspective, consider the recommended daily allowance is currently 60 milligrams, 50 times less than he was taking. He claimed to have felt healthier and that his colds were less frequent and less severe. To the dismay of the medical establishment, he started publicly preaching the benefits of taking mega doses of vitamin C. In 1970 he published *Vitamin C and the Common Cold,* which emphasized studies that showed a positive benefit of taking large amounts of vitamin C and refuted opposing studies. The book became a bestseller and consumers began purchasing large quantities of vitamin C to prevent and treat the common cold. The National Institutes of Health conceded that a few studies did suggest that taking vitamin C might prevent the onset or shorten the duration of a cold, but the majority of research showed no effect.

Pauling began collaborating with Scottish physician Ewan Cameron, who believed vitamin C was beneficial to cancer patients. They published a paper in the *Proceedings of the National Academy of Sciences* in 1976 titled "Supplemental Ascorbate in the Supportive Treatment: Prolongation of Survival Times in Terminal Human Cancer," and a book in 1979 titled *Cancer and Vitamin C.* Again, the public embraced their claims, anxious to believe something as simple as taking vitamins could successfully treat cancer, but the medical establishment was not pleased. No scientific studies have verified a beneficial effect of vitamin C on cancer.

In 1973, with the help of Arthur B. Robinson, Pauling established the Linus Pauling Institute of Science and Medicine in Palo Alto, California. In 1996 the institute moved to Oregon State University, where the major areas of research are heart disease, cancer, aging, and neurodegerative diseases.

The death in 1981 of his wife of almost 60 years from stomach cancer devastated Pauling. He published another popular health book titled *How to Live Longer and Feel Better* (1986), but he was diagnosed with prostate cancer in 1991. Though Pauling lived for three more years, taking massive amounts of vitamin C, he died on August 19, 1994, at his ranch in Big Sur, California.

Linus Pauling was a passionate, charming, conceited, and brilliant Renaissance man, interested in and knowledgeable about many subjects. A master of chemistry, he had an extraordinary ability for building molecular models within his own head, as if he could see the actual atoms interacting with one another. To make it easier for the rest of world to understand what came to him naturally, he condensed his expansive knowledge into a few simple

rules to explain chemical bonding and predict molecular structures. Pauling's numerous and substantial contributions to science include his explanation of chemical bonding in terms of hybridization, character, and resonance, his elucidation of the architecture of proteins and other molecular structures, and his description of sickle cell anemia as the first identified molecular disease. He also is credited with the foundation of molecular biology, molecular medicine, and even molecular evolution. He was able to flawlessly merge physics and biology with chemistry and was an expert at combining theoretical and experimental data, but Pauling admitted that he took his greatest pleasure in the award of the Nobel Peace Prize. Learners in every field can benefit from the inspirational advice he gave to a group of students at the 1954 Nobel award ceremony in Stockholm, words he obviously took to heart himself:

> When an old and distinguished person speaks to you, listen to him carefully and with respect—*but do not believe him. Never put your trust in anything but your own intellect.* Your elder, no matter whether he has gray hair or has lost his hair, no matter whether he is a Nobel Laureate, *may be wrong.* The world progresses, year by year, century by century, as the members of younger generations find out what was wrong among the things that their elders said. So you must always be skeptical—*always think for yourself.*

See also BIOMOLECULES; CHEMICAL BASIS OF LIFE; GENETIC DISORDERS.

FURTHER READING

Brownlee, Christen. "The Protein Papers." *PNAS at 100: Classics of the Scientific Literature.* National Academy of Sciences, 2007. Available online. URL: http://www.pnas.org/misc/classics1.shtml. Accessed on January 25, 2008.

Dunitz, Jack D. "Linus Carl Pauling (February 28, 1901–August 19, 1994)." In *Biographical Memoirs. National Academy of Sciences.* Vol. 71. Washington, D.C.: National Academy Press, 1997.

Hager, Tom. *Force of Nature: The Life of Linus Pauling.* New York: Simon & Schuster, 1995.

———. *Linus Pauling and the Chemistry of Life.* New York: Oxford University Press, 1998.

The Nobel Foundation. The Nobel Prize in Chemistry 1964. Available online. URL: http://nobelprize.org/nobel_prizes/chemistry/laureates/1954/. Accessed January 25, 2008.

photosynthesis Photosynthesis is a process in which cells use radiant energy from sunlight to synthesize chemical compounds, such as carbohydrates, from simple inorganic carbon sources. All organisms require organic compounds to build proteins, carbohydrates, nucleic acids, and lipids—the biomolecular components of cells, membranes, and organelles. While heterotrophic organisms—all animals, fungi, some protists, and some bacteria—must fulfill their nutritional and energy requirements using external sources of organic compounds, autotrophic organisms have the ability to synthesize organic molecules using carbon dioxide (CO_2) as their principal carbon source. The conversion of inorganic carbon compounds into organic compounds demands a lot of energy.

Photoautotrophs possess specialized pigments that harvest energy from a light source and convert it to a chemical form of energy that the organism uses to synthesize reduced carbon compounds using CO_2 from the atmosphere. Plants, algae, and different types of bacteria are capable of carrying out photosynthesis, and therefore form the foundation of the food chain. Photosynthesis produces an estimated 160 billion metric tons of carbohydrates each year, and generates the oxygen in our atmosphere that supports aerobic life-forms.

The process of photosynthesis consists of two major stages. The first stage encompasses the light-dependent reactions, during which electrons absorb light energy from the sun. During the second stage, the light-independent reactions (sometimes loosely referred to as the dark reactions), the cell uses the high-energy electrons to form covalent bonds between carbon atoms, building simple carbohydrates $(CH_2O)_n$. Water (H_2O) serves as the original source of low-energy electrons, and molecular oxygen (O_2), a compound tremendously important to many life-forms, is a waste product. The overall process can be summarized as follows:

$$CO_2 + H_2O \xrightarrow{\text{light}} (CH_2O) + O_2$$

Simply, photosynthesis accomplishes the reverse of cellular respiration, the process of breaking down organic compounds in the presence of O_2, generating H_2O and CO_2 as waste products, for the purpose of releasing energy stored in chemical linkages.

LIGHT-DEPENDENT REACTIONS

In eukaryotic cells, photosynthesis occurs in an organelle called a chloroplast. A double membrane envelopes the chloroplast, with the outer membrane containing porins that allow for the passage of certain molecules and the inner membrane being relatively impermeable. The stroma, or the space bounded by the double membrane, contains thylakoids, flattened sacs organized into structures called grana that

resemble stacks of pancakes. The membrane system of the thylakoids contains pigments, colored molecules that absorb light of specific wavelengths. Chlorophylls absorb light with wavelengths characteristic of violet-blue and red light, thus they reflect green light, which is why plants and algae appear green. Other types of pigments, such as carotenoids, absorb different wavelengths of light, and give plants or algae red, yellow, or orange coloring.

The chlorophyll pigments work together with other molecules in arrangements called photosystems embedded in the thylakoid membranes. When light hits a chlorophyll molecule, the pigment absorbs a photon, a packet of radiant energy, and enters an excited state. The excited pigment transfers the energy to a series of other pigments and eventually to a reaction center that contains a primary electron acceptor. As the light energy reaches the reaction center, it boosts an electron of a special chlorophyll molecule near the primary electron acceptor to a higher energy level. The chlorophyll molecules all return to their ground (resting) state, while the excited electron procedes to drive the synthesis of nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP). Two types of photosystems cooperate in the light reactions: photosystem I (PS I) and photosystem II (PS II). The chlorophyll molecules are both the same type, chlorophyll a, but different molecules surrounding them affect their electron distribution, giving them slightly different properties. PS I absorbs light with a wavelength of 700 nanometers and PS II absorbs light with a wavelength of 680 nanometers.

The pathway of an excited electron can be either noncyclic or cyclic. Noncyclic electron flow is more common and begins when a primary electron acceptor captures an excited electron from a PS II chlorophyll a molecule. An enzyme cleaves a molecule of H_2O into one oxygen atom (O), two hydrogen ions (H^+), and two electrons (e^-). The oxygen atom combines with an oxygen atom liberated from another water molecule to form O_2, a waste product of the light reactions. The electrons from the water molecule replace electrons lost from the chlorophyll of PS II. Meanwhile, the original electron has been traveling down an electron transport chain, being passed from one electron acceptor to another at a lower energy level. The molecules that carry the electron from PS II include plastoquinone, a cytochrome complex, and plastocyanin. The electron eventually reaches a PS I chlorophyll molecule that lost an electron when it captured a photon of light. The excited electron from PS I also makes its way down an electron transport chain that includes the electron carrier protein ferredoxin. At the second chain's end, the enzyme $NADP^+$ reductase transfers two electrons to $NADP^+$, generating the reduced form NADPH and accomplishing one of the goals of the light reaction. Light-dependent reactions also produce ATP. As the electron falls down successively lower energy levels, the released energy is used to transport the H^+, or protons, released from the splitting of H_2O, from the stroma of the chloroplast across the thylakoid membrane into the thylakoid space. In a process called chemiosmosis, the gradient powers the transport of H^+ back across the membrane into the stroma via channels that have an ATP synthase activity. As the H^+ diffuse back across the membrane, ATP synthase attaches an inorganic phosphate to a molecule of adenosine diphosphate (ADP) to generate ATP.

Noncyclic electron flow results in equal molar quantities of NADPH and ATP, but the light-independent reactions utilize more ATP than NADPH. Cyclic electron flow compensates for this extra ATP demand. By rerouting the electron traffic from PS I back to the cytochrome complex located in between PS II and PS I, $NADP^+$ reductase never gains access to the electrons. However, the transport of the electrons through this cyclic pathway still allows for a gradient to be created, driving the production of ATP by chemiosmosis.

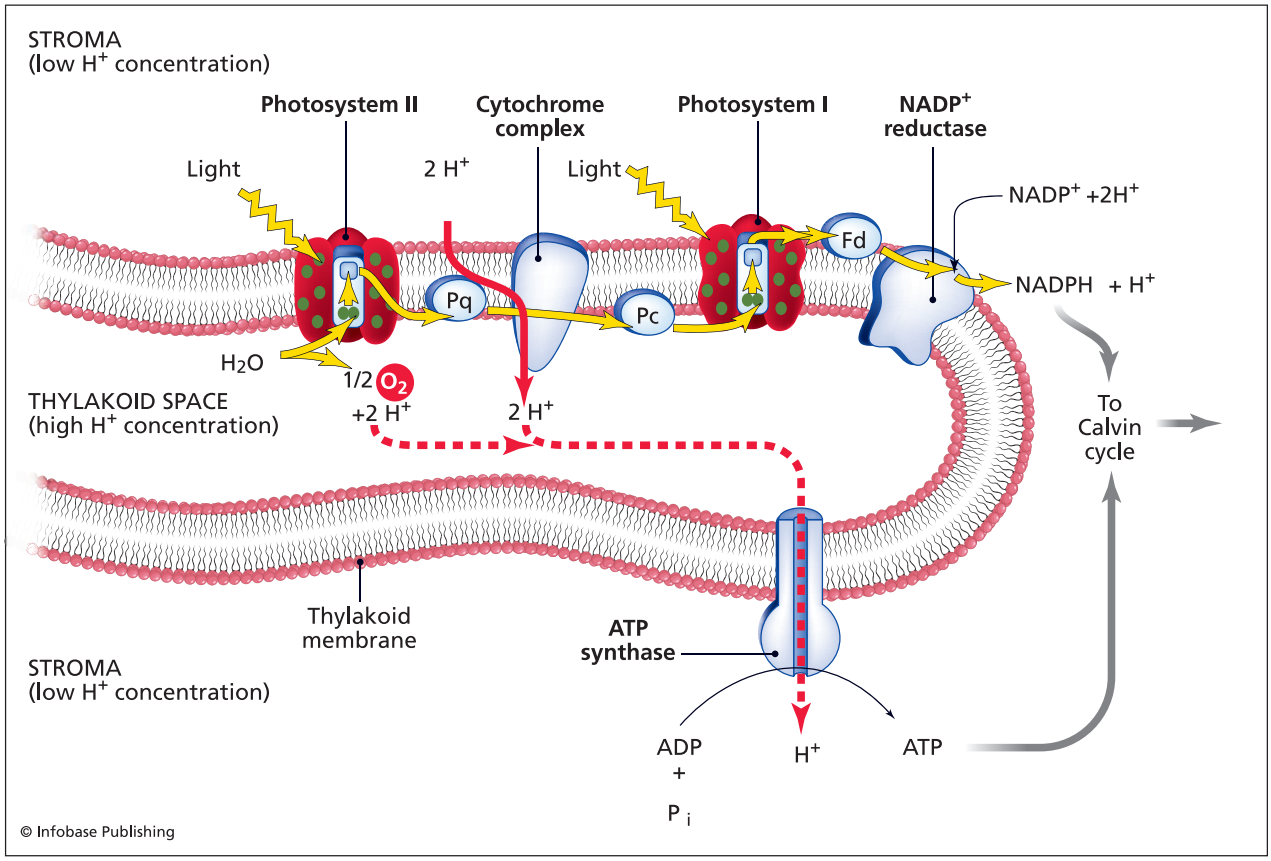
Photosynthetic prokaryotic organisms have some slight differences in their light-dependent reactions. The cyanobacteria, green and purple sulfur bacteria, and green and purple nonsulfur bacteria are all capable of photosynthesis, but as prokaryotic cells, they do not have chloroplasts or any other membrane-bound organelles; photosynthesis takes place in internal membrane systems formed by extensive invaginations of the cell membrane. This increases the area in which the photosynthetic pigments and electron transport chain participants are embedded. The internal membrane system can consist of vesicles, tubular membranes, or lamellae (a thin flat layer of membrane) that may or may not be continuous with the cell membrane. Green sulfur and green nonsulfur bacteria have chlorosomes, ellipsoidal structures consisting mostly of light-harvesting pigments surrounded by a lipid monolayer. Two types of photosynthesis occur in prokaryotes, oxygenic and anoxygenic. Cyanobacteria carry out oxygenic photosynthesis, meaning water serves as the photosynthetic electron donor and chlorophyll a plus phycobiliproteins (a unique type of pigment attached to proteins) harvest the light energy. The remaining photosynthetic bacteria are all anoxygenic: they utilize reduced compounds other than water (such as hydrogen sulfide, sulfur, hydrogen, or reduced organic molecules) as the electron donors and therefore do not produce oxygen as a by-product. Anoxygenic photosynthetic bacteria primarily utilize bacteriochlorophylls rather than chlorophylls.

LIGHT-INDEPENDENT REACTIONS

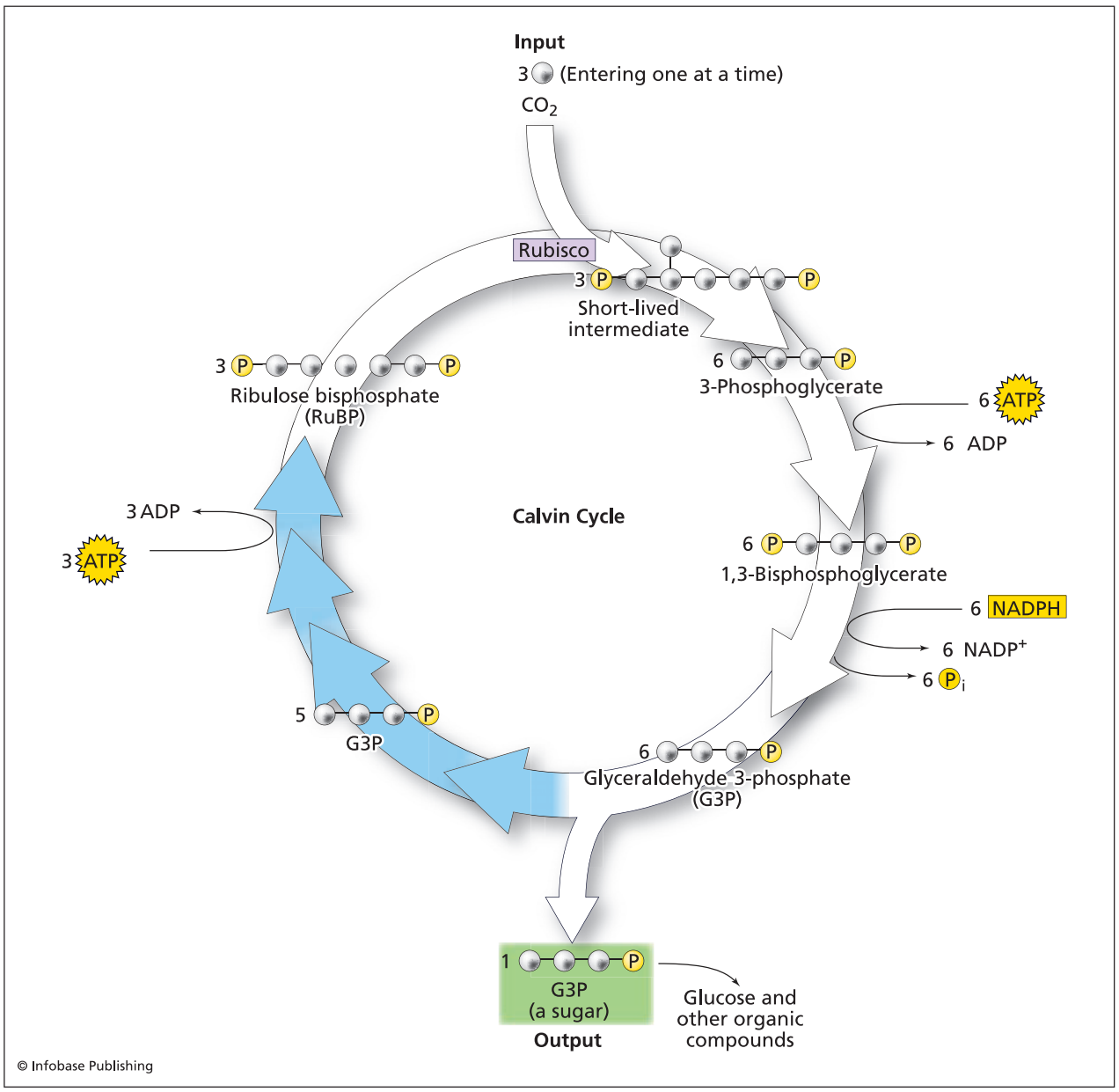
During the light-dependent reactions, the chloroplast harvested energy from sunlight, electron transport moved low-energy electrons from water to a higher potential energy state in NADPH, chemiosmosis synthesized ATP, and O_2 was produced as a waste product. The subsequent steps, the light-independent reactions, are sometimes called the dark reactions, but they can take place in the presence or absence of light. The reactions occur in the stroma of the chloroplasts. During the light-independent reactions, the chloroplasts use chemical energy created by the light-dependent reactions to synthesize sugars from CO_2. The incorporation of carbon from an inorganic source (CO_2) into an organic molecule (like sugar) is called carbon fixation, and it requires a lot of energy.

Cellular enzymes can easily access the chemical energy stored in ATP by hydrolyzing the high-energy bond that links the last phosphate group to the adjacent phosphate group. Breaking that bond releases energy that can immediately be utilized for other cellular needs. The energy stored in NADPH and other electron carriers is in the reduction potential of the molecule. Reduction is the gain of electrons, and oxidation is the loss of electrons. One is always accompanied by the other—electrons move from one molecule (the donor that is oxidized as it loses its electrons) to another molecule (the acceptor that gains the electrons). When carrying electrons, NADPH has the ability to reduce other molecules, that is, it has high-energy electrons available to donate to another molecule. The high-energy electrons contributed to the electron acceptor participate in a chemical bond, which stores the energy until catabolic processes break the newly formed bond. Organic molecules, such as glucose, are reduced carbon compounds that store energy for the cell. When the cell needs ATP, the organic molecules are oxidized, in other words, they accept electrons, and the released energy is used to generate ATP.

The reductive pentose phosphate cycle, more commonly known as the Calvin cycle, is a cyclical biochemical pathway that results in the production of a three-carbon carbohydrate, glyceraldehyde 3-phosphate. The cycle regenerates all the other intermediates but consumes three molecules of CO_2, six



During the light-dependent reactions of photosynthesis, photosystems embedded in the thylakoid membranes harness solar energy and convert it into chemical form by creating NADPH using water molecules as the ultimate source of electrons and synthesizing ATP via chemiosmosis.



The Calvin cycle utilizes the ATP and NADPH made in the light-dependent reactions of photosynthesis to create organic compounds from CO_2.

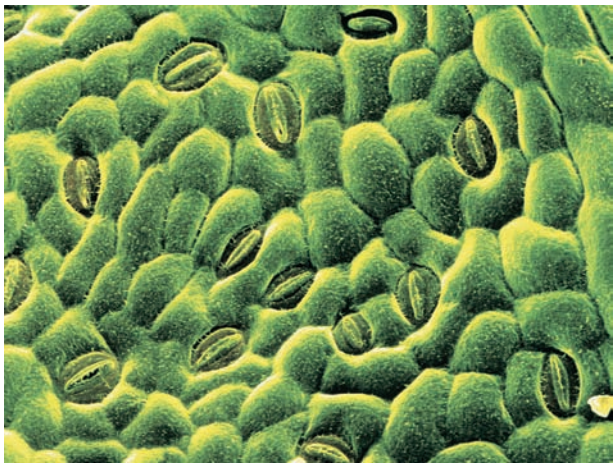
molecules of NADPH, and nine molecules of ATP for every molecule of glyceraldehyde 3-phosphate produced. Three main stages include carbon fixation, reduction, and regeneration of ribulose bisphosphate. The Calvin cycle begins when the enzyme ribulose bisphosphate carboxylase (rubisco) adds one molecule of CO_2 to the five-carbon sugar ribulose bisphosphate (RuBP), forming a six-carbon sugar that quickly degrades into two molecules of 3-phosphoglycerate, a three-carbon sugar.

The fixation of three successive CO_2 molecules results in the formation of six three-carbon molecules, the ultimate reduction of which leads to the creation of one three-carbon molecule of glyceraldehyde 3-phosphate. Five of these three-carbon molecules continue cycling and participate in the regeneration of RuBP, to which more CO_2 is added. Once the carbon is fixed, or incorporated into the organic compound glyceraldehyde 3-phosphate, other enzymes convert it into glucose and other organic compounds. The process of transferring energy from sunlight into chemical energy stored in organic compounds is now complete. The cell can use the organic compounds as fuel that can be burned as needed to generate ATP or as structural components to build new cell parts during growth.

ALTERNATIVE MECHANISMS OF CARBON FIXATION

The CO_2 utilized during the light-independent reactions enters the leaf through stomata, openings in the epidermis of the plant tissue. Transpiration, the process by which water vapor exits the plant, also occurs through the stomata. Having a shared passageway presents a problem in hot, dry conditions. Stomata close to prevent water loss, but this restricts the CO_2 from entering. As a result CO_2 levels decline and O_2 produced by photosynthesis builds up since it cannot exit through the closed stomata. Under these circumstances, the enzyme rubisco binds O_2 and adds it to RuBP rather than adding CO_2. The resulting product splits into one three-carbon compound and one two-carbon compound instead of two three-carbon compounds. The two-carbon compound exits the chloroplast and mitochondria and peroxisomes metabolize it and releases CO_2. This process, called photorespiration, resembles cellular respiration since O_2 is consumed and CO_2 is produced; however, in contrast to cellular respiration, no ATP is synthesized in photorespiration. Like photosynthesis, photorespiration occurs in the presence of light, but no sugars are produced since the products leave the Calvin cycle and exit the chloroplast. Photorespiration does not confer any obvious benefits to the plant and is a wasteful means of using resources. Some scientists suspect it is an evolutionary artifact.

Most plants are C_3 plants, meaning the first product produced following carbon fixation is a three-carbon compound (3-phosphoglycerate). C_4 plants have an evolutionary adaptation that avoids the wastefulness of photorespiration, an advantage for plants that live in arid conditions. C_4 plants contain two distinct cell types involved in photosynthesis: bundle-sheath cells and mesophyll cells. The tightly packed bundle-sheath cells surround the plant's vascular system and are responsible for carrying out the Calvin cycle. The mesophyll cells loosely surround the bundle-sheath cells. The light-dependent reactions occur in the mesophyll cells, as does the first step of carbon fixation, which is accomplished by a slightly different method than in C_3 plants. The enzyme phoshoenolpyruvate (PEP) carboxylase adds a CO_2 to the three-carbon molecule PEP, forming a four-carbon molecule (hence the designation C_4). The four-carbon compound moves into the bundle-sheath cells, releases CO_2 that can promptly enter the Calvin cycle, and the three-carbon molecule moves back to the mesophyll cells where PEP carboxylase can join it to another CO_2. PEP carboxylase works more efficiently than rubisco in hot, dry conditions because it has a higher affinity for CO_2 and a lower affinity for O_2. So when the atmospheric CO_2 cannot enter through the stomata that are closed or partially closed in order to conserve water, the plant can still fix carbon from CO_2 using this supplementary method for CO_2 uptake and produce sugars via the Calvin cycle. The mesophyll cells keep the bundle-sheath cells supplied with plenty of CO_2 for rubisco to stay active. Examples of C_4 plants include crabgrass, corn, and sugarcane.



Closed stomata are scattered among the epidermal cells on the surface of this rose leaf. *(Andrew Syred/ Photo Researchers, Inc.)*

Whereas the two stages of light-dependent reactions (carbon fixation and carbohydrate synthesis) occur in cells that are spatially separated in C_4 plants, another adaptation temporally separates the steps. Plants that store water, such as cacti and pineapples, open their stomata at nighttime. During the daytime, CO_2 is not available, so the plants uptake it overnight and temporarily incorporate it into various organic acids stored in vacuoles. The next day, when solar radiation furnishes the light-dependent reactions with energy to produce ATP and NADPH, the organic acids release CO_2, the remaining ingredient necessary to fuel the Calvin cycle. Plants with this adaptation are called crassulacean acid metabolism (CAM) plants, after a family of succulent plants in which this process was first discovered.

See also BOTANY; CALVIN, MELVIN; CELLULAR METABOLISM; ECOSYSTEMS; EUKARYOTIC CELLS; INGENHOUSZ, JAN; PLANT FORM AND FUNCTION; PRIESTLEY, JOSEPH.

FURTHER READING

Blankenship, Robert E., Govindjee, Gerald A. Berkowitz, Archie R. Portis, Jr., Robert E. Blankenship, and R. J. Shopes. "Photosynthesis." In AccessScience@ McGraw-Hill. Available online. URL: http://www.accessscience.com, DOI 10.1036/1097-8542.511700. Accessed January 15, 2008.

"Photosynthesis." In *Encyclopaedia Britannica*. 2008. From Encyclopedia Britannica Online School Edition.

Available online. URL: http://school.eb.com/eb/article-9108553. Accessed January 15, 2008.

Stern, Kingsley R., James Bidlack, and Shelley Jansky. *Introductory Plant Biology*. 11th ed. New York: McGraw Hill, 2008.

physiology Physiology is the branch of life science concerned with the function of organisms. Physiologists explore the mechanisms by which organisms accomplish a task (a mechanistic approach) while considering the purpose or need fulfilled by performing the function (the teleological approach). A major goal of physiology is to understand how different cells, tissue types, organs, and organ systems work together to carry out life processes, including homeostasis (the maintenance of relatively stable conditions inside an organism's body despite external and internal fluctuations), organization, metabolism, growth, adaptation, response to stimuli, and reproduction. The study of how organisms function is closely tied with their form or structure. The branch of life science that focuses on the structure of organisms is anatomy, a subject often taught simultaneously with physiology.

Because human physiology is the major discipline related to understanding health and disease, the applications to medicine are obvious. Knowing how the body works helps to maintain health and to determine the cause when something goes wrong in order to correct or treat the malady. Health-care professionals, including physicians, nurses, dentists, physical therapists, occupational therapists, speech and language pathologists, optometrists, chiropractors, pharmacists, athletic trainers, and others, all receive training in physiology. Veterinarians and agriculturists who raise farm animals or who are involved in animal husbandry or other animal sciences rely on knowledge of the physiology of the animals with which they work. Studies related to plant physiology are important for food production and for commercial industries, but also for maintaining healthy ecosystems and even global climate since plants consume carbon dioxide (a greenhouse gas) during photosynthesis.

Society directly benefits from applications of physiology as a discipline, but biologists also study physiology simply to gain a better understanding of the living world. Examining the mechanisms by which different organisms, even across kingdoms, carry out different life processes provides information about evolutionary relationships. In addition to evolutionary biology, the field of physiology integrates concepts from other branches of life science such as ecology, the study of the interactions of organisms and their surroundings. The environment in which an organism resides strongly influences how that organism will perform physiological tasks such as maintaining a stable body temperature or obtaining nutrition. For example, two types of animals, lynxes and sponges, both must fulfill their nutritional needs by taking in nutrients in the form of presynthesized organic molecules. Sponges are sessile, marine invertebrates—they live in the ocean, remain attached to rocks or other surfaces, and feed by filtration. Ocean currents carry plankton (microorganisms such as algae, bacteria, and protozoans that drift with the currents), which the sponge brings in through pores in its body wall. Specialized collar cells called choanocytes within the sponge have flagella that whip back and forth to force water through the sponge while trapping the nutritious bits, which are then picked up by amoebocytes, cells that transport the nutrition throughout the sponge. In contrast, lynxes are predators, and they must be able to run quickly to chase down prey such as snow hares, for food. Their skeletal systems must be strong and their musculature must allow for quick bursts of metabolic activity to accompany the powerful movements associated with running after, capturing, and then tearing apart and chewing their food. Evolution will favor physiological adaptations that benefit an organism in its quest for food. As evidenced by the predator-prey relationship between lynxes and snow hares, interactions with other organisms also determines the degree to which a physiological mechanism benefits a species in a particular environment. Filter-feeding works well for sponges living in the ocean, while this physiological adaptation would obviously not work for a terrestrial animal such as a lynx.

In addition to integrating other branches of life science such as ecology and evolution, studies in physiology also incorporate information gained by studying organisms at different levels, from the chemical level up through the level of the organism. Cell physiology examines the functions occurring at the cellular level. Systemic physiology focuses on one organ system at a time. The body systems include the circulatory, digestive, respiratory, excretory, skeletal, muscular, integumentary, immune, nervous, endocrine, and reproductive systems. One can also study the physiology of organisms that do not exhibit so many levels of organization. For example, bacterial physiology aims to elucidate the mechanisms by which bacterial cells carry out all of life's processes such as growth, maintaining a constant intracellular environment, fulfilling nutritional and energy needs, and reproduction, despite the fact that the whole organism comprises a single cell.

See also ANATOMY; ANIMAL FORM; CIRCULATORY SYSTEM; DIGESTIVE SYSTEM; ENDOCRINE SYSTEM; EXCRETORY SYSTEM; HOMEOSTASIS; HOST DEFENSES;

(continues on page 610)

THE CHILDHOOD OBESITY EPIDEMIC

by Sharon Brown, Ph.D.
Transylvania University

Many leading health officials consider the current epidemic of childhood and adolescent obesity in the United States to be the greatest public health problem facing us today. Currently, 18.8 percent of children (ages six to 11) and 17.4 percent of adolescents (ages 12 to 19) are overweight or obese. This translates into nearly one out of every six young people in the United States, and this number is growing. Comparing these figures with those from the previous generation reveals why so many experts are alarmed. In 1980 the percentage of obese children was 7 percent and the percentage of obese adolescents was 5 percent. In just one generation, obesity in young people has more than doubled.

What do we mean when we say someone is obese? Obesity is more than being just overweight—it means being excessively overweight. Currently the most common way to classify whether someone is obese or not is to use BMI, or body mass index. A BMI is calculated by taking someone's weight in kilograms and dividing it by the square of his or her height in meters. The formula can be written as

$$\text{BMI} = (\text{weight in kg})/(\text{height in m})^2$$

A BMI of 18.5 to 24.9 is considered normal weight. A person with a BMI of 25.0 to 29.9 is overweight. Someone with a BMI of 30.0 to 39.9 is classified as obese. Another way to define obesity is by the percentage of a person's body that is fat. Doctors generally agree that males with more than 25 percent body fat and females with more than 30 percent body fat are obese. Health care professionals use these measurements to help identify and recommend children and adolescents for medical assessment because their excess weight is likely to cause health problems. Obesity often persists into adulthood, when the health risks become even more critical.

Today the obesity epidemic includes young people in the United States of both genders from every race, ethnic group, economic background, and region. Certain groups have a higher risk for developing obesity than others. These include young people from low socioeconomic-status families; those from Hispanic, African American, and Native American families; and children and adolescents who live in southern states.

One of the major concerns for obese young people is their appearance. Certainly looks are an important issue, but obesity-related problems go beyond appearance. Serious health consequences can result from having too much body fat. Some of these problems occur in youth, but many will not appear until much later in an obese person's life.

HEALTH CONCERNS

Being obese can make a person feel tired and uncomfortable and puts extra stress on the joints and on other parts of the body. More seriously, being obese also puts children and adolescents at an increased risk for heart disease (also called cardiovascular disease). Because of this, obesity can reduce not just the quality of a person's life, but his or her life expectancy as well.

Cardiovascular disease, as the name suggests, refers to diseases that involve the heart and blood vessels (arteries and veins). While the term can be used for any disease that affects the cardiovascular system, its most common usage means atherosclerosis, the narrowing of the vessels that supply blood to the heart or the brain. Since heart attack (caused by a lack of blood to the heart) and stroke (caused by a lack of blood to the brain) are the leading causes of death in the United States, the high percentage of children and adolescents with obesity have a life-threatening problem. Approximately 60 percent of obese young people have at least one physiological cardiovascular disease (CVD) risk factor—such as high cholesterol, high blood pressure, or insulin resistance—and 25 percent have two or more of these conditions. Persistently high blood pressure levels (this condition is also known as hypertension) occur about nine times more frequently among obese children and adolescents than in their nonobese peers.

In healthy bodies, insulin helps covert consumed sugars and starches into energy. Over time, obesity can lead to a condition known as insulin resistance, in which this process becomes ineffective. Insulin resistance often develops into a serious sickness known as type 2 diabetes. Formerly referred to as adult-onset diabetes, type 2 diabetes now affects significant numbers of children and adolescents as well. Type 2 diabetes, a medical problem itself, is also a major risk factor for heart disease, kidney failure, nerve damage, and amputation, and is the leading cause of blindness.

SOCIAL AND EMOTIONAL PROBLEMS

In addition to the physical risks, obese young people face social and emotional difficulties. Children and adolescents who are obese are often stigmatized because of their appearance. They may encounter intentional weight-related teasing and name calling, as well as less-direct but still potentially hurtful comments from their friends, classmates, family members, teachers, employers, and even strangers.

Others often make negative assumptions about obese children and adolescents. Because they are so overweight, they are often seen as being lazy or undisciplined, or even being unclean. Young people who are obese may have trouble keeping up with their friends or walking very far. Because of these factors, obese young people are more likely to suffer from low self-esteem and depression.

DIRECT CAUSES AND INDIRECT FACTORS

Because of the health problems associated with childhood and adolescent

obesity, society must take steps to reduce the percentage of young people in America who are obese or who may become obese in the future. The causes of the modern epidemic of childhood and adolescent obesity are both simple and complex.

The two direct causes of obesity are improper eating habits and lack of physical activity—people gain weight when they eat more calories than they burn. However, in America, many indirect factors influence young people's consumption of too many calories and insufficient exercise. What about genes and heredity? Although genetics may predispose someone to become obese, the genetic characteristics of the human population have not changed in the last three decades, while, as mentioned earlier, obesity has more than doubled. This suggests that the modern obesity problem has been caused primarily by eating and activity patterns, not by genetics. Obese parents often have children who also end up being obese, but this is typically because the children have picked up their parents' unhealthy eating habits and inactive lifestyle.

A number of factors that promote eating high-fat, high-sugar, high-calorie foods contribute to the increase in childhood obesity. Advertising that is specifically geared toward encouraging young people to consume junk food, fast food, or sugary drinks has contributed to the problem. While watching television, the average child sees 10,000 food advertisements a year, and over 80 percent of those commercials are for high-sugar or high-fat foods. Just recently, under pressure from parents and medical groups, the leading U.S. food and beverage companies have agreed to cut in half their junk food advertisements to children and to increase their marketing for healthy food choices.

The fact that families seem to have less and less time also has helped to fuel the obesity epidemic. Families in a hurry often eat more convenience foods, which are typically high in calories from sugar or fat. Skipping breakfast due to a lack of time can lead to overeating later in the day.

Emotions are yet another of the many indirect factors that add to the obesity problem. People, young and old, eat more when they are stressed, lonely, anxious, or bored. This pattern can become a hard cycle to break because overeating may trigger more negative emotions, prompting more overeating.

Poor eating habits are one side of today's obesity problem. The other side is a sedentary lifestyle. In spite of the recent boom in youth sports, in general young people today are more inactive than ever before.

Recent technological developments such as video games, cell phones, and even remote controls mean that young people engage in less physical activity than in the past. Cars have become the dominant form of travel, so fewer young people are walking or riding their bikes. The amount of time children spend watching television has significantly increased, averaging more than four hours per day. A typical child in the United States spends 900 hours in school each year and 1,023 hours each year in front of a television. The American Academy of Pediatrics recommends no more than one to two hours a day of television and/or computer and video game playing a day. The amount of time watching television directly correlates with increased risk of obesity because young people who watch a lot of television are not only less active, but also tend to snack more often.

The amount of regular activity has decreased while the amount of time spent on sedentary activities has increased. The National Association of Sport and Physical Education (NASPE) recommends that all children accumulate at least 60 minutes, and up to several hours, of age-appropriate physical activity on all, or most days of the week. In spite of this, according to the Centers for Disease Control, in 2006 only 3.8 percent of elementary schools, 7.9 percent of middle schools, and 3 percent of high schools provided daily physical education for the entire school year for students in all grades in the United States.

SOLUTIONS

The keys to reducing and preventing obesity in young people are regular activity and good eating habits. To encourage this, society needs to take action to ensure healthier school, community, and home environments.

By passing into law the Child Nutrition and WIC Reauthorization Act of 2004, the U.S. Congress required all schools that have federally funded meals programs to create and implement wellness programs in order to educate their students, to increase the amount of activity time for recess and physical education, and to add more playground space. Another important action taking place in schools involves eliminating junk food from vending machines and school cafeterias and offering more nutritious options.

Healthy communities can also help combat childhood and adolescent obesity. Neighborhoods need to provide safe places for young people to walk, bike, run, skate, and play. Communities should encourage healthy food options, including farmers' markets and community gardens. Fast-food restaurants need to offer more healthy choices while changing the focus from high quantity of food to high quality.

Because young people spend much of their time at home, home environment plays a critical role in helping children learn to make healthy choices. Parents and caregivers need to provide children with healthy foods and beverages, giving them choices that are low in sugar and rich in nutrients. Instead of fast food, convenience food, and junk food, young people should eat more vegetables, fruits, and foods made from whole grains—selections that provide lots of fiber, complex carbohydrates, vitamins, and minerals. When they eat meat, young people should be encouraged to select low-fat, leaner options. Parents should teach children what healthy-sized portions are, should encourage and support regular physical activity, and should

(continues)

(continued)

monitor television and video playing time. Most importantly, parents and caregivers should serve as healthy role models themselves.

SUMMARY

Because of the growing rates of obesity in young people, for the first time in history, the younger generation may have a shorter life expectancy than their parents. Americans spend over 74 billion dollars a year treating obesity-related illnesses. Since eight out of 10 obese children become obese adults, the most effective treatment for obesity clearly is preventing it in the first place. Because of the physical and emotional problems it causes, the prevention of obesity in children and adolescents has become a national public health priority.

FURTHER READING

American Pediatric Society/Society for Pediatric Research home page. Available online. URL: http://www.aps-spr.org. Updated February 6, 2008.

Centers for Disease Control and Prevention home page. Available online. URL: http//cdc.gov. Updated February 28, 2008.

Koplan, J. P., C. T. Liverman, and V. I. Kraak. *Preventing Childhood Obesity: Health in the Balance.* Washington, D.C.: The National Academies Press, 2005.

National Association for Sport and Physical Education (NASPE) home page. Available online. URL: http://www.aahperd.org/NASPE/. Accessed March 1, 2008.

The Obesity Society homepage. Available online. URL: http://www.obesity.org. Accessed March 1, 2008.

(continued from page 607)

HUMAN REPRODUCTION; INTEGUMENTARY SYSTEM; MUSCULOSKELETAL SYSTEM; NERVOUS SYSTEM; RESPIRATION AND GAS EXCHANGE; REPRODUCTION; SENSATION.

FURTHER READING

Bailey, Jill. *Animal Life: Form and Function in the Animal Kingdom*. New York: Oxford University Press, 1994.

Hill, Richard W., Gordon A. Wyse, and Margaret Anderson. *Animal Physiology*. Sunderland, Mass.: Sinauer, 2004.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. New York: McGraw Hill, 2006.

Taiz, Lincoln, and Eduardo Zeiger. *Plant Physiology*. 3rd ed. Sunderland, Mass.: Sinauer, 2006.

plant diversity Throughout history, the biological grouping of plants has, at times, included fungi, various protists, different kinds of algae, and even some animals that superficially resemble plants, like corals or sponges. Modern classification schemes include flowering plants, cone-bearing plants, ferns, bryophytes, and their relatives in the eukaryotic kingdom Plantae.

Some botanists also consider green algae, or at least charophyceans, the green algae that are most closely related to plants, a member of this kingdom. Others prefer the traditional classification scheme, limiting plants to embryophytes, plants in which the embryo is retained within maternal tissue. Though botanists may disagree regarding the distinction, they generally agree that extant land plants are derived from an algal ancestor. Plants and green algae both have chlorophylls a and b, carotenoids, store food reserves as starch, and have cellulose in their cell walls. Charophyceans and land plants both have rosette-shaped arrays of proteins that synthesize the strands of cellulose, and they contain a higher percentage of cellulose in their cell walls. The peroxisomes of both types of organisms contain unique enzymes that protect organic compounds from damage by photorespiration. Charophycean sperm resemble the flagellated sperm of some land species, and charophyceans and land plants are the only organisms to create a phragmoplast, a complex of microtubules vesicles, during cell division. Though extant charophyceans are not the actual plant ancestors, studying their structure and physiology can help botanists imagine what the earliest land plants were like.

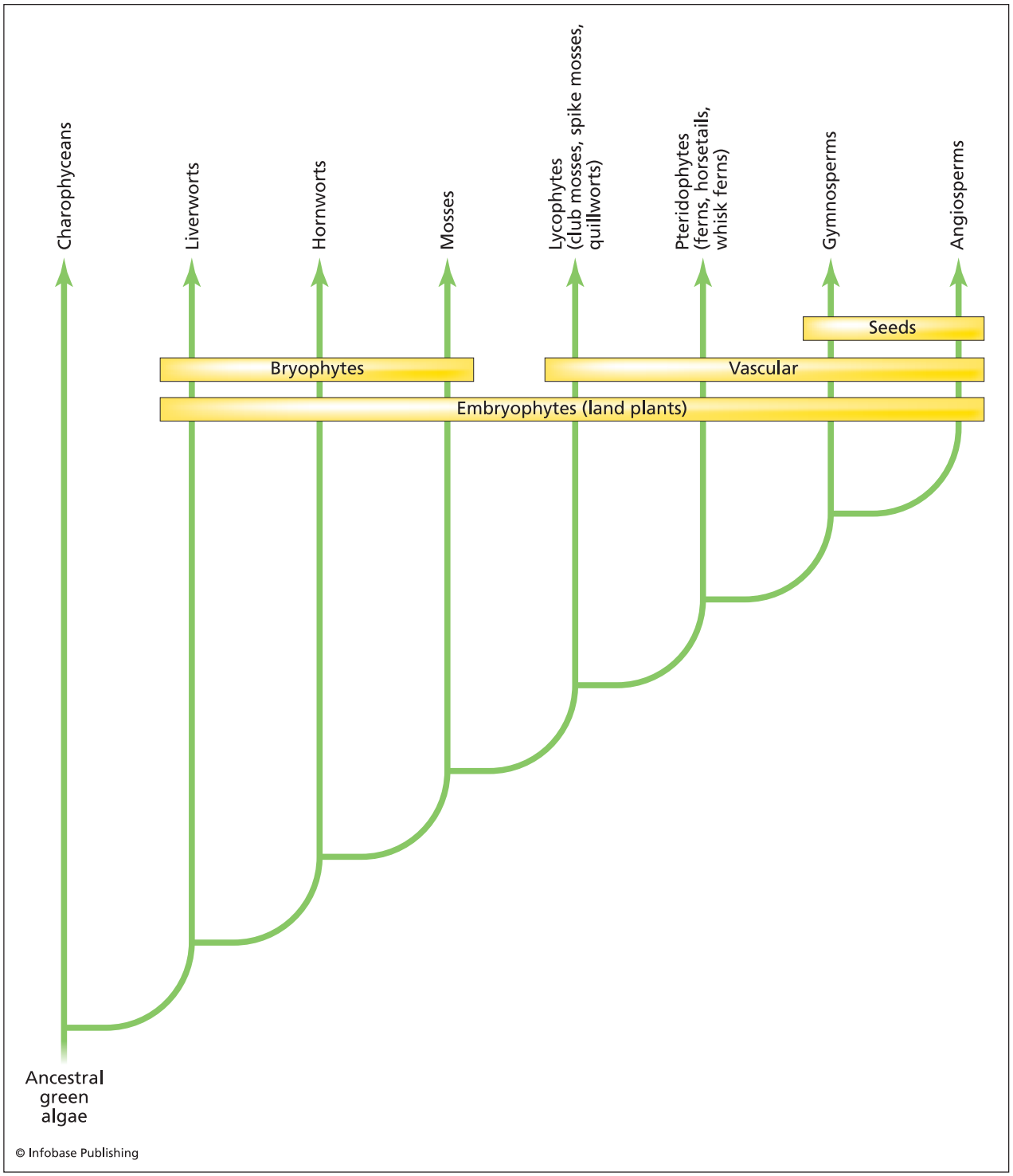
PLANT ADAPTATIONS

Fossil evidence suggests the first land plants appeared about 460 million years ago. These organisms were derived from aquatic green algae that lived in symbiotic relationships with fungi. Moving out of the water meant that sunlight necessary for photosynthesis was not absorbed by the water, and carbon dioxide was more abundant. Over time, plants developed at least five traits that distinguished them from their evolutionary ancestors: apical meristems, alternation of generations as part of their life cycle, sporangia that produce walled spores, multicellular gametangia, and multicellular, dependent embryos. These characteristics are common to embryophytes, despite their numerous diversities.

Plants are not motile, but they have clusters of cells at the tips of their roots and shoots that retain the ability to multiply and differentiate into specialized tissue. Activity at these regions, called apical meristems, results in elongation of the roots to reach farther and deeper into the soil to seek out the nutrients and water and the growth of stems and leaves to provide more area for photosynthesis to generate

more chemical energy to support metabolism and continued growth.

All organisms that sexually reproduce create haploid single-celled gametes that fuse during fertilization, regenerating the diploid state. Plant life cycles alternate between two multicellular stages: the haploid gametophyte stage and the diploid sporophyte stage. The gametophyte creates haploid gametes by mitosis, a form of nuclear division that preserves the number of chromosomes such that the resulting daughter cells are identical to each other and to the parent. Gametes fuse in a process called



All embryophytes, or land plants, emerged from an ancestral green alga, then some lineages developed adaptations such as vascular tissues and seeds, giving rise to the many diverse forms present today.

fertilization, forming a diploid zygote. The zygote undergoes numerous rounds of mitosis to create a multicellular sporophyte stage. The sporophyte has specialized reproductive structures called sporangia that contain cells called sporocytes that produce haploid gametes through meiosis, a form of nuclear division that results in daughter cells that have only half of the original chromosome number. The haploid spores germinate to form a multicellular gametophyte, whose cells are all haploid, and the cycle begins again. Life cycles characterized by the alternation of generations evolved independently in certain types of algae other than the charophyceans.

The spores of land plants have cell walls made of the polymer sporopollenin, which protects the contents in harsh conditions, allowing for further dispersal through the air and increasing the chance of survival until conditions are favorable for growth. Because sporocytes produce the spores inside of a sporangium, the spores are protected until they are ready for release and dispersal.

In the gametophyte, gamete production occurs inside multicellular organs called gametangia. These structures protect the single-cell gametes from desiccation and their surroundings. Female gametangia are called archegonia, and male gametangia are called antheridia. Archegonia are shaped like a vase and contain a single egg. Antheridia produce sperm that often fertilize the egg internally within the archegonium. Some seed plants that have severely reduced gametophyte forms have lost the antheridia and archegonia.

After fertilization the zygote develops into a multicellular embryo within the maternal structures. The maternal tissues provide nutrients for the embryo, which is said to be dependent. Because of this characteristic, land plants are called embryophytes.

All land plants share the above derived traits, or are derived from lineages that did. Other characteristics that have boosted the success of terrestrial plants include the presence of a waxy cuticle that coats the surface of land plants and functions to prevent water loss and microbial infection. Other plants have developed alternate metabolic pathways that result in the production of secondary compounds. These organic molecules perform a variety of functions such as repelling herbivores or parasites, absorbing excess ultraviolet radiation, or chemical signaling to potentially beneficial symbiotic species. Some plant species have returned to living in aquatic environments but are often still referred to as land plants. This distinguishes them from green algae and serves as a reminder of their evolutionary history.

BRYOPHYTES

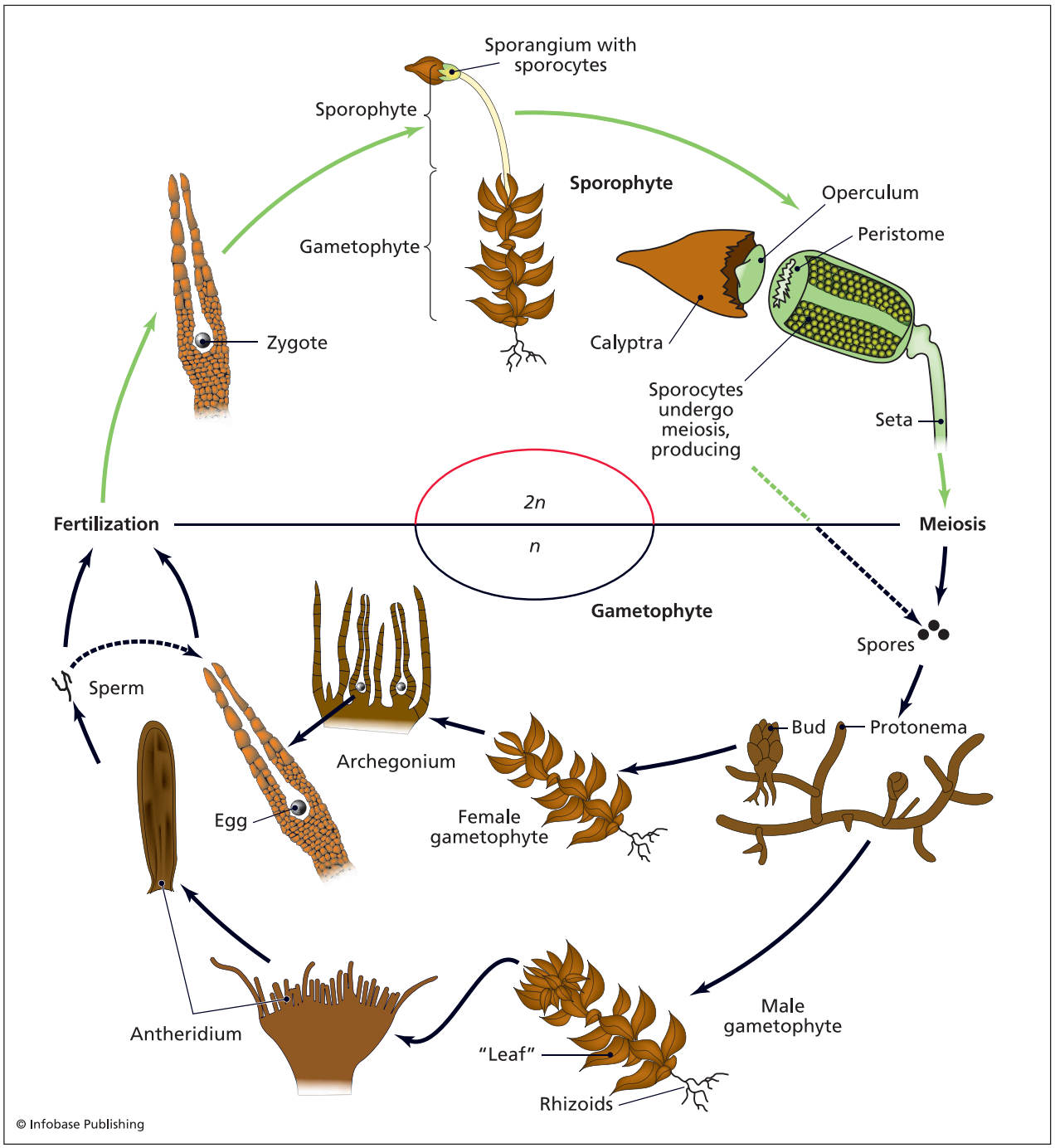
The term bryophytes, when not capitalized, refers to three phyla of nonvascular land plants: the liverworts, the hornworts, and the mosses. These groups are the most primitive of the embryophytes. Bryophytes share a few common features: the haploid gametophyte stage is dominant, the sperm must swim through a fluid-filled tube to fertilize eggs, and structures called rhizoids anchor the plant to the ground. Rhizoids resemble roots, but they do not function in water or nutrient absorption as the roots do in more complex land plants.

The liverwort *Marchantia polymorpha* bears structures called gemma cups that contain gemmae, which can develop into clones of the parent. *(Dr. Morley Read/ Photo Researchers, Inc.)*

The liverworts, phylum Hepatophyta, are a sister group to other land plants consisting of between 8,000 and 9,000 species. Unlike most land plants, they do not possess stomata, openings through which carbon dioxide, oxygen, and water vapor are exchanged. The most common liverworts, such as *Marchantia,* have flat but thick leaflike bodies called thalli. The most abundant liverworts have a leafy appearance similar to that of mosses, but the structure and arrangement of the leaves differ. *Frullania* and *Plagiochila* are examples of leafy liverworts. Liverwort gametophytes develop from spores that in some cases grow into short filaments called protonema before reaching the mature gametophyte stage. Rhizoids are made from single cells, and growth occurs by spreading outward rather than upward. *Marchantia* thalli have tiny cuplike structures that contain gemmae, pieces of tissue that can grow into complete organisms if they are removed from the cup as by a hard rain. *Marchantia* gametophytes produce umbrella-shaped gametangia that produce either sperm or eggs, depending on whether the plant is male or female. When a sperm fertilizes an egg, a microscopic diploid sporophyte forms and remains attached by a stalk called the seta. The sporophyte depends on the gametophyte for sustenance. Meiosis occurs inside the capsule of the sporophyte, and when the spores are mature, the capsule breaks open

and releases the spores. If the spores land in a suitable environment, they will germinate and develop into male or female gametophytes.

Hornworts, plants belonging to the phylum Anthocerophyta, consist of approximately 100 species of small fleshy, thalloid plants. Unlike most plants, their cells contain a single chloroplast. Like all other plants except liverworts, hornworts do have stomata. Photosynthetic bacteria called cyanobacteria live in spaces of the thalli filled with mucilage (a gelatinous substance containing proteins and polysaccharides) and assimilate nitrogen from the atmosphere into organic compounds that the plant can use. The gametophyte generation is dominant as in all bryophytes, but the sporophyte stage is more apparent because it grows up to two inches (5 cm)



The dominant gametophyte stage of mosses produces haploid gametes that unite to form a diploid, dependent sporophyte that contains a sporangium filled with sporocytes that undergo meiosis to generate haploid spores. The spores germinate to form male and female gametophytes and the cycle continues.

high and multiple sporophytes can grow from the same plant.

The mosses include 15,000 species that belong to the phylum Bryophyta, which includes three different classes: the peat mosses, the true mosses, and the rock mosses. Other plants and organisms that resemble plants are informally called mosses but do not belong to the phylum Bryophyta. Mosses live all over the planet including in extreme environments such as Antarctica and deserts, but they are most common in forests and wetlands. Mosses differ from other bryophytes in that they grow upward more than outward, but they typically reach only two to three inches (5 to 8 cm) tall. The leaves of mosses have primitive structures, with no mesophyll tissue, stomata, or veins. Except for in the middle, the leaves are one-cell layer thick and grow in a spiral arrangement around the stemlike axis. Depending on the species, male and female gametangia may grow on the same plant. Archegonia grow upward from the tips of gametophytes, and an egg grows in a cavity at the base (called the venter). The neck, the cylindrical region extending above the venter, contains a canal. Hairlike sterile filaments called paraphyses grow among archegonia. Moss antheridia are rounder then archegonia, and tissue within them gives rise to sperm cells. The antheridium absorbs water and swells, forcing the sperm out of the top. Individual sperm swim down the canal in the neck of an archegonium and fertilize the egg, which develops into an embryo inside the venter. Eventually the embryo grows large enough to poke through the top. The mature sporophyte consists of a slender stalk called a seta and a capsule. Even when the sporophyte is mature and can produce its own food through photosynthesis, it depends on the gametophyte for water and minerals. Sporocytes form within the capsule and produce haploid spores by meiosis. When they germinate, they form filamentous protonemata (plural for protonema), which produce buds that grow into gametophytes.

This fan club moss is a common forest ground cover, also used for making holiday wreaths. *(Audrey M. Vasey, 2007, used under license from Shutterstock, Inc.)*

Whisk ferns (*Psilotum nudum*), here shown in the Kao Desert of Hawaii, have no true roots or leaves, but rather scalelike outgrowths that resemble small leaves and rootlike stems that anchor the plants in place. *(© David Cavagnaro/Visuals Unlimited)*

Mosses play an important role in ecological succession and are often the first plants to appear after a volcanic eruption or glacial retreat. They accumulate minerals and help retain moisture, eventually making the environment more hospitable for other organisms. The peat mosses, such as the genus *Sphagnum,* are extremely absorptive. Gardeners often add dried peat moss to soil as a conditioner. Because peat moss naturally secretes compounds that inhibit bacterial and fungal growth, it has antiseptic properties and has been used to pack wounds. Acidic secretions also inhibit bacterial growth, and peat bogs can preserve mummified corpses for thousands of years.

SEEDLESS VASCULAR PLANTS

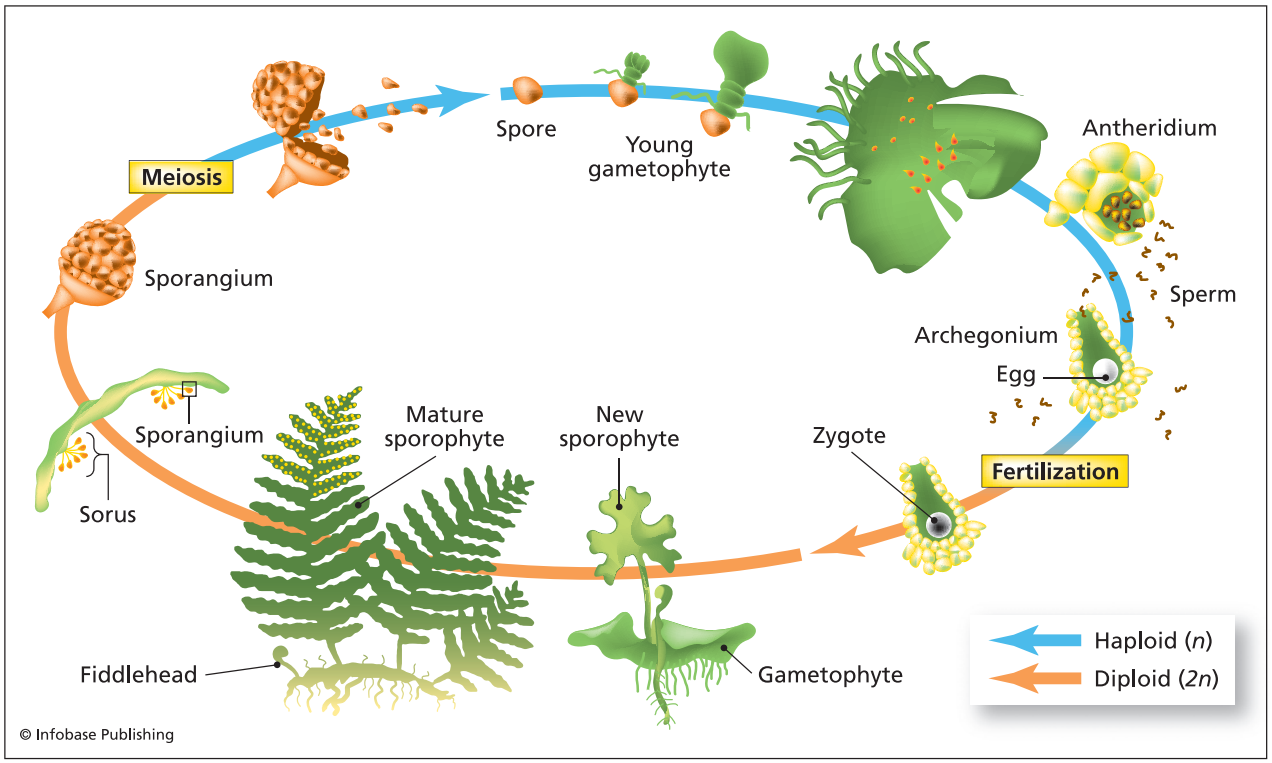
The seedless vascular plants dominated the Carboniferous (354 to 290 million years ago). The evolution of vascular tissue brought about structural and metabolic adaptations that enabled these plants to reach great heights. Dense forests covered the Earth and significantly decreased the carbon dioxide levels in the atmosphere. Physical and geological processes converted the remains of these plants into the coal used today.

An ancestral bryophyte gave rise to vascular plants, the plants that dominate the land today. First seedless forms appeared, and then later seed-bearing plants. Four characteristics that made vascular plants successful are a dominant sporophyte stage, xylem and phloem, roots and leaves, and sporophylls. Vascular plants continued to undergo an alternation of generations, but the diploid sporophyte stage grew larger and began producing multiple sporangia. This increased the plant's success because if an

animal eats one sporangium, other sporangia from the same plant may survive to reproduce. Two types of vascular tissue allowed plants to grow higher above ground: xylem and phloem. Xylem transports most of the water and minerals through tube-shaped cells called tracheids. A polymer called lignin helps strengthen the walls of the tracheids and prevents drooping. Phloem carries sugars, amino acids, and other organic nutrients throughout the plant. Vascular tissue also extends into the roots, structures unique to vascular plants that allow them to absorb water and nutrients from the soil. Leaves increase the surface area where photosynthesis occurs. Microphylls are leaves that contain a single unbranched vein, and megaphylls contain complex, branched vascular networks and are therefore usually larger than microphylls. Sporophylls are specialized leaves that bear sporangia. Homosporous plants have one type of sporophyll that makes one type of spore that develops into a bisexual gametophyte. Heterosporous plants have two types of sporophylls: megasporophylls that make the female megaspores and microsporophylls that make male microspores. Most seedless vascular plants are homosporous, and most seed plants are heterosporous.

The extant seedless vascular plants consist of two major clades, Lycophyta and Tracheophyta. The lycophytes, which are more primitive, have microphylls and include the clubmosses, the spike mosses, and the quillworts. Lycopoda, the clade that includes the clubmosses, once boasted trees that grew up to 130 feet (40 m) tall. Living clubmosses resemble mosses, but they are sporophytes rather than gametophytes and are homosporous. The types that inhabit the tropics live as epiphytes up in trees. Those that live in temperate climates live on forest floors and remain green in the winter. The spike mosses, belonging to the genus *Selaginella,* generally inhabit moist environments though there are exceptions. *Selaginella* species are heterosoporous and require water for reproduction. The quillworts, consisting of the genus *Isoetes,* have leaves shaped like quills, are also heterosporous, and grow mostly in water.

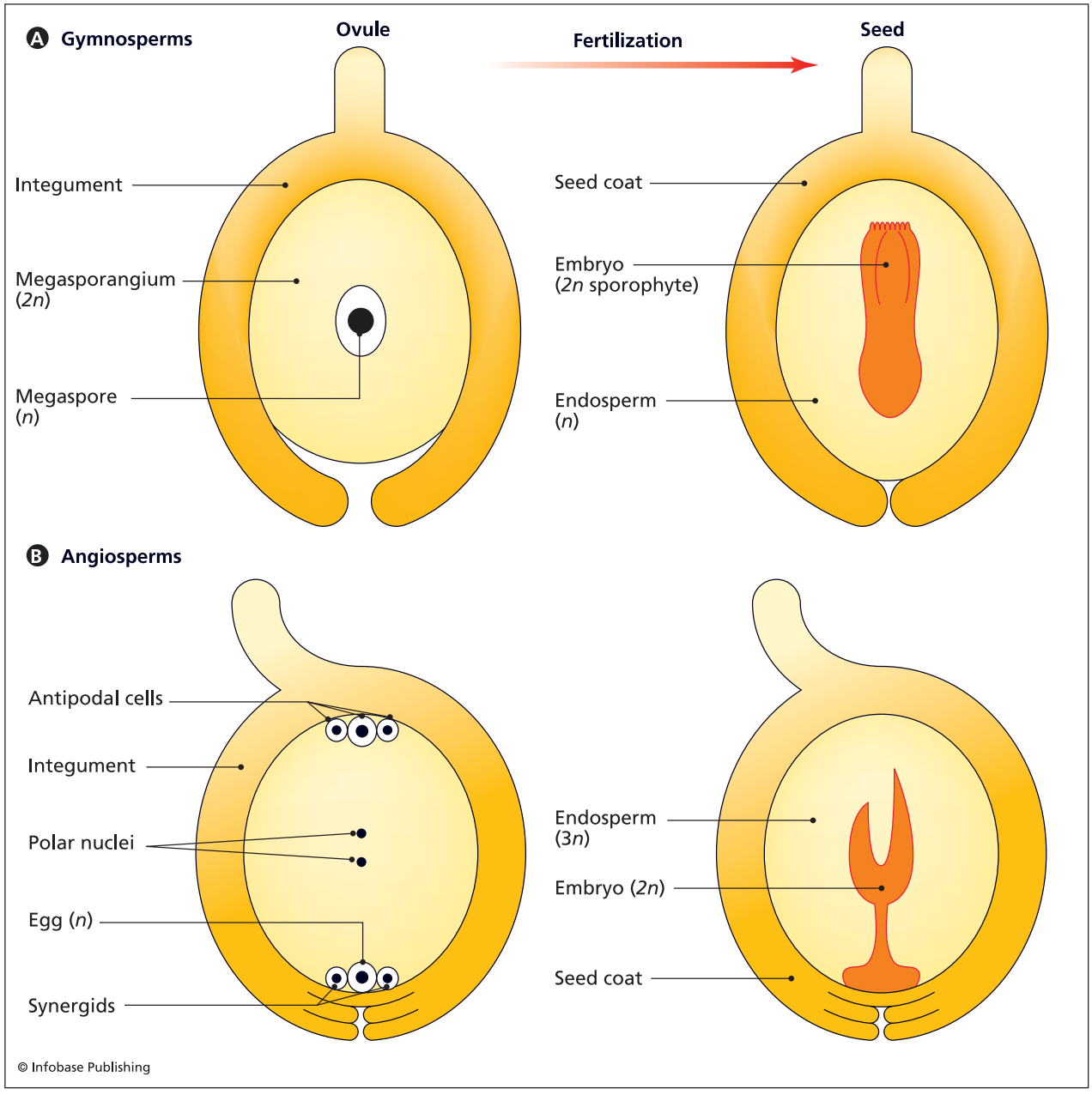
The other major group of seedless vascular plants is the Pteridophytes, which includes 12,000 species of whisk ferns, horsetails, and ferns. Some textbooks place horsetails and whisk ferns in separate divisions or phyla, but plant taxonomists now believe they are included in the fern lineage. Psilophytes, commonly known as whisk ferns, are unique in that they have no roots or leaves, but molecular evidence suggests they lost them rather than never developed them. Only two genera of whisk ferns exist, *Psilotum* and



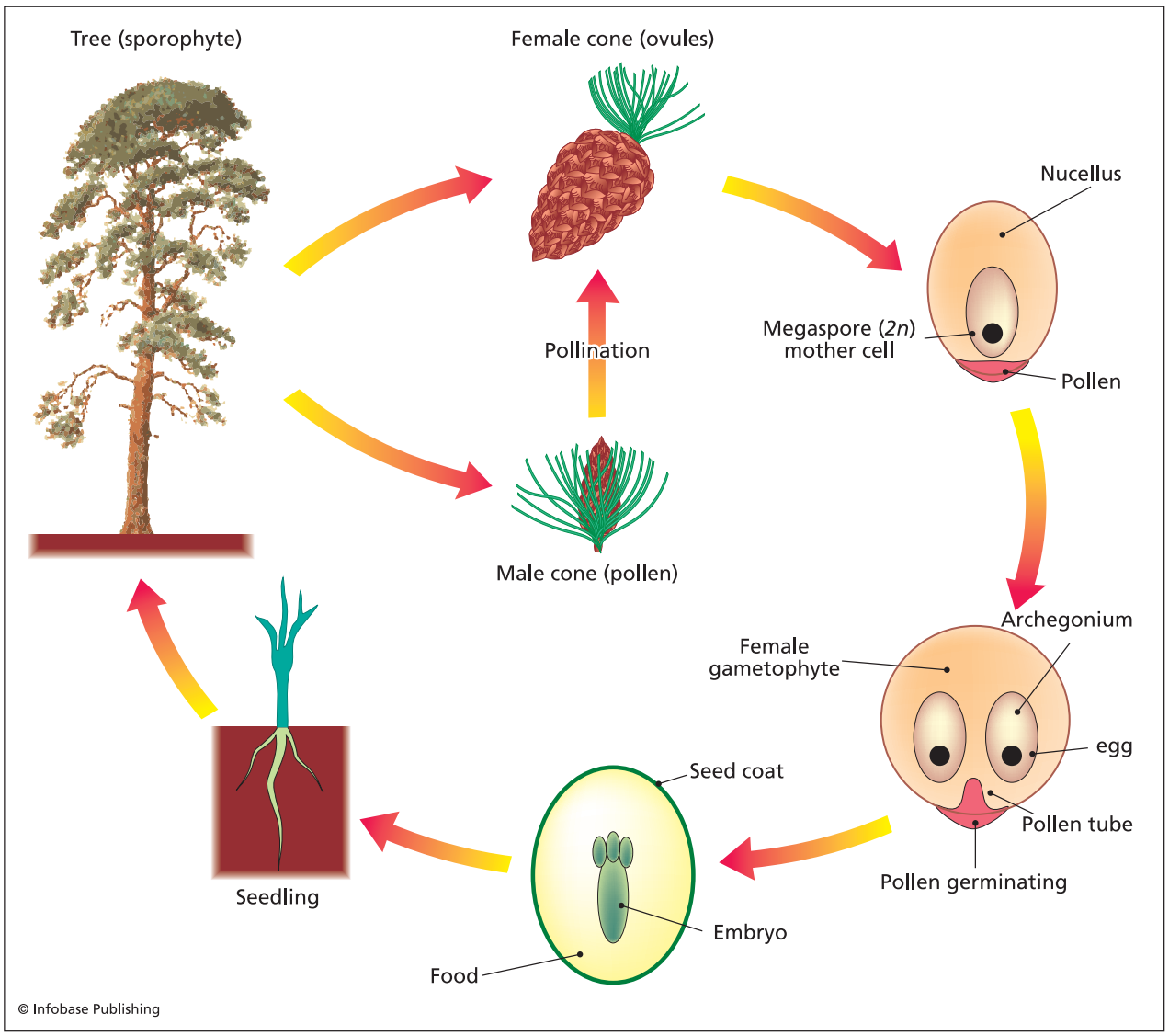
The dominant sporophyte stage of ferns produces haploid spores by meiosis. The spores are released from inside sporangia of sori that form underneath leaves. The spores mature into haploid, free-living gametophytes that make egg and sperm. After fertilization, the zygote develops into a sporophyte that matures and eventually produces haploid spores to complete the cycle.

Tmesipteris. Sphenophytes, the horsetails, consist of a single genus, *Equisetites*. They live in damp places such as marshes or by streams. The pterophytes, more commonly known as ferns, have adapted to different habitats but are most diverse in the tropics. Unlike other seedless vascular plants, they have megaphylls. Some species can grow up to about 80 feet (24 m) tall. Most ferns are homosporous, and the sporophytes have sporangia that can send spores several feet when they burst open.

The life cycle of a fern is representative of many seedless vascular plants. Though the sporophyte generation dominates, the gametophytes are still visible with the naked eye, whereas in seed plants



A) In gymnosperms, a megaspore develops into a multicellular gametophyte. Pollen grains (not shown here) containing the male gametophytes germinate to form a pollen tube that transports the sperm through the opening in the integument. After fertilization, the ovule transforms into a seed, consisting of the embryo, the endosperm, and a seed coat. B) Angiosperms undergo a process called double fertilization that involves two sperm nuclei. One sperm nucleus fuses with the egg, forming a zygote that develops into the embryo. The other sperm nucleus combines with two polar nuclei to create the endosperm that nourishes the embryo. A seed coat encloses the embryo in both gymnosperms and angiosperms.



Pine trees have both ovulate and pollen cones. When a pollen grain has contact with an ovule, it germinates to form a pollen tube, inside which two sperm develop. Meanwhile, the female gametophyte undergoes meiosis, resulting in a single surviving megaspore. A female gametophyte in the megaspore forms two archegonia, each with an egg inside. One sperm unites with each egg to form zygotes, which develop into the embryo within a seed. A new tree grows from the seedling.

one must use a microscope to observe the gametophyte generation.

SEED PLANTS

The appearance of the seeds about 370 million years ago allowed plants to survive harsh conditions and to disperse their offspring over greater distances. Unlike spores, seeds can lay dormant for months or years before germinating. Covered by a tough, protective coating, seeds are multicellular and contain enough nourishment for the sporophyte embryo until it can carry out photosynthesis on its own. All seed-bearing plants are heterosporous and have reduced gametophyte stages. Megasporangia each give rise to a single megaspore that remains inside the parent sporophyte tissue, protected by integuments that surround it. A microscopic female gametophyte develops from the megaspore, and the entire structure, including the integument, megasporangium, and megaspore, is called an ovule.

The microsporangia (pollen sacs) produce the microspores that develop into male gametophytes, called pollen grains. Because the pollen grains have a protective coat, after being released by the sporophyte, the pollen can be carried away by wind or animals. Pollination is the process of bringing the

pollen into contact with an ovule, which can result in germination of the pollen, the release of sperm, and fertilization. Successful fertilization initiates the formation of a seed from the ovule. Thus the seeds characteristic of seed plants are simply fertilized ovules covered by a protective covering called a seed coat.

The ability of seed plants to carry out fertilization in the absence of water greatly increased their fitness. Seed plants can be divided into two major groups based on whether or not the sporophyte encloses the seeds in an ovary. Gymnosperms produce naked seeds, and angiosperms produce flowers and mature ovaries, also known as fruits.

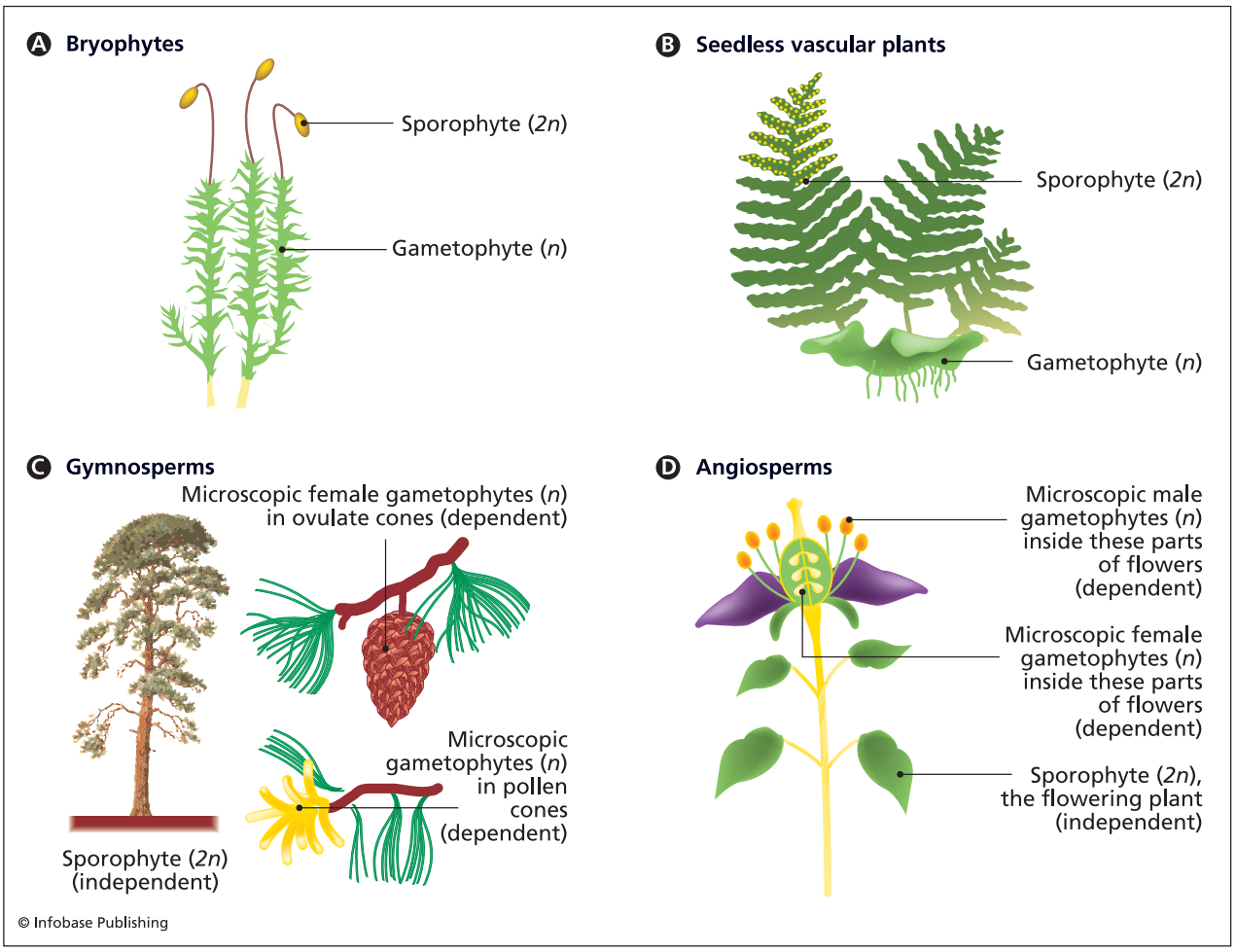
Gymnosperms are a polyphyletic group of vascular plants that produce naked or exposed seeds that usually form on cones. Four extant divisions include the cycads, ginkgos, conifers, and gnetophytes. Phylum Cycadophyta arose about 320 million years ago. Cycads have large cones and their leaves resemble palms, but they are not palms. Phylum Ginkgophyta has only a single species, *Ginkgo biloba,* also known as the maidenhair tree. Ginkgos are popular in cities and parks because they are quite resistant to pollution and their fan-shaped leaves turn a pretty gold color in the fall. The males and females grow as separate plants. Some people cook and eat the fleshy ovules. The seed coats contain butanoic acid and smell rancid when they decay, so ornamental ginkgos are usually pollen-producers. Most of the extant gymnosperms belong to the phylum Coniferophyta, which contains approximately 600 species. Their name means cone-bearing. Cones can be male or female, though a single tree can produce both types. In pines, 15 months pass between pollination and fertilization. The seeds develop inside the cones and when mature, the scales on the cone open up to release them. Conifers dominate landscapes in the Northern Hemisphere, and most are evergreen, meaning they keep their leaves all year round. Many but not all have needlelike leaves, arranged in a spiral manner. This phylum boasts the tallest living organisms, the giant sequoia that grow to more than 360 feet (110 m), and the oldest, the bristlecone pines that can reach ages of more than 4,600 years. The last gymnosperm phylum is Gnetophyta, which seems to be a sister group to the angiosperms. The extant species have very diverse morphologies. For example, the southwestern African desert plant *Welwitschia mirabilis* has two wide, straplike leaves that can reach 6.5 feet (2 m) in length. Another gnetophyte is *Ephedra viridis,* a drought resistant branched shrub with tiny, scaly leaves at the nodes.

Orchids, like the one shown here, make up the largest and most diverse of the angiosperm families. *(JJJ, 2007, used under license from Shutterstock, Inc.)*

Angiosperms, more commonly known as flowering plants, first appeared between 141 and 100 million years ago, and approximately 260,000 known species exist. They dominate most of the world's landmasses, including tropical forests, deciduous forests at higher latitudes, and grasslands, and they inhabit almost all others at least to some extent. In addition to producing reproductive structures called flowers, angiosperms have ovules that are completely enclosed by parental tissues. To reach the megaspore, during fertilization a pollen tube grows from the male gametophyte down the style of the female carpel to the ovule. The gametophyte stage of angiosperms is very reduced. The male gametophyte consists of only three cells (one forms the pollen tube and two are sperm cells), and the female gametophyte has only seven, one of which is the egg cell. Two sperm nuclei enter the ovule—one fertilizes the egg nuclei to form a zygote and the other combines with another gametophyte cell to form the endosperm, which will provide nutrition for the developing embryo inside the seed. This process is called double fertilization. One other feature shared by angiosperms is the presence of sieve cells, cells that conduct sugars and other organic nutrients in the phloem. (See PLANT FORM AND FUNCTION.)

Botanists have traditionally split up the angiosperms into two main groups, the monocots and dicots. Systematists have realized that this division does not accurately reflect the phylogenies and are working to elucidate the true relationships. Meanwhile, plants can be categorized into four main groups: the basal angiosperms, magnoliids, monocots, and eudicots.

Of the basal angiosperms, *Amborella trichopoda* is a shrub found only on the island of New Caledonia in the South Pacific and is the only species in a sister group to the rest of the angiosperms. The *Nymphaeales,* consisting of the water lilies and their relatives,



Embryophytes exhibit gametophyte and sporophyte stages. A) The gametophyte stage is dominant in bryophytes, and the sporophyte is dependent on the gametophyte for nourishment. B) The sporophyte stage is dominant in seedless vascular plants, and the gametophyte is free-living. C) In gymnosperms, the gametophyte is reduced and is dependent on the sporophyte. D) In angiosperms, the gametophyte is also reduced and is dependent on the sporophyte.

diverged next, followed by the group represented by the single species *Illicium floridanum*, commonly known as star anise.

The woody magnoliids share some characteristics in common with basal angiosperms, such as spiral-formed flowers, but they are now thought to be a sister group to the eudicots. One well-known species is the *Magnolia grandiflora*, commonly known as the Southern magnolia, which has flowers that can reach one foot (0.3 m) across.

The monocots include familiar plants such as orchids, lilies, the grasses (including the cereals), bananas, and palms. All the members of this group have embryos that develop a single first leaf, or cotyledon, hence their name. Other characteristics in common include parallel venation in the leaves, scattered vascular tissue, no main root, a pollen grain with a single opening, and floral organs arranged in multiples of three.

The majority of all angiosperms are eudicots, consisting of about 170,000 species. In contrast to monocots, eudicot embryos develop two cotyledons. This group used to be called dicots, but since that grouping proved to be polyphyletic, systematists removed some of the lineages (the basal angiosperms and magnoliids) and renamed the group eudicots. Other common features include branched venation, a circular arrangement of vascular tissue, the presence of a taproot (main root), several openings in the pollen grains, and floral organs arranged in groups of four or five. Poppies, oaks, legumes, cacti, birches, squashes, apple trees, potatoes, peonies, and marigolds are all examples of eudicots.

See also ALGAE; EUKARYA; EUKARYOTIC CELLS; PHOTOSYNTHESIS; PLANT FORM AND FUNCTION; REPRODUCTION.

FURTHER READING

Ingrouille, Martin, and Bill Eddie. *Plants: Diversity and Evolution*. New York: Cambridge University Press, 2006.

Stevens, P. F. Angiosperm Phylogeny Web site. Version 8, June 2007. Available online. URL: http://www.mobot.org/MOBOT/research/APweb/. Updated June 8, 2007.

Tudge, Colin. *The Variety of Life: A Survey and a Celebration of All the Creatures That Have Ever Lived*. New York: Oxford University Press, 2000.

plant form and function Members of the kingdom Plantae are eukaryotic, multicellular organisms that are photosynthetic, immotile, contain no nervous system or sensory organs, and have cell walls composed of cellulose. An estimated 400,000 plant species have been identified and include organisms such as trees, shrubs, flowering plants, grass, herbs, and moss. Fungi, lichens, and algae share some plantlike characteristics, but they are not members of the kingdom Plantae. Plant life is a crucial component of the Earth's ecosystems, as all animals eat plants or eat other animals that eat plants. In addition to supplying much of the energy and nutritional requirements for life on Earth, plants also release oxygen, a gas that animals need to breathe and live. Plants evolved from algae, photosynthetic protists that live in water or extremely moist habitats. Three unique adaptations that allowed plants to survive in terrestrial environments are a means of absorbing nutrients from their surroundings, a way to prevent desiccation, and a mechanism for reproducing that does not require water for sperm transport. Though aquatic plants have lost some of their terrestrial adaptations, they are descendents of land plants.

GENERAL MORPHOLOGY

Plants vary tremendously in size and shape. The most familiar plants belong to the group called embryophytes, in which the embryo is retained within maternal tissue. Embryophytes include most of the green land plants such as trees, flowers, ferns, and mosses. Though exceptions certainly exist, the majority of land plants consist of three main structures: roots, stems, and leaves. Examination of the basic structure of flowering plants, called angiosperms, demonstrates the basic structures common to most plants.

Three types of tissue make up the roots, stems, and leaves of plants: dermal, vascular, and ground tissue. Dermal tissue encloses the entire plant like skin covers a human being. Composed of a layer of tightly packed cells called the epidermis, the main function of the dermal tissue system is to protect against dehydration and disease. Woody plants such as trees also have tissue called periderm surrounding the epidermis. In different organs, epidermis performs specialized functions. The root hairs at the tips of roots that function in nutrient and water absorption are epidermis. Epidermis in leaves and stems secretes a fatty substance called cutin that forms a waxy coating called the cuticle to help prevent against dehydration. The vascular tissue system is responsible for transporting materials throughout the plant body and consists of two main types: xylem and phloem. Xylem carries water and dissolved substances from the roots to the shoots, generally in an upward direction. Phloem carries the products of photosynthesis, namely, organic nutrients, from the leaves where they are synthesized to areas of the plant that need the energy for growth. The term *stele* refers to the cylindrical bundle of vascular tissue that runs along the length of a stem or into a root. The ground tissue system encompasses all the plant tissue that is neither dermal nor vascular. Pith, the spongy material inside stems, internal to the vascular tissue, contains many unspecialized cells called parenchyma cells, though some perform photosynthetic functions or storage roles. Cortex is the ground tissue found in between the vascular and epidermis tissue.

Though some plants live in aquatic habitats or on other plants, most plants must absorb water and minerals from the soil, a function carried out by the root system. Roots anchor vascular plants into the soil (or other growth medium). A taproot is a vertical root that develops from an embryonic root. Other smaller roots branch laterally from the taproot to penetrate into the soil. Some taproots, such as sweet potatoes, are modified to store energy for later use during flowering or fruit production. Sweet potato crops must be harvested before the plants flower, or the plant will begin to break down the starch stored in the taproot, and the sweet potato will shrivel up. In other plants, such as seedless vascular plants and monocots like grass, the embryonic root dies and a fibrous root system grows. Unlike taproot systems that penetrate downward vertically, fibrous root systems spread out like a mat under the surface of the soil. Both types of root systems efficiently anchor the plant to the ground. Modifications to some plant roots help support large plants or tall tree trunks, project into the air to increase the absorption of oxygen in some environments, or store nutrients or energy.

Besides anchoring, another main function of roots is to absorb water and nutrients from the soil for the plant. The larger a plant's root system, the more surface area through which the plant can

absorb materials. Most absorption occurs at the root tips, which are covered by tiny root hairs that reach out to absorb water and minerals. When digging up a plant for transplantation, one must be careful to not break and destroy the root hairs. Symbiotic associations with fungi or bacteria can also act to increase absorptive efficiency.

Stems are part of the shoot system, the aboveground portion of a plant that also includes leaves, flowers, buds, and fruits containing seeds. The stem consists of nodes and internodes. Nodes are locations where leaves are attached to the stem. Internodes are the regions in between nodes. Axillary buds, found at nodes, have the potential to form a lateral branch from the main stem. Growth by elongation occurs at the tips, which terminate in buds that contain developing leaves. In a phenomenon called apical dominance, growth occurs at the terminal buds and inhibits growth of nearby axillary buds. Removal of the terminal bud will relieve the inhibition of the dormant axillary buds, which will then form lateral shoots, making the plant appear fuller. Just as modified roots perform specialized functions, so do modified stems. Horizontal stems called stolons allow plants to reproduce asexually. The stolons grow aboveground along a surface, root at the nodes, and develop entire new plants. Strawberries are an example of a plant that reproduces asexually by stolons. Rhizomes are also stems that grow horizontally, but usually just below or at the surface of the ground and often containing nutrient stores. Tubers, such as potatoes, are enlarged ends of rhizomes that store energy for the plant in the form of carbohydrates. Bulbs, such as onions, are underground shoots with enlarged leaf bases of several overlapping layers.

The structure of the stem tissue differs in monocots and eudicots. Vascular tissue runs along the stem length in both types, but in monocots the vascular bundles are scattered throughout the ground tissue, whereas in eudicots the vascular bundles form a ring around the circumference of the stem. The xylem faces the pith in the center of the stem, and the phloem faces the cortex, located just under

Plants have a variety of shapes and sizes, ranging from the giant sequoias (A) in northern California to the simple mosses *(National Park Service)* **(B), such as those covering the rocks in this mountain stream.** *(Martin Holek, 2007, used under license from Shutterstock, Inc.)*

The venation in leaves can form a complex branched pattern, as shown here, or run parallel to each other along the length of the blade. *(Mark William Penny, 2007, used under license from Shutterstock, Inc.)*

the epidermis. The ground tissue consists mostly of unspecialized parenchyma in both plant types.

Leaves are the main photosynthetic organs of plants. Leaves come in a variety of shapes and sizes, each appropriately adapted to withstand certain environmental conditions such as intense sun exposure or strong winds. In many plants, the leaf joins the stem at a node by a petiole, a stalk that connects the leaf to the stem, but other plants, such as many monocots, lack petioles. The blade of a leaf is the flattened portion through which veins run. In monocots the veins form a parallel pattern, and in eudicots, the veins form a more complex, branched pattern. Leaf shape can be with simple, compound, or double compound. Simple leaves contain one undivided blade, though the blade may contain several lobes. Compound leaves consist of multiple leaflets branching from the petiole. Doubly compound leaves are further subdivided such that the leaflets branching from the petiole themselves branch into multiple leaflets.

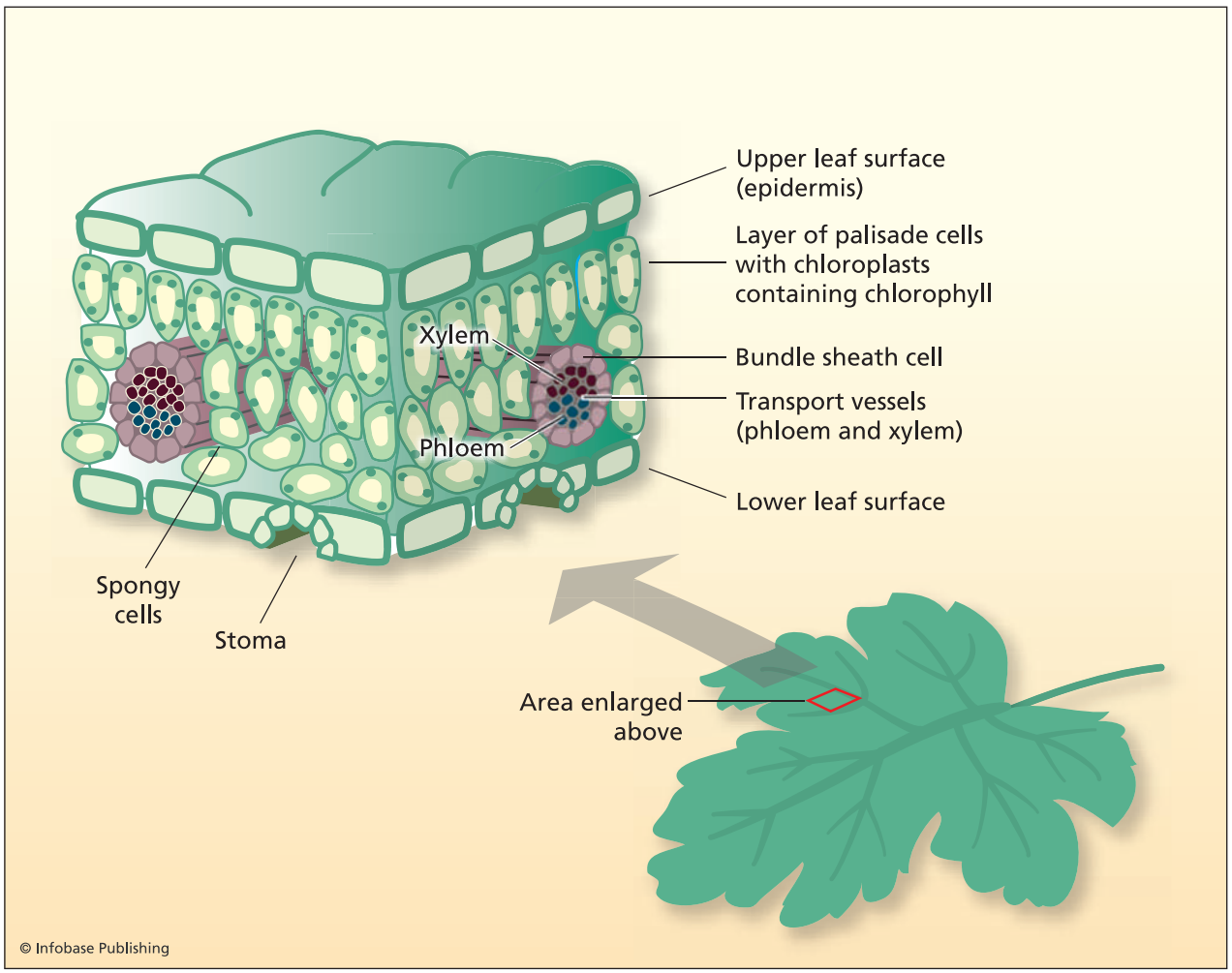
Because photosynthesis and transpiration (the evaporation of water into the air) both occur through the leaves, the anatomy of a leaf is complex. Stomata are small openings in the epidermis of a leaf through which gases move into and out of the leaf tissues. Two guard cells flank the pore and control opening and closing of the opening. The pore leads to air spaces surrounding the cells of the leaf tissue. Two layers of epidermis sandwich the ground tissue, which contains mesophyll, the location of the photosynthetic cells of the plant. In some plants the mesophyll consists of two subtypes: paslisade and spongy mesophyll. Palisade mesophyll has elongated cells and is closer to the upper epidermis, whereas cells in the spongy mesophyll are loosely packed to allow gases (carbon dioxide and water vapor) to flow throughout the tissue. The palisade cells house numerous (as many as 50) chloroplasts, organelles that carry out photosynthesis. Veins containing xylem and phloem branch out from the main stem into the leaf mesophyll so the phloem can pick up organic products of photosynthesis and xylem can bring water and dissolved minerals and substances to the tissue for exchange.

Flowers, buds, fruits, and seeds are more specialized plant structures. Buds are young shoots that can produce new stems with leaves and flowers. Flowers are the reproductive structure of angiosperms, or flowering plants, and consist of several organs derived from leaf tissue. While flowers persist only for one season, buds of some plants (perennials) can survive winter conditions and begin growing when the weather becomes warm once again. The buds contain leaves in a halted developmental state and are protected by tougher leaves called scales. Seeds contain dormant embryonic root and shoot tissue and a food source all wrapped in a tough seed coat derived from the parent plant. Fruit-bearing plants enclose the seeds in structures called fruit derived from the ovaries after pollination and fertilization have occurred.

GROWTH AND DEVELOPMENT

Like animals, since plants are multicellular, they must develop from a single reproductive cell into fully grown organisms consisting of different tissue types with specialized functions. A plant's genes control its growth, but environmental factors shape the manner in which it grows. For example, an oak tree growing in a wide open space will grow thick and the branches will spread outward, but an oak tree growing in a crowded woodland will be taller and thinner.

Plant growth consists of the production of new cells and the enlargement of existing cells. A meristem is a tissue that is composed of cells that can divide and give rise to new cells or that can differentiate into specialized cells. Roots and shoots have primary apical meristems, meristems that are located at the apex of a root or shoot and that are capable of growth by elongation. Primary apical meristems are found at the root tips, axillary buds, and shoot tips. At a root tip, a cap covers the fresh apical meristem tissue, where new growth occurs. Moving away from the tip of the root inward toward the plant body, three zones at different stages of development exist. The zone of cell division at the root tip is where mitosis occurs. Cells in the zone of elongation elongate, causing the root to penetrate deeper into the soil. In the zone of maturation, cells complete the differentiation process and become fully functional. Apical meristem at the ends of terminal buds of shoot tips give rise to fingerlike projections that develop into leaves. Axillary buds form from apical meristem tissue left behind at the bases of leaves. Inside



The leaf consists of epidermis, palisade cells, spongy cells, transport vessels, and air spaces, shown here in this cross section.

the buds the distance between nodes is short, so the leaves are crowded until elongation occurs within the internodes.

Plants grow taller by the production of new nodes, followed by elongation of internodes. Woody plants, such as trees, shrubs, and woody vines, have secondary meristems called the vascular cambium and cork cambium that produce wood and bark to increase the diameter of the stems and roots. When viewed in cross section, the vascular cambium appears as a ring. The individual cells of plants can grow by increasing the size of their cytoplasms, but also by taking in water into central vacuoles, accompanied by expansion of the cell wall. Plants can grow rather quickly by this mechanism.

A variety of hormones regulates the growth of plants. Young leaves produce auxins, organic compounds that enable cell walls to expand, allowing growth. Auxins are responsible for apical bud dominance, induce the formation of roots on plant cuttings, inhibit fruit and leaves from dropping, stimulate ripening, stimulate ethylene synthesis, and promote growth toward light. Cytokinins, chemicals that stimulate growth in plants, induce mitosis in meristems, aid in the development of vascular tissue, and delay aging of leaves. Florists often spray cytokinins on cut flowers to help them last longer after cutting. Giberellins, produced by apical buds, roots, and young leaves, stimulate growth by inducing cell division and cell growth, promote seed germination by inducing the breakdown of starch and seed storage proteins, and stimulate the development of flowers in some plants. Ethylene is a gas that promotes the ripening of some fruits, promotes leaf and flower aging, and affects cell elongation and seed germination. Many other hormones act to protect the plant from heat, cold, salt, chemicals such as herbicides, and water loss.

Differentiation, or the specialization of cells to perform certain functions and to assume unique

structures, occurs by two main mechanisms in plants. Unequal cell division, in which the cytoplasmic contents are disproportionately split between the two daughter cells, can cause cells to develop differently. Positional effects also influence plant cell differentiation, as some cells might be exposed to more chemicals or other environmental factors.

The natural life span of plant species ranges from a few months to more than 1,000 years. Plants called annuals grow, flower, and die within one year. Biennial plants grow and store energy one year and reproduce and die the following year. Perennial plants typically grow and flower every year. Creosote bush grows in clumps called clones that can persist for more than 10,000 years.

TRANSPORT

Plants must transport water and nutrients throughout their bodies. Water and dissolved substances such as salts and minerals enter the plant through the root system. The epidermis at the root tips and root hairs, which are simply extensions of root tip epidermis, is permeable to water. Many plants participate in a symbiotic relationship with fungi to form mycorrhizae, structures that increase the absorptive surface area of the root system. After the water crosses over the hydrophilic cell walls of the roots, it flows through the apoplast, the continuum of cell walls and extracellular spaces, eventually making its way into cells by diffusing through the cell membranes. Water can also enter the plant by diffusing through the cell membranes at the root tip into the symplast, the continuum of cytoplasm between cells. Plasmodesmata, open channels that connect the cytoplasm of adjacent cells, allow the water and dissolved contents to flow from cell to cell until it reaches the vascular tissue. Fluids traveling along the apoplastic route must diffuse through the cell membranes en route to the vascular tissue. An endodermis surrounds the vascular tissue, and a strip of waxy materials within this layer blocks water from entering the vascular tissue except through plasmodesmata. The endodermal cells transport the water and minerals into the xylem vessels.

The content of the xylem is called sap. In the tallest plants, xylem carries the sap upward against gravity for more than 330 feet (about 100 m). Root pressure from water flowing into the roots provides some of the force necessary to push the sap up the xylem. When more water enters the plant via the roots than leaves via transpiration, droplets of water collect at leaf tips, an event called guttation. In many plants, however, root pressure alone is not sufficient to transport enough water up the xylem. As water is lost by transpiration, a negative pressure at the top of the plant pulls water from below, a condition called transpirational pull. The adhesion and cohesion of water molecules assists in this process. Adhesion holds the water molecules to the hydrophilic walls of the xylem cells, and cohesion allows the water molecules in the sap to pull or tug each other up, since they stick to one another. By causing evaporation, sunlight drives transpirational pull, a process that is particularly effective when the air is dry, since transpiration causes the loss of more water vapor than on wet days.

Plants lose a lot of water through transpiration, the evaporation of water through the leaves of the plant, thus plants require a lot of water, much more than they actually utilize in metabolic processes. The stomata, openings in the leaf tissue that allow the entry of carbon dioxide necessary for photosynthesis, also allow water to escape through transpiration, accounting for approximately 90 percent of water lost from a plant. The cuticle prevents additional water loss. Though large quantities of water are lost by transpiration, especially in hot or dry environments, this process is vital to plant health, as it creates the negative pressure that pulls water and nutrients up from the roots through the plant body. Plants that live in dry habitats have fewer stomata, and they open at night when it is cooler. They also store water in their roots, stems, and leaves.

While xylem transports water and minerals from the roots to the rest of the plant body, the other main vascular tissue, phloem, carries sugar and other organic nutrients throughout the plant. The direction of travel usually opposes that of xylem. This process of transporting organic nutrients is called translocation.

NUTRITION

All living organisms require a source of energy and nutrients. Cells need chemical energy to perform many cellular activities such as anabolic processes, active transport, or movement. Adenosine triphosphate (ATP) is the cell's most immediate source of cellular energy because its bonds store large amounts of energy that breaking releases. Whereas animals derive their energy from eating plants or plant-eaters, plants are phototrophic, meaning they obtain their energy from the sunlight. They are also autotrophic, meaning they can synthesize their own organic nutrients from carbon dioxide (CO_2). By carrying out photosynthesis, plant cells convert the light energy from the sun into chemical energy, then use the chemical energy to synthesize their own organic nutrients in the form of carbohydrates that can be used to synthesize other organic molecules, or broken down to generate ATP. Carbon dioxide and water are required for photosynthesis to make carbohydrates. Plants obtain water through absorption through their root systems, and they obtain carbon dioxide from the

atmosphere through their stomata. The following equation summarizes photosynthesis:

$$6H_2O + 6CO_2 + \text{sunlight} \rightarrow C_6H_{12}O6 + 6O_2$$

Plants require nine nutrients in large quantities: carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, potassium, calcium, and magnesium. CO_2 supplies most of the carbon and oxygen. The hydrogen atoms and some of the oxygen atoms come from water. The soil provides the remaining nutrients in the form of inorganic ions. Present in nitrate (NO_3^-) and ammonium ions (NH_4^+), nitrogen is required for building proteins, nucleic acids, hormones, chlorophyll, and coenzymes. Phosphorus, found in phosphates (PO_4^-), is needed for nucleic acids, phospholipids, ATP, and several coenzymes. Sulfur, present in sulfate (SO_4^{2-}), is a component of proteins and coenzymes. Potassium (K^+) assists in protein synthesis, functions in maintaining proper water balance, and controls the opening of stomata. Calcium (Ca^+) helps form cell walls, functions in cell membrane structure and function, serves as a cofactor for some enzymes, and helps regulate other cell responses. Magnesium (Mg^{2+}) is a component of chlorophyll and acts as a cofactor for many enzymes. Other nutrients required in smaller quantities are chlorine, iron, manganese, boron, zinc, copper, nickel, and molybdenum. Nutrient deficits can stunt plant growth and cause discoloration of leaves.

As a component of proteins, nucleic acids, chlorophyll, and other biomolecules, nitrogen availability has the greatest impact on plant growth. Though the atmosphere is composed of more than 79 percent nitrogen gas (N_2), most organisms cannot use nitrogen in this form. While many other nutrients in the soil originate from the weathering of rock, available nitrogen, in the form of NO_3^- and NH_4^+, comes from specialized bacteria. Ammonifying bacteria decompose organic matter, converting the nitrogen from organic molecules into NH_4^+. Nitrogen-fixing bacteria convert atmospheric N_2 into ammonia (NH_3), which combines with hydrogen ions in the soil to form NH_4^+. Nitrifying bacteria metabolize NH_4^+ into NO_3^-. Nitrogen then enters plants through the roots in the form of either NH_4^+ or NO_3^- dissolved in water. The plant transforms NO_3^- back into NH_4^+ before it incorporates the nitrogen into organic compounds.

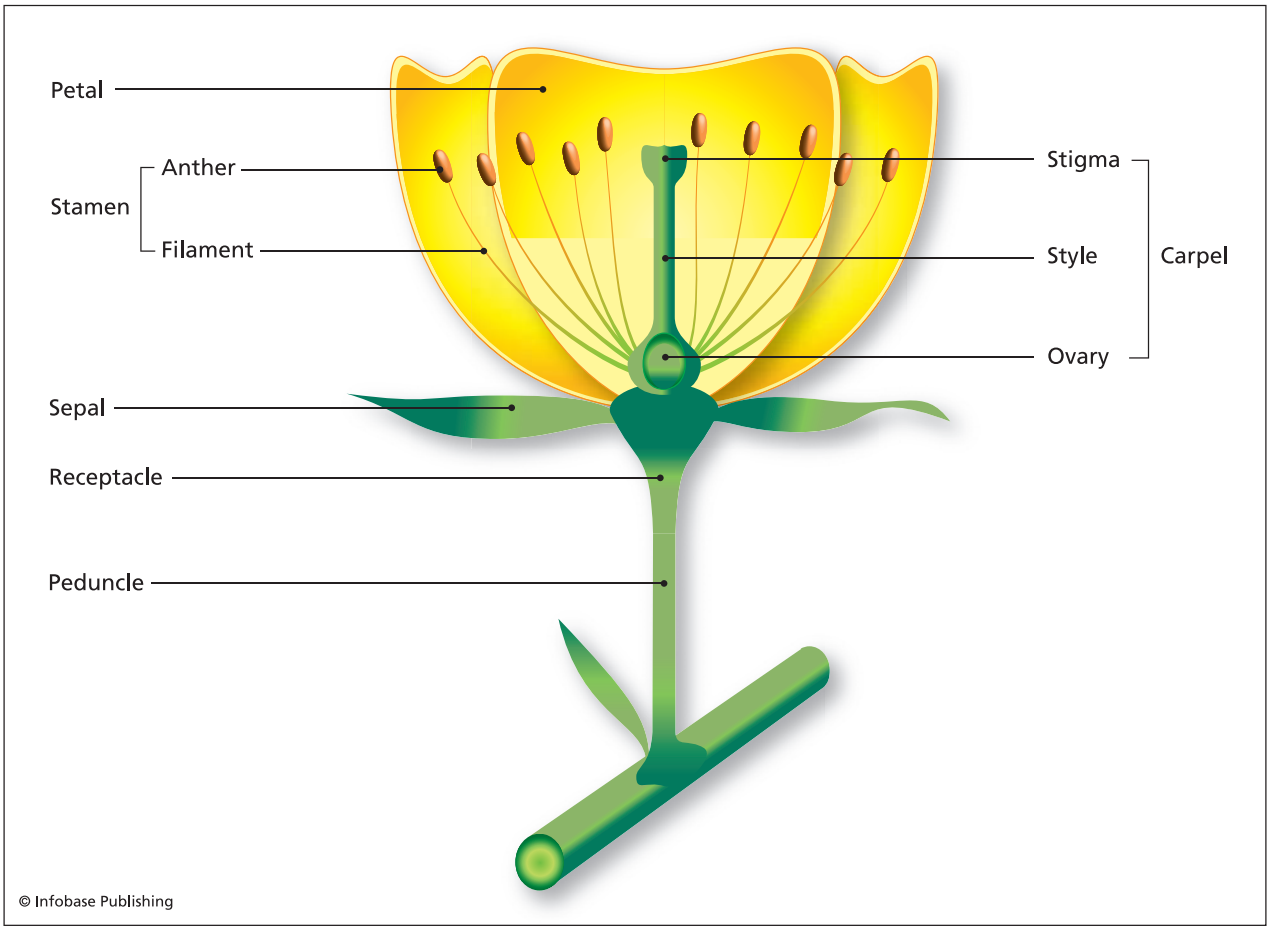
Some plants participate in symbiotic relationships with microorganisms that fix atmospheric nitrogen, providing the plants with a constant, readily available supply of ammonium. Leguminous plants, such as peas, beans, and clover, have root nodules, swellings that house nitrogen-fixing *Rhizobium* bacteria. The bacteria fix nitrogen for the plant, and, in return, the plant provides carbohydrates and other organic compounds for the bacteria.

REPRODUCTION

Most plants can reproduce both sexually by growing flowers and making seeds and asexually by vegetative propagation. Asexual reproduction is accomplished by regeneration, the replacement or growth of new tissue from a small segment. Individual organisms generated in this manner are called clones because they are genetically identical to the parent plant. Many nurseries take cuttings of stems as a means of propagating plants indefinitely. Vegetative propagation also occurs naturally. For example, a broken cactus stem can take root after falling to the ground and develop into a new cactus plant. Tubers, swollen underground stems that store starch for the plant, contain eyes, or buds, that can each develop into a new plant.

In addition to developing from an excised segment of a viable plant, plants can grow from zygotes or spores. A zygote forms from the union of an egg and a sperm cell and is the product of sexual reproduction. Following fertilization, repeated mitotic divisions increase the cell number. Spores are haploid, single-celled structures that have the potential to develop into multicellular organisms. Spores develop into a plant body type called a gametophyte that produces haploid gametes. The product of the union of two gametes, a zygote, grows into an embryo that develops into a diploid plant body type called a sporophyte, which produces spores. These two plant body types alternate during the plant's life cycle and have different numbers of chromosomes.

In angiosperms, flowering plants, the sporophyte generation dominates. The flower is a shoot of the sporophyte of a plant that is modified for reproduction. An ideal flower consists of modified leaves arranged in whorls at the end of a peduncle, or stalk. The end of the peduncle swells to form a receptacle, the structure to which the flower parts are attached. Thick, green sepals protect the bud when it is forming and open up to surround the base of the flower after blooming. The petals can be fused or separate, are thinner than the sepals, and are often brightly colored to attract insects or birds for pollination. Together, the whorl of sepals is called the calyx, and the whorl of petals is called the corolla. Though these parts of the flower play a role in forming the reproductive structure, they are sterile. The male reproductive organs are called the stamens, each consisting of a stalklike filament that terminates in an anther, which contains the pollen sacs that produce the pollen. Grains of pollen are covered in a tough protective coating. The female reproductive parts are called the carpels. Sometimes the term *pistil* is used to



Typical flowers contain a peduncle, receptacle, sepals, petals, stamens, and carpels

refer to a single carpel or group of fused carpels. The bottommost part of the carpel is rounded and contains the ovary, the location of the ovules. The style extends up from the ovary and ends in a stigma.

The male and female gametophytes develop within the anthers and ovules, respectively. Male gametophytes are pollen grains, and female gametophytes are embryo sacs. The pollen sacs, also called the microsporangia, contain diploid microsporocytes. By meiotic division, the microsporocytes generate four haploid microspores that mature into pollen grains. The microspores divide by mitosis, forming a tube cell and a generative cell, enclosed by a spore wall with a shape distinctive of the species. The generative cell moves inside of the tube cell.

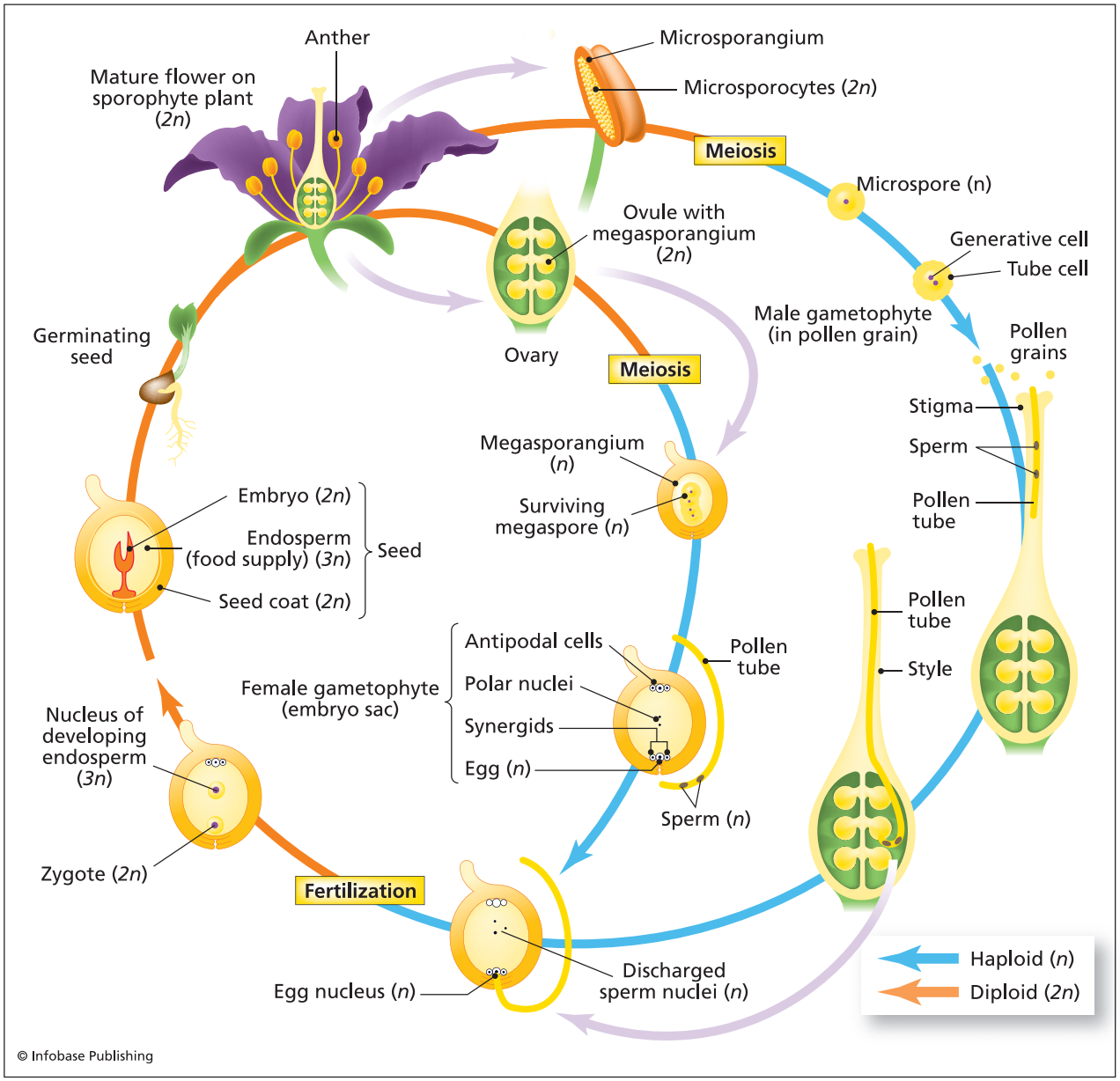
Within the ovary, the ovules contain megasporangia that house megasporocytes, which undergo meiosis, resulting in four megaspores. Usually only a single megaspore survives and divides mitotically but does not undergo cytokinesis. The result is a single cell that has eight haploid nuclei and matures into an embryo sac. One egg cell and two cells called synergids lie on one end of the embryo sac, and three antipodal cells, whose function is unknown, lie on the other end of the sac. The other two nuclei are referred to as polar nuclei.

Pollination occurs when pollen comes into contact with the female gametophyte. An insect, a bird, or even the wind might carry pollen to the flower of a new plant. Colorful and aromatic flowers attract birds and insects toward the flower where they become dusted with pollen. After the bird or insect leaves, it carries the pollen with it to another plant. After the pollen lands on the stigma, it absorbs moisture and germinates. The first step is the formation of a long, slender pollen tube by the tube cell. The generative cell produces two sperm cells while the tube cell elongates down the length of the style toward the ovary of the female carpel. When it reaches the ovary, it penetrates through an opening in the coverings, or integument, of the embryo. In a process called double fertilization, one of the sperm fertilizes the egg to form a zygote, and the other sperm combines with both of the polar nuclei to form a triploid nucleus. An endosperm will form from the resulting large cell and serve to provide nutrition for the growing embryo inside of the seed. By requiring double fertilization to reproduce, an organism avoids

wasting nutrients to form endosperm in ovules where the egg has not been fertilized. This process of double fertilization is unique to flowering plants.

The fertilized ovule develops into a seed, and the ovary develops into a fruit. In many plants, the endosperm of the seed stores proteins, oils, and starch to feed the seedling after germination. In other plants, the endosperm is used up to form seed leaves, cotyledons, before the seed is fully mature. As the zygote divides by mitosis, some of the daughter cells form a filamentous suspensor that anchors the embryo to the endosperm, and other cells form the proembryo. Protrusions from the proembryo develop into cotyledons—one forms in monocots, and two form in eudicots. The embryo grows longer, and shoot apical meristem and root apical meristem develop so they will be ready to grow as soon as the seed germinates. Near maturation, the seed dehydrates and the embryo becomes dormant, a condition characterized by an extremely low metabolism. A hard seed coat protects the contents of the seed until conditions are suitable for germination.

Meanwhile, the ovary surrounding the seeds develops into a fruit. The wall of the ovary becomes the thick wall of the fruit, the pericarp, which consists of three regions: the endocarp, the part closest to



Most flowering plants possess both male and female reproductive organs and undergo a unique process called double fertilization.

the seed; the mesocarp, the fleshy tissue in the middle; and the exocarp, or the skin of the fruit. One means of categorizing fruits is by whether or not the mesocarp is fleshy or at least partly fleshy. Fleshy fruits include drupes, berries, and pomes. Drupes possess a single seed enclosed by a hard endocarp and include fruits such as coconuts, apricots, cherries, olives, and almonds. Berries, such as strawberries, raspberries, cucumbers, tomatoes, and bananas, contain more than one seed and develop from a compound ovary consisting of two or more fused carpels. Pomes are fleshy fruits, the bulk of which is derived from the receptacle that grows around the ovary, such as apples and pears. Another way to categorize fruits is by the number of carpels and flowers from which the fruit develops. Simple fruits develop from one carpel; examples include peas and lemons. Aggregate fruits develop from several carpels of one flower; examples include raspberries and blackberries. Multiple fruits develop from many carpels of many flowers; examples include pineapples and figs.

Seeds spread by different mechanisms. Fruits attract animals who eat the fruits, ingest the seeds, and spread them via waste elimination at a potentially distant location. Some dry fruits pop open when they ripen, causing the seeds to scatter about. Other dry fruits are carried by the wind, such as feathery dandelion fruits that are easily blown away.

Germination occurs when the seed experiences favorable conditions, particularly with respect to moisture and temperature. The seed case breaks open, and a young root grows downward into the soil. The cotyledons (seed leaves) provide food, and a stem pushes its way up through the ground. The first true leaves open up and begin making food by photosynthesis. Meanwhile, the cotyledons shrivel up, and the seed case decomposes.

RESPONSE TO ENVIRONMENTAL STIMULI

As do all living things, plants must detect changes in the environment and respond accordingly. Cells have protein receptors embedded in their cell membranes that respond to specific stimuli. Certain receptors are designed to recognize specific stimuli; for example, photoreceptors detect light of particular wavelengths, and chemical receptors detect the presence of specific molecules. The receptor transmits the signal to the interior of the cell, where relay molecules or second messengers initiate an appropriate response. The cell might activate different biochemical pathways, synthesize new proteins, or mount a defense against a dangerous chemical.

Hormones, chemical signals produced by an organism that travel throughout the body and communicate with other parts of the organism's body, help coordinate processes involved in growth and development. Very small amounts of a hormone can cause significant changes in a plant's cellular activities, and often two or more hormones act together to bring about a response. Hormones are an example of a chemical stimulus from the plant's internal environment.

This mint geranium plant exhibits phototropism, the tendency of a plant to grow toward a light source. *(Maryann Frazier/Photo Researchers, Inc.)*

One common response of plants to a stimulus is curved growth toward or away from the stimulus, a phenomenon called a tropism. If the growth is toward light it is called positive phototropism, if the plant curves away from the light it is called a negative phototropism. Auxins are plant hormones that promote cell elongation in plant shoots. Other chemical substances inhibit cell growth or elongation. One hypothesis to explain the mechanism of phototropism is that light causes an asymmetrical distribution of these two substances, which results in more rapid growth on one side of a shoot than another, causing the shoot to curve as one side grows faster than the other. Plants also use light-sensing to control seed and spore germination and to determine the amount of daylight (or length of the night) as an indicator of

the seasons in order to regulate the timing of flowering in the spring and dormancy in the winter.

Plants also have means for detecting and responding to stimuli such as gravity, mechanical stimuli, drought or flood conditions, and temperature. Gravitropism, an organism's response to gravity, causes roots to grown downward into the soil and stems to grow upward. Structures called statoliths inside plant cells contain dense granules of starch that cause them to settle due to gravity. The position of the statoliths inside the cells affects the distribution of growth hormones. Thigmomorphogenesis is a change in form due to mechanical disturbances. For example, many viny plants have coils that wrap around structures such as other stems, fenceposts, tree branches, or whatever else they might come into contract with while growing. The mechanical stimulation of growing into another object makes the tendrils grow more rapidly on one side than the other, causing them to wrap around the object as they grow. Another example of a plant responding to a mechanical stimulus is a Venus flytrap plant that closes its trap when a fly or other insect mechanically stimulates hairs attached to sensory cells inside the trap. In hot, dry conditions, plant cells droop from loss of turgor pressure, the pressure exerted by the fluid contents inside a plant cell against the cell wall. One response to a slight loss in turgor is as the guard cells lose turgor, it causes the stomata to close and prevent additional water loss by transpiration. The leaves also produce abscisic acid, a compound that acts on guard cells to keep stomata closed. The presence of too much water can also be damaging because water replaces the air pockets in the soil that normally carry oxygen needed for cellular respiration to the roots. Oxygen deprivation from too much water stimulates the production of ethylene, which causes changes in the root structure that compensate for the lack of oxygen. Temperatures that are too high stimulate the production of heat shock proteins, proteins that temporarily enable cells to withstand stress due to harsh conditions. In response to cold temperatures, cells change the composition of their membranes to avoid loss of fluidity. This response takes several hours to days to implement, thus rapid drops in temperature cause plants more damage than a gradual lowering.

Because plants are immotile, they have developed unique adaptations to defending against herbivores, animals that eat plants. Thorns, chemicals that taste bad, and poisonous substances are a few mechanisms for keeping herbivores away, but plants mount other defenses in direct response to stimulation. In one example, chemicals in the saliva of a caterpillar, in conjunction with the mechanical stimulus of destruction from the caterpillar eating the leaf, causes the plant to release a volatile compound that attracts a wasp that parasitizes the caterpillar. Some plants also respond to the release of volatile compounds from neighboring plants by mounting their own defenses. The epidermis serves to protect plants from infection by pathogens such as viruses, bacteria, or fungi. Damage to the cell wall by a pathogen causes the release of molecules called oligosaccharins that stimulate the production of antimicrobial compounds called phytoalexins. Plants can respond to a microbial attack by sealing off the infected area to prevent spreading, and then self-destroying the cells in that area.

See also ALGAE; BIOGEOCHEMICAL CYCLES; BIOLOGICAL MEMBRANES; CELL COMMUNICATION; CELLULAR METABOLISM; CELLULAR REPRODUCTION; EUKARYA; EUKARYOTIC CELLS; PHOTOSYNTHESIS; PLANT DIVERSITY; REPRODUCTION; WATER, ITS BIOLOGICAL IMPORTANCE.

FURTHER READING

Burnie, David. *e.guides: Plant*. New York: DK, 2006.

Graham, Linda E., James M. Graham, and Lee W. Wilcox. *Plant Biology*. 2nd ed. Upper Saddle River, N.J.: Prentice Hall, 2006.

Stern, Kingsley R., James E. Bidlack, and Shelley Jansky. *Introductory Plant Biology*. 11th ed. New York: McGraw Hill, 2008.

point mutations Mutations are relatively permanent changes in the deoxyribonucleic acid (DNA), either physical changes to the chromosomes or changes to individual deoxyribonucleotides, the building blocks of DNA. Point mutations specifically refer to changes to single nucleotides in the DNA sequence. The DNA contains all the genetic information in living organisms. The sequence of four different deoxyribonucleotides within a gene encodes for the specific amino acid sequence of a protein. The synthesis of a protein involves several steps, the first of which is transcription, the synthesis of messenger ribonucleic acid (mRNA) from the DNA. Ribosomes then translate the sequence of ribonucleotide subunits in the mRNA into a sequence of amino acids in a polypeptide. Given a specific sequence of a gene or its mRNA transcript, one can predict the amino acid sequence using the genetic code, the key for interpreting the triplet codons. The ribosomes read the mRNA three ribonucleotides at a time, and specific tRNA molecules carry the correct amino acid to the ribosome for incorporation into the new polypeptide molecule based on the codon sequence.

An alteration of the nucleotide sequence in the DNA of a gene will lead to an alteration in the mRNA sequence, and may or may not lead to a different amino acid sequence. Point mutations can occur by the replacement of one nucleotide for another, the

chemical modification of a nucleotide, or the insertion or deletion of one or more nucleotides. Genetic variations are persistent differences in the nucleotide sequence of a gene between individuals. The different forms are called alleles, the foundation of biological diversity. Genetic variation provides flexibility that allows some organisms to thrive in different environments where others fail. Adaptive evolution by natural selection is based on this phenomenon.

TYPES OF MUTATIONS

Frameshift mutations and replacement mutations are two different types of point mutations. The addition or deletion of a single base causes a frameshift mutation, the most devastating type of point mutation. Normally, recognition of the first triplet codon (AUG) determines the reading frame of the coding region for the gene. After the positioning of the first amino acid, methionine, the ribosome "reads" the mRNA transcript three nucleotides at a time. The insertion or removal of a nucleotide disrupts this reading frame. For example, the following mRNA specifically encodes for a sequence of particular amino acids:

AUG	GUC	GAG	UUU	ACC	CCG	CCA	UAA
met	val	glu	phe	the	pro	pro	(stop)

The insertion of a single base, such as the bolded A below, throws off the entire amino acid sequence from the point of insertion onward.

AUG	GU**A**	CGA	GUU	UAC	CCC	GCC	AUA	A. . .
met	val	arg	val	tyr	pro	ala	ile	

The deletion of a single nucleotide would have a similar effect, also throwing off the reading frame. Frameshifts can also result in the inappropriate termination of translation. A stop codon might occur in an altered frame and cause premature termination, or the ribosome might not encounter one in the new reading frame until after passing the original stop codon.

The replacement of a single base usually has less severe consequences than a frameshift mutation, and sometimes no observable effect. Because of redundancy in the genetic code, several codons can encode the same amino acid. A base change in a codon that results in the same amino acid being encoded is called a silent mutation. The protein encoded by the mutated gene will have the same amino acid sequence, and therefore will display no change in its function. The effects of missense mutations, when a base in a codon changes such that a different amino acid is indicated, are variable. Many of the 20 amino acids have side chains with similar structures and therefore properties. For example, a codon that originally encodes an amino acid with hydrophobic properties might mutate so it encodes a different amino acid, but one that is still hydrophobic. Though the amino acid sequence differs, the mutation might not affect the function of the protein at all. Conversely, the new amino acid might have radically different properties and completely destroy the protein's ability to function properly, or the effect might be intermediate. In the case of nonsense mutations, a codon that originally coded for an amino acid mutates to become one of the three stop codons (called nonsense codons): UAG, UGA, or UAA. A nonsense mutation results in early termination of translation; the ribosome halts translation before reaching the original stop codon. If this occurs at a codon near the end of the mRNA, the effect might not be devastating, but if this occurs shortly after the start codon, the effects are usually severe.

MECHANISMS OF MUTAGENESIS

Mutations can occur spontaneously or be induced. Spontaneous mutations happen naturally at a rate of approximately one in 100 million in prokaryotic genomes, and eukaryotic genomes mutate at rates between 1/10,000 to 1/100,000,000. On occasion, DNA polymerase simply incorporates an incorrect nucleotide during DNA replication for no apparent reason, but often something causes DNA polymerase to add an incorrectly paired nucleotide to the new strand. If replication happens to occur while one of the nitrogenous bases is in its rare tautomeric form, then the polymerase might accidentally incorporate the wrong base in the complementary strand. Tautomers are structures that contain the same elements, but the arrangement of chemical bonds shifts. Adenine and cytosine both contain an amino group ($-NH_2$) as a side chain, but occasionally the amino converts to the imino form (=NH). Guanine and thymine normally exist in the keto form (C=O), but their tautomers are in the enol form (COH). When the rare tautomeric forms are present, the hydrogen bonds that normally form between A–T and C–G base pairs cannot form; instead, A will pair with C, and T will pair with G. Chemical alterations also can cause spontaneous mutations. For example, if guanine oxidizes, it will pair with A instead of C. Another common cause of spontaneous mutations occurs when a sequence or a nucleotide is repeated. As replication proceeds, either the template strand or the newly synthesized strand can become misaligned, leading to the addition of an extra nucleotide or the deletion of a nucleotide. On occasion, within an intact double-stranded DNA molecule, a nitrogenous base will spontaneously break off, causing an apurinic site (AP) because the purines adenine and

guanine are most often involved. If replication passes through an apurinic site before it is repaired, DNA polymerase may insert an incorrect base.

Induced mutations result from the exposure to certain environmental conditions or chemicals. Ionizing forms of radiation, such as X-rays, have short wavelengths and are very energetic. This type of radiation can penetrate tissues and bump electrons off of atoms within biomolecules, forming reactive groups that can affect the DNA and lead to mutations. Ultraviolet (UV) light, a type of nonionizing radiation, causes the formation of pyrimidine dimers, covalent linkages between the nitrogenous bases of adjacent thymines or cytosines. Some chemicals act as base analogues, in other words, they mimic the structure of purines or pyrimidines, and DNA polymerase mistakenly incorporates them into a growing chain. The problem is that the analogues can induce tautomeric shifting and often form unconventional base pairs with other bases. Examples of base analogues include 5-bromouracil and 2-amino purine. Alkylating agents are chemicals that add methyl ($-CH_3$) or ethyl ($-CH_2-CH_3$) groups to the keto or amino side chains of bases, leading to the formation of altered base pairs. Some chemicals cause frameshift mutations. For example, acridine orange and ethidium bromide contain planar aromatic rings and can slide in between base pairs of a DNA molecule, distorting it slightly, causing DNA polymerase to slip and miss adding a nucleotide or adding an extra one.

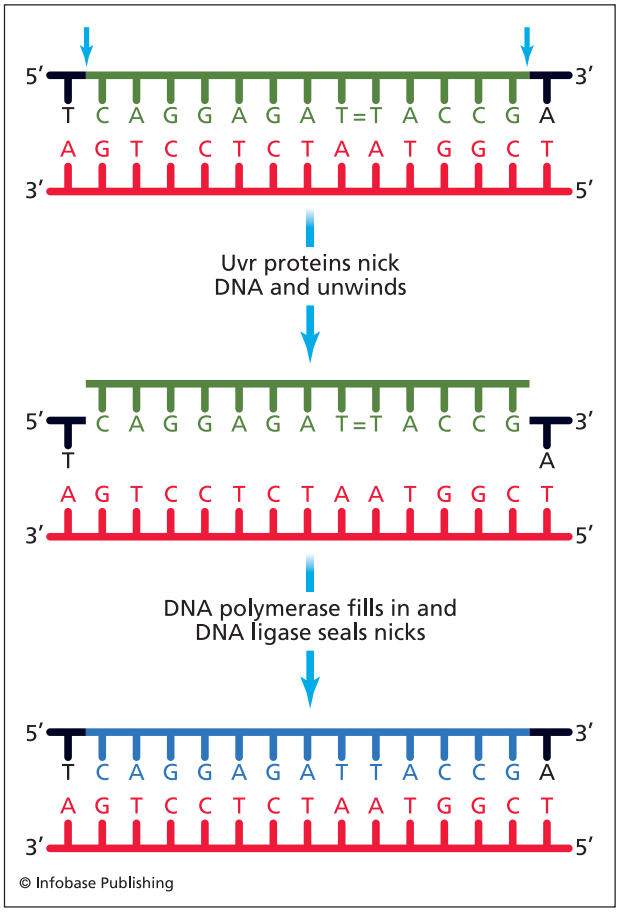
REPAIR MECHANISMS

Because of the importance in maintaining the integrity of the DNA for proper cell function and in order to pass hereditary information down from one generation to the next, cells have evolved several mechanisms for repairing mistakes made during replication, spontaneous base alterations, and even induced mutations. DNA repair falls under four general categories: proofreading and mismatch repair, photoreactivation repair, excision repair, and recombinatorial repair.

During DNA replication, DNA polymerase "proofreads" as it synthesizes the new strand. When it sees an incorrect base pairing, it removes the last base added, and replaces it with the correct one. Though this form of repair decreases the error rate from 1/10,000 to 1/10,000,000, additional mechanisms operate to further increase the fidelity. Mismatch repair acts by recognizing mismatches after replication and removing and replacing the incorrect pair with a proper pair. Newly replicated strands look slightly different from the original template strands, as the original template strands contain methyl groups added to certain adenine residues. This allows the cell to know which strand contains the correct deoxyribonucleotide and which one should be removed.

Photoreactivation repair reverses the effects of pyrimidine dimer formation caused by exposure to UV light. In the presence of light in the blue range of the spectrum, an enzyme called photoreactivation enzyme (PRE) simply cleaves the covalent linkages formed between the pyrimidine bases, restoring the normal structure.

Excision repair also repairs damage from UV light exposure, but involves many more steps and proteins. First an enzyme recognizes the distortion in the double helix caused by the presence of a pyrimidine dimer and cleaves the region immediately surrounding it from that strand of the DNA. Characterization of UV-sensitive mutant bacteria strains led to the identification of the *uvr* genes, *uvrA*, *uvrB*, and *uvrC*, that play a role in this step. The enzyme DNA polymerase I reads the exposed strand of DNA and inserts the complementary deoxyribonucleotides to fill the gap, and then DNA ligase seals the nicks to complete the repair process. Apurinic sites and accidental incorporation of uracils in DNA can also



In excision repair, the Uvr proteins recognize and remove single-stranded regions of DNA that contain lesions, DNA polymerase I fills in the excised area, and then DNA ligase seals together the ends.

be repaired by excision repair after other specialized enzymes recognize the sites and begin the excision process.

Recombinatorial repair involves the function of a protein called RecA that becomes activated when the damage to the DNA is great enough to interfere with DNA synthesis so that DNA polymerase just jumps over the affected areas, leaving gaps containing single-stranded DNA after synthesis. The RecA protein interacts with a regulatory protein called LexA, which then allows numerous other repair proteins to be expressed, a postreplicative response called the SOS response. In order to obtain a template, after the homologous daughter duplex is formed, the RecA protein mediates an exchange with the original second DNA strand from the homologous duplex DNA, bringing the two parental strands together again and leaving the newly synthesized strand unpaired. Other repair enzymes can then come in and create two intact daughter double-stranded DNA molecules. The presence of single-stranded DNA, as when damage is excessive and DNA polymerase skips a lot, activates the SOS response.

See also BIOMOLECULES; DEOXYRIBONUCLEIC ACID (DNA); DNA SEQUENCING; GENE EXPRESSION; GENETIC DISORDERS; GENETICS; GENOMES; VARIATION, GENETIC VARIATION.

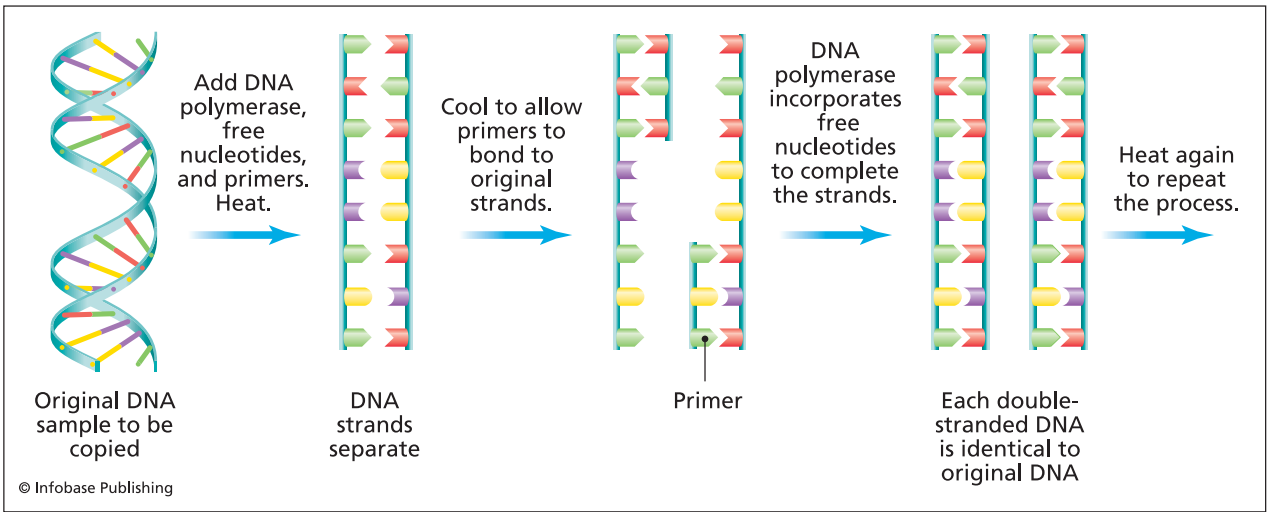
FURTHER READING

Brooker, Robert J. *Genetics: Analysis and Principles.* 2nd ed. New York: McGraw Hill, 2005.

polymerase chain reaction The polymerase chain reaction (PCR) is a technique used to amplify, or make multiple copies of a specific segment of deoxyribonucleic acid (DNA). Theoretically, PCR can amplify a piece of DNA one billionfold within a few hours. Kary Mullis, a chemist working for Cetus Corporation, invented the technique in 1985. The power and utility of PCR has revolutionized the biological sciences to such a degree that Mullis was awarded the Nobel Prize in chemistry in 1993 for his discovery.

DNA is a long chain of nucleotides containing one of four different nitrogenous bases: adenine, guanine, cytosine, and thymine. The sequence encodes for the synthesis of proteins within the cell. Each nucleotide specifically pairs with another nucleotide (A with T and C with G) through hydrogen bonding in a manner that links two strands of DNA to one another. This specific pairing means that if the sequence of one strand of DNA is known, one can determine the sequence of the complementary strand to which it is bound. In the cell, an enzyme called DNA polymerase performs the task of synthesizing new DNA molecules by reading one strand and incorporating complementary nucleotides to build the other strand.

The appropriately named polymerase chain reaction involves a series of cycles, or a chain of reactions, that employ DNA polymerase. The goal of PCR is to increase the concentration of a specific piece of DNA. From information about the sequence of the template DNA (the DNA to be copied) a scientist can design specific oligonucleotides that hybridize only to selected locations on the template. Oligonucleotides are short pieces of DNA, approximately 20 to 40 nucleotides in length. The two specially designed oligonucleotides, called primers because they prime or initiate the polymerization reaction, must bind to opposite strands of the dou-



The polymerase chain reaction can theoretically increase the amount of a DNA sequence by more than 1 billion times.

ble-stranded DNA template with the segment to be copied located between them. Heating breaks the hydrogen bonds holding together the two strands of the template DNA, causing them to separate in a process called denaturation. As the sample cools, the primers hybridize to the chosen sites on the now single-stranded template DNA. DNA polymerase then extends the primers, adding new nucleotides to them, creating strands complementary to the templates and completing the first cycle. As the second round of PCR begins, the double-stranded DNA that now consists of one original strand and one newly synthesized strand is heated, so that denaturation occurs. As the temperature decreases, new primers bind to complementary DNA, this time on both the original and the newly synthesized strands. Once again, DNA polymerase extends the primers, creating new double-stranded molecules of DNA. By the completion of 20 cycles of PCR, the amount of DNA theoretically has increased by one million times (2^{20} = 1,048,576), and by 30 cycles, one billion times (2^{30} = 1,073,741,824).

With the advent of PCR, researchers have available a virtually unlimited supply of any DNA sequence. The invention of PCR has advanced research in numerous fields including medicine, genetics, biotechnology, and forensics. The wide range of applications includes the detection of infectious organisms, diagnosis of genetic disorders, determination of paternity, analysis of DNA for taxonomic classification, ecological studies of seed dispersal, genetic fingerprinting to identify criminal suspects, and assistance in limiting the illegal trade in endangered species. New uses are discovered constantly.

See also DEOXYRIBONUCLEIC ACID (DNA); ENZYMES; MOLECULAR BIOLOGY; MULLIS, KARY.

FURTHER READING

Mullis, Kary, and Fred A. Faloona. "Specific Synthesis of DNA in Vitro via a Polymerase Catalyzed Chain Reaction." *Methods in Enzymology* 255 (1987): 335–350.

Mullis, Kary, Fred Faloona, Stephen Scharf, Randall K. Saiki, Glenn T. Horn, and Henry A. Erlich. "Specific Enzymatic Amplification of DNA in Vitro: The Polymerase Chain Reaction." *Cold Spring Harbor Symposia on Quantitative Biology* 51 (1986): 263–272.

Rabinow, Paul. *Making PCR: A Story of Biotechnology*. Chicago: University of Chicago Press, 1996.

population ecology Populations, groups of individuals belonging to the same species and living in the same geographical area, are dynamic: the number of individuals rises and falls, the density and dispersion changes, and the demographics fluctuate. Just as communities and individuals within them interact with and influence their environment, so do populations. The study of how the environment, including both abiotic and biotic factors, affects features of a population of organisms and how the population influences the environment is called population ecology.

POPULATION SIZE AND DENSITY

The density of a population is the number of individuals per unit of area. Ecologists define the geographical boundaries based on the focus of their specific investigation. A study may examine the honey bee population in North America or a population of truffles in a certain forest. Direct counting of individuals in a population is rarely possible, so ecologists rely on a variety of sampling techniques to estimate the true densities. For example, they might count the number of a certain species of spider on several plots of a field, then take the average number found within the plot size and extrapolate to estimate the density in a larger area. A method for determining the density of an animal population called the mark-capture technique involves trapping and marking or tagging the animals, such as with collars or spots of dye. The researcher releases the animals, waits for a while, then sets up traps again. This time the traps will catch new animals as well as previously trapped animals. Based on comparison of these numbers and assuming the researcher waited for a sufficiently long time period in between trappings for the trapped animals to mix with the rest of the population, the researcher can use statistical analysis to estimate the density of that population.

Density changes due to the addition of new members and the removal of members from a population. New births and immigration both add to a population, whereas deaths and emigration decrease the size of a population. Physical conditions of the environment and other members of the population as well as populations of other species within a community affect these events. Because members of a species fulfill the same ecological niche within a community, the individuals all compete for the same resources. For example, the availability of food limits the density of a population. As the availability of food decreases, so will the population size, but when food is abundant, other factors may play a larger role in influencing population dynamics. Other organisms that consume the population of interest through herbivory or predation also control the population size. When the population size of species at the higher trophic level decreases, the focus population may increase. Community ecologists examine these sorts of interspecific interactions. Changes in weather or in the availability of

inorganic nutrients are examples of abiotic factors that affect population size.

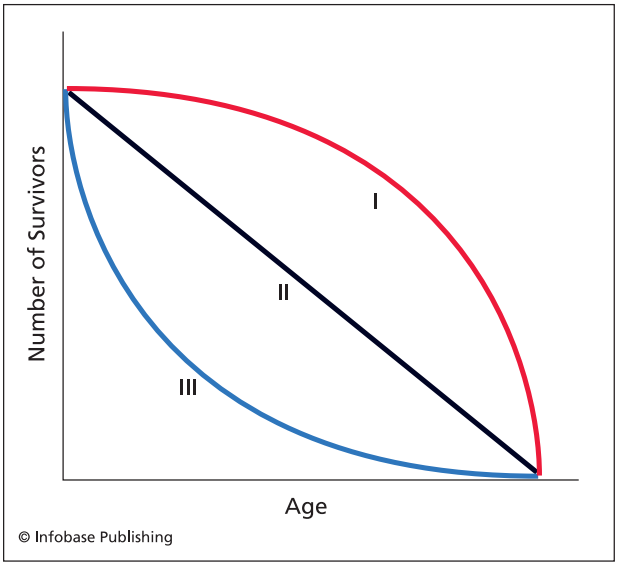
For animals in general, population density decreases with increasing body mass. Though this trend is true across a broad range of animal groups, slight differences do exist. For example, compared with other animals of similar body mass, aquatic invertebrate populations are slightly denser. Likewise, mammals have higher population densities than birds. The same relationship is true for plants, though the characteristic population density may change during the life cycle of a plant. Seedlings can survive denser populations than larger, mature plants, especially for large trees.

The dispersion of a population is the distribution or spacing of the individuals over the defined area. Dispersion studies investigate how the density of a population varies within localized regions of the set boundaries. The different patterns reveal information about the social interactions and behaviors of the species. Three major patterns of dispersion are clumped, uniform, and random. Clumping is the most common, and often relates to conditions that are more favorable in a particular setting. For example, insects may clump under rocks or fallen logs within a forest due to a cooler and moister microclimate. Or clumping of predators may result from increased success at hunting when the individuals stay close together. Uniform or evenly distributed dispersion patterns often indicate that members of the species interact aggressively with other members of the same species. Thus, each seeks its own space or territory for foraging and reproducing. Random dispersal occurs when neither strong attraction nor repulsive forces are in place. The interactions are neutral, and individuals have an equal chance of being anywhere in an area.

DEMOGRAPHICS

Demography is the study of populations and how they change with respect to features such as size, density, age, gender, birth rates, and death rates. These statistics describe a population and may reveal relevant information about the organism. By tracking a large group of individuals, called a cohort, a population ecologist can create a life table to depict the age-specific survival summaries of a population. Such a table provides information such as the average age of death and life expectancy for members of that cohort and gives insight as to the dynamics of that population. Reproductive tables summarize the birth rates, including number of male and female offspring born to different aged individuals. Birth rate is a measure of how many young are born, how many eggs are laid, or how many seeds are produced by one female per unit time. Such data gives information about the reproductive strategies for the species and reveal information such as whether organisms have overlapping (e.g., mammals) or nonoverlapping generations (e.g., annual plants). Life and reproductive tables may indicate whether a population is growing, stable, or declining and highlight historical periods of high or low birth and death rates. Survivorship curves reveal patterns of survival in a population. The number of survivors on a logarithmic scale is plotted on the *y*-axis as a function of age, which is plotted on the *x*-axis. Different types of animals have different shaped curves that give insight into the organism's life history, survival, and reproductive strategies. Three major categories summarize different survival patterns. Type I curves show high survival among the young and high frequency of death among older individuals. Populations that exhibit roughly equal survival and death rates among all ages have type II survivorship curves. Type III curves represent high mortality among the young and much lower death rates among the older members of a population. Together with survivorship patterns and the life tables, population ecologists can surmise the life history strategies of an organism, which will help ecologists better relate an organism's reproductive patterns to the organism's evolutionary history.

To illustrate the type of information that can be gained from demographic information, consider the



Most survivorship curves follow one of three general patterns, though natural populations exhibit all sorts of intermediate patterns. In type I survivorship, the young have a high survival rate and the older individuals have a higher mortality rate. In type II, individuals of the population die at equal rates regardless of age. In type III, the young have a high mortality rate and the older individuals have a high survival rate.

following examples. Humans are an example of a species with a type I survivorship curve. The number of offspring born to individuals is low, but the parents invest a lot of energy into parental care, which contributes to the relatively high survival rate among the young compared to the older members of a population. Squirrels, bees, large birds, and corals are examples of species with type II survivorship curves, which do not reflect an age-dependent mortality. The major causes of death are hunting and disease, which can strike at any age. Species that exhibit type III survivorship curves produce many offspring but very few survive. Much energy is spent producing large numbers of eggs or seeds, but very little is expended on parental care. Unpredictable and variable environmental conditions largely determine the survival rate of the young. Those individuals that survive their risky juvenile years have long life expectancies. Plants and many marine invertebrates such as oysters are examples.

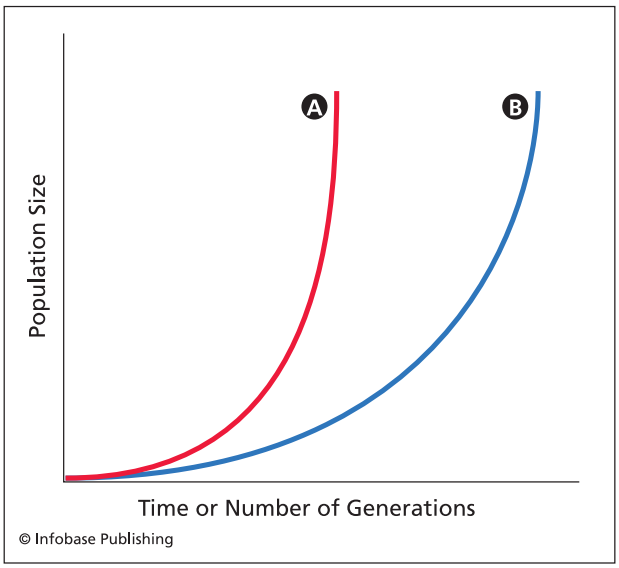
POPULATION GROWTH

While characteristics inherent to the species influence populations, so do factors such as dispersal, climate change, and changes in the food supply. When predators are not abundant, food is readily available, and an organism's adaptations suit the climate, then a population may grow geometrically or exponentially. In geometric population growth, each generation differs in size by a constant ratio. Species that exhibit this type of growth in favorable conditions include organisms that grow in annual pulses, such as insects or annual plants that reproduce once per year.

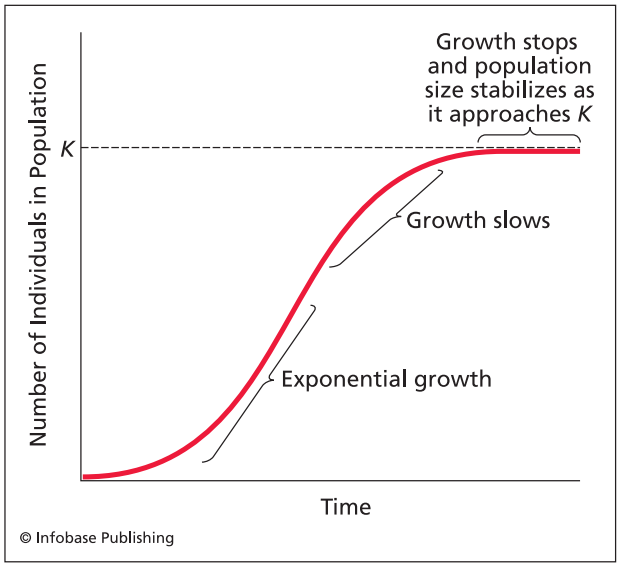
Exponential growth occurs when no restrictions limit the increase in a population's size other than the organism's physiology. The per capita intrinsic rate of increase, or the average number of offspring produced per unit time by an individual, is constant and generates a J-shaped curve when the population size is plotted over time or over generation.

In the real world, exponential growth cannot continue indefinitely. As a population's density increases, so does the competition for food and other resources. Carrying capacity (*K*) is the maximum number of individuals that the environment in a defined area can support. At the carrying capacity for a species, the birth rate and death rate are approximately equal. A variety of complex interactions between biotic and abiotic factors influence population size. Competition for resources is one, but a limit on available territories, a decline in health characteristic of dense populations, interspecific interactions such as predator-prey relationships, and the accumulation of toxic metabolic waste products all work together to regulate population size.

The human species is no different from other populations, and over the past few centuries population growth has declined. According to the U.S. Census Bureau, in August 2007 the world population was greater than 6.6 billion and is estimated to reach 9 billion in the early 2040s. The dynamics of the local regions varies tremendously. The less developed countries have approximately 81 percent of the world's population, with an average growth rate of



When the physiological capacity (of a species) is the only limitation on its reproduction rate and the environmental conditions are optimal for survival, the population grows exponentially, as demonstrated by plotting the population size over time. The curve labeled A represents a population with a higher maximal intrinsic rate of increase than the population in B.



When a population reaches its carrying capacity (*K*), the growth curve assumes a sigmoidal or S shape.

1.38 percent. In comparison, the average growth rate in more developed countries is about 0.23 percent. In the United States, the growth rate was about 0.89 percent, and in the United Kingdom, about 0.28. The age structure of different countries' populations gives insight as to the future growth trends.

The concept on an ecological footprint helps population ecologists understand the land and water uses of a population. Estimates suggest that the human population's ecological footprint is more than 23 percent larger than what the planet can regenerate. This scary statistic has prompted the environmental movement toward sustainability. Again, different populations have different footprints, which are expressed in land area (in hectares) per person. Some countries, such as the United States, are above their carrying capacity, whereas others, such as New Zealand, are below theirs.

See also COMMUNITY ECOLOGY; CONSERVATION BIOLOGY; ENVIRONMENTAL CONCERNS, HUMAN-INDUCED.

FURTHER READING

Molles, Manuel C., Jr. *Ecology: Concepts and Applications*. 4th ed. New York: McGraw Hill, 2008.

U.S. Census Bureau. International Data Base (IDB). Available online. URL: http://www.census.gov/ipc/www/idb/. Updated June 18, 2008.

Priestley, Joseph (1733–1804) English *Chemist*

Joseph Priestley was an 18th-century chemist who helped delineate photosynthesis, the process that renders the planet Earth habitable by aerobic life-forms. He is also credited with the discovery of oxygen and 10 other gases. This tenacious researcher was a trained minister who felt that studying natural philosophy (the natural sciences including physics, chemistry, and biology) was one way to honor God. Priestley's contributions also advanced the fields of political science, language and grammar, philosophy, history, and many more.

CALVINIST UPBRINGING

Joseph Priestley was the oldest of six children born on March 13, 1733, to Jonas Priestley and his first wife, Mary Swift. They lived in Fieldhead, England, near Yorkshire, where his father was a cloth merchant. As the family grew, Joseph was sent to live temporarily with his maternal grandparents. His mother died during the birth of her sixth child in as many years, and at age nine Joseph was sent to live with his aunt, Sarah Priestley Keighley, until adulthood.

The members of the Keighley household were strict Calvinists, which meant they believed in salvation by God's grace alone, God's omnipotence, predestination, and original sin, among other things. Joseph was exposed to several religious viewpoints, as dissenting ministers were often invited to their home. Dissenters were people who did not accept the doctrines of the Church of England. While simply having nonconformist beliefs was not illegal, there were specific laws that limited the privileges of those who did not subscribe to the state religion.

By the time he was a teenager, Joseph had already mastered several languages and received private tutoring in algebra, geometry, and Newtonian mechanics. He felt called to the ministry, but, as a young adult, he was denied admission into the church in which he was raised because he could not accept the doctrine of original sin, which purported that all humanity was inherently evil and brought suffering upon itself. Since he was not a communicant of the Church of England, he could not seek admission to the universities at Oxford or Cambridge, England's most renowned. At age 19 he became the first student to enroll in the dissenting academy at Daventry.

MINISTER AND TEACHER

He left the academy in 1755, taking a position as an assistant minister at Needham Market in Suffolk. As Priestley's personal religious beliefs matured, his congregation and senior minister became uncomfortable with his viewpoints. In particular, Priestley no longer accepted the trinity, or the union of the Father, the Son, and the Holy Ghost. When he had fulfilled the obligations of his term, he anxiously accepted a position as a minister at Nantwich, in Cheshire. To supplement his income there, he opened a successful school for the girls and boys in his congregation. His own interests in natural philosophy increased, and he purchased an air pump and an electrical machine for his students to perform experiments.

His reputation as a teacher grew, and, in 1761, Priestley was invited to join the faculty at the dissenting academy at Warrington, in the county of Lancashire. He began to write and publish successful texts on language, grammar, and education, and he conducted electrical experiments. In 1764 he was awarded a doctorate degree from the University of Edinburgh for his studies on education. Priestley remained at Warrington for six years, until the income was no longer enough to support his growing family. He had married Mary Wilkinson, the sister of one of his former students, on June 23, 1762, and they already had one daughter. Later they would have three more children, all sons.

While traveling to London in 1766, Priestley met Benjamin Franklin, an American statesman who was representing the colonies in discussions with the British government. Franklin was a respected scientist and had already made his famous discovery that

lightning was an electrical phenomenon. Priestley took advantage of this opportunity to discuss his electrical experiments with Franklin, who encouraged Priestley to write *History and Present State of Electricity, with Original Experiments,* a text that gave an up-to-date accounting of all related research. One new observation from Priestley's own experiments was that carbon, a nonmetal, could conduct electricity. He reported his deduction that the electrical attraction between bodies has an inverse-square relationship to the distance between them. He also recorded the first description of an oscillatory discharge, which was the same principle used in wireless telegraphy as deduced by the Italian inventor Guglielmo Marconi. Even before the book, which was published in 1767, reached the shops, news of Priestley's experiments had spread among other English scientists. In 1766 he was elected a fellow of the Royal Society on the basis of his electrical work, quite an honor for someone with no formal training in science.

His position at Warrington did not offer the Priestley family the financial security they desired, and Joseph missed the ministry. In 1767 he moved his family to Leeds and took over the Presbyterian parish at Mill-Hill Chapel.

CHEMISTRY OF AIRS

A minister at heart, but also fully a scientist, Priestley could not ignore the unique odor that came from the brewery next door to their new home. He became particularly interested in the air over the fermentation vats and obtained permission from the brewery owner to explore this phenomenon. This initiated his experiments in pneumatic chemistry, the chemistry of airs. (The term *gas* had not yet been coined.)

Priestley climbed up and over the large fermenting vats and made observations. He saw a cloud of unknown composition hanging over the vats. When he waved at it, the cloud sank to the ground, demonstrating that it was heavier than ordinary air. He also found that when he put a candle to it, the air extinguished the flame. To see if this air could be dissolved in water, he put a pan of water very close to one of the vats and let some of the heavy air fall into it. Then he mixed it up and let more fall in until he was sure no more of the heavy air could dissolve in the water. The gas was mostly soluble, but some tiny bubbles floated to the top, reminding him of the expensive seltzer water harvested from natural springs. When he sipped some, it tasted like seltzer water; Priestley had invented carbonated beverages.

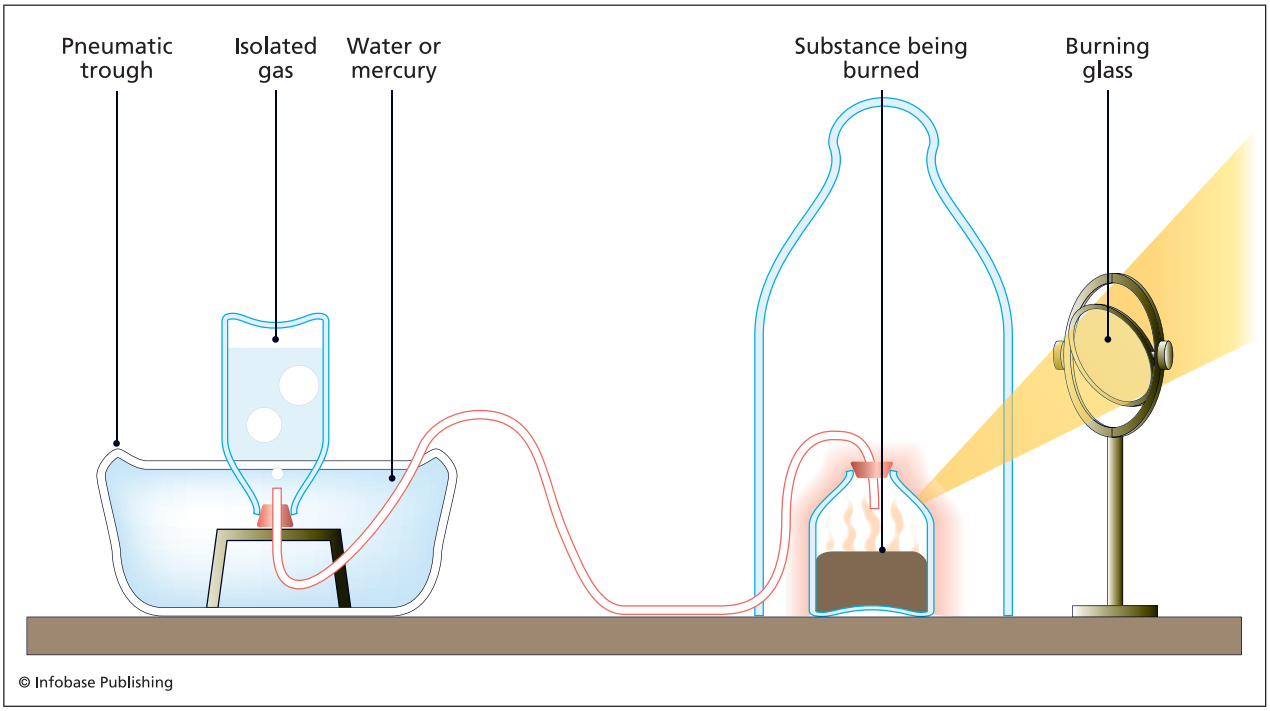
After days of going back and forth between the brewery and his home trying to collect the unusual air hanging over the vats in bottles to examine further at home, he thought perhaps he could produce this special air on his own. He recalled reading that a Scottish chemist named Joseph Black made what was called "fixed air" by heating limestone. The fixed air, which today is called carbon dioxide, also had the characteristic of extinguishing flames. Priestley tried Black's method, but he found that he had more success heating chalk with water and adding "marine acid" (today called hydrochloric acid).

To collect the carbon dioxide, he filled a tub with water, inverted a bottle filled with water with the mouth covered, and then removed the cover after setting the inverted bottle in the tub. He connected a tube running from the apparatus in which he mixed chemicals or heated substances to the opening of the inverted bottle. If a gas was formed and it was lighter than water, it would rise up the inverted bottle and displace some of the water down out of the mouth. The amount of gas produced was related to the amount of water displaced. This apparatus is called a pneumatic trough.

Priestley demonstrated his method of producing carbon dioxide and homemade seltzer water at the next meeting of the Royal Society of London. The members were fascinated, especially when Priestley challenged one of them to taste it. He was asked to repeat these experiments for the College of Physicians. One month later, Priestley received a letter from the Royal Society recording their favorable impressions. His impregnated water was demonstrated to the Lords of Admiralty (the British navy) in hopes of preventing scurvy. Today we know that scurvy is caused by a vitamin C deficiency, and this treatment would not have worked at all. In 1773 the Royal Society awarded Priestley the Copley Medal, their highest scientific honor.

While researching carbonated water, Priestley also performed experiments to learn about how plants breathed. Others had previously demonstrated that a mouse placed in a sealed bottle would use up all the "good air" and soon die. To see if plants reacted similarly, he placed some mint in inverted bottles kept in a tub of water to prevent any outside air from entering the plant's environment. Mint was a good specimen for examining this phenomenon because the sprigs could live in water. The bottles were set up outside in his garden. Surprisingly, the plants survived for weeks. He decided to test the air inside the jars. When a candle was placed inside a bottle of "used-up" air, such as one in which a mouse had died, the flame was quickly smothered. However, when he placed a lighted candle into a bottle in which the mint had been growing, the flames became brighter and lasted longer.

After pondering this, Priestley tried putting a small mouse in a bottle that had been used for growing mint. The mouse did not seem to suffer any ill



Joseph Priestley used a pneumatic trough to collect the gases he produced by heating different substances.

consequences. He depleted all the good air in a bottle by burning a candle in it until the flame went out and then quickly replaced the candle with a sprig of mint. The mint thrived in such a bottle, despite the absence of any good air. Just as surprising, when he took the mint out after 10 days and put in a lit candle once again, it burned brightly. The mint plant seemed to have refreshed the air inside the bottle. Furthermore, he found that a mouse could survive in a bottle whose bad air had been renewed by a mint sprig. From all this he concluded that plants take in bad air and release good air. It should be noted that the Dutch physician Jan Ingenhousz examined these same phenomena at about the same time as Priestley.

At the time, chemists had identified only three different airs: ordinary common air, fixed air (now called carbon dioxide), and inflammable air (now called hydrogen gas). Scientists thought that ordinary air was an element and could not be broken down any further. Today scientists know that air is made up of several types of gases, but primarily nitrogen (78 percent) and oxygen (21 percent). Fueled by his successes with fixed air, Priestley decided to see what other airs he might produce, mostly by heating different substances and examining the emissions.

When heating liquids, he stuffed a cork with a hole in it into the mouth of the heated bottle and fed a tube through the hole. The tube led to inverted bottles in his pneumatic trough. He sealed any leaks with cement. When heating a solid, he used heat to dry it out as much as possible, and then he introduced the solid into the barrel of a gun, filled it with sand, and heated the barrel over a fire. The bottom was sealed off, but the top was connected to a tube that led to his pneumatic trough. Any contaminating particles would be held back by the sand, and only the pure gas would be able to escape. If even hotter temperatures were required, the solid was placed inside a glass jar and a burning lens was used to concentrate sunlight onto the solid, burning it. The released gas was fed into the pneumatic trough. Priestley determined that even though his new method for collecting airs was efficient, airs that were highly soluble in water would escape collection; so he used liquid mercury in place of water in the inverted bottle and the basin. Knowing the breathable portion of air reacted with nitric oxide, he developed a method using this substance to test for the purity of airs he studied.

Using these methods, Priestley was able to produce and identify the gases that are now called nitric oxide, nitrogen dioxide, nitrous oxide (laughing gas), ammonia, hydrogen chloride, sulfur dioxide, silicon tetrafluoride, nitrogen, and carbon monoxide. Some of these experiments were presented in 1772 to the Royal Society in his paper "Observations on Different Kinds of Air." Other discoveries were published in his six-volume series titled *Experiments and Observations on Different Kinds of Air and Other Branches of Natural Philosophy*. These volumes were published during the period 1774–86. Because of his

advancements in the field of pneumatic chemistry, the French Academy of Sciences elected Priestley to membership.

Once again, the financial strain of supporting his family lured Priestley into new employment in 1773. William Fitzmaurice Petty, the second earl of Shelburne, hired him as his librarian, literary companion, and supervisor of his sons' education. Lord Shelburne promised him not only a generous salary, but also a well-equipped lab and an extra stipend to purchase chemicals and supplies for experiments. Unfortunately, taking this position also meant moving his family to Calne, in Wiltshire. Priestley spent the summers with his family and the winters with Lord Shelburne in London. His years with Shelburne (1773–80) were by far his most productive in advancement of chemistry. Most of the work presented in his *Airs* series was performed during these years.

DISCOVERS OXYGEN

In August 1774, for no obvious reason, Priestley burned mercurius calcinatus (red mercuric oxide) using a large magnifying lens. He collected the resultant gas using his usual method of passing it through mercury into an inverted bottle. Shiny globules of elemental mercury were left behind. He collected three bottles full of the gas released. Because he had a lighted candle nearby, he held it to the gas in one bottle. The flame burned brighter. He took a glowing ember of wood and held it to the second bottle, and it immediately burst into flames. Even more remarkable was that a mouse could live entrapped in a bottle with this air longer than with ordinary air.

During this time, the widely accepted theory explaining how materials burned involved a substance called phlogiston. If a substance had a lot of phlogiston, it burned easily. If a substance had little phlogiston, then it was more resistant to burning. When all the phlogiston had left a substance, burning would cease. The fact that a candle flame would be extinguished if kept under a jar was explained by the air becoming saturated with phlogiston, thus it could absorb no more, and the flame would die. Because the new air that Priestley had extracted from mercurius calcinatus allowed the candle and the ember to burn more brightly, he reasoned that the air produced by burning the mercurius calcinatus had little to no phlogiston present in it. Thus it was able to suck the phlogiston out of the candle and the wood much more readily. He called this air "dephlogisticated." Today it is called oxygen, and Priestley is credited with its discovery. Priestley inhaled some himself and found that it made him feel light and easy. He predicted that breathing this new air would constitute a good medical treatment for people with respiratory problems. He wrote up his results the next year and sent his findings to the Royal Society in March 1775.

During fall 1774, Priestley accompanied Lord Shelburne to continental Europe. One night they dined with other famous scientists, including the French chemist Antoine Lavoisier who was much younger than Priestley but already very respected in the field. Lavoisier asked Priestley about his current experiments, and Priestley openly shared his exciting discovery of dephlogisticated air. The other scientists were impressed and peppered him with questions, but Lavoisier just listened silently. Unbeknownst to Priestley, Lavoisier was already trying to incorporate this new knowledge into a set of experiments he would perform over the next few months. Lavoisier later repeated Priestley's experiments and presented his own results to the French Academy of Sciences in April 1775 without giving any prior credit to Priestley for his intellectual contribution. Lavoisier called the dephlogisticated air "oxygen," from the Greek word *oxys,* which means sharp (like an acid), and *gen,* which means to be born. He also showed that ordinary air is made up of approximately 20 percent oxygen and then went further by using this information to blast the entire phlogiston theory. He systematically measured the weights of several materials before and after burning and found that the weight always increased after burning, a result inconsistent with the suggestion that phlogiston was released when something burned. Lavoisier correctly hypothesized that burning, or combustion, is the result of the combination of a substance with oxygen. Candle flames were extinguished in enclosed spaces due to the oxygen being used up. Mice died after a while under a jar for the same reason. Priestley could not accept this. He believed that phlogiston had a quality called levity, a sort of negative weight. He thought the explanation of phlogiston saturation was sufficient to explain why candle flames burned out after a while. Another problem with the phlogiston theory was that phlogiston had never been isolated. To this, Priestley responded that neither gravity nor electricity nor magnetism had been isolated. He responded that phlogiston resembled a power more than a substance.

Priestley did not make a fuss after Lavoisier tried to steal the rights to the discovery of dephlogisticated air, or oxygen. He believed that the fact of discovery was what mattered, and that benefits could be derived no matter who got credit for it.

STUDIES PHOTOSYNTHESIS

After identifying so many different airs, Priestley began to wonder about how ordinary air was purified. His research at Leeds told him that green plants

were capable of cleaning bad air and replacing it with pure air. To figure out how, he filled several bottles with water and inverted them over bowls of water. Some of the bottles had green pond scum (probably algae) in the water, and others did not. He placed them all outside in the sunlight, and, by the end of the day, the water had been displaced in the bottles over the scummy water but not in the bottles over the plain water. A gas had been produced in the presence of the pond scum. When he placed a glowing ember in the gas and it burst into flames, he realized that the green plants (or algae) had produced oxygen. To determine if the sunlight required by green plants for survival was related to their oxygen-producing capability, Priestley repeated the experiment, but this time he put some of the bottles in the dark. No oxygen was produced in those bottles, and he concluded that sunlight was required for the plants to carry out the process that changed the composition of the air. Ingenhousz researched this phenomenon more thoroughly and, in the 20th century, the American chemist Melvin Calvin delineated the associated biochemical pathways. This well-researched process is now termed photosynthesis, the conversion of light energy into chemical energy stored as organic compounds.

The process of photosynthesis supplies energy in the form of food for almost all living organisms, either directly or indirectly. Even carnivores, or meat-eaters, ultimately depend on organisms that directly obtain their energy from organisms that undergo photosynthesis. The chloroplast is the structure capable of undergoing photosynthesis in plants and algae. Chloroplasts are specialized membrane-bound organelles that contain the pigment chlorophyll, which absorbs the red and blue light of the visible spectrum. Because green light is reflected, most photosynthetic organisms are the color green, or at least have green parts. When energy in the form of light is absorbed by chlorophyll, electrons of the chlorophyll molecules jump to higher energy levels and begin a cascade of falling step by step to sequentially lower energy levels in a chain of molecules. Water molecules, which supply the electrons from their hydrogen atoms, are split, and the oxygen is released as a by-product. The hydrogen atoms donate electrons to the chlorophyll molecules, and the remaining protons are used to create a proton gradient. The energy harvested from the sunlight, now in the form of a chemical and electrical gradient, is used to drive the synthesis of adenosine triphosphate (ATP), a form of chemical energy readily useable by the cell, and nicotinamide adenine dinucleotide phosphate (NADPH), an electron carrier molecule. The cells then use both the ATP and the NADPH to reduce carbon, supplied in the form of carbon dioxide (CO_2), synthesizing carbohydrates, or sugars. In summary, photosynthetic organisms use sunlight, CO_2, and water to make sugars, releasing oxygen in the process.

$$6CO_2 + 12H_2O + \text{sunlight} \rightarrow C_6H_{12}O_6 \text{ (sugars)} + 6O_2 + 6H_2O$$

LATER CAREER AND CONTROVERSY

Meanwhile, Priestley's radical political and religious opinions caused his relationship with Lord Shelburne to slowly cool. In 1780 Priestley accepted a position as minister at the New Meetinghouse in Birmingham, Warwickshire. By original agreement, despite their parting, Priestley continued to be paid an annuity from Lord Shelburne. While at Warwickshire, Priestley joined the Lunar Society, a group of men who met on nights when the moon was full. Other members included James Watt (of steam engine fame), Josiah Wedgwood, John Smeaton, Matthew Boulton, and Erasmus Darwin as well as others of high reputation. They freely discussed religious, scientific, and political matters, and Priestley benefited from the intellectual stimulation.

In January 1781 Priestley carried out some experiments attempting to convert air to water and vice versa. He put a piece of minium, red lead (Pb_3O_4), into a container filled with inflammable air over water. When he focused the rays from a burning lens on it, the minium turned black and then into pure lead while the air volume decreased and the water ascended in the receiver. The water seemed to be absorbing the air. The next day he combined inflammable air with common air and sent an electrical spark through it. A crackle resulted, and moisture appeared on the inside of the container. Thinking he might have turned air into water, he wrote to Henry Cavendish, the French chemist who had discovered inflammable air. Cavendish examined this phenomenon further, and due in part to Priestley's initial observation and willingness to share scientific knowledge, Cavendish was able to determine the composition of water to be two parts hydrogen and one part oxygen, H_2O.

In addition to carrying out his scientific research, Priestley continued to publish controversial religious works, some of which vehemently attacked the doctrines of the Church of England. On July 14, 1791, the night of the second anniversary of the storming of Bastille (a key event of the French Revolution), several friends, including Priestley, planned to meet at a local inn for dinner. For some reason, Priestley remained home, playing backgammon with his wife that evening. Suddenly they heard noises outside. A neighbor was rushing to tell them that the inn had been stormed by a "Church-and-King" mob. He feared that Priestley was the real target, so Priestley's

family immediately fled to a friend's house, and then Joseph snuck out of town to London. By the end of that night the mob had destroyed his house, his laboratory, and his church.

Shortly thereafter, Priestley took a position preaching at Gravel Pit Meeting in Hackney, but their life was not comfortable. Priestley had defended the rights of Americans to break away from England, he sympathized with the French revolutionaries, he had consistently chipped away at the doctrines of the nation's official religion, and his former colleagues at the Royal Society now shunned him. In April 1794 Joseph and Mary Priestley set sail for the United States, where their three sons had moved a few years earlier. Josiah Wedgwood and others from England sent several pieces of equipment from England so Priestley could set up a laboratory in his new home in Philadelphia. Within a few years after moving to America, his youngest son and his wife died.

Though most of his time was spent puttering in his lab, writing religious texts, and corresponding with colleagues, including Thomas Jefferson, Benjamin Rush, and John Adams, Priestley did continue to keep up with new developments in chemistry. He hoped to determine the amount of phlogiston in various metals. In 1799 he discovered yet another new gas, carbon monoxide, by heating coal in a small amount of air. Though poisonous, this gas has many industrial uses. He continued to passionately defend the phlogiston theory despite the fact that it had been demolished, ironically, as a result of his own discovery. His last scientific paper was titled "The Doctrine of Phlogiston Established," which it was not. Years before, Lavoisier had finally vindicated Priestley for his discovery of oxygen, but from that discovery Lavoisier had given birth to a new revolution in chemistry, one that had no place for phlogiston.

Priestley's health began to weaken in 1801. By 1803 he was mostly bedridden. On February 6, 1804, he died with his son by his side. To recognize his distinguished services to chemistry, the American Chemical Society established the Priestley Medal in 1922, honoring the father of pneumatic (gas) chemistry.

See also Calvin, Melvin; Ingenhousz, Jan; photosynthesis.

FURTHER READING

Schofield, Robert E. *The Enlightenment of Joseph Priestley: A Study of His Life and Work from 1733 to 1773*. University Park: Pennsylvania State University Press, 1997.

Schwartz, A. Truman, and John G. McEvoy, eds. *Motion toward Perfection: The Achievement of Joseph Priestley*. Boston: Skinner House Publications, 1990.

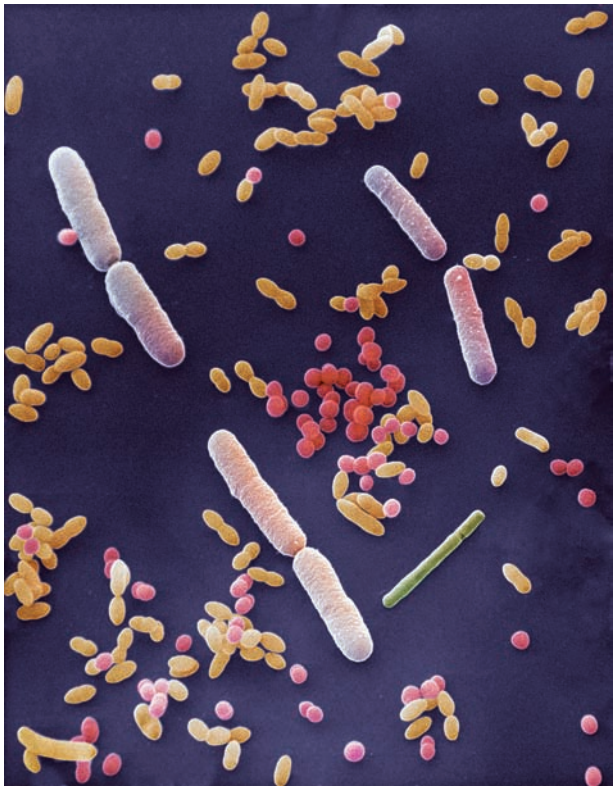
prokaryotic cells All cells are categorized into two main types based on major structural differences. Eukaryotic cells contain a nucleus that encases the genetic material, a complex cytoskeleton, and an endomembrane system. All of the organisms belonging to the domain Eukarya are composed of eukaryotic cells, including animals, plants, fungi, and protists. Prokaryotic cells lack a distinct nucleus and other membrane-bound organelles. According to the traditional five-kingdom classification system proposed by Robert Whittaker, prokaryotic organisms belonged to the kingdom Monera. Since the late 1970s, when Carl Woese and his colleagues discovered that prokaryotic organisms consisted of two entirely separate groups, prokaryotic organisms have been classified into two domains, Archaea and Bacteria.

Members of the prokaryotic domains are unicellular, so a single cell is a complete organism. Though often referred to as the simplest of life forms, prokaryotic cells are complex structures that are capable of carrying out all the functions necessary to sustain life and reproduce within a single cell that is typically smaller than one cell of a multicellular eukaryotic organism. Prokaryotic cells range from about 7.87×10^{-6} to 7.87×10^{-3} inches (0.2 to 200 µm), though cells smaller and larger have also been observed. A typical *Escherichia coli* cell measures about 3.937×10^{-5} inches (1.0 µm) in width. Whether one considers prokaryotes structurally simple or complex, bacteria and archaeans are functionally very successful life forms, having adapted to live in an extremely diverse range of environments.

CELL SHAPE AND ARRANGEMENT

Prokaryotic cells exhibit great diversity in their morphology. Within a species, cells have the same general shape, governed by the cell wall, but variations between individuals do exist. Most prokaryotic cells are coccus (spherical) or bacillus (rod) shaped, but they can also be vibrio (curved), spiral, helical (spirochetes), or irregularly shaped. Some cell types are combinations, such as coccobacilli, which are short, plump, and cylindrical.

The arrangement of the cells, or the way they are grouped, is also characteristic of a species. After division, the progeny cells can remain attached to one another. If two cells stick together, the prefix diplo- is used, as in diplococcus. If the cells occur as a random cluster, like a bunch of grapes, the prefix staphylo- is used, as in staphylococcus, meaning a cluster of spherical cells. Strepto- indicates the cells form a chain, like streptobacillus. Cocci can also exist as tetrads, groups of four, or in cubical packets, a grouping called a sarcina. Some bacterial colonies resemble molds because they grow as filamentous, branched rods.



Bacteria have a variety of shapes and sizes, as shown by this scanning electron micrograph. (photograph taken at a magnification of 8,000×) *(David M. Phillips/ Photo Researchers, Inc.)*

Unless otherwise specified, the following descriptions refer to bacterial cells.

CELL ENVELOPE

The cell envelope consists of the cell wall, the cell membrane, and the outer membrane if one is present. The cell wall of prokaryotic organisms consists of peptidoglycan, a compound made of long polysaccharide chains that are bridged together by tetrapeptide side chains. The polysaccharide chains consist of alternating repeated units of *N*-acetylglucosamine (NAG) and *N*-acetyl muramic acid (NAM). The chains are cross-linked either directly between amino acids or through another peptide interbridge, giving rigidity to the layer. The number of layers of peptidoglycan differs between bacterial groups. The main function of the cell wall is to provide structural support and maintain conformation of the cell. Because most bacteria live in aqueous environments, water is constantly diffusing into the cell by osmosis. Without the rigid cell wall, the internal osmotic pressure of the cell would cause it to burst open, or lyse, killing the cell.

Some bacteria have atypical cell walls. *Mycobacterium* and *Nocardia,* two pathogenic bacteria, have mycolic acids, long chain fatty acids, in their cell walls. Mycolic acid gives these bacteria a waxy nature that makes staining them by conventional methods difficult and also contributes to their pathogenicity. Mycoplasmas are a unique group of bacteria that have no cell wall. The presence of sterols in their cell membrane compensates by providing sufficient structural support to prevent lysis. Without a rigid covering, these tiny bacteria are pleomorphic, meaning they exhibit a variety of cell shapes. Archaea do not share the cell wall structure typical of bacteria. Their cell walls are composed of either polysaccharides or protein.

The cell membrane lies at the boundary of the cytoplasm, just underneath the cell wall. Consisting of a phospholipid bilayer with proteins embedded in it, the cell membrane performs numerous functions for the bacterial cell. The major function is to regulate what materials are allowed into and out of the cell. Nutrients must be transported across the cell membrane into the cell, while waste products must be transported out of the cell. Reactions for cellular respiration and adenosine triphosphate synthesis also occur in the cell membrane, as do reactions necessary for photosynthesis in photosynthetic bacteria. In some bacteria, the cell membrane folds inward to form mesosomes, protrusions of the membrane into the cytoplasm. The purpose of mesosomes is to increase the surface area over which cellular respiration can occur. Whereas bacterial membrane lipids have bonds called ester linkages connecting the glycerol and fatty acids, archaean membranes have bonds called ether linkages and substitute isoprene for the fatty acids in the membrane phospholipids.

In 1884 Hans Christian Gram discovered a staining procedure that allowed one to distinguish between cells with significant physical differences in the structure of their cell envelopes. The procedure involves an initial staining step with crystal violet, a purple dye that nonselectively binds and coats the surface of all bacterial cells. The addition of a mordant, Gram's iodine, causes large complexes with the crystal violet to form. These get trapped in the peptidoglycan of the cell wall. The next step is a decolorization step. Alcohol dissolves the lipids of the outer membrane and the crystal violet-iodine complexes wash out, unless there are too many layers of peptidoglycan present, which is the case in gram-positive cells. During the final step, a counterstain, safranin, stains the cells from which the crystal violet-iodine complexes were washed free, coloring them red. Cells that end up red are called gram-negative, and the purple ones are gram-positive. This staining procedure is the first step in the identification of a bacterial specimen in the laboratory.

Gram-positive cells have numerous (up to 40) layers of peptidoglycan lying external to their cell membrane. The cell walls of gram-positive bacteria also contain teichoic acids and lipoteichoic acids that are believed to function in cell wall maintenance and to regulate the cation (positively charged ion) levels at the cell's surface. The cell walls of gram-negative bacteria consist of a single peptidoglycan layer, making them more flexible than gram-positive cells. As in gram-positive cells, the cell wall lies just external to the cell membrane, but in gram-negative cells, external to the cell wall lies another membrane, the outer membrane. The structure of the outer membrane is a phospholipid bilayer, but it contains lipopolysaccharide (LPS) molecules arranged throughout it. The polysaccharide chains of LPS act as antigens, and the lipid portion of LPS acts as an endotoxin. In gram-negative infections, the endotoxin can cause fever and shock. The outer membrane is selectively permeable, but special channels called porins allow larger molecules to penetrate. The space in between the outer membrane and the cell wall is called the periplasmic space, and it is filled with hydrolytic enzymes and binding proteins.

EXTERNAL STRUCTURES

Some bacteria have appendages that aid in motility or attachment, allowing them to live or even thrive in certain conditions. Flagella are long, helical filaments, made primarily of the protein flagellin, that function to propel bacterial cells through an aqueous medium. They consist of three main parts: the basal body, the hook or sheath, and the filament. The basal body anchors the flagellum into the cell envelope and consists of a stack of rings embedded in the cell membrane and cell wall. A rod passes through the middle of the rings and attaches at the exterior end in an L-shaped hook. This structural arrangement allows the filament, which is attached to the hook, to rotate 360°, like a propeller of a boat motor, moving the cell forward as it spins. Flagella vary in number and arrangement. When located at one or both ends of a cell, the arrangement is termed polar. Monotrichous describes single, polar flagella; lophotrichous flagella occur in bunches extending from the one end; amphitrichous flagella emerge from both ends of the cell; and peritrichous flagella are spread over the whole surface of the cell.

The presence of flagella allows a bacterial cell to move in response to environmental stimuli, a behavior called chemotaxis. For example, a bacterial cell might move toward a desired nutrient, or away from a toxin. Clusters of receptors on the cell surface near the basal body detect the presence of the chemical agents and direct the basal body to rotate accordingly. Movement results from a combination of runs, smooth linear progressions, or tumbles, when the flagellum abruptly reverses direction or causes the cell to change direction. Some spirochetes have flagella that are enclosed between the cell membrane and the cell wall and make the cell move in a twisting motion.

A pilus is a single, long, hollow appendage that extends from a bacterial cell and functions in the exchange of genetic material. Only cells that have a certain genotype can participate in this type of genetic exchange called conjugation. The pilus, made of the protein pilin, originates from the cell membrane and provides a cytoplasmic connection between the two cells through which DNA can be transferred.

Fimbriae are smaller appendages made mostly of protein that protrude from all over a cell surface. They function in adherence of the bacterial cell to other surfaces such as rocks or host tissues during infection.

Bacteria often have a protective coating called a glycocalyx that lies external to the cell wall or the outer membrane of gram-negative bacteria. A loose covering is called a slime layer and protects against drying out and nutrient loss. Thicker coverings called capsules bind more tightly to the cell and give the colonies a mucoid or smooth appearance. Composed of repeating units of polysaccharides or proteins or both, capsules can also play a role in adherence and also in protection of pathogenic bacteria from phagocytosis and destruction.

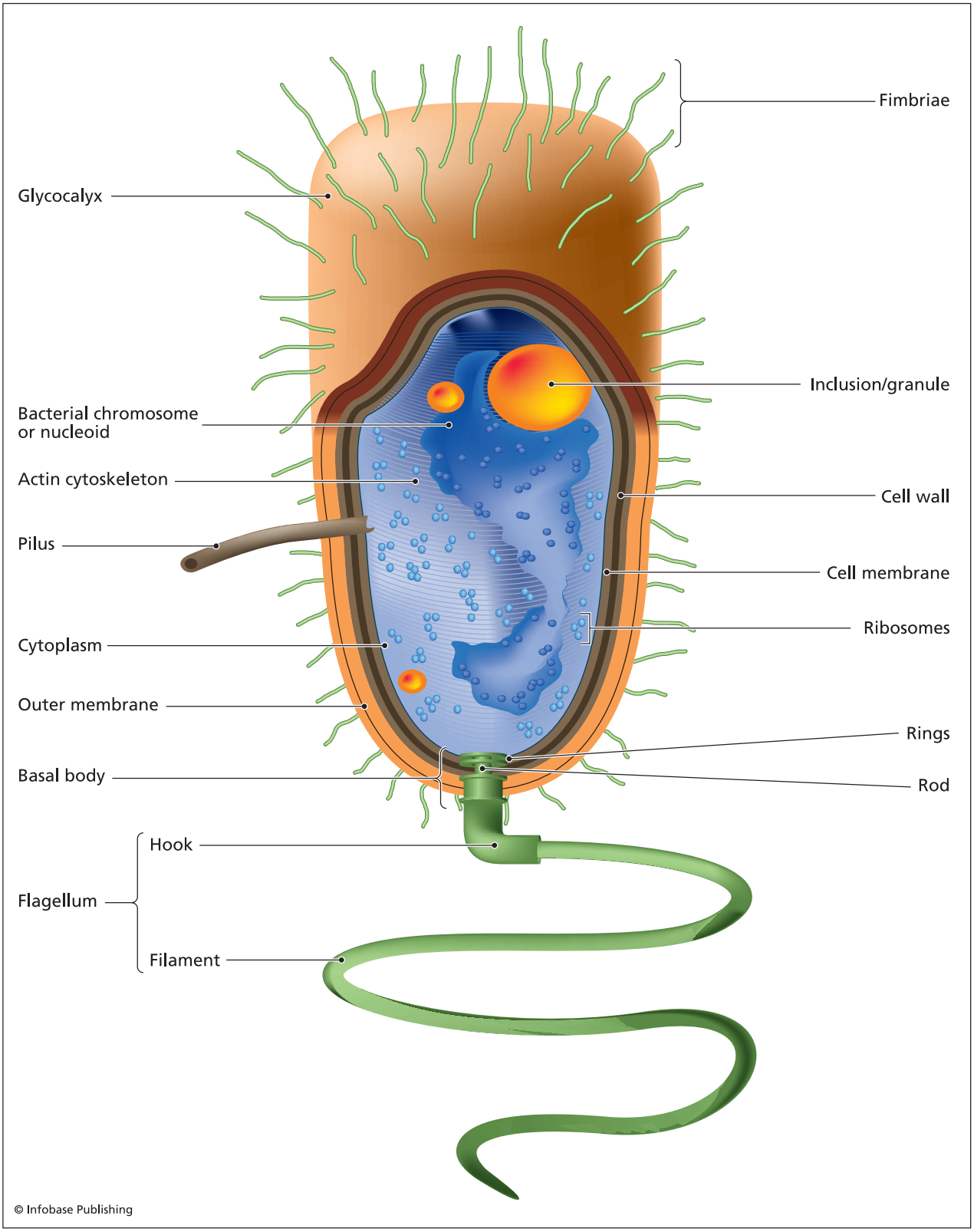
INTERNAL STRUCTURES

The gelatinous substance found inside the cell membrane is called the cytoplasm. Composed of 70 to 80 percent water, the cytoplasm serves as a solvent in which all the cell's biochemical reactions occur. Its components include amino acids, monosaccharides, ions, buffers, vitamins, and the cellular organelles and structures.

The nucleoid is the region that stores the genetic material. Bacteria typically possess one circular chromosome, approximately 5 million base pairs in length. The chromosome is not enclosed by a membrane, but rather condenses and aggregates in one area of the cytoplasm. Some bacteria also possess smaller circular plasmids that carry genes for traits such as antibiotic resistance, production of various toxins, or pili-mediated DNA transfer.

All prokaryotic cells must have ribosomes, the cellular apparatus responsible for protein synthesis. Ribosomes are made up of 60 percent ribosomal ribonucleic acid (rRNA), 40 percent protein, and consist of two subunits. A 30S subunit and a 50S subunit combine to form the 70S ribosome typical of prokaryotic organisms. Thousands of ribosomes are present at one time in a cell.

Bacteria can store excess nutrients such as polysaccharides or lipids in enclosures called inclusions or inclusion bodies. Other inclusion bodies store inorganic compounds like sulfur in photosynthetic bacteria or iron that functions to orient cells in a magnetic field. Structures similar to inclusions called



A single prokaryotic cell contains all the necessary structures and components to carry out the processes necessary to sustain life and reproduce.

gas vacuoles retain gases to give aquatic bacteria buoyancy. This maintains their position near the surface where the intensity of sunlight and levels of nutrients and oxygen are optimal.

In addition to the support provided by the rigid cell wall, some rod- and spiral-shaped bacteria receive additional support from an actin cytoskeleton located just under the cell membrane.

In adverse conditions such as nutrient deprivation, the absence of sufficient moisture or the buildup of acidic waste products, some bacteria (*Bacillus, Clostridium,* and *Sporosarcina*) can form endospores. During the vegetative portion of their life cycle, these bacteria actively grow and divide, but when conditions are no longer favorable, they undergo sporulation. The cells can exist as inactive, resistant endospores for an indefinite length of time until the environmental conditions return to favorable. The cell's return to an actively metabolizing, growing, and reproducing cell is called germination. As endospores, the bacterial cells are extremely resistant to desiccation, heat, and ultraviolet radiation, making sterilization difficult.

See also ARCHAEA; BACTERIA (EUBACTERIA); BIOLOGICAL MEMBRANES; BIOMOLECULES; CELLULAR METABOLISM; EUKARYOTIC CELLS; GENE EXPRESSION; MICROBIOLOGY; WHITTAKER, ROBERT; WOESE, CARL.

FURTHER READING

Panno, Joseph. *The Cell: Evolution of the First Organism.* New York: Facts On File, 2004.

Sullivan, James A. "Cells Alive!" Available online. URL: http://www.cellsalive.com/. Accessed January 15, 2008.

Talaro, Kathleen Park. *Foundations in Microbiology.* 6th ed. New York: McGraw Hill, 2008.

Tortora, Gerald J., Berdell R. Funke, and Christine L. Case. *Microbiology: An Introduction.* 9th ed. San Francisco: Benjamin Cummings, 2006.

protozoa According to the traditional five-kingdom classification system, the eukaryotic kingdom Protista consists of two major groups: protozoa and algae. Newer classification schemes do not combine all of the organisms that formerly belonged to the kingdom Protista into a single kingdom, and some propose as many as 18. Because classification of the domain Eukarya is in transition and not all biologists agree as to how this should be done, this entry will consider protists to be an informal category encompassing eukaryotic unicellular or colonial organisms that lack true tissues, thus including both protozoa and algae. The major difference between the two is that algae are photosynthetic whereas protozoa are heterotrophic, meaning they must obtain their nutrition from organic molecules in the environment. Though they are unicellular, protozoa exhibit a wide range of strategies for movement, feeding, and other behaviors. Of the 65,000 identified protozoan species, most are free-living and inhabit aquatic environments or moist soil, and only a few are pathogenic to humans. All protozoa can reproduce asexually and most can also undergo some form of sexual reproduction.

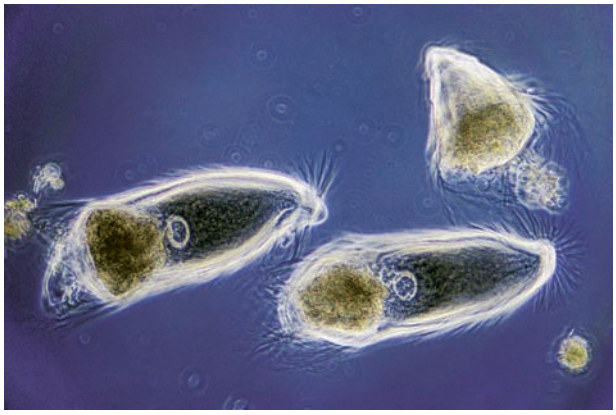
The average size of a protozoan cell ranges from about 10 to 200 micrometers (μm, 1 μm = 10^{-6} m) though some are as long as 0.16 inches (4 mm). Protozoa possess all the typical eukaryotic cell structures except chloroplasts. Their cytoplasm consists of the ectoplasm and the endoplasm. The outer ectoplasm functions in feeding, motility, and protection. The endoplasm is the inner portion of cytoplasm that houses the nucleus and other organelles. Some protozoa have unique structures for feeding, while others absorb food directly through their cell membranes. Motility is accomplished through flagella, cilia, or pseudopods. Structures called contractile vacuoles remove excess water from the cell, especially in freshwater species.

Many protozoa exhibit a life cycle that includes trophozoite and cyst stages. The trophozoite, or vegetative feeding form, rounds up and becomes immotile if adequate nutrients or moisture are not available. A tough wall forms around the cell, which becomes a dormant, resting structure called a cyst that persists until moisture and nutrient levels are sufficient. When the conditions return to favorable, the cyst wall breaks open and releases an active trophozoite. Some protozoa cannot form cysts, and therefore are less likely to survive changes in environmental conditions.

TYPES OF PROTOZOA

Due to their tremendous diversity, classification of protozoa is complex. The traditional approach groups organisms together based on common means of motility, rather than evolutionary history or relationships. In fact, molecular data suggests that the evolutionary distance between some organisms grouped together based on motility mechanisms appears to be greater than the evolutionary distance between plants and animals.

The Mastigophora, or the flagellates, share the distinguishing characteristic of having one or more flagella. Locomotion occurs when the flagella beat in an undulating motion, with a wave initiating at the junction of the cell body and the flagellum and traveling toward the end of the flagellum. The flagella of some mastigophora are arranged in a unique pattern. Flagellated protozoa that are sessile use their flagella to gather food, rather than to move from one location to another. Because the system for grouping protozoa based on locomotory organelles is not phylogenetic,



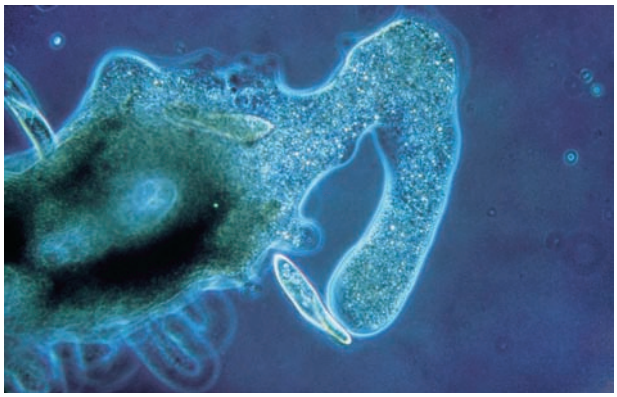
Trichonympha is a flagellated protozoan that lives in the gut of termites and feeds on ingested wood particles. *(Michael Abbey/Photo Researchers, Inc.)*

the flagellates actually consist of two vaguely distinct groups, the plantlike flagellates (Phytomastigophora) and the animal-like flagellates (Zoomastigophora). The plantlike flagellates are considered algae because they have chloroplasts that carry out photosynthesis. Flagellates reproduce asexually by binary fission, in which a cell splits into two genetically identical, equally sized daughter cells, and some also reproduce sexually by syngamy, the fusion of two gametes. Most flagellates are free-living, but some are parasitic—for example, *Trypanosoma* (a blood pathogen), *Giardia* (an intestinal parasite), and *Trichomonas* (causes vaginitis). In a tripartite symbiosis, some flagellates (for example, *Trichonympha*) live inside the digestive tract of wood-eating termites and digest cellulose from the wood. Living within the flagellates are prokaryotic endosymbionts that probably perform tasks such as nitrogen fixation, ammonium assimilation, and hydrogen metabolism.

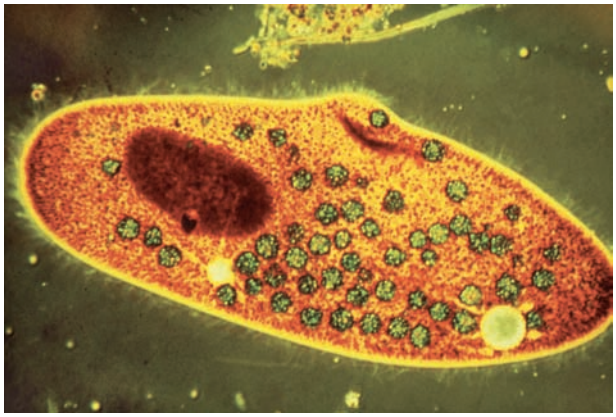
Members of the phylum Sarcodina, or amoebas, possess unique structures called pseudopodia, meaning false feet, cytoplasmic protrusions that function in locomotion and in gathering food. The pseudopodia of some sarcodines are blunt, lobelike cytoplasmic projections, and others are long, firm, needlelike projections supported by microtubules. Amoebas that are encased in shells extend their pseudopodia through pores. The calcareous shells, or tests, from amoebas such as foraminifers and radiolarians form layers of chalk deposits in the ocean. To move, an amoeba extrudes a pseudopodium from the cell body, anchors it to a surface, and then pulls the rest of the cell in that direction by contracting its body. When an amoeba senses nearby food such as bacteria, algal cells, or other protozoa, the pseudopodia reach around to envelope the particle, form a vacuole around it, and digest it into nutrients the organism can use. Indigestible material leaves the cell by exocytosis. Most amoebas are free-living, but a few parasitic forms exist, such as *Entamoeba histolytica* (causes dysentery). Protozoa reproduce by fission and can form cysts.

The phylum Ciliophora includes the protozoa, such as *Paramecium,* that possess hundreds of cilia, short, dense, hairlike structures used for locomotion and feeding. Cilia usually occur in organized rows called kineties but also in tufts. The cilia beat in a coordinated fashion to propel through the environment and to move food particles toward the mouth. Most ciliates produce cysts, have a mouth, are harmless, and contain two types of nuclei: one large macronucleus and several micronuclei. The macronucleus contains thousands of short pieces of DNA and is involved in regulation of the cell cycle. The diploid micronuclei contain two copies of each chromosome and divide by mitosis. Micronuclei are necessary for sexual reproduction by conjugation, a process during which individuals exchange genetic information but do not increase in numbers. Ciliates reproduce asexually by binary fission. The only ciliate known to be a human pathogen is *Balantidium coli,* which infects the intestine and causes diarrhea.

Members of the protozoan phylum Apicomplexa are parasitic and contain no specialized locomotory structures but sometimes move by bending or creeping. This phylum was formerly called Sporozoa because many members produce spores, but Apicomplexa is preferred since they all have specialized organelles that form an apical complex at their anterior end. Microtubules extend from the apical complex to provide structural support for the cell, and enzymes produced in organelles of the complex assist in penetration of host tissues during infection. Apicomplexans have complicated life cycles that include both asexual and sexual stages and that involve at



Amoeba feed on other organisms such as *Paramecium* by extending pseudopodia around the organisms and engulfing them. *(Eric Grave/Photo Researchers, Inc.)*



Paramecium caudatum displays cilia characteristic of protozoa that belong to the phylum Ciliophora. *(Eric Grave/Photo Researchers, Inc.)*

least one host species. Following sexual reproduction, apicomplexans form thick-walled zygotes called oocysts that undergo meiosis, giving rise to sporozoites, specialized sporelike cells that play a role in the transmission of infection. Asexual reproduction occurs by repeated fissions to form many cells. Human diseases caused by apicomplexans include malaria, caused by *Plasmodium*; toxoplasmosis, caused by *Toxoplasma gondii*; and cryptosporidiosis, caused by *Cryptosporidium*.

PATHOGENIC PROTOZOA

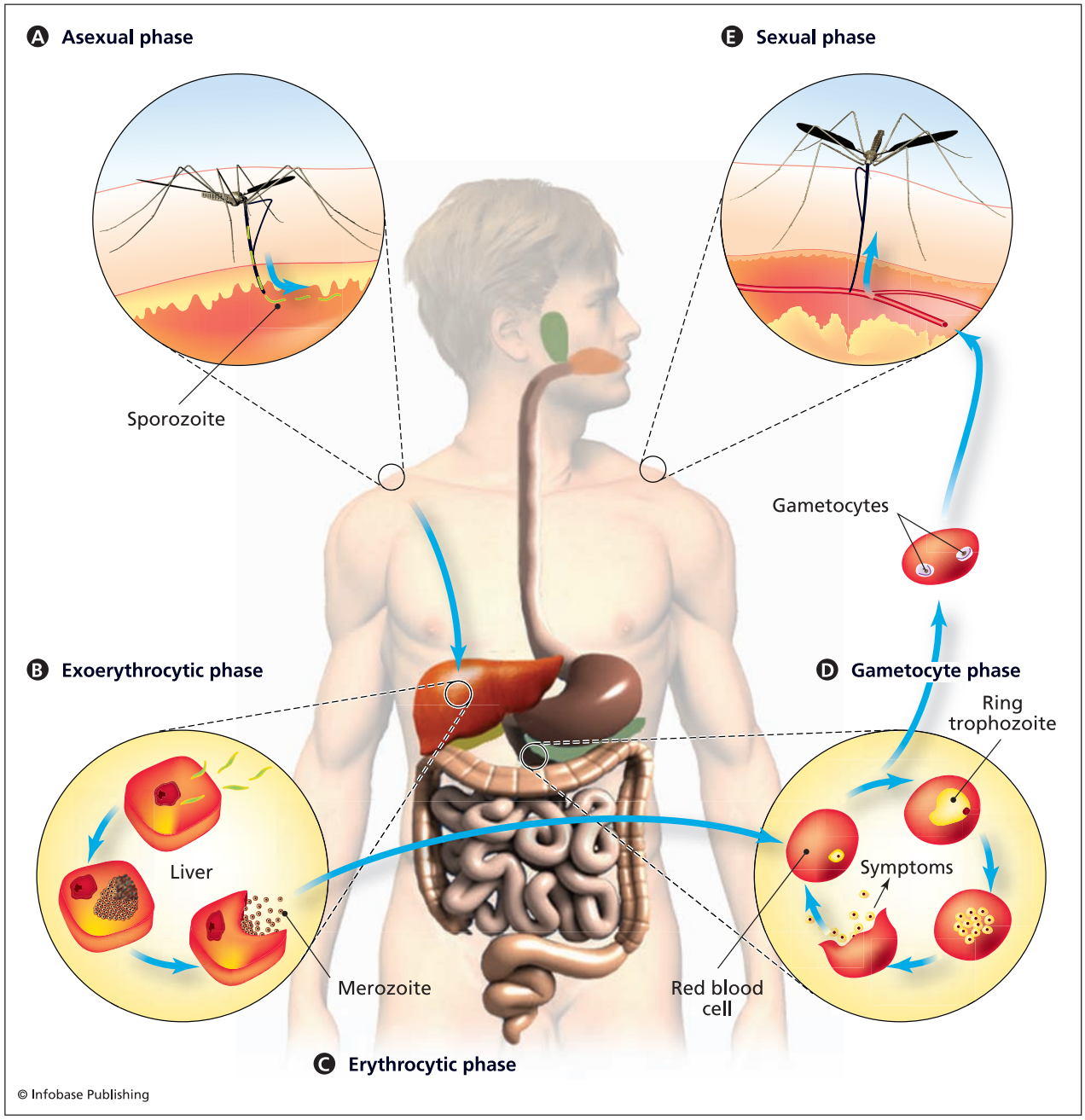
Though relatively few species of protozoa are pathogenic to humans, the ones that are affect millions of people each year. The four mentioned groups of protozoa all contain species that are pathogenic to humans or animals, but all of the members belonging to the phylum Apicomplexa are parasitic.

A common infective amoeba is *Entamoeba histolytica,* the causative organism of amebiasis and its more severe version, amoebic dysentery. An infected individual can be asymptomatic, or have mild symptoms including diarrhea, stomach aches, and cramping. More severe cases are called ameobic dysentery and involve bloody stools, intense stomach pain, and fever. A person becomes infected by ingesting resistant cysts, which germinate upon entering the small intestine. In the trophozoite stage, the amoebas migrate to the large intestine where they feed, grow, and divide. The trophozoites can penetrate the intestinal lining and travel to the liver, lungs, or brain, but this is rare. Some of the trophozoites in the intestine will form cysts that exit the body in feces. Treatment usually consists of prescribed antibiotics. Lack of sewage treatment facilities, improper treatment of sewage, and poor sanitation practices cause the spread of this disease. The cysts can persist in soil and water or on contaminated foods.

Giardiasis and trichomoniasis are examples of diseases caused by flagellates that are common in the United States. Giardiasis is caused by *Giardia lamblia,* also called *Giardia intestinalis,* a protozoa that normally inhabits the digestive tract of many animals and even humans. When the organism overgrows, gastroenteritis results. Symptoms include diarrhea, fatigue, bloating, cramping, and flatulence, caused when a thick layer of *Giardia* coats the lining of the small intestine and prevents nutrient absorption. Many people never show any symptoms and fight the infection without treatment, but some cases require antibiotics. Transmission of *Giardia* occurs by the fecal-oral route. Eating anything that has come into contact with the organism or swallowing contaminated water (even from recreational sources such as pools or hot tubs) can cause infection. Trichomoniasis is a common sexually transmitted disease that affects the urethra or vagina and can be asymptomatic or can cause painful urination, itching, and discharge. The causative organism is the flagellate *Trichomonas vaginalis,* and treatment includes antibiotics.

Four different species of *Plasmodium,* belonging to the phylum Apicomplexa, cause malaria. More than 41 percent of the world's population lives in areas threatened by malaria (parts of Africa, Asia, the Middle East, Central and South America, Hispaniola, and Oceania). After becoming infected, a person develops malaise, nausea, and sometimes diarrhea, followed by chills, fever, and sweating. These symptoms cycle every 48 to 72 hours. Malaria causes anemia from lysis of red blood cells and leads to liver, spleen, and kidney damage resulting from the accumulation of cellular debris. In some types of malaria, vessels in the brain can become blocked, causing death.

The life cycle of *Plasmodium* is complex, involving an asexual stage carried out in a human host and a sexual stage in the *Anopheles* mosquito. When a female *Anopheles* mosquito bites a human host, she injects an anticoagulant into the blood before sucking up her meal. If the mosquito is carrying the protozoa, sporozoites are also injected. The sporozoites enter the blood through a capillary and travel to the liver where they asexually reproduce to form thousands of merozoites per cell. After five to 16 days, infected liver cells burst open, releasing the merozoites into the bloodstream. The merozoites invade red blood cells, in which they develop into ring-shaped trophozoites and further asexual reproduction occurs, leading to the infection of more red blood cells. A pathologist can easily identify the ring-shaped trophozoites in a blood smear under the microscope. Microgametocytes and macrogametocytes form from the merozoites, and persist in the person's bloodstream until another mosquito bites the human host.



The complex life cycle of the apicomplexan that causes malaria, *Plasmodum vivax,* involves two hosts and asexual and sexual reproductive stages.

While feeding, the mosquito ingests both types of gametocytes, and inside the insect host the microgametocytes release spermlike gametes that fertilize the macrogametocytes, creating oocysts. After multiple mitotic divisions, the oocysts release sporozoites that can infect another human host when the mosquito seeks its next blood meal.

The use of bed netting dipped in insecticide and the elimination of standing water where mosquitoes can breed are two effective preventative measures for the spread of malaria. Quinine and its derivatives can be used to treat malaria caused by nonresistant strains of *Plasmodium*. The type of chemotherapeutic treatment prescribed depends on the strain *Plasmodium,* its drug-resistant status, the geographical region, the condition of the patient, and any other accompanying illnesses or conditions.

See also ALGAE; EUKARYA; EUKARYOTIC CELLS; INFECTIOUS DISEASES; MICROBIOLOGY; REPRODUCTION.

FURTHER READING

Cowan, Marjorie Kelly, and Kathleen Park Talaro. *Microbiology: A Systems Approach*. New York: McGraw Hill, 2006.

International Society of Protistologists home page. Available online. URL: http://www.uga.edu/~protozoa. Accessed January 22, 2008.

Lee, John J., Gordon F. Leedale, and Phyllis Bradbury, eds. *An Illustrated Guide to the Protozoa: Organisms Traditionally Referred to as Protozoa, or Newly Discovered Groups*. 2nd ed. 2 vols. Lawrence, Kans.: Society of Protozoologists, 2000.

Rohde, Klaus, ed. *Marine Parasitology*. Wallingford, England: CABI, 2005.

Sigee, David C. *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. Hoboken, N.J.: John Wiley & Sons, 2005.

Prusiner, Stanley (1942–) American *Biochemist, Neurologist* Stanley Prusiner discovered a new type of infectious particle, called prions, that consists only of protein. Though at first many doubted his claim asserting the existence of an infectious agent with no genome, today the prion theory is the leading explanation for the cause of neurodegenerative diseases such as Creutzfeldt-Jakob disease.

EARLY CAREER

Stanley Prusiner was born on May 28, 1942, in Des Moines, Iowa, to Lawrence Prusiner and the former Miriam Spigel. They later had another son named Paul. The family moved around a bit due to his father's naval career. They eventually settled in Ohio, where Stanley attended Walnut Hills High School and his father worked as an architect. Stanley studied Latin for five years, but otherwise claims his high school education was unremarkable.

After graduation, he majored in chemistry at the University of Pennsylvania, where he also joined the crew team. During the summer before his senior year, he participated in a research project on hypothermia in the laboratory of Sidney Wolfson in the department of surgery.

Prusiner obtained his bachelor's degree in 1964 and continued at the university to study medicine. As a medical student in collaboration with Britton Chance, he researched brown adipose tissue in hamsters awakening from hibernation. Brown adipose tissue is a type of fatty tissue that undergoes nonshivering thermogenesis, a metabolic process in which energy obtained from the breakdown of lipids is used to generate heat. Part of this research was performed in Stockholm at the Wenner-Gren Institute with Olov Lindberg. This experience awakened in Prusiner an interest in biomedical research.

In 1968 Prusiner returned to the United States, and, after completing his medical studies, he began a year-long internship sponsored by the National Institutes of Health at the University of California in San Francisco (UCSF). There he met his wife, a high school math teacher named Sandy Turk, with whom he has two daughters, Helen and Leah. Afterward, he joined Earl Stadman's laboratory at the NIH, where he studied glutaminases in the bacterium *Escherichia coli*. Glutaminases are enzymes that remove an amino group from the amino acid glutamine to form another amino acid, glutamate. They play an important role in nitrogen and energy metabolism. Prusiner attributes much of his scientific success to his three years spent at the NIH. He learned about the scientific process, many types of biochemical techniques and methods, and how to write research papers.

DISCOVERY OF PRIONS

In 1972 Prusiner began a residency in neurology at the UCSF. One of his early patients suffered from an infection called Creutzfeldt-Jakob disease (CJD).

Stanley Prusiner received the Nobel Prize in physiology or medicine in 1997 for discovering prions. *(AP Images)*

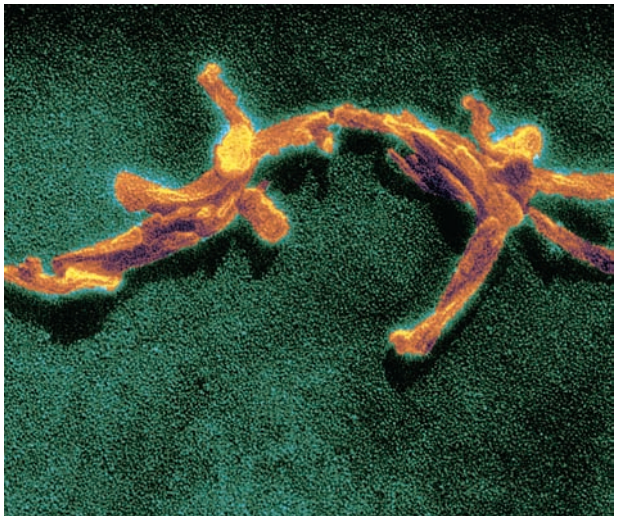
Symptoms included progressive memory loss, dementia, muscle incoordination, personality changes, impaired thinking, loss of the ability to move or speak, and eventual death. Scientists suspected that a "slow-virus" caused CJD. The causative agent, if a virus, had some unusual properties. Research suggested similarities between CJD and two other diseases: kuru of the Fore-people of New Guinea and scrapie of sheep. Scrapie was first described during the 18th century.

Prusiner was very interested in learning more about these diseases and read as much as he could find about them during his two-year residency. When UCSF offered him an assistant professorship in neurology, he accepted, anxious to start researching the structure of the "slow viruses." The fact that scrapie assays were tedious and expensive did not deter Prusiner, who also wrote a grant to obtain financial support from NIH for research on glutaminases.

His initial results suggested that the scrapie agent preparations contained protein but not nucleic acid. This confused him because all viruses minimally consisted of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) as their genetic material and a protein coat surrounding the nucleic acid. In fact, all other known infectious agents also had genomes: bacteria, fungi, parasitic protozoans, and worms. To compound his worries, Prusiner's financial support was discontinued, and UCSF denied him tenure. With support from colleagues, he ended up receiving tenure in 1980.

After convincing himself that his data was not artifactual, Prusiner wrote an article describing his findings that the causative agent of scrapie was a "prion," a term he invented by combining protein and infectious. The article, titled "Novel Proteinaceous Infectious Particles Cause Scrapie," appeared in the journal *Science* in 1982. Skeptical, many virologists performed intensive searches for the elusive nucleic acid of the scrapie virus, but none have been successful.

Meanwhile, others in Prusiner's lab isolated the scrapie protein and determined part of the amino acid sequence. In collaboration with Charles Weissmann, a Hungarian-born Swiss molecular biologist who currently heads the Scripps Florida Department of Infectology, the prion protein (PrP) gene was cloned. The researchers synthesized antibodies that recognized PrP, facilitating many of their biochemical studies. Stephen DeArmond, currently a professor of pathology at UCSF, studied the pathogenesis of the disease in transgenic mice, into which they had inserted the PrP gene. Others examined incubation time, uncovered a related genetic mutation, and explored the structure of the prion proteins. By the 1990s, the scientific community had learned much about prions, though the mechanism by which normal PrP transforms into disease-causing PrP is still being elucidated.



Prions are infectious molecules of protein that are often associated with illnesses of the nervous system. This transmission electron micrograph shows prions isolated from the brain of a hamster infected with scrapie-associated fibril. *(Eye of Science/Photo Researchers, Inc.)*

Prion proteins normally exist in a harmless form. Scientists have discovered that nascent prions form either by the spontaneous mutation of a host protein or by the exposure to an exogenous source of the prion protein, designated PrPSc. It should be noted that the normal and abnormal prion protein forms have identical amino acid sequences; they simply have different three-dimensional structures. Even though prions have no nucleic acid genome, they replicate by converting the normal endogenous PrPC, present in nervous tissue, into the infectious form. This transformation is characterized by a diminished presence of the alpha helices, one type of secondary structure assumed by polypeptides, and an increase in the amount of beta sheets, another type of secondary structure. As the abnormal form, PrPSc, accumulates, long aggregated filaments form and damage nervous tissue, causing the related symptoms. Because destruction of the nerve cells causes the tissue to take on a spongelike appearance, prion diseases are called spongiform encephalopathies. Prusiner's lab and others are working to elucidate the molecular mechanism by which PrPSc can instigate this structural transition of PrPC.

Prion diseases, such as scrapie, kuru, bovine spongiform encephalopathy (BSE), or CJD, arise in three different ways: they may be transmitted from one infected animal to another animal; mutations in the prion protein gene may be inherited from a parent; or a spontaneous mutation may occur.

Three categories of CJD exist in humans: sporadic, hereditary, and acquired. The most common form is sporadic CJD, for which the cause is unknown. In hereditary CJD, the patient either has a family history of the disease or tests positive for the mutation. Acquired CJD results from the horizontal transmission of the disease through exposure to infected tissue. PrPSc is resistant to treatments that typically destroy other infectious agents such as high temperatures, enzymatic degradation, and ultraviolet irradiation, making it difficult to establish preventative measures for people who come into contact with it. Absolute diagnosis of prion diseases can be made only by brain biopsy or autopsy. No treatment is available for patients suspected to have prion diseases, so brain biopsies are only performed in order to rule out other suspected causes of a patient's symptoms.

Prusiner became a full professor of neurology and of virology at UCSF in 1984, and a professor of biochemistry in 1988. The 1990s brought him numerous honor and awards, including the Potamkin Prize for Alzheimer's Disease Research of the American Academy of Neurology in 1991, the Christopher Columbus Quincentennial Discovery Award in Biomedical Research of the NIH in 1992, the Metropolitan Life Foundation Award for Medical Research in 1992, the University of Pittsburgh Dickson Prize in Medicine in 1992, the Charles A. Dana Award for Pioneering Achievements in Health in 1992, the Richard Lounsbery Award for Extraordinary Scientific Research of the National Academy of Sciences in 1993, the Gairdner Foundation Award for Outstanding Achievement in Medical Science in 1993, the Bristol-Meyers Squibb Award for Distinguished Achievement in Neuroscience Research in 1994, the Albert Lasker Award for Basic Medical Research in 1994, the Paul Ehrlich Prize of the Paul Ehrlich Foundation and the Federal Republic of Germany in 1995, the Wolf Prize in Medicine of the Wolf Foundation and the State of Israel in 1996, and the Keio International Award for Medical Science of Keio University in 1996. In 1997 Stanley Prusiner won the Nobel Prize in physiology or medicine "for his discovery of prions—a new biological principle of infection."

Prusiner continues researching prions at UCSF, where he serves as director of the Institute for Neurodegenerative Diseases and remains a full professor of neurology and biochemistry.

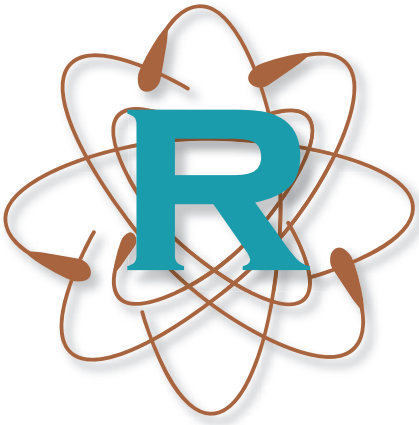
See also VIRUSES AND OTHER INFECTIOUS PARTICLES.

FURTHER READING

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1997." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1997. Accessed January 25, 2008.

Prusiner Laboratory Web page. Available online. URL: http://prusinerlab.ucsf.edu/. Updated September 26, 2008.

Prusiner, Stanley B. "Novel Proteinaceous Infectious Particles Cause Scrapie." *Science* 216 (1982): 136–144.



radioactivity The physical phenomenon of radioactivity is characterized by the spontaneous emission of energy from an unstable atomic nucleus. As the basic unit of matter, an atom is the smallest particle of a chemical element that retains the properties of that element. The central nucleus of an atom holds positively charged protons and neutral neutrons, while negatively charged electrons orbit the nucleus in discrete energy levels. The number of protons in an atomic nucleus (called the atomic number) determines what element an atom is; for example, an atom with six protons is always carbon, and an atom with 15 protons is always phosphorus. Both protons and neutrons contribute to the atomic mass.

Because protons are all positively charged, they naturally tend to repel each other. Neutrons help provide some distance between protons in a nucleus and also attract other neutrons and protons within a short range, helping to keep the atomic nucleus intact. The number of neutrons can vary between atoms of the same element. Isotopes are atoms that have the same number of protons but different numbers of neutrons; they are the same element, but have different atomic masses. Two isotopes of carbon, carbon 14 and carbon 12, can be represented as follows:

$$^{A}_{Z}C_{N} : {}^{14}_{6}C_{8} \text{ or } {}^{12}_{6}C_{6}$$

where C is the abbreviation for the chemical element carbon, A equals the atomic mass, Z equals the number of protons, and N equals the number of neutrons. Atoms are electrically neutral, so the number of protons equals the number of electrons. Since the arrangement of electrons in an atom, particularly the composition of the valence or outermost electron shell, determines the chemical properties of an atom, different isotopes generally have the same chemical properties. A nuclide is a nucleus with a specific number of protons and neutrons. The terms nuclide and isotope are sometimes used interchangeably, but nuclide refers to the contents of the nucleus, and isotope refers to the entire atom. In order to be stable, the ratio of neutrons to protons of a nuclide must fall within a specific range. When the ratio falls outside of that narrow range, the nuclide is unstable and releases radioactive emissions in an attempt to gain stability. This process is known as radioactive decay.

Most elements have naturally occurring isotopes, but many are very unstable and do not persist for long. The half-life of an isotope is a measure of the stability of that isotope and equals the amount of time it takes for half of the original amount of that isotope to decay by spontaneous disintegration of the unstable nuclide. This is an exponential function; after one half-life, half of the original material will remain, but only one-fourth will remain after two half-lives, and only one-eighth after three half-lives. Chemical or physical factors do not affect the half-life of a radioisotope. Some naturally radioactive elements have extremely long half-lives; for example, uranium 238 has a half-life of 4.5 billion years. In contrast, some have extremely short half-lives; oxygen 22 has a half-life of 2.25 seconds.

The three main types of radioactive decay include the emission of alpha particles, beta particles, and gamma rays. In alpha decay, the number of protons is too great, and in order to reduce the repulsion of the positive charges, the nucleus emits two protons and two neutrons, in other words, a helium nucleus. This reduces the mass of the atom by four atomic

mass units. Alpha particles are slow and heavy and cannot penetrate through a sheet of paper. Because they have a strong positive charge, they can ionize surrounding atoms by stealing electrons from them, causing damage. An example of alpha decay is the conversion of uranium 235 to thorium 231.

$$^{235}_{92}U_{143} \rightarrow {}^{231}_{90}Th_{141} + {}^{4}_{2}He_{2}$$

Beta decay can occur by three different methods. If the number of neutrons is too high, a neutron converts to a proton and an electron, and the electron (referred to as a beta particle in this context) is emitted. This does not change an atom's mass, but the atomic number does increase by one. Beta particles are faster and lighter than alpha particles and have greater penetrating powers, but they do not cause ionization of other atoms as strongly as alpha particles. One example of beta decay is the decay of carbon 14 to nitrogen 14.

$$^{14}_{6}C_{8} \rightarrow {}^{14}_{7}N_{7} + \beta^{-}$$

where β^{-} indicates the emission of a beta particle with a negative charge, in other words, an electron. Another type of beta decay involves the conversion of a proton to a neutron and the accompanying emission of a positron (represented by β^{+}), a subatomic particle with a positive charge but the mass of an electron (essentially no mass). Positron emission occurs when the neutron to proton ratio is too low and results in a decreased atomic number, but the atomic mass stays the same.

$$^{7}_{4}Be_{3} \rightarrow {}^{7}_{3}Li_{4} + \beta^{+}$$

Alternatively, in electron capture, the third type of beta decay, the nucleus may capture an electron in order to remedy a low neutron to proton ratio. The captured electron converts a proton to a neutron, thus the atomic number decreases by one, but the mass stays the same.

$$^{7}_{4}Be_{3} + \beta^{-} \rightarrow {}^{7}_{3}Li_{4}$$

In gamma decay, the energy contained within the nucleus is too high, so the nucleus emits a high-energy, massless particle called a photon. Gamma rays have high penetrating powers, thus must be blocked by a thick sheet of lead or concrete. Though gamma rays do not ionize other atoms directly, they can induce other atoms to emit particles that can ionize other atoms. Heavy elements spontaneously decay by fission, in which the nucleus splits into two roughly equal parts and releases an enormous amount of energy.

USES OF RADIOACTIVITY IN THE LIFE SCIENCES

Life science research depends on the use of radioactive isotopes in many ways. Cell and molecular biologists and biochemists use radioactive isotopes such as sulfur 35 to label proteins, or phosphorus 32 or tritium (hydrogen 3) to label nucleic acids, allowing the researchers to follow the movement of the labeled molecules throughout cells, to identify specific molecules, and to locate their positions on a gel after electrophoresis. Physiologists can follow the absorption and metabolism of different minerals and nutrients using radioisotopes such as calcium 45 and iodine 131.

Medical science uses radioactive isotopes in diagnostic testing and treatments. X-rays, a form of gamma radiation, are useful for situations in which the physician needs to observe structures inside of the body, for example, in order to identify tubercles in the lungs in the case of tuberculosis, diagnose broken bones, detect colon cancer, or look for dental cavities. Radioisotopes such as iodine 123 can be used to evaluate problems such as blocked kidneys. Radiation is also used to sterilize medical instruments that cannot withstand heat and to prevent spoilage of food by destroying bacteria and mold. Cancer treatments include targeting gamma radiation at tumors or placing pellets or liquids containing radioactive isotopes inside the body to kill cancer cells.

Carbon dating is a technique dependent on naturally occurring radioisotopes that allows paleontologists to estimate the age of fossils. Living organisms continually cycle the element carbon throughout their bodies and contain a known proportion of carbon 14 to carbon 12. After death, an organism no longer brings in or eliminates carbon from the body, yet the carbon 14 that is present will continue to decay to carbon 12. The decrease in the ratio of carbon 14 to carbon 12 allows one to estimate how much time has passed since the organism stopped bringing in new carbon 14. Because the half-life of carbon 14 is only about 5,730 years, this particular isotope is useful only for dating remains that are 70,000 to 35,000 years old or less.

Radioisotopes and radiation are also used for many applications outside of the life sciences. Smoke alarms incorporate radioisotopes that emit alpha particles, which ionize the air. This ionization creates a weak electric current that the presence of smoke disrupts by absorbing the alpha particles, causing the alarm to sound. Nuclear power stations convert the energy liberated during fission reactions (the splitting of radioactive nuclides) to useable electrical energy. The source of the destructive power associated with nuclear weapons is the enormous amount of energy released during fission of heavy radioactive elements

such as uranium 235. The injection of low levels of radioisotopes into pipes helps locate underground leaks, and gamma radiation helps detect weak spots after welding. Because radiation is dangerous and can cause radiation sickness and cancer, people who work with radioactivity must take preventative measures to limit exposure and monitor their exposure carefully.

See also CHEMICAL BASIS OF LIFE.

FURTHER READING

L'Annunziata, Michael F. *Handbook of Radioactivity Analysis*. 2nd ed. San Diego, Calif.: Academic Press, 2003.

Lowenthal, G. C., and P. L. Airey. *Practical Applications of Radioactivity and Nuclear Radiations: An Introductory Text for Engineers, Scientists, Teachers, and Students*. New York: Cambridge University Press, 2001.

Magill, Joseph, and Jean Galy. *Radioactivity, Radionuclides, Radiation*. New York: Springer-Verlag, 2005.

Myers, Rusty L. *The Basics of Physics*. Westport, Conn.: Greenwood Press, 2006.

Strathern, Paul. *Curie and Radioactivity*. New York: Anchor Books, 1997.

recombinant DNA technology

In the early 1970s, an American biochemist at Stanford University, Paul Berg, revolutionized the life sciences when he pioneered recombinant DNA technology. Sometimes referred to as gene splicing or genetic engineering, recombinant DNA technology refers to a set of methodologies used to cut up DNA, isolate fragments, and recombine genetic material from different organisms. Since their initial development, these tools have allowed genetic researchers to describe the function of thousands of genes, examine the effects of various mutations, determine the sequence of genomes from hundreds of organisms, synthesize drugs and chemicals for medicinal and commercial use, and advance agriculture by genetically modifying plants and animals to improve their yield, hardiness, nutritional value, and marketability.

Paul Berg began his studies on mammalian gene expression and regulation using SV40, a tumor virus whose DNA persists as a minichromosome in the nuclei of infected host cells. Scientists had already taken advantage of the fact that viruses often pick up DNA from a host cell during packaging and carry it to a new host. This naturally occurring phenomenon, called transduction, had provided much information regarding the organization and expression of prokaryotic genes, but information about eukaryotic genomes lagged behind. Scientists had even used transduction to clone and amplify segments of DNA from bacteria for further study. Berg wondered if a similar process would allow for the transfer of genes into mammalian cells. To examine the likelihood of this possibility, he first set out to synthesize an artificial SV40 genome, a task that necessitated the joining together of different molecules of DNA. With colleagues David Jackson and Robert Symons, Berg first used restriction enzymes to produce cohesive ends on the different molecules of DNA. This allowed the SV40 DNA to anneal with the other DNA molecule, specifically a bacterial plasmid that contained bacteriophage lambda (λ) DNA and three *E. coli* genes for enzymes necessary for galactose utilization. Berg also treated the molecules with an exonuclease, followed by an enzyme to add adenine residues to the end of one strand and thymine residues to the end of the other strand. This allowed the artificially created tails to anneal to one another, as well as the cohesive ends created by the endonuclease activity to anneal to one another, forming a complete circle. DNA polymerase was used to fill in gaps, and ligase formed the final phosphodiester bonds. By these methods, Berg successfully constructed the first recombinant genome. The development of this technology has enabled researchers to analyze in detail the relationship between the chemical structure of DNA and its function. Berg received the Nobel Prize in chemistry in 1980 for his studies on nucleic acids and for developing recombinant DNA technology.

Recognizing the possible implications and dangers of having the ability to manipulate DNA from virtually any organism, Berg initiated a worldwide discussion concerning the hypothetical risks of recombinant DNA technology. Global dialogue about human safety and environmental effects ensued, and the debate over the creation and release of genetically modified organisms continues today. Because of its usefulness, however, recombinant DNA technology has permeated all fields of life science research, ranging from molecular genetics to population ecology and from cell biology to health and medicine.

TOOLS OF RECOMBINANT DNA TECHNOLOGY

Working with nucleic acids in the laboratory is relatively easy. DNA is soluble in aqueous solutions, and methods for isolating it from different sources and for purifying it have been well established. Depending on the source and characteristics of the DNA being isolated, slightly different procedures might be necessary. When working with eukaryotic cells, one might first need to break open the cells and isolate certain organelles (nuclei, mitochondria, or chloroplasts). Bacterial cells do not contain any nuclei or other membrane-bound organelles. Breaking open cells might involve treatment with enzymes,

harsher chemicals, or mechanical efforts. Once the nucleic acid is released, treatment with proteinases and RNases will break down any unwanted proteins and RNA that are present. Extraction with chemicals such as phenol and chloroform will also remove proteins or other organic substances, while leaving the nucleic acid in the aqueous phase. The addition of salt and alcohol will cause DNA to precipitate out of solution and then form a pellet at the bottom of a tube during centrifugation. The pellet is typically resuspended in a buffer solution containing Tris, which maintains a slightly basic pH, and often also a chemical reagent for chelating any divalent cations that might encourage contaminating nucleases to chew up the desired DNA.

Enzymes are indispensable tools in molecular biological research. Restriction endonucleases are a class of enzymes that recognize specific sequences of DNA and cleave the DNA at those sites. Bacteria naturally produce these enzymes to destroy viral or other DNA, but their own DNA is protected from cleavage by methylation, the addition of methyl groups to certain nucleotide bases. The names of the enzymes often reflect the source organism. For example, the restriction enzyme *Eco*RI is derived from *E. coli,* and *Pvu*I comes from *Proteus vulgaris* (*P. vulgaris*). The name restriction endonuclease comes from the fact that the presence of these enzymes in bacteria restricts the host range of a virus, and the fact that the enzyme digests the DNA from within the molecules, rather than from the end. Enzymes that digest DNA from the ends are called exonucleases, rather than endonucleases. The target sequences of restriction endonucelases are usually between four and eight nucleotide base pairs long and are palindromic, meaning they read the same backward and forward. Because DNA exists as double strands and the strands are antiparallel, one must follow the same directionality to detect the symmetry. For example, the previously mentioned enzyme *Eco*RI recognizes and cuts at the asterisks on both strands.

5′ - G*A A T T C - 3′
3′ - C T T A A*G - 5′

EcoRI always recognizes the same six nucleotide base pair sequence, and always cuts between the G and the first A. This leaves overhanging single-stranded ends, as depicted below.

--- G - 3′ 5′ - A A T T C ---
--- C T T A A - 5′ 3′ - G ---

Termini that overhang like this are called cohesive or sticky, and they may anneal with other DNA fragments that have been cleaved by the same enzyme, since the second molecule will have the same cohesive terminal sequences. Other restriction endonucleases leave blunt ends; for example, *Alu*I cleaves the following sequence at the asterisk.

5′ - A G*C T - 3′
3′ - T C*G A - 5′

Endonucleases that recognize shorter target sequences cleave more frequently than those that recognize longer sequences. For random DNA sequences, a four-base pair (bp) cutter will encounter its recognition site once every $4^4 = 256$ bp. In contrast, an eight-bp cutter will encounter its recognition site only once every $4^8 = 65{,}536$ bp. Sequences in an organism's genome are not completely random, however, so these values are simply estimates.

After a restriction digest, a laboratory worker often performs agarose gel electrophoresis to separate the DNA fragments based on their molecule weights. The DNA samples are placed within wells of a semisolid agarose matrix and subject to an electric current. Because DNA is negatively charged, the fragments migrate through the gel toward the positive electrode. The larger fragments move through the gel more slowly than smaller fragments, so, after a period of time, the DNA fragments will appear as a set of parallel bands that have traveled different distances from their original position in the wells. Running a molecular weight marker in one lane of the agarose gel allows the researcher to determine relative weights of the DNA fragments in the sample of interest.

Vectors are vehicles for carrying fragments of DNA in a bacterium or other type of host cell during a cloning procedure. Plasmids are small, closed circular DNA molecules that commonly serve as vectors. They exist naturally in many bacterial species, and range in size from a few thousand to about 14,000 bp. Useful vectors share several characteristics. They must have a selectable marker to facilitate detection of organisms that contain the vector. Antibiotic resistance genes are common selectable markers. Bacteria that contain the vector DNA will be able to grow in the presence of an antibiotic such as ampicillin or tetracycline. Vectors must also have a system for self-replication to ensure that the bacteria maintain the cloned DNA over several generations. The multiple cloning site is a region on the vector that contains numerous restriction endonuclease recognition sequences into which the researcher inserts the cloned fragment. In order to indicate clones that contain a vector that has the insert, the multiple cloning site is often inserted within another gene. If the cloned fragment is present, then the gene will

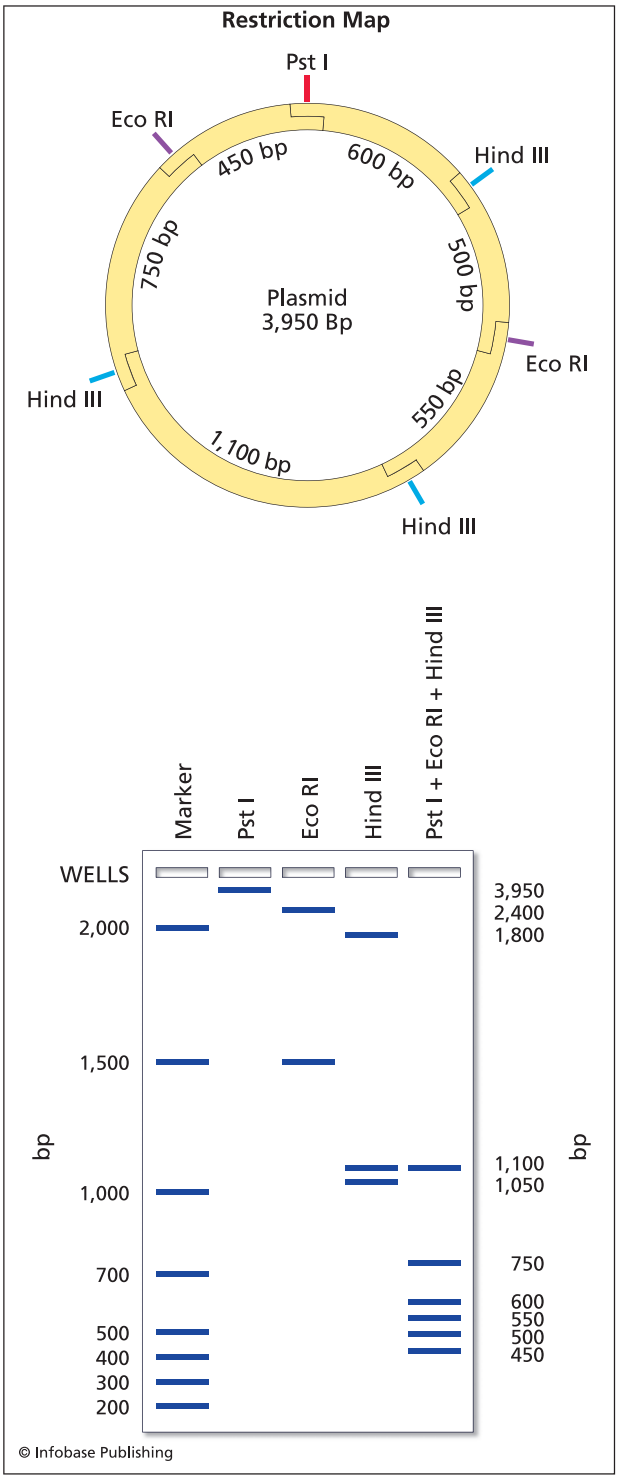
be disrupted and not express functional protein. For example, the β-galactosidase enzyme is necessary for a bacterium to utilize lactose. If the cloned gene has been inserted into the vector in the middle of the β-galactosidase gene, the bacterium will not be able to ferment the carbohydrate lactose.

The simplest method for cloning a fragment of DNA into a vector is to digest the cloned fragment and the vector with the same restriction endonuclease. After digestion, treatment of the vector sample with alkaline phosphatase will remove the phosphate groups. Plasmids must be dephosphorylated to prevent the plasmid ends from reannealing and closing the plasmid. The restricted DNA to be cloned is combined with the prepared vector, and the enzyme DNA ligase is added to form phosphodiester bonds between the cohesive termini on the vector and the insert.

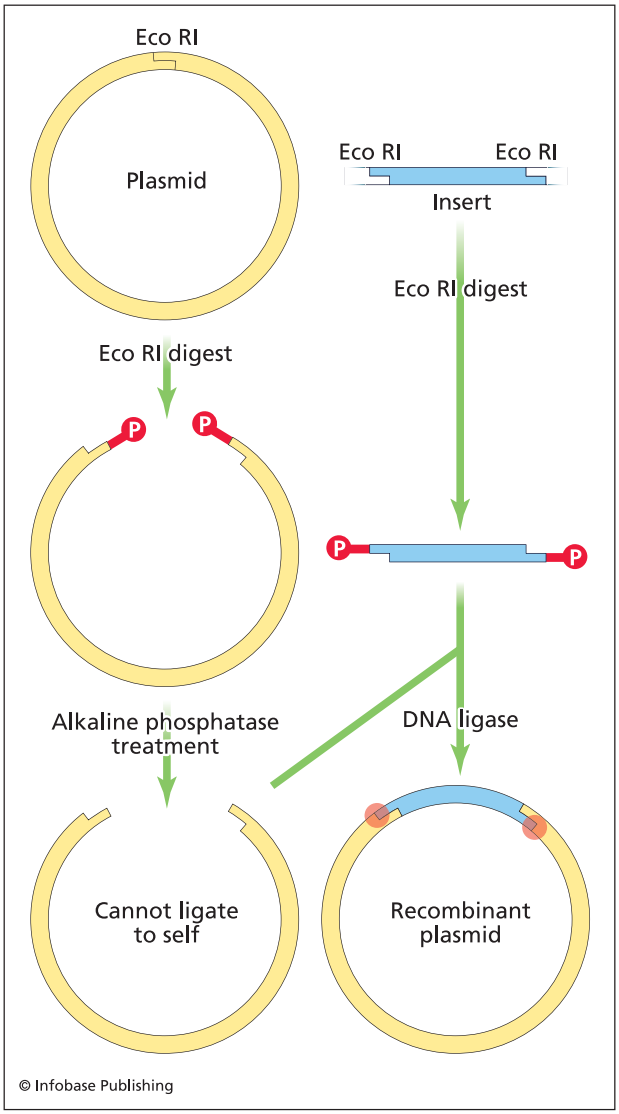
After successful insertion of the DNA, the recombinant DNA must be introduced into a bacterium for propagation. In order to get a plasmid vector into bacterial cells, the cells must be artificially treated to encourage them to uptake the naked plasmid DNA. The treatment typically involves calcium and cold temperatures; alternatively, providing a jolt of electricity also works, a technique called electroporation. Cells that have been treated in such a manner are called competent, and the procedure that causes the bacteria to take in the plasmid DNA is called transformation. Following the transformation, the bacteria are plated onto media that contains an antibiotic for which the plasmid contains a resistance gene. Thus, only bacteria resulting from successful transformations should grow. The colonies that do grow must then be screened for the presence of the insert. Transformation efficiencies typically decrease as plasmid size increases, with the upper limit approaching 20 kb.

Phage, viruses that infect bacteria, can also serve as vectors, but they are more difficult to deal with than plasmids. Lambda (λ) phage are common. The recombinant DNA is generated in the same fashion, but phage naturally infect bacterial cells, so less artificial manipulation is necessary to get the vector into the bacterial cells for propagation, and the efficiency is higher. Another advantage to using phage vectors is that they can carry larger pieces of DNA than a plasmid can, in the range of 12 to 20 kb. Other specialized vectors are required for cloning larger genomic DNA fragments. Cosmids, vectors that contain features of both plasmids and of lambda phage, can handle up to 40 kb inserts, and yeast artificial chromosomes can accommodate up to 1,000 kb. When phage-infected bacteria are plated onto an agar plate, plaques form. The plaques appear as a clearing on the plate, resulting from lysis of the bacterial cells that were in that location.

Growing up the organism that contains the recombinant DNA allows the researcher to isolate and purify large quantities of the DNA for further use or analysis. One can confirm the presence of the



Digesting the plasmid DNA with various enzymes alone or in combination allows one to map the restriction cut sites relative to one another on the plasmid. Gel electrophoresis reveals the number and size of fragments released by cutting DNA with a particular enzyme.



To insert DNA into a plasmid vector, after opening up a circular plasmid with an enzyme, the phosphate groups are removed, and the linear DNA is mixed with the insert DNA, which has been cleaved with the same enzyme. The complementary ends anneal and DNA ligase seals the gaps.

insert in the vector by several methods. Based on the strategy the researcher used to insert the DNA into the vector, the researcher may be able to directly sequence the DNA. Sequencing is a very definitive method for demonstrating the DNA is what one predicts. A quicker method is to perform a restriction digest and gel electrophoresis on the DNA. The researcher should be able to make predictions regarding the number and size of fragments depending on the specific endonuclease used. Most cloning systems have useful features that facilitate the screening process. While a selectable marker such as antibiotic resistance only allows bacteria that contain the vector to grow, one cannot know whether or not the vector contains the insert. Other tricks are necessary. The fermentation of lactose results in acid production. If the multiple cloning site is inside the β-galatosidase gene, which allows for lactose fermentation, then inclusion of lactose to the media will result in a decreased pH at colonies that do not contain the insert. Addition of a pH dye indicator to the media will color the colonies that contain the insert a different color from those that do not contain the insert.

Once sufficient quantities of the desired DNA have been obtained and purified, a researcher may perform further manipulations and analyses. Site-directed mutagenesis is a technique that allows a scientist to introduce mutations to a DNA sequence at specific positions using the polymerase chain reaction. Hybridization procedures, such as southern analysis or northern analysis, allow one to look for sequences that are complementary to other specific sequences. Gene mapping studies help reveal the relative locations of specific DNA markers along the length of a chromosome. The DNA may be sequenced, and bioinformatics analyses may aid in the identification of the gene or the protein it encodes. If the gene is cloned into an expression vector, one can generate large quantities of the gene product for investigation. The nucleic acid may also be used in assays for studying the function of DNA-binding proteins.

See also BIOINFORMATICS; BIOTECHNOLOGY; CLONING OF DNA; DEOXYRIBONUCLEIC ACID (DNA); DNA SEQUENCING; ELECTROPHORESIS; GENETIC ENGINEERING; MOLECULAR BIOLOGY; POLYMERASE CHAIN REACTION.

FURTHER READING

Birren, Bruce, Eric D. Green, Sue Klapholz, Richard M. Myers, and Jane Roskams. *Genome Analysis: A Laboratory Manual Series*. 4 vols. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1997.

Sambrook, Joseph, and David W. Russell. *Molecular Cloning: A Laboratory Manual*. 3rd ed. 3 vols. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2001.

reproduction One of the hallmarks of life is the ability to reproduce, to create new individuals of the same species. Any factor that inhibits the ability of a species to reproduce could result in the extinction of that species. While life-forms exhibit a wide variety of strategies for producing offspring, mechanisms can be divided into two main categories: asexual processes that involve a single parent and sexual processes that involve two parents. In general, simple organisms reproduce by asexual methods, whereas more complex organisms

undergo sexual reproduction. Prokaryotic organisms generally reproduce asexually by a process called binary fission. Most eukaryotic organisms reproduce sexually, but eukaryotic cells also reproduce asexually. Both forms of reproduction employ numerous strategies to overcome different environmental challenges and to ensure success in perpetuating the species.

In order to create new individuals, the genome, or the genetic material of an organism, must pass from one generation to the next. All organisms possess deoxyribonucleic acid (DNA) as their molecular carrier of genetic information. Contained within the DNA are the general blueprints or the instructions for how to build an organism of that species in addition to the characteristics unique to each individual within a species. The DNA exists on chromosomes. Prokaryotic organisms typically have one closed circular chromosome, while eukaryotic organisms have linear chromosomes. The number varies depending on the species. For example, alfalfa has 16 chromosomes, mosquitoes have six chromosomes, and humans have 46 chromosomes. The chromosome or chromosomes must be duplicated before being passed on to the next generation. In asexual reproductive methods, the parent organism copies and passes all of its genes to the offspring. In sexual reproduction, only half of a parent's genome passes to the offspring. Because two parents participate, the offspring still receives a complete genome.

ASEXUAL REPRODUCTION

In asexual reproduction, only one parent is involved, and all the offspring are genetically identical to the parent—in other words, they are clones. While asexual reproduction is simpler, faster, and requires less energy than sexual reproduction, it does not increase the genetic variability, making the population more vulnerable to changes in environmental conditions. Genetic diversity within a population is associated with the ability of that population to adapt to new environments. Some genetic differences can arise by mutations to the DNA of organisms that reproduce asexually.

Prokaryotic cells reproduce by binary fission, a process in which one parent cell divides into two daughter cells of the same size. The daughter cells are identical to each other and to the parent cell. After growing in overall size and increasing its cell membrane and cell wall components, a prokaryotic cell develops indentations in the middle of the cell. The chromosome attaches to a certain location on the cell membrane and is replicated. The indentations grow inward to form a transverse septum, a plane that divides the contents of the cell into two portions. As the cytoplasmic contents are divided, the separating cells also each receive one copy of the duplicated chromosome. New cell membrane components assemble on both sides of the septum, and eventually, constriction at the septum leads to the complete separation of the two cells. Theoretically, each generation results in a doubling of the total cell number. Under optimal conditions, binary fission can proceed very rapidly. A population of *Escherichia coli* can double every 20 minutes in the laboratory. Unicellular eukaryotes such as protozoa also reproduce by fission. In multiple fission, the nucleus divides several times before the cell divides, forming numerous daughters at one time.

Eukaryotic cells can reproduce asexually by mitotic cell division, a process similar to binary fission in that it results in the production of two identical daughter cells from a single parent cell, but different in that eukaryotic mitosis involves a highly orchestrated alignment and division of the multiple chromosomes carried out by a complex mitotic apparatus. In mitosis, all of the chromosomes are duplicated and divided into equal sets. When the cell divides into two daughter cells, each receives a complete set of chromosomes. Mitotic division increases the cell number during cleavage of early development as a multicellular organism grows and develops from a single cell. In fully developed organisms, mitotic division also replaces old or damaged cells. Some unicellular, eukaryotic organisms asexually reproduce by mitotic cell division.

Budding is another form of asexual reproduction that occurs when a small outgrowth of an organism's body develops into a smaller version of the adult organism and eventually detaches, giving rise to a new individual. Yeast and hydra are two examples of organisms that reproduce by budding. Some sponges can reproduce by gemmulation, in which a reproductive bud called a gemmule develops. The gemmule consists of an aggregate of cells surrounded by a hardened capsule and can survive within a dehydrated or frozen parent sponge until the following spring.

Fragmentation is a means of asexual reproduction in which an individual organism breaks into parts (fragments) that develop into whole new individuals. Animals capable of reproducing by fragmentation include sponges, cnidarians, and annelids. The process of regeneration per se is not a form of asexual reproduction, though it is often discussed in the same context. Regeneration is the restoration of a body part after injury or removal. A whole new organism is not produced with regeneration; rather, a part simply regrows. An example of regeneration is when a lizard loses part of its tail while running from a predator, and the tail grows back.

Hydra reproduce asexually by budding. *(Biophoto Associates/Photo Researchers, Inc.)*

Vegetative propagation in plants is a type of asexual reproduction that does not involve the formation of seeds or spores. The creation of new plants by vegetative propagation occurs naturally but can also be induced for the purpose of growing new plants genetically identical to the parent plant. Fragmentation is one of the most common mechanisms of vegetative propagation in which pieces or parts of a parent plant give rise to whole new plants. This can happen naturally as when the root systems may develop numerous shoots that each gives rise to a new plant. Rhizomes, modified plant stems that grow horizontally just below the surface of the ground, often grow roots and shoots from the nodes. These can give rise to new individuals. Similar structures, called stolons or runners, sprout from preexisting stems and grow horizontally along the surface of the ground. Plants such as strawberries and ivies grow stolons, which can give rise to adventitious roots (roots that form from branches, stems, or leaves rather than other roots) that penetrate into the soil.

Strawberry plants can reproduce asexually by sending out runners. *(Alan and Linda Detrick/Photo Researchers, Inc.)*

Adventitious buds (buds that form at any location other than apical meristem at the tip of a stem) also form along the stolon and grow, forming new stems and leaves. Tubers are fleshy, food-storing structures formed from stolons that sprout in the springtime. Some plants reproduce asexually by forming bulbs, modified buds used for storing food when a plant becomes dormant, as in the winter. In the spring, the bulbs form stems and roots that develop into a new plant.

Cutting and grafting are other means for vegetatively propagating plants. Stem cuttings are the most common, although other plant parts can also be used. The cutting uses stored energy in the form of carbohydrates to form roots and shoots. In grafting, a small twig from one plant, the scion, is inserted into a cut of another plant, the stock.

Some plants, such as dandelions, can form seeds asexually, without fertilization, by a process called apomixis. A diploid cell in the ovule gives rise to the embryo, dispersed by seeds, as in sexual reproduction.

SEXUAL REPRODUCTION AND MEIOSIS

In sexual reproduction, which only occurs in eukaryotes, two parents contribute to the genome of the offspring, thus the offspring contain genes from both parents. Whereas asexual reproduction is efficient and successful in a stable environment, sexual reproduction results in the production of new genotypes, an advantage when organisms are challenged by new environmental conditions. Almost all animals reproduce sexually, despite the fact that it is more energetically expensive, more complicated, and takes longer. Sexual reproduction involves the formation of gametes, a process that involves the costly process of meiosis. In eukaryotes, chromosomes exist in homologous pairs, pairs of chromosomes that encode the same genes, though the individual chromosomes might have different forms or alleles of the gene. The term diploid describes a cell or an individual that has two sets of chromosomes and therefore two copies of each gene. In contrast, haploid describes a cell or an individual that possesses only one of each type of chromosome and therefore only one allele for each gene. Gametes, the cells that unite during sexual reproduction, are haploid. Males produce sperm, and females produce eggs. Because the adult form of most eukaryotes is diploid, the organisms must have a mechanism for reducing the genetic material in the gametes by twofold. That way, the joining of two haploid cells together during fertilization restores the diploid number. Meiosis is a specialized form of cell division that halves the number of chromosomes in the cells that make gametes. The process involves two cellular divisions, meiosis I and meiosis II, and

results in the production of haploid cells from a diploid cell.

Meiosis resembles mitosis in many ways. Both processes involve cell division, and before either process begins, the chromosomes must be replicated during interphase. Following DNA synthesis, the chromosomes comprise two sister chromatids, exact copies of one another. The centrosome, a cytoplasmic structure consisting of two centrioles that helps organize microtubules, also duplicates, forming two pairs of centrioles. The sister chromatids remain attached at the centromeres. Though mitosis involves a single cell division, meiosis consists of two cell divisions, termed meiosis I and meiosis II. The first stage of meiosis is reductional division because the number of chromosomes is reduced. The second stage is equational division, because the total number of chromosomes remains the same after the division.

During prophase I, the first step of meiosis I, the replicated chromosomes condense, becoming visible when stained and viewed under the microscope. The nuclear envelope disassembles, and homologous chromosomes pair up with one another, a process called synapsis. A synaptonemal complex holds the homologous chromosomes together temporarily. While they are paired, crossing over or recombination can occur. During crossing over, a segment of one chromatid physically exchanges with the corresponding region of one chromatid of its homologous partner by breaking and then rejoining with the other chromatid's DNA. Following crossing over, the sister chromatids of one chromosome will no longer be exactly identical. Chiasmata are the regions where the exchange occurs, and they serve to hold the homologous chromosomes together in a tetrad (named so because four chromatids participate in the formation) even after the synaptonemal complex disassembles in late prophase I. The centrosomes move toward opposite poles, and microtubules form spindle fibers that extend inward from the centrosomes at the poles, eventually connecting with a specialized structure located at the centromere of each chromosome, a kinetochore.

While the steps of meiosis are contiguous, meaning no point in time marks the definite end of one step and the beginning of the subsequent step in the sequence, the next phase can be easily recognized under the microscope. Metaphase I involves the alignment of all the homologous pairs at the equator, a plane traversing the center of the roughly spherical cell in between the two poles. The kinetochore of each chromosome in a homologous pair is attached by microtubules to a pole, so that each homologue is attached to a different pole. The manner in which the chromosomes become associated with one pole or the other and therefore with other chromosomes also associated with that pole is completely random, as described by the law of independent assortment, proposed by Gregor Mendel in the mid-1800s.

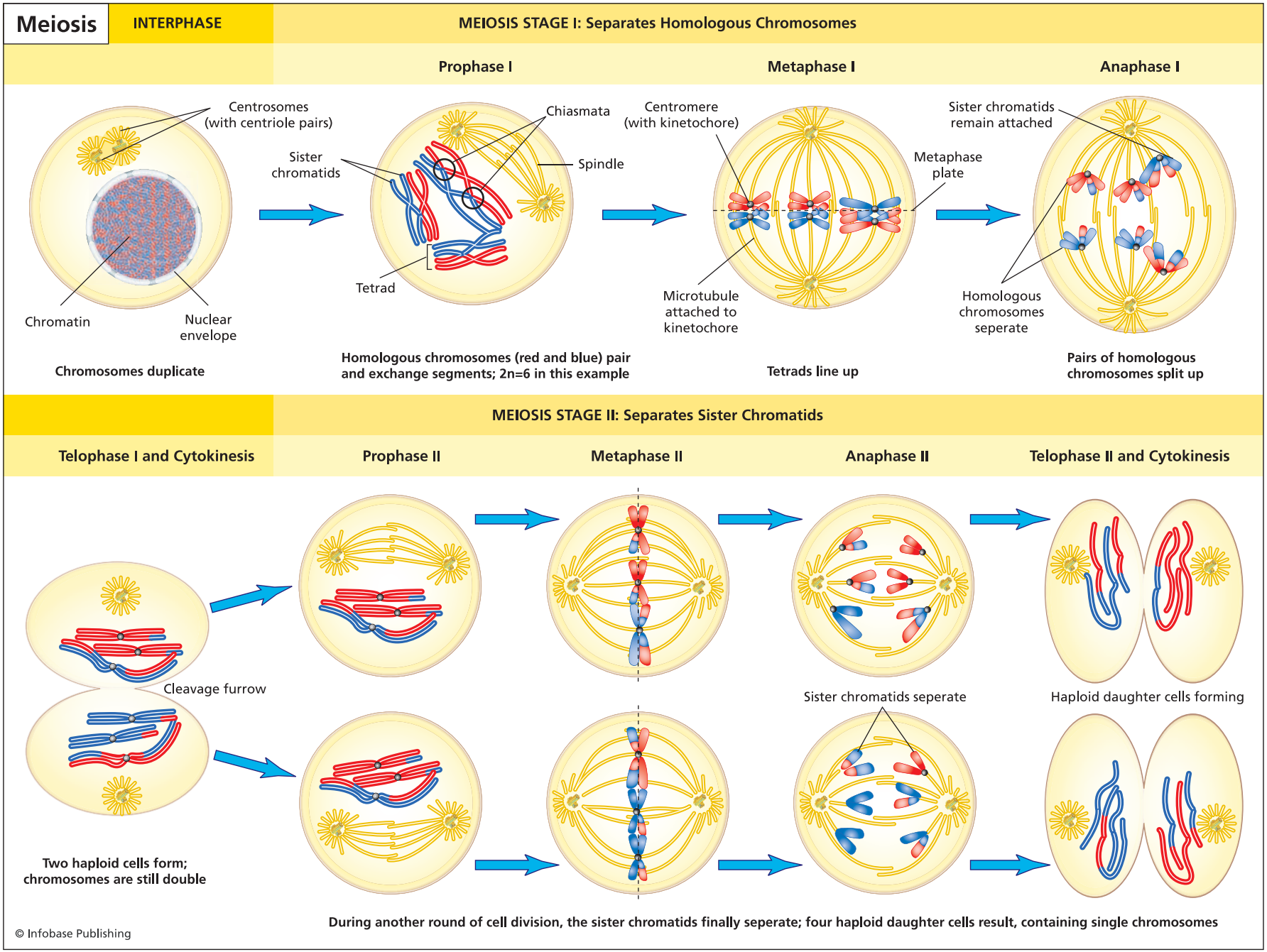
During anaphase I, the homologous chromosomes of a pair move toward opposite poles, ensuring that one complete haploid set of chromosomes ends up at each pole. The spindle apparatus assists in moving the chromosomes, which still consist of two linked sister chromatids, though, due to crossing over, the sister chromatids are no longer identical.

Telophase I begins with the formation of a cleavage furrow (in animals) or a cell plate (in plants) that divides the cytoplasmic contents, with each half containing one haploid set of chromosomes. Cytokinesis, the division of the cell itself, occurs, forming two haploid daughter cells. In some organisms, the nuclear envelope reforms before meiosis II begins, and in others it does not.

The second stage of meiosis, meiosis II, begins with prophase II. Roughly, the same events that occur in mitotic prophase occur in prophase II. The centrosomes duplicate, a new spindle forms, and the chromosomes begin to move toward the equator once again. At metaphase II, all the chromosomes are positioned at the equator, just as in mitosis. Microtubules extending from the centrosomes at opposite poles attach to the kinetochores of conjoined sister chromatids. The centromeres holding together the sister chromatids divide in anaphase II, allowing the sister chromatids to move to opposite poles of the cell. Once they separate, they each become a single chromosome. Telophase II brings about the reformation of the nuclear envelope, decondensation of the chromosomes, and the onset of cytokinesis. By the end of telophase II and the completion of cytokinesis, four haploid cells result, all originating from one single diploid cell, and all possessing different haploid genomes.

In summary, meiosis results in genetically variable haploid cells. Through independent assortment and crossing over, meiosis can create a practically unlimited number of different genetic combinations in the gametes made by an individual. The random process of fertilization during sexual reproduction increases the already tremendous variability, providing the raw material upon which natural selection acts, resulting in evolution.

(opposite) The cellular process of meiosis occurs in two stages and results in the production of haploid cells.


Meiosis
INTERPHASE
MEIOSIS STAGE I: Separates Homologous Chromosomes
Prophase I
Metaphase I
Anaphase I
Centrosomes (with centriole pairs)
Sister chromatids
Chiasmata
Spindle
Tetrad
Centromere (with kinetochore)
Metaphase plate
Microtubule attached to kinetochore
Sister chromatids remain attached
Homologous chromosomes seperate
Chromatin
Nuclear envelope
Chromosomes duplicate
Homologous chromosomes (red and blue) pair and exchange segments; 2n=6 in this example
Tetrads line up
Pairs of homologous chromosomes split up
MEIOSIS STAGE II: Separates Sister Chromatids
Telophase I and Cytokinesis
Prophase II
Metaphase II
Anaphase II
Telophase II and Cytokinesis
Cleavage furrow
Sister chromatids seperate
Haploid daughter cells forming
Two haploid cells form; chromosomes are still double
During another round of cell division, the sister chromatids finally seperate; four haploid daughter cells result, containing single chromosomes
© Infobase Publishing

While all sexually reproducing organisms have meiosis in common, gametogenesis, the process of making gametes, involves a bit more complexity. The eggs and sperm of each species are unique, and the process of their formation in addition to their final structure varies depending on the living conditions of the organism as well as differences in reproductive strategies. For example, in mammals, oogenesis, the formation of eggs within the ovaries, involves an unequal distribution of the cytoplasm during cytokinesis, such that one of the four resultant haploid cells contains practically all of the contents. The other three become polar bodies and basically serve as repositories for the extra genetic material. Spermatogenesis involves the development of specialized structures, like flagella for motility and acrosomes to aid in the penetration of the egg during fertilization.

PARTHENOGENESIS AND HERMAPHRODITISM

Parthenogenesis is the development of a new individual from an unfertilized egg; thus, this mechanism of reproduction requires meiosis, but not necessarily two partners. The resulting offspring are clones of the parent, meaning they are genetically identical. Some fishes, lizards, and frogs can asexually reproduce by parthenogenesis. Whiptail lizards reproduce only by parthenogenesis. No males of the species exist, but the females exhibit mating behaviors that increase the chance of ovulation. Rotifers, some flatworms, crustaceans, and insects undergo ameiotic (asexual) parthenogenesis, in which the egg is formed by mitosis. Meiotic parthenogenesis involves the formation of a haploid egg, and a male may or may not be required to induce its further development. The eggs of many flatworms, rotifers, annelids, mites, and insects can develop even in the absence of any male. In some social insects such as bees, wasps, and ants, males develop parthenogenetically from unfertilized eggs, whereas females develop from fertilized eggs. In other animals, such as some fish, mating with a male is required to activate the egg into development even though fertilization need not occur.

Organisms that rarely come into contact with other members of their species may benefit from hermaphroditism, the condition of one individual having gonads of both sexes. Being able to contribute either eggs or sperm during sex ensures that an individual can mate with every other member of the same species encountered and, if both partners are hermaphroditic, then hermaphroditism results in double the number of eggs fertilized. Some hermaphrodites are capable of fertilizing themselves, but this reproductive strategy does not introduce any new genetic material.

Some species, such as earthworms, that employ hermaphroditism to reproduce can produce both eggs and sperm throughout their adult life stage, a phenomenon called simultaneous or synchronous hermaphroditism. Earthworms cannot self-fertilize, however, and they exchange both types of gametes when they mate. Tapeworms are hermaphrodites that can self-fertilize. The hamlet demonstrates an alternate strategy of simultaneous hermaphroditism; this fish takes turns playing the male or female role when mating with another individual several times over the course of a few days. Sperm-sharing, another behavioral strategy displayed by simultaneous hermaphrodites, occurs when an individual acting as a male during copulation fertilizes a mate with sperm obtained from a previous mating.

Other animals exhibit sequential hermaphrotidism, meaning they function as either a male or a female at one time but can switch sex roles at different times. Obviously, sequential hermaphrodites are not capable of self-fertilization. In protogyny, an animal begins as a female, but becomes a male later in life. For example, wrasses belong to a family of bony marine fish, all of which are protogynous. In some species, the conversion from female to male occurs when an individual reaches a certain age and size. Other species live in harems consisting of a single supermale and numerous females. When the supermale dies or is removed from the harem by other means, the largest female of the harem changes into a male and starts producing sperm. Protandry, which is less common than protogyny, is a type of sequential hermaphroditism in which an animal is first a male and then becomes a female. In protandrous oysters, larger size is advantageous for the female, as size correlates to the number of eggs produced.

Gonadal dysgenesis, a condition formerly referred to as true hermaphrotidism, can occur in mammals including humans, but it is a developmental anomaly rather than a reproductive strategy. The reproductive organs are rarely fully developed and therefore are incapable of producing gametes.

Most flowering plants are hermaphroditic. Plants with flowers that contain both male and female reproductive organs, stamens and carpels, are said to have perfect flowers and be monoclinous. Monoecious plants have flowers that contain either male reproductive structures or female reproductive structures, but not both on the same flower. Only a few plants are dioecious, meaning each individual plant is either male or female. Hermaphroditic plants often produce the male and female elements at different times to avoid self-fertilization, though some regularly self-fertilize.

FERTILIZATION AND REPRODUCTIVE STRATEGIES

Fertilization is the union of haploid gametes to produce a diploid zygote. Sexually reproducing species utilize different strategies for ensuring that fertilization occurs. External fertilization takes place outside

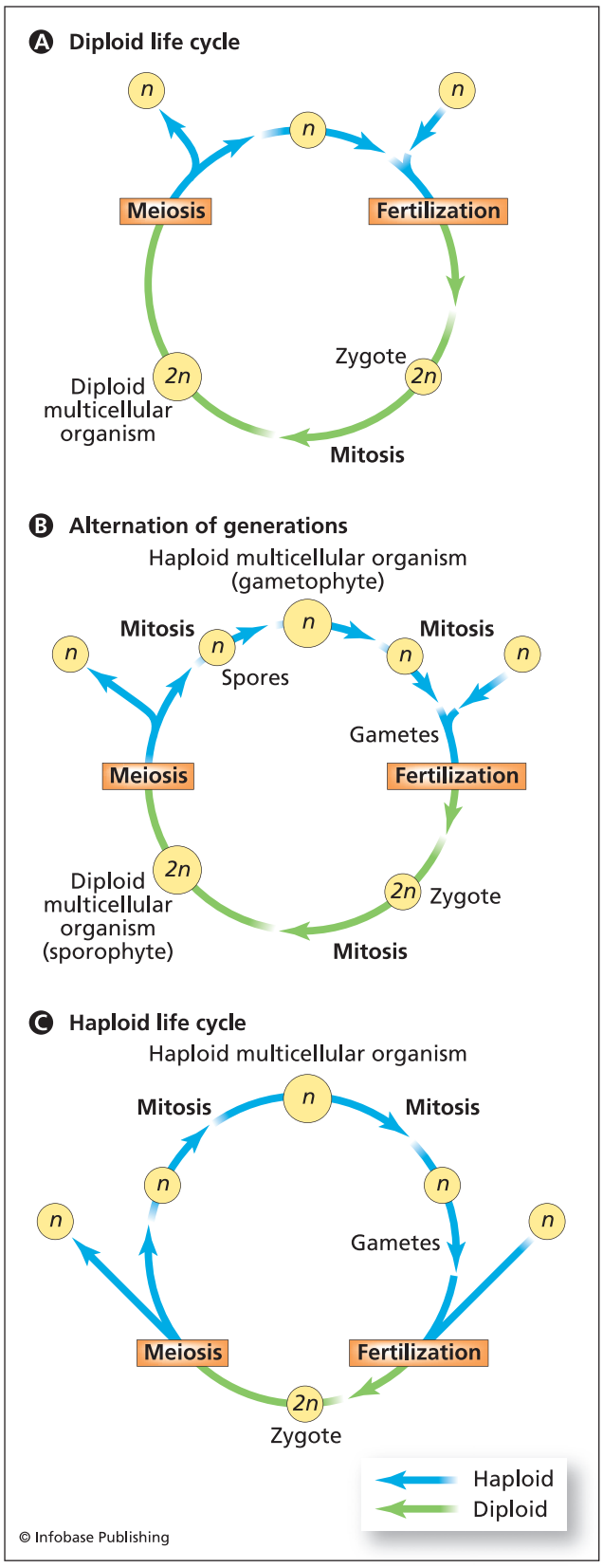
the body of an animal; thus, it requires a moist or aquatic habitat to prevent dehydration of the gametes. Typically, females release eggs and males release sperm into the surrounding environment, where they unite. Whole populations may shed gametes simultaneously in response to environmental cues such as chemicals secreted by other members of the species, day length, or temperature. A disadvantage of external fertilization is that many gametes, which are energetically expensive to produce, are wasted. To reduce the amount of waste, individuals of some species require certain mating behaviors to trigger the release of gametes, a strategy that ensures the opposite sex is present and that fertilization will occur. Courtship behaviors often include mounting or other physical contact. External fertilization usually involves the production of copious amounts of gametes to overcome the high mortality rate of the embryos and young.

Internal fertilization requires that the male deposit sperm inside the female reproductive tract, where fertilization occurs. This act necessitates specialized structures, copulatory organs such as a penis and a vagina, that facilitate sperm delivery, storage, and transport, in addition to cooperation between both sexes. In some species, the male deposits packages of sperm into the external environment, and then places them inside of a female, or the female picks them up. Though internal fertilization is much more complicated than external fertilization, it enables fertilization in terrestrial or dry environments. One trade-off of this mechanism is the production of fewer eggs for a greater chance of survival. The energy is invested in protecting and caring for the young rather than producing an excessive quantity of gametes, as in external fertilization.

Following fertilization, development ensues, and animals have a variety of patterns for embryonic development. The eggs of oviparous creatures develop externally, though they may be fertilized internally or externally. The degree of care or protection offered by the parent during development varies. Examples of oviparous animals include fish, amphibians, reptiles, and birds. In ovoviviparous animals, the eggs develop inside the mother following fertilization, obtaining nourishment from the yolk of the egg, and hatch within the mother or immediately after being laid. Many fish, reptiles, and invertebrates exhibit ovoviviparity. Viviparous animals develop inside the body of the mother, where they obtain nourishment directly from the mother, and are born as juveniles. Placental mammals are viviparous.

LIFE CYCLES OF SEXUALLY REPRODUCING ORGANISMS

Sexual reproduction involves the formation of haploid gametes that unite during fertilization to restore



Sexual reproduction is characterized by the alternation of the haploid and diploid conditions during an organism's life cycle, but the relative prominence of each stage varies among different species.

the diploid number. The sperm and egg each contain one set of chromosomes, and the zygote and all the cells produced from it by mitosis contain two sets of chromosomes. This alternation of the haploid and diploid condition is common to all sexually reproducing organisms, though the timing and exact stages in the life cycles vary between species. In general, sexually reproducing eukaryotes undergo life cycles dominated by the haploid stage, the diploid stage, or alternate equally between generations. The major difference in the three types of cycles is which form exists as the multicellular organism: the haploid form, the diploid form, or both.

Many simple eukaryotes such as algae and fungi have haploid life cycles, in which the majority of their lives is spent in the haploid state. Only when two gametes fuse, producing a zygote, is the organism diploid. Soon afterward, the zygote undergoes meiosis, generating haploid cells or spores that divide by mitosis to give rise to the mature multicellular organism, sometimes referred to as a gametophyte. Gametes are produced by mitosis, and the cycle begins again.

Most animals, including humans, have diploid life cycles, in which the diploid state occupies the majority of the life cycle. Mature adults produce haploid gametes by meiosis, and usually these are the only haploid cells of the organism. The sperm and egg cells join by fertilization, and the diploid zygote undergoes numerous rounds of mitotic divisions to give rise eventually to the multicellular offspring.

Plants, algae, and some protists undergo an alternation of generations, in which they regularly switch from the haploid stage to the diploid stage. The mature diploid form is called a sporophyte, because it forms spores by meiosis. The haploid spores germinate to give rise to the sexually mature gametophyte stage of the organism, which produces gametes by mitosis. The haploid gametes then fuse during fertilization, and the diploid zygote develops into the sporophyte form.

See also ANIMAL BEHAVIOR; CELLULAR REPRODUCTION; HUMAN REPRODUCTION.

FURTHER READING

Hill, Richard W., Gordon A. Wyse, and Margaret Anderson. *Animal Physiology*. Sunderland, Mass.: Sinauer, 2004.

Maxwell, Kenneth E. *The Sex Imperative: An Evolutionary Tale of Sexual Survival*. New York: Plenum Publishing, 1994.

Stern, Kingsley R., James E. Bidlack, and Shelley Jansky. *Introductory Plant Biology*. 11th ed. New York: McGraw Hill, 2008.

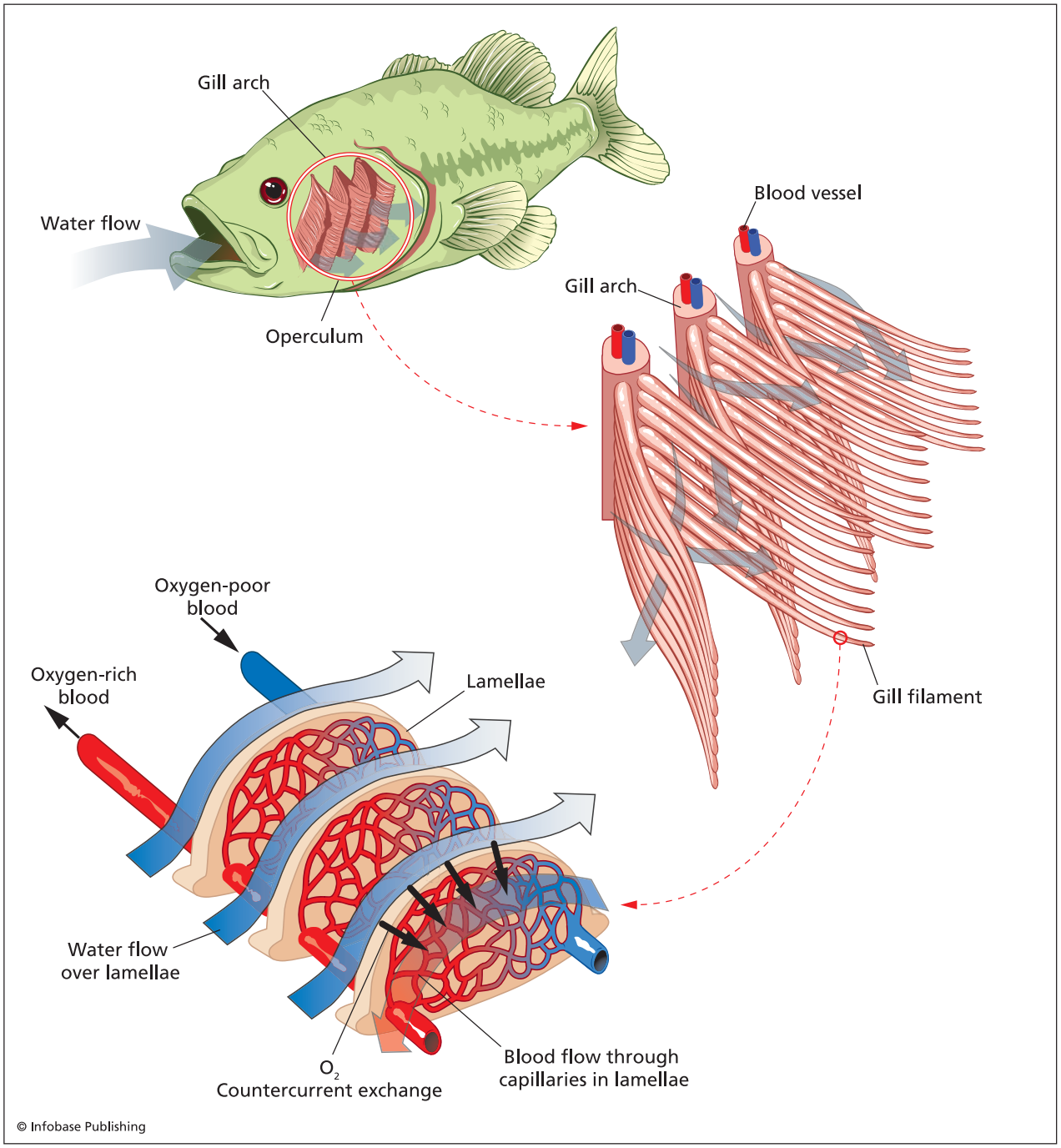
respiration and gas exchange

Living cells need molecular oxygen (O_2) to carry out cellular respiration, the mechanism by which cells produce adenosine triphosphate (ATP) through the breakdown of food molecules. In the process, the cells generate carbon dioxide (CO_2) as a waste product. In single-celled animals and primitive invertebrates, the uptake of O_2 and the discharge of CO_2 occur directly with the environment. Both O_2 and CO_2 are small enough to move across phospholipid membranes via simple diffusion. Some small animals such as earthworms respire, or exchange gases, directly through their skin, but many cells of multicellular animals are too far removed from the external environment for simple diffusion to accomplish gas exchange efficiently. In complex multicellular animals, a respiratory system accomplishes the exchange of gases with the external environment, a circulatory system transports the gases between the body tissues and the organs where gas exchange occurs, and internal respiration allows exchange of the gases between the circulatory system and the body tissues.

DIVERSITY OF RESPIRATORY SYSTEMS

The surface area across which gas exchange occurs must remain moist. For aquatic animals, the exterior environment provides the moisture. In terrestrial animals, extracellular fluid bathes the cells of the body tissues. Skin sometimes acts as the respiratory organ for small animals that live in moist environments, including annelids (segmented worms) and amphibians, but the surface area of the skin alone cannot sufficiently support respiration in animals that have high metabolic rates, so gills or lungs often share the task. Animals that live in aquatic environments, including mollusks, some arthropods, echinoderms, and fish, possess gills, external respiratory organs that remove oxygen from the water. Because gas exchange occurs across a surface, structural adaptations that increase the total surface area of the gills maximize respiratory efficiency. Animals have a diverse variety of structures that function as gills: sheetlike gills as in fish, numerous tubular protrusions as in sea stars, projections such as parapodia in marine worms, or feathery appendages as in crayfish.

Gills are external structures, meaning they come into direct contact with the external environment even if they have protective coverings. Animals that live in aquatic environments do not require special mechanisms for keeping their respiratory surfaces moist. A disadvantage, however, is the low concentration of dissolved oxygen in water. Diffusion from the atmosphere, the action of wind and waves, and photosynthesis all provide available oxygen in the water, but at much lower concentrations than in



Countercurrent exchange maximizes the exchange of gases in fish gills.

the atmosphere. To obtain sufficient quantities of oxygen from the water, animals must circulate water over the gills by flapping them or swimming through the water. This process, called ventilation, prevents a buildup of low O_2 and high CO_2 levels in the area immediately surrounding the gills.

Most bony fish have four pairs of gills located in chambers on the sides of the head and underneath a protective gill cover. When the fish swims, water flows in through the mouth and over rows of gill filaments attached to gill arches. A fish that is not moving must pump water through its mouth in order to breathe. Numerous flattened structures called lamellae on the filaments contain capillaries that bring oxygen-poor blood into the lamellae and converge into arteries that carry newly oxygenated blood to the body tissues. As the water flows over the lamellae, O_2 diffuses into the capillaries, and

CO_2 diffuses out of the capillaries and exits the body through gill openings. A unique arrangement called countercurrent exchange allows the water to flow in the opposite direction as the blood moving through the capillaries in the lamellae. As blood moves through a capillary, more O_2 diffuses into it from the water, so the O_2 concentration increases. Countercurrent exchange ensures that as the blood O_2 levels increases, the water it encounters also has an increasing concentration of O_2, facilitating efficient continued exchange of O_2 throughout the entire length of the capillary The reverse situation is true for carbon dioxide levels. As blood moves through a capillary, the CO_2 concentration decreases, but relatively lower levels of CO_2 in the freshwater entering the gills ensure that the CO_2 continues to diffuse from the blood as it travels through the capillary.

Terrestrial animals exchange gases with air, which contains higher relative concentrations of oxygen than water. Gases diffuse more rapidly through air than water, but the respiratory surface loses a great deal of water through evaporation, a problem that terrestrial animals solve by housing respiratory organs inside of their bodies. Many arthropods, including insects and some spiders, respire using tracheal systems. Tracheae are hollow tubes that branch into smaller tubes that extend to all the cells of the body. Air flows into the tracheae through spiracles, tiny openings on the sides of the abdomen. Valves control air flow through the spiracles and prevent water loss. Insects that are larger or that have higher metabolisms have mechanisms to ventilate or move air through their tracheal systems. For example, during flight, when metabolic needs are greater, ventilation occurs by the contraction and relaxation of the muscles involved in flight.

Other terrestrial animals such as reptiles, birds, and mammals rely on lungs for respiration. Because amphibians also respire through their skin, species that have lungs at all have small lungs. Aquatic vertebrates that live in water with very low O_2 levels or that live on land part-time possess lungs and breathe air. Lungs do not branch or extend throughout the body, so respiration and circulation function in close association. The respiratory system exchanges gases with the external environment, and the circulatory system brings O_2 to the body tissues and carries CO_2 to the lungs to be expelled.

MAMMALIAN RESPIRATORY SYSTEM

In mammals, the respiratory system consists of a nasal cavity, pharynx, larynx, trachea, and two bronchi that branch into bronchioles inside the lungs and terminate in alveoli. Air enters through the nose, where tiny hairs filter out large particulate matter. The temperature and the humidity of the air increase during its passage through the nasal cavity toward the pharynx, located behind the mouth, where the respiratory and digestive systems meet. Air then flows through the larynx, also called the voicebox because during exhalation it can produce sound such as speech, song, or other noises. The cartilaginous trachea, or windpipe, follows the larynx and branches into two bronchi that branch into successively smaller bronchiole tubes. Epithelial cells lining the respiratory tract secrete a thick substance called mucus that traps particulate matter from the air. Cilia that line the larger tubes of the respiratory tract beat to move the mucus containing trapped substances up to the pharynx where it can be swallowed down the esophagus.

Millions of air sacs called alveoli exist at the ends of the bronchioles and are the sites for gas exchange. Networks of capillaries surround the alveoli. When inhaled air reaches the epithelium of the alveoli, oxygen diffuses through the moist respiratory surface and into the capillaries. At the same time, carbon dioxide present in the capillary blood diffuses into the alveoli and is exhaled.

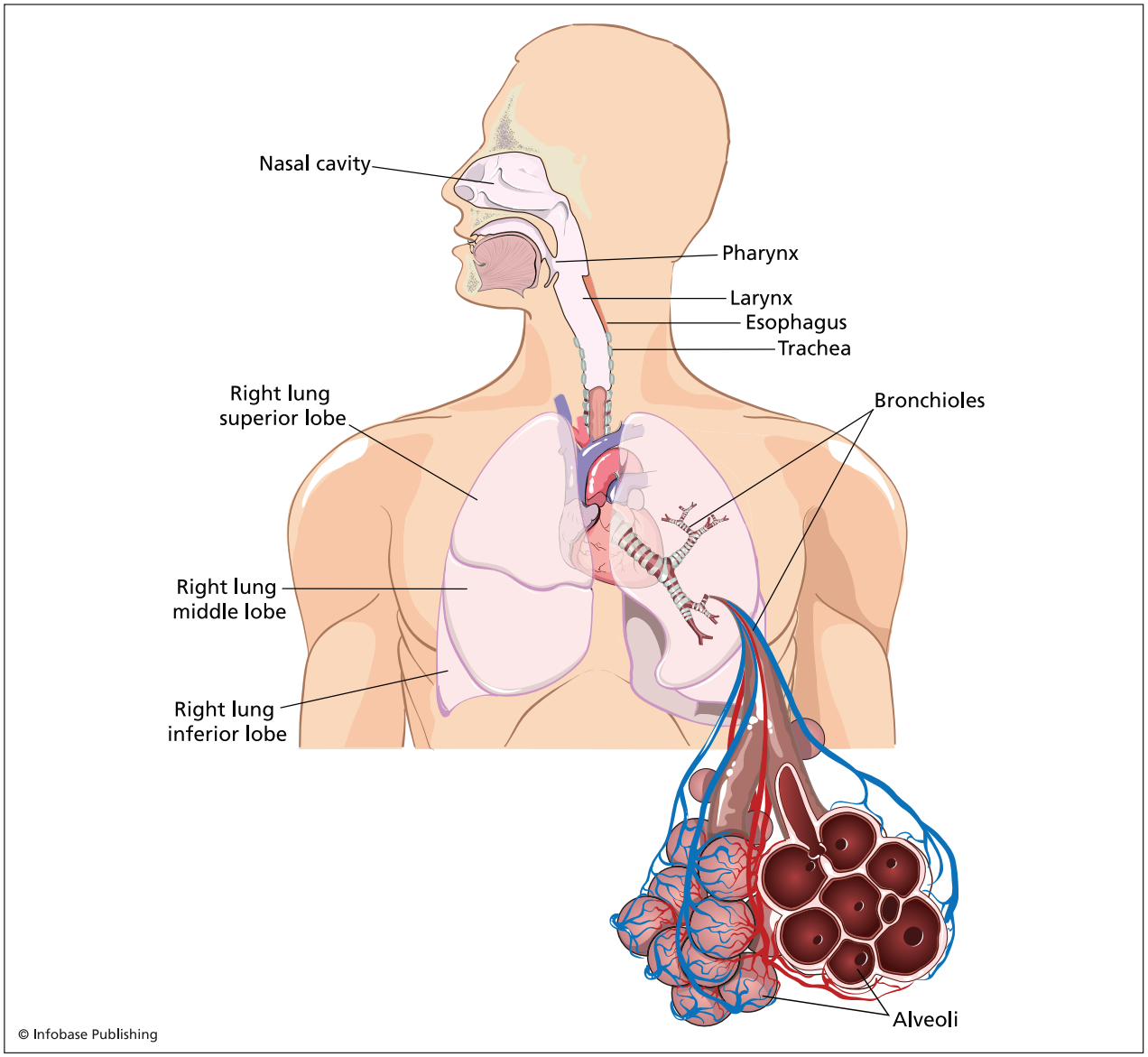
As in fish and insects, mammals must ventilate their lungs to expose the respiratory surface of the alveoli to fresh air. Two types of muscles function in inhalation and exhalation: the diaphragm and the intercostal muscles. The diaphragm is a sheetlike muscle located at the intersection of the chest and abdominal cavities, and the intercostal muscles are in between ribs. When contracted, the diaphragm moves downward and the intercostals cause the rib cage to expand, creating a region of negative pressure inside the lungs, meaning the air pressure is lower relative to the external atmospheric pressure. Gases naturally move from regions of higher pressure to regions of lower pressure. During inhalation, air rushes inward to fill the extra space created by contraction of the diaphragm and the intercostals muscles. After gas exchange occurs, the muscles relax, compressing the space of the chest cavity. This causes an increase in the air pressure inside the lungs, and air rushes out through the same path it entered. Birds and amphibians ventilate using different mechanisms.

Humans can exert temporary voluntary control over inhalation and exhalation, but involuntary or autonomic mechanisms usually control breathing. An adult human breathes in and out between 10 and 18 times in one minute depending on changing metabolic needs. Children and infants breathe at a much faster rate. The medulla oblongata and the pons of the brain monitor the levels of O_2 and CO_2 in the blood and respond by regulating autonomic control over breathing. During periods of physical activity, the breathing rate increases because the body's cells are consuming more O_2 and producing more CO_2

through cellular respiration. The brain coordinates an accompanying increase in the heart rate to circulate the gases throughout the body. Emotional stimuli such as stress or chemical stimuli such as caffeine can increase the breathing rate.

GAS TRANSPORT

The pulmonary arteries carry blood from the heart to the lungs, where it enters the alveolar capillaries. After O_2 diffuses into the bloodstream, red blood cells pick it up and transport it throughout circulation. The blood's capacity for dissolved O_2 is lower than could adequately supply a mammal's metabolic needs. Respiratory pigments, such as hemoglobin in humans or hemocyanin in other animals such as arthropods and mollusks, increase the amount of O_2 that the blood can carry. In humans, each red blood cell contains a few hundred million molecules of hemoglobin, a protein consisting of four polypeptide chains, each of which contains a heme group with an attached iron atom. Each heme group reversibly binds one molecule of O_2. Pulmonary veins carry the oxygenated blood back to the left side of the heart, which pumps it into the aorta and into the major arteries. The O_2 concentration is lower in the body's tissues than in the arterial blood, and the concentration of CO_2 is higher in the tissues than in the blood. The CO_2 and other waste products of metabolism make the blood more acidic, causing the hemoglobin to change shape slightly. The structural alteration



In mammalian respiratory systems, air inhaled through the nasal cavity or mouth enters the pharynx, then passes through the larynx, trachea, bronchi, and bronchioles on the way to the alveoli, where gas exchange occurs.

releases the O_2 molecules, and the O_2 diffuses out of the red blood cells, out of the capillaries, into the extracellular fluid of that tissue, and eventually into the tissue's cells. The CO_2 diffuses from the extracellular fluid of the tissue into the capillaries. The capillaries converge into venules and then veins, which carry the oxygen-poor blood back to the right side of the heart. The blood returns to the lungs via the pulmonary artery, and the cycle begins again.

In the cytoplasm of red blood cells, the enzyme carbonic anhydrase catalyzes the formation of carbonic acid (H_2CO_3) from CO_2 and water. Because slight acidity causes hemoglobin to release oxygen, the active tissues, that is, the tissues that are utilizing O_2 and producing CO_2, will receive more O_2 and be able to continue cellular respiration. Hemoglobin also helps transport CO_2 in the blood and prevents the blood from becoming too acidic. All but approximately 7 percent of the CO_2 from the tissues diffuses into the capillaries and then into the red blood cells. Inside the cytoplasm of the red blood cells, H_2CO_3 dissociates into a hydrogen ion (H^+) and a bicarbonate ion (HCO_3^-). Approximately 70 percent of the CO_2 in the blood is in the form of HCO_3^-, which is more soluble then CO_2. Thus, the formation of HCO_3^- enables the blood to carry greater quantities of CO_2. Hemoglobin absorbs some CO_2 (approximately 23 percent) and the H^+, preventing the pH from becoming too acidic. The HCO_3^- diffuses out of the red blood cells and travels in the blood plasma. When it reaches the lungs, the HCO_3^- diffuses back into the red blood cells and combines with H^+ released by the hemoglobin to form H_2CO_3, which converts back to CO_2 and H_2O. The CO_2 diffuses out of the red blood cells, out of the capillaries, into the fluids surrounding the alveoli, and into the alveolar space to be exhaled.

See also ANATOMY; ANIMAL FORM; BIOLOGICAL MEMBRANES; CIRCULATORY SYSTEM; INVERTEBRATES; PHYSIOLOGY; VERTEBRATES.

FURTHER READING

Seeley, Rod, R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

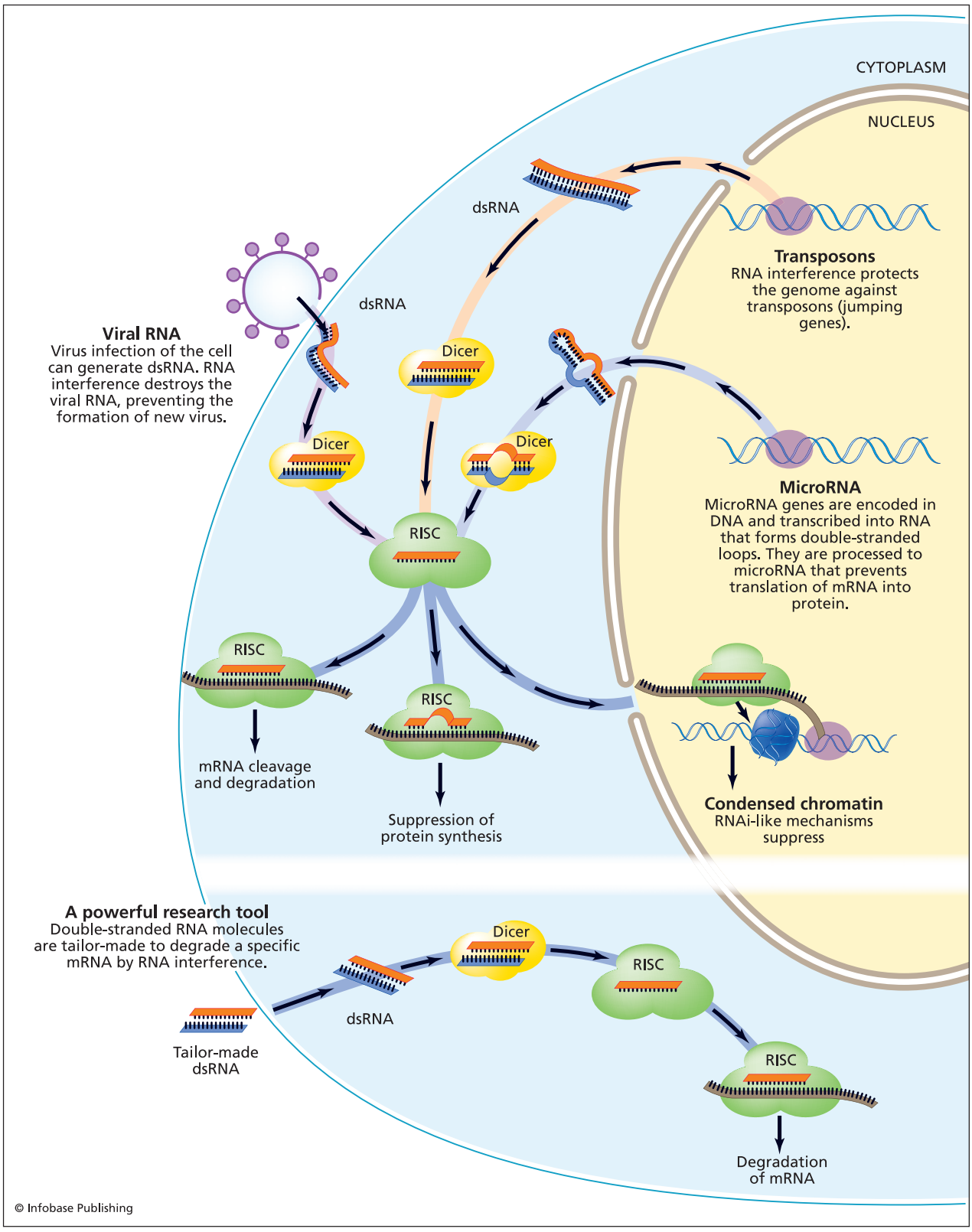
RNA interference

Ribonucleic acid (RNA) molecules that do not encode for proteins, transfer RNA (tRNA), or ribosomal RNA (rRNA) are abundant in both prokaryotic and eukaryotic cells. In bacteria they are commonly referred to as small RNAs, and in eukaryotic cells they are referred to as noncoding RNAs. These types of RNA appear to have a variety of functions, including roles in the regulation of transcription, the structure and replication of chromosomes, RNA processing, the stability of messenger RNA (mRNA), the inhibition and activation of translation, and protein degradation and translocation across membranes. RNA interference (RNAi) is a posttranscriptional mechanism used by plant and animal cells to regulate gene expression. Molecular biologists exploit this phenomenon, using it as a tool to prevent the expression of specific genes in order to study their effects.

In order to synthesize a protein, first a cell must create an mRNA transcript from the deoxyribonucleic acid (DNA) in a process called transcription. Then ribosomes read the sequence of nucleotides on the mRNA and synthesize a chain of amino acids from it in a process called translation. The RNA interference pathway is activated by long pieces of double-stranded (ds) RNA, more than 200 nucleotides long, that silence target genes in several types of eukaryotic organisms. The presence of the ds RNAs inside the cell activates cellular mechanisms for degrading mRNA from a gene homologous to the ds RNA. An enzyme referred to as dicer breaks the longer pieces of ds RNA into fragments between 20 and 25 nucleotides long called small interfering RNAs (siRNAs). The siRNAs assemble with protein components into complexes called RNA-induced silencing complexes (RISCs), leaving portions unwound and available for binding with complementary RNA. The exposed RNA seeks and recognizes complementary sequences in mRNA present in the cytoplasm. After forming hydrogen bonds with the target mRNA transcripts, the RISCs cleave the target mRNAs, leaving ribosomes unable to translate them into proteins.

DISCOVERY OF RNA INTERFERENCE

In 2006 two American scientists, Andrew Fire from Stanford University in California and Craig Mello from the University of Massachusetts at Worcester, received the Nobel Prize in physiology or medicine "for their discovery of RNA interference—gene silencing by double stranded RNA." While studying the regulation of gene expression in the nematode *Caenorhabditis elegans,* Fire and Mello observed the effect of injecting several types of RNA into the worms. Sense RNA is defined as the sequence of RNA that encodes for the synthesis of a protein. The complementary sequence is called antisense RNA. When Fire and Mello separately injected sense or antisense mRNA that encoded for a muscle protein, they observed no effect. When they injected both simultaneously, the worms twitched in a manner similar to



RNA interference is a process that triggers the degradation of mRNA for specific genes by a mechanism that involves the presence of double-stranded homologous RNA. Scientists use RNA interference as a tool for molecular biological research.

worms that had mutant copies of the muscle coding gene. After hypothesizing that the injected sense and antisense RNA strands bound to one another and silenced the very gene that they encoded, they examined the effect of injecting ds RNA with sequences of other proteins. The ds RNAs inhibited translation of the mRNA whose sequences were similar to the ds RNA sequences. Fire and Mello published their observation that ds RNA could silence, or prevent the expression of specific genes in 1998. The fact that only eight years passed between the report of their discovery and the Nobel award is a testament to the far-reaching implications of their work.

Recognizing RNA interference as a potential fundamental mechanism for controlling gene expression, scientists rapidly identified and characterized the cellular machinery that functioned as part of the RNAi pathway. The protein complex dicer digests the ds RNA molecules into smaller fragments. One strand from the smaller fragments binds to another complex, the RISC complex, which seeks out mRNA molecules with sequences complementary to the sequence of the single strand of RNA it carries. RISC then cuts the mRNA molecule, silencing it.

SIGNIFICANCE

Scientists believe that RNA silencing pathways evolved as a defense mechanism against invading nucleic acids. Many viral pathogens have ds RNA genomes. After the viral nucleic acid penetrates the host cell, dicer could bind it, leading to RISC activation and destruction of the viral RNA. Transposons are genetic elements that have the intrinsic ability to insert themselves at different locations throughout a genome. Depending on where they insert, their effects can cause damage to the cell. RNA interference may protect against transposons that replicate themselves via an RNA intermediate. Recent studies have shown that RNA silencing also regulates the expression of endogenous genes and plays a role in the formation of heterochromatin, inactive, highly packaged areas on chromosomes. Experiments have shown that RNAi plays a role in the regulation of certain genes during development and biologists are currently exploring that role in plants, worms, and mammals. Humans and worms both contain genes for small noncoding RNAs that share sequences with the transcribed sequences of other genes. Estimates of the number of these small RNAs in mammalian cells approach 500, and scientists believe they might be involved in the regulation of expression of 30 percent of all genes.

RNAi has been a valuable tool for molecular biologists researching the functions of certain genes. By silencing specific genes, they can observe the effects that not having that specific protein causes to cells or model organisms. This helps to determine the normal function of a protein.

Understanding the mechanism of RNAi has led to a better understanding of normal cell function, and it also carries medical implications. For example, in 2004 a team of scientists led by Markus Stoffel, who is now at the Swiss Federal Institute of Technology in Zurich, discovered that overproduction of a specific small noncoding RNA involved in insulin secretion caused diabetes in mice. Medical researchers are currently exploring the use of RNAi in gene therapies, which aims to cure or treat diseases by introducing normal copies of genes to cells that have defective copies. RNAi for gene therapy would introduce nucleic acid in order to prevent the selective expression of the defective gene, rather than try to replace it. Preliminary trials in mice using one method of RNAi gene therapy resulted in fatal liver damage. Clinical trials are underway using RNAi to treat macular degeneration, a condition that can cause blindness, and respiratory syncytial virus, which can cause pneumonia.

See also BIOMOLECULES; DEOXYRIBONUCLEIC ACID (DNA); GENE EXPRESSION.

FURTHER READING

Filipowicz, Witold. "RNAi: The Nuts and Bolts of the RISC Machine." *Cell* 122, no. 1 (2005): 17–20.

Fire, Andrew, SiQun Xu, Mary K. Montgomery, Steven A. Kostas, Samuel E. Driver, and Craig C. Mello. "Potent and Specific Genetic Interference by Double-Stranded RNA in *Caenorhabditis elegans*." *Nature* 391, no. 6669 (1998): 806–811.

The Nobel Foundation. The Nobel Prize in Physiology or Medicine 2006. Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/2006/index.html. Accessed January 22, 2008.

Sontheimer, Eric J., and Richard W. Carthew. "Silence from Within: Endogenous siRNAs and miRNAs." *Cell* 122, no. 1 (2005): 9–12.

Storz, Gisela. "An Expanding Universe of Noncoding RNAs." *Science* 296, no. 5571 (2002): 1,260–1,263.

Zamore, Phillip D., and Benjamin Haley. "Ribo-gnome: The Big World of Small RNAs." *Science* 309, no. 5740 (2005): 1,519–1,524.

Schleiden, Matthias (1804–1881) German *Botanist* Matthias Schleiden contributed to the formulation of the cell theory by reporting in 1838 that plant tissues are constructed from individual cells. Other major contributions to the cell theory include Theodor Schwann's similar statement regarding animal cells and Rudolf Virchow's assertion that all cells arise from preexisting cells.

Matthias Schleiden was born on April 5, 1804, in Hamburg, Germany. His father was a successful physician. Schleiden attended the University of Heidelberg from 1824 to 1827, obtaining a doctorate in law. Unhappy with this profession, he initiated studies in natural science at Göttingen in 1833, then transferred to Berlin, where he focused on botany. While working in the laboratory of the respected German physiologist and comparative anatomist Johannes Müller, he met Theodor Schwann, cofounder of the cell theory.

Schleiden made his mark on the history of life science early in his botanical career. Most botanists of the day simply described and named plants based on their physical characteristics. In contrast, Schleiden studied them under the microscope. This approach to botany led to his realization that all plants were composed of cells. He thought that the cell served as the basic unit of life and proposed that plant growth (i.e., phytogenesis) occurred by the multiplication of the cells. In 1838 he published his ideas in an article titled "Beiträge zur Phytogenesis" (Contributions of phytogenesis) in *Müller's Archiv,* one of the leading journals of the time. This paper was immediately translated into English and French and stimulated numerous debates. Schleiden recognized that the nucleus played an important role in cell division. He suggested that the growth of new cells initiated with the formation of a bud from the nucleus of the parent cell. These conclusions were based on his erroneous interpretation of the pollen tube. While his conclusion that plants consist of cells has stood the test of time, his ideas regarding plant growth have not. Nevertheless, he is considered the founder of plant cytology.

In 1839 Schwann published his assertion that animals were composed of cells. Thus biology had found a principle unifying the study of plants and animals—the cell theory. Originally the cell theory had two basic tenets: all organisms consist of cells, and the cell is the basic structural and functional unit of life. Rudolf Virchow formalized the cell theory in 1858, adding the aphorism that all cells arise from preexisting cells to Schleiden and Schwann's contributions.

After obtaining a doctorate at Jena in Germany in 1839, Schleiden was appointed extraordinary professor of botany at the University of Jena, where he was a popular lecturer. In 1850 he became a titular professor of botany. In 1862 he moved to Dorpat (now Tartu in Estonia), but he stayed there only for one year. Afterward, he moved from city to city, settling finally in Frankfurt, where he taught privately.

Schleiden's other contributions to life science include his observations on protoplasmic streaming and his study of mycorrhize. Protoplasmic streaming, more commonly called cytoplasmic streaming, is the movement of the cytoplasm, the colloidal complex of organic and inorganic compounds inside the cell. Mycorrhizae are symbiotic associations of fungi with plant roots. Schleiden also authored several books, including a popular botany texbook *Grundzüge der wissenschaftlichen Botanik* (1842–43), and a compilation of several well-attended lectures he delivered,

Die Pflanze und ihr Leben: Populäre Vorträge (*The Plants and Their Lives: Popular Lectures,* 1948). The second and third editions of his botany textbook were subtitled *Die Botanik als inductive Wissenschaft behandelt* (English translation published as *Principles of Scientific Botany: Or, Botany as an Inductive Science,* 1849). The textbook was the first to include a discussion of plant cytology, and the volume introduced new pedagogical methods involving inductive reasoning that set the standard for teaching botany for decades.

Matthias Schleiden died on June 23, 1881, in Frankfurt am Main, Germany. Summarizing his career, the Belgian botanist Léo Abram Errera said of Schleiden, "As a popularizer he was a model; as a scientist, an initiator."

See also CELL BIOLOGY; SCHWANN, THEODOR; VIRCHOW, RUDOLF.

Schwann, Theodor (1810–1882) German *Physiologist* Theodor Schwann is one of the founders of the cell theory. He reported in 1839 that animal tissues are constructed from individual cells. Matthias Schleiden had published similar conclusions regarding plants the year before. Together with Rudolf Virchow's claim that all cells arose from existing cells, the cell theory emerged, and it has guided the study of life ever since.

Theodor Schwann was born on December 7, 1810, in Neuss, Germany. His father worked as a goldsmith and a printer. Schwann was a good-natured and intelligent child. In 1826 he entered the Jesuit College of the Three Crowns in Cologne, planning to enter the church, but he changed his mind a few years later. In 1829 he enrolled at the University of Bonn, where he prepared for a medical career. During his time there he assisted in the laboratory of Johannes Müller, a renowned physiologist and comparative anatomist. Schwann moved to Würzburg in 1831, then two years later to Berlin, where Müller had been appointed to teach anatomy and physiology. After receiving his doctorate in medicine in 1834, with a dissertation on air and the development of chick eggs, he formally joined Müller's laboratory at the University of Berlin.

Schwann's early investigations involved the nervous, muscular, and digestive systems. Neurolemmocytes, cells that provide myelin insulation to axons of neurons, are commonly referred to as Schwann cells in his honor. He measured muscle length during contraction as a function of different loads and found that the stimulus affected the intensity of contraction. In 1836 he isolated the digestive enzyme pepsin, the first enzyme isolated from animal tissue. He also studied fermentation, the biochemical conversion of sugars into alcohol and other organic substances. At the time many other scientists were also examining the process of fermentation, which they believed was a chemical process catalyzed by oxygen. To test this, Schwann bubbled air through a boiled suspension of yeast in a sugar-based medium, and fermentation occurred. When he heated the air before bubbling it through the boiled yeast-sugar suspension, fermentation did not occur. He concluded that the unheated air contained a live organism that carried out the process of fermentation. Other experiments he performed helped to disprove spontaneous generation, though he never received much credit for this.

Müller was a vitalist, meaning he believed that living organisms contained a vital force distinct from physical or chemical forces. This did not satisfy Schwann, who determined to find a more scientific explanation for life through his microscopic examinations. He was the first of Müller's pupils to break with tradition by disagreeing with this principle.

A conversation in 1837 with Matthias Schleiden, who was also working in Müller's lab at the time, revealed their common observation that nuclei occurred both in plant cells and in cells of the notochord, a rodlike structure that is found in chordates and that develops into the spinal column of vertebrate animals. Realizing the importance of this commonality, both men soon confirmed that cells constituted both plants and animals. Schleiden published his finding that plants are composed of cells in a paper published in 1838. The following year, Schwann reported that animals were made of cells and more clearly enunciated what has come to be known as the cell theory in *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen.* This book was translated into English in 1847 as *Microscopic Investigations on the Accordance in the Structure and Growth of Plants and Animals.* The book explained that in frog larvae, the notochord cells were derived from structures similar to plant cells in that they contained a nucleus, a membrane, and a vacuole. Tissues, with varied "elementary parts," resulted from cellular differentiation. He concluded by philosophizing that the source of life originated from within the physical matter of the organisms, such as the arrangement of molecules that form the body structures, and the chemical activities of the molecular components. Functions and activities characteristic of the mysterious phenomenon of life were simply the actions of atoms and molecules obeying natural physical laws. The fundamental unit of the cell merged the molecules of chemistry with organisms of biology.

As set forth by Schwann and Schleiden, the cell theory stated that all living organisms consisted of

cells and that cells were the fundamental unit of life. Rudolf Virchow built on this and asserted that all cells arise from preexisting cells. Together these principles compose the basis of the cell theory, one of the most important historical developments in the life sciences.

The remainder of Schwann's scientific career was mostly uneventful. By the time his famous book was published, he had grown weary from the attacks of his colleagues who mocked his views on alcoholic fermentation. After being rejected for a chair at the University of Bonn, he abandoned rationalism and his enthusiasm for research science waned. He became a professor of anatomy at Louvain in 1839. While there, he developed a unique method for studying the role of bile in digestion, concluding that bile secretion was critical for survival. For this work he received the Sömmering Medal in 1841. In 1848 he moved to Liège as a chair in anatomy and became professor of physiology and general anatomy and embryology in 1858. He gave up teaching general anatomy in 1872, gave up embryology in 1877, and retired altogether in 1878.

After suffering a stroke while visiting his brother and sister in Cologne, Theodor Schwann died on January 11, 1882.

See also CELL BIOLOGY; SCHLEIDEN, MATTHIAS; VIRCHOW, RUDOLF.

scientific investigation Though the subject matter comprising the main areas of natural science encompasses a broad range of topics, the means of obtaining new knowledge unifies life science, physical science, and Earth and space science. All scientists use similar methods to increase their understanding of the world around them. The stepwise process begins with the collection of observations about a natural phenomenon. The scientist then asks related questions about the phenomena, develops a hypothesis that attempts to explain the observations, tests the proposed hypothesis by designing and performing a controlled experiment, analyzes the data, and draws conclusions concerning the validity of the hypothesis.

The first stage of scientific investigation involves collecting observations. A good scientist pays careful attention to the behavior of matter and organisms and the events surrounding them. From these observations, the scientist asks questions relating to the event and may seek additional information. The next step in the process is the development of a hypothesis, an educated, testable, tentative explanation of the event. Formulation of the hypothesis may stem from the scientist's creative imagination or from knowledge of similar events, but ultimately, validation of the suggested explanation requires the implementation of a set of procedures common to scientific investigation. The hypothesis must be testable in a manner that allows it to be proven incorrect, though an experiment could never prove a hypothesis to be true.

Comprehension of a phenomenon allows one to make predictions related to its behavior. Because a hypothesis attempts to explain a certain event, one can use the hypothesis to predict the outcome of an experiment designed to test that hypothesis. A well-designed experiment includes both a control group and an experimental group. The control group serves as a standard for comparison with an experimental group that varies from the control by a single factor. The factor that the experimenter deliberately alters is called the independent variable, and the factor that changes as a result of the manipulation is called the dependent variable.

After defining experimental treatments, the investigator must identify appropriate methods for collecting and analyzing the data. The investigator then performs the experiment, collects and analyzes the data, interprets the results, and draws conclusions that relate back to the original hypothesis. A well-designed experiment often leads to the identification of more refined questions and a repeat of the entire process.

The scientific method can be used to solve everyday problems. For example, consider a man who plants sunflower seeds around his house, and then observes that the sunflowers on one side of his house grow faster than those on the other side. He wonders why this is so. From previously gained knowledge, he knows that plants need both sunlight and water to grow. He considers the possibility that the sun might shine for a longer period of time on one side of the house compared to the other. Careful record keeping reveals that the total hours of sunlight that each side of the house receives each day are approximately equal. Based on this information, he rejects the notion that an increased amount of sunlight caused the sunflowers to grow faster on one side of the house compared to the other.

He then wonders if the observed effect is due to the amount of water received by plants on each side of the house. He waters both sides for an equal amount of time each morning, but he has only one hose and always waters one side in the mornings before leaving for work, and the other side when he comes home for lunch each day. After realizing that the side he waters first each day grows faster, he considers the possibility that the time of day might affect the growth rate of the plants. When the faster growing plants are watered, the sun is not out yet, but it is high overhead by the time the slower growing plants

(continues on page 676)

MODEL ORGANISMS FOR LIFE SCIENCE RESEARCH

by Michael L. Goodson, Ph.D.
University of California at Davis

In 2000 Dr. Eric Kandel, an Austrian-born psychiatrist turned neurobiologist, received the Nobel Prize in physiology or medicine for his discoveries of how neuronal synaptic changes are involved in learning and memory. He performed much of this research on the sea slug *Aplysia californica*—on the surface an odd choice of organism to study. However, unlike human beings, which have over 100 billion (10^{11}) neurons in their central nervous systems, *Aplysia* have only about 20,000 neurons that organize into nine groups or ganglia. *Aplysia* also have a number of readily observable simple behaviors, such as the gill withdrawal reflex in response to siphon stimulation, that can be conditioned. This greatly simplified system allowed Dr. Kandel's laboratory to make key discoveries about how neuronal signals pass within and between neurons and how both short- and long-term memories form. The lessons learned in *Aplysia* permitted Dr. Kandel and other researchers to make testable predictions about how the nervous systems of more complex organisms operate.

Experimental biologists frequently use model organisms and model systems because they make it possible to study some aspect of a biological phenomenon that cannot be studied experimentally in the organism of interest. For example, it is generally not possible to study and develop cures for human disease directly in people. Instead, scientists develop model systems such as isolated cells or simpler model organisms for the disease. Also, by focusing intense study on a limited number of carefully chosen organisms, scientists are often able to infer which phenomena are unique to a particular organism and which are conserved between many organisms. In general, a model system or organism must recapitulate the biological phenomenon (or at least some aspect of it) from the organism of interest. Ideal model systems also tend to have a short reproductive or life cycle, are genetically manipulable, and are relatively easy and inexpensive to grow and maintain. Also, less complex model organisms typically raise fewer ethical concerns and considerations associated with the use of the organism experimentally.

One of the simplest model organisms is the enteric bacterium *Escherichia coli.* Because of its ease of growth, quick generation time (often as short as 20 minutes under optimal laboratory conditions), and the relative simplicity of genetic manipulation, *E. coli* provided the foundation for much of modern molecular biology and molecular genetics. The ability to produce large amounts of *E. coli* quickly and inexpensively allowed scientists to identify and purify many of the enzymes and other factors involved in cellular metabolism. Furthermore, by making a large number of mutant *E. coli* strains, scientists were able to deduce the metabolic pathways that cells use to convert the nutrients they consume into necessary cellular constituents and to learn a great deal about how cells replicate and repair their DNA. By studying how *E. coli* respond to different sources of carbon (such as glucose versus lactose), molecular biologists began to understand how expression of genes is regulated. Because gene regulation in eukaryotes differs significantly from prokaryotic gene regulation, researchers needed other model systems to study eukaryotic-specific phenomena.

Unlike eukaryotes, *E. coli* and other prokaryotes do not have a nucleus or other membrane-bound organelles. Also, *E. coli* have a single circular chromosome rather than multiple linear chromosomes, as found in eukaryotes. The baker's and brewer's yeast *Saccharomyces cerevisiae* serves as a useful genetic model for studying many eukaryotic-specific cellular functions, including regulation of the cell cycle, DNA replication, and cellular and organellar structure and dynamics. Like *E. coli, S. cerevisiae* are single-celled organisms that can be grown easily and quickly (a four-hour cell cycle, or less, depending on the medium) and are genetically manipulable. The ability to make a wide variety of sophisticated mutations, including temperature sensitive mutations and compensatory mutations, have allowed scientists to tease apart many complex regulatory systems and pathways, such as the regulation of the progression through the cell cycle by the cyclic expression of a series of regulatory proteins called cyclins.

Because entire populations of genetically identical *E. coli* and *S. cerevisiae* can be grown under precisely defined conditions, scientists are able to perform controlled experiments that are simply not possible with more complex metazoan organisms. The additional advantage of being able to produce larger amounts of biological material than would be possible from more complex sources has facilitated the isolation and characterization of cellular components from these cells that are not sufficiently abundant to study in other organisms. Access to a virtually unlimited supply of the biological material facilitates the biochemical characterization of the proteins and factors.

The unicellularity of *E. coli* and *S. cerevisiae* limits their usefulness as models for multicellular development and organization. The free-living nematode (roundworm), *Caenorhabditis elegans,* serves as a particularly useful model for studying metazoan development. Cell biologists have determined the developmental lineage of every one of the approximately 1,000 somatic cells in *C. elegans.* The lineage of each of these cells does not vary between individual worms, a phenomenon that is very different from more complex organisms. Another advantage of using nematodes is that they exist both as hermaphrodites and as males, a trait that makes it possible both to produce populations of genetically identical worms and to test the effect of a given mutation in the background of other mutations. The invariant nature of cell fate in *C. elegans* enabled the identification of

a plethora of mutants that affect specific cell fates, an accomplishment that earned the noted British molecular biologists Sydney Brenner and John Sulston shares of the 2002 Nobel Prize in physiology or medicine. The extremely simplified nervous system (comprised of only 302 neurons, the connections between which are all known) made it a useful model for early neurobiological studies as well.

The relative simplicity and the lack of plasticity in cell fate have made *C. elegans* extremely powerful, but also limit the insights into nongenomic determinants of cell fate and the processes involved in complex organogenesis. Another powerful model of development is the fruit fly, *Drosophila melanogaster,* which has served extensively as a model for segmented embryonic development and symmetry and limb development. Scientists have been able to identify mutations in the intercellular signaling pathways involved in the development of complex organ and body structures such as wings, legs, and compound eyes. Studies on *Drosophila* embryonic development have revealed a great deal about the role of both embryonic and maternal expression of genes, called homeobox genes, in establishing the body segment patterns during early embryogenesis.

While all of the previously described model organisms (and many others) have provided keen insights into a wide range of biological phenomena, they are all invertebrate organisms. One of the vertebrate models for development is the African clawed toad, *Xenopus laevis.* Detailed analysis of the development of the frog from egg to tadpole through metamorphosis into mature frogs has led to the discovery of many of the factors that control the timing of morphogenic signals. The *Xenopus* oocytes have also proven extremely useful. Because of their large size (approximately 0.039 inches [1 mm] in diameter), researchers can mechanically inject DNA directly into the oocyte.

Mammalian model organisms provide information most relevant to human biology and human disease. Because rats and mice are closely related to humans and many processes are conserved between humans and other mammals, researchers have been able to develop strains of mice or rats that recapitulate human diseases. In the past, many of these model systems came about by spontaneously occurring mutations that lead to diseases that were homologous to human disease in the model organism. In the 1950s at the Jackson Labs, a strain of morbidly obese mice was isolated and found to possess mutations in the *ob* gene. Later work determined that the *ob* gene encodes a regulatory protein hormone, called leptin, that controls appetite and metabolism. Another strain of mice arose from the spontaneous mutation of the *db* gene, which was subsequently identified as the leptin receptor gene. In addition to being obese, the *db* strain of mice develops diabetes very early. Medical researchers still use the *ob* and *db* mice as models for studying obesity, diabetes, and the metabolic syndrome.

Today researchers can create very specific disease models and test the role of genes and mutations in specific diseases by selectively inserting, deleting, or replacing genes in mice. This methodology was used to create models for a wide range of diseases including several models for cystic fibrosis. The ability to construct model mouse strains allows scientists to directly test the role of specific genes or mutations in diseases. Pharmaceutical research utilizes these strains in order to develop drugs that more selectively target specific disease-related gene products, resulting in more effective therapies with fewer side effects. One example of this is the HER-2 growth factor receptor. About 20 to 40 percent of breast cancers express HER-2 at higher than normal levels, and patients with this type of tumor have a poor survival prognosis. Mice that express the HER-2 protein spontaneously develop mammary tumors. These mice allowed scientists to develop and test a HER-2 targeted antibody therapy that significantly increases the survival rate of patients with HER-2 positive breast cancer.

Scientists also frequently use cells isolated from either humans or other organisms as models for diseases such as cancer as well as other biological processes. By using cells in isolation many scientists are able to ask questions using a very reductionalist approach. One application of this involves fibroblast cells (cells that form fibrous connective tissue) isolated from mice. The cultured cells can be induced to differentiate into adipose (fat) cells; thus, this system has been widely used as a model to study adipogenesis, a key process involved in heart disease and diabetes. By studying these cells in culture (rather than in the whole organism), researchers discovered many of the extracellular signals that are required to cause fibroblasts to differentiate into adipocytes, as well as many of the changes in gene expression that occur throughout this process.

Model systems allow scientists to design and perform experiments to test hypotheses that provide insights into biological phenomena that would otherwise not be directly testable. This is possible because many biological processes and components are conserved between living organisms. Information gained from experiments using the model systems allows for more insightful predictions that can be used to design experiments that are directly testable in nonmodel organisms.

FURTHER READING

Cold Spring Harbor Laboratory. "Model Organisms." In *Unwinding DNA: Life at Cold Spring Harbor Laboratory.* Available online. URL: http://www.exploratorium.edu/origins/coldspring/tools/index.html. Accessed February 2, 2008.

Kandel, Eric R. *In Search of Memory: The Emergence of a New Science of Mind.* New York: W. W. Norton, 2006.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 2000." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/2000/index.html. Accessed February 2, 2008.

———. "The Nobel Prize in Physiology or Medicine 2002." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/2002/index.html. Accessed February 2, 2008.

TheScientist.com. Model Organisms. Vol. 17, supplement 1 (June 2, 2003). Available online. URL: http://www.the-scientist.com/supplement/2003-6-2/. Accessed February 2, 2008.

(continued from page 673)
are watered at midday. This realization leads him to hypothesize that the plants on one side of his house grow faster because the water has a chance to soak into the soil where the roots can access it, whereas the water sprayed on the slower growing sunflowers evaporates before much of it can be absorbed by the soil, and therefore the roots of the sunflowers. To test whether or not the time of day that he waters the plants (the independent variable) causes different growth rates (the dependent variable), he designs an experiment in which he switches the time of day that he waters each side of the house. If the hypothesis is true, then the plants that are watered in the morning will grow faster than plants that are watered at midday, no matter which side of the house they are on. The man collects data by measuring the height of the plants every other day. At the end of two weeks, the data shows that the plants watered in the morning grew faster than the ones at lunchtime. These results support his hypothesis, and he concludes that the plants on one side of his house grew faster due to an increase in the amount of available water.

This single experiment does not prove his hypothesis to be true, though the data are convincing. To gain additional supportive evidence for the same hypothesis, the man could perform another experiment in which he waters the lunchtime plants for a longer period of time to account for the increased evaporation rate of the water, effectively increasing the amount of available water. If the increased watering time led to a faster growth rate than the original watering time, those results would also support his hypothesis.

After amassing an overwhelming amount of supportive evidence, a hypothesis can grow into a theory. By definition, a scientific theory is a hypothesis that has withstood repeated experimental testing. Common use of the word theory often implies assumption, a hunch, or a conclusion based on a feeling not necessarily supported by any scientific evidence. For example, someone might claim to have a theory about why television shows on Friday and Saturday nights are more violent than shows that appear during the week. Scientifically, the word theory is only used when overwhelming evidence has already been gathered to support a claim. Examples of widely accepted theories that explain many observable phenomena in the life sciences are the theory of evolution, the germ theory of disease, and the cell theory.

See also SCIENTIFIC THEORY.

scientific theory The word *theory* can imply distinctly different meanings depending on the context in which it is used. *Theory* can refer to a set of ideal principles; for example, someone may declare that they believe that socialized medicine, a Montessori eduction, or communism sounds good in theory. In a slightly different context of everyday conversation, a person may claim to have a theory about why family members disappear from the kitchen so quickly after eating dinner. In this case, a theory means a guess. The so-called theory is simply speculation, not necessarily based on any facts—the polar opposite of what the word theory means to the scientific community. When used in scientific discourse, the word *theory* implies a strongly supported, widely accepted explanation for a set of related observations or natural phenomena. Thus, a statement such as "evolution is just a theory" has no place in scientific discussions.

Nonscientists often confuse scientific theories, hypotheses, and laws or use the terms interchangeably, but the terms all have different meanings. Scientific laws, also called natural laws, are simple universal truths that describe a natural phenomenon. Laws have a much narrower focus than theories. Observations repeatedly confirm laws to be reality. They are not debatable and do not require inference or human interpretation; they simply state what has happened in the past and will happen given a future similar situation. Laws govern a limited circumstance, and many scientific laws can be summarized by a mathematical expression. Unlike laws that govern society, one cannot choose to follow or not to follow a scientific law. Scientists can make predictions about future events with certainty based on a scientific law. For example, according to the law of gravitation, if a woman steps off a diving board over a pool, she will fall into the water below. Every person who has ever stepped off a diving board has fallen downward, and tomorrow if a diver who has never before stepped off a diving board does so, that person too will fall into the water. Because scientific laws do not change, they form the foundation of science. Examples of other scientific laws, also called natural laws, include the law of conservation of energy and the law of partial pressure.

A hypothesis is an educated guess, a tentative explanation that can be tested. Scientific experiments test hypotheses, and the data may support or refute the hypothesis. If the data refutes a hypothesis, a scientist will reexamine the data and consider previous observations to formulate a new hypothesis. As long as an idea is still subject to investigation, it remains a hypothesis.

By the time a scientific theory has achieved the status of a theory, multiple scientists have gathered enough data from numerous experiments and observations to support it beyond a reasonable doubt. Although one scientist can initially formulate and

propose a theory, one person cannot alone elevate an idea to the status of a scientific theory. The majority of the scientific community must generally agree with the evidence and rationale supporting the theory. New observations may demand that a certain aspect of the theory be slightly modified, but theories are rarely overturned, just fine-tuned. Though it would be rare for a scientific theory to be disproved, by nature a scientific theory must be falsifiable. For example, the so-called theory of creationism is not a scientific theory because it cannot be disproved.

Scientific theories are broader in scope than scientific laws and may encompass several laws. A theory provides a rational explanation for the regularity of an observation or event described by a scientific law. Individual laws fit into the structure or framework of a theory. To illustrate the relationship between a law and a theory, consider the law of gravitation, as mentioned above. In 1916 the renowned theoretical physicist Albert Einstein published his general theory of relativity, the most successful theory of gravitation. The *law* of gravitation describes the acceleration of one object in a direction toward another closely spaced body, such as any object falling in the direction from the sky toward Earth or a diver falling into the water in the above example. The *theory* of general relativity explains the effect of gravitation while describing the trajectory of the body in space-time—explicating that one large mass (such as the planet Earth) curves the shape of the surrounding space (and warps time), causing a second mass within the affected space to accelerate toward the first mass, making it look like one mass attracts the other mass. Other examples of scientific theories include the big bang theory, the theory of relativity, the electromagnetic theory, the atomic theory, the cell the theory, the theory of evolution, the chromosomal theory of inheritance, and the germ theory of disease.

See also SCIENTIFIC INVESTIGATION.

sensation A unifying theme of biology is interaction with the environment. Living organisms have mechanisms for obtaining information about environmental variables such as temperature, light, or sound. Even the most primitive organisms display mechanisms for receiving input from the environment and coordinating appropriate responses. Many bacteria and protists have chemoreceptors that detect the presence of certain chemicals such as oxygen and direct their movement by following an increasing chemical gradient toward the substance. This process, called chemotaxis, can also result in movement away from toxic substances. Plants also have the ability to respond to information about conditions of the external environment, such as drought, flooding, mechanical pressure, or the amount of sunlight, and respond to the conditions appropriately.

Animals with developed nervous systems have unique mechanisms for receiving information from the external environment and from within their bodies. Electrical signals travel through the nervous system as action potentials along neurons and across junctions via chemical signals, but first, the action potential must originate somewhere in response to something. An action potential, a momentary reversal in electrical potential across the membrane of a neuron occurs when a cell has been activated by a stimulus. The initial stimulus can be in the form of a chemical, mechanical, or energy change and can be from the external environment (seeing a brightly colored flower) or from inside the body (experiencing a pain in the abdomen). No matter what the trigger is, an action potential is the same, so the difference in how an organism perceives the sensation is due to the location in the brain that receives the signal. Action potentials that travel down sensory neurons are called sensations; they originate from specialized receptors activated by a form of energy and travel along a neural pathway toward the brain. Perception occurs when the brain receives the sensory signal and the organism becomes aware of the sensation. The brain then instructs the body how to respond to the information by initiating new action potentials down motor neurons, neurons that carry impulses from the brain to muscles, resulting in movement. Much of the information relayed to the brain from internal receptors contributes to homeostasis, the maintenance of a constant internal environment. The brain processes the received input, integrates it with other information, and coordinates a response based on physiological needs, past experiences, and resource availability.

SENSORY RECEPTORS AND PROCESSING

Sight, smell, hearing, taste, and touch are types of sensations grouped together based on the associated well-known sense organs: the eyes, nose, ears, mouth, and skin. This classification system does not include sensory perceptions of body positioning, temperature, pain, or other sensations. Another means of categorizing the senses relies on the nature of the detected stimulus. Five types of sensory receptors include mechanoreceptors, pain receptors, thermoreceptors, chemoreceptors, and electromagnetic receptors. Each type transduces, or converts, a form of energy into a change in membrane potential. Mechanoreceptors detect physical stimuli such as touch, pressure, or vibration—all forms of energy that lead to a physical deformation in the receptor or surrounding cells. An example is a hair cell such as in the inner ear of vertebrates. Specialized cilia or microvilli bend in

response to an environmental stimulus, initiating an action potential in the membrane of the hair cell. Pain receptors, also called nocireceptors, respond to stimuli that cause physical or chemical damage to tissues and are potentially dangerous, such as extreme heat or excessive pressure. Thermoreceptors monitor the internal and surface temperature of the body and relay the information to the body's thermostat in the hypothalamus. Chemoreceptors specifically recognize the presence of certain chemicals in the air, in food, or in bodily fluids, or more generally receive information about the surroundings such as the overall solute concentration of the blood. The binding of the chemicals results in a change in membrane permeability. Taste and smell receptors are specific types of chemoreceptors. Electromagnetic receptors detect and respond to electromagnetic energy such as light, electricity, or magnetism. Photoreceptors are a class of electromagnetic receptors that responds to particular wavelengths of visible light and are necessary for vision.

After detection, sensory receptors convert the stimulus into a change in membrane potential, a process called sensory transduction. Alteration of the membrane potential results from altered permeability of the cell membrane to ions. Specific ion channels open or close, making the membrane more or less permeable to the charged particles. The change in membrane potential is called the receptor potential, and it is graded, meaning the degree of change in the membrane potential depends on the intensity of the stimulus. After sensory transduction, the action potential travels to the central nervous system (CNS) for processing. The original energy stimulus is amplified during transmission.

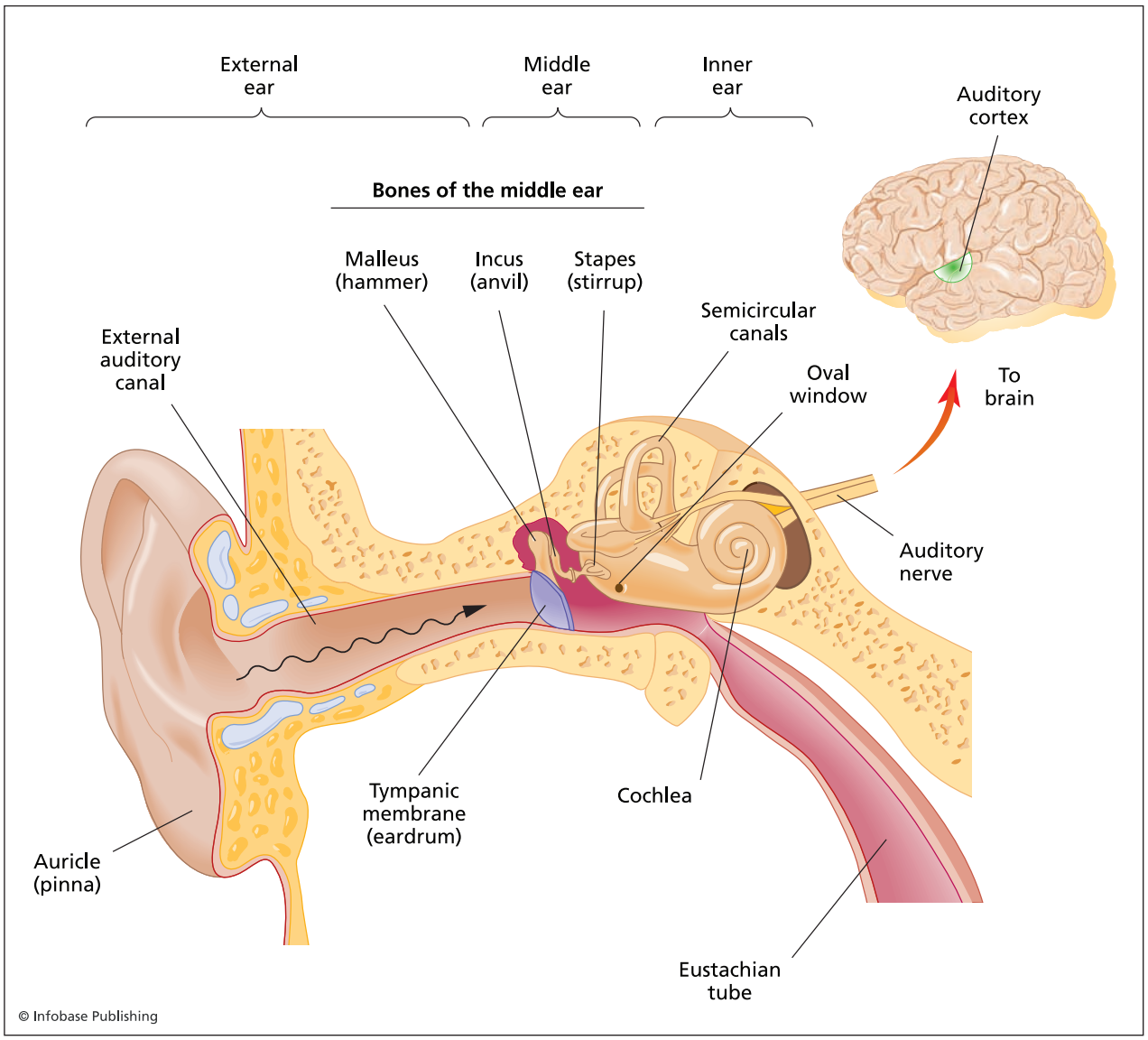
The thalamus receives the sensory input and directs the signals to the appropriate regions of the cerebrum, the largest region of the brain. The cerebral cortex, the convoluted outer layer of the cerebrum, is responsible for sensory and motor integration. Specialized regions process different types of sensory input. Primary sensory areas contain groups of neurons that receive related sensory input and process information from the sensory organs, while association areas integrate information from different parts of the brain to help one develop a more complete understanding of the sensory input. The cerebral cortex consists of a right and a left hemisphere, each of which contains four lobes named for the skull bones under which they are found: the frontal, temporal, parietal, and occipital lobes. The frontal lobes interpret sensory input, organize responses to that input, and control motor output in addition to regulating emotions, monitoring behavior, helping in making decisions, and retrieving information from memory. Damage to the frontal lobes can cause symptoms that could be interpreted as immature behavior, such as impulsiveness, displaying extreme emotions, repeating mistakes, not being able to sequence tasks to accomplish a larger goal, and difficulty retrieving and applying information. Temporal lobes are located on the lower sides and play a role in sensory perception, spatial organization, and forming memories. If the temporal lobes do not function properly, a person might struggle with words, language comprehension, context clues, and visually oriented memories. Located in the middle of the hemispheres, the parietal lobes compile sensory data such as sights and sounds to assign meaning to the information. For example, the parietal lobes help someone recognize that the sound coming from the front of the classroom is the teacher's voice and that she is calling someone's name. The occipital lobes, located at the back of the brain, are responsible for registering visual sensory information, which is then sent to the parietal lobes for processing.

HEARING AND BODY EQUILIBRIUM

Hearing, balance, and awareness of body position involve mechanoreceptors. Many invertebrates have statocysts, fluid-filled vesicles lined with cilia that detect the position of statoliths, grains of sand or other calcareous material whose positioning aids in body equilibrium. Gravity causes the statoliths to sink to the bottom of the statocyst, stimulating mechanoreceptors attached to the cilia at that location. The animal gains information about its body position based on which mechanoreceptors the statoliths stimulate. In vertebrates, position sense receptors located inside of muscles and tendons monitor muscle length and degree of tension. During rest or movement, these receptors keep the brain informed regarding the positions of all body limbs and body orientation.

Hearing in most insects also involves cilia that move, but the movement occurs in response to sound waves, vibrations in the air. Different hairs have different characteristics with respect to length and thickness, and therefore respond to different frequencies. The movement stimulates mechanoreceptors that send the information to the brain for processing. Some insects also have tympanic membranes, thin membranes that cover the opening of an air chamber, that vibrate in response to sound waves and can sense movement nearby.

In mammals including humans, the sensory organs for balance and hearing are both located in the ears, paired structures located on either side of the head consisting of three parts: outer, middle, and inner. The pinna of the outer ear acts as a funnel that collects sound and transmits it through an auditory canal to the tympanic membrane (the ear-



The human ear functions in both hearing and balance and consists of three parts: the outer ear, the middle ear, and the inner ear.

drum). Sound waves cause the tympanic membrane to vibrate. Three tiny bones in the middle ear, the malleus (hammer), incus (anvil), and stapes (stirrup), transfer the energy from the vibrating membrane to the inner ear, which is composed of a complex arrangement of tubes and chambers: the vestibule, the semicircular canals, and the cochlea. Three separate fluid-filled canals comprise the cochlea, which contains thousands of hairs, or cilia, attached to hair cells. Movement of the bones converts the vibrations of the eardrum into pressure waves that cause fluid movement within the cochlea, which ultimately results in deflection of the hairs. In response, the hair cells initiate action potentials in the neurons attached to the hair cells, sending nervous impulses through auditory nerves to the thalamus, which relays the signals to auditory processing centers in the temporal lobe of the cerebral cortex.

Two chambers inside the vestibule, the utricle and the saccule, function in maintaining balance by detecting gravity. They contain tiny particles of calcium carbonate called otoliths that function similarly to statoliths. The otoliths rest on top of hair cells in the utricle and saccule, sending information regarding body positioning to the brain. Changes in body positioning moves the fluid, causing the hairs to bend, and stimulate the hair cells lining the canals to release neurotransmitters that activate sensory neurons across a chemical synapse. The sensory neurons send electrical impulses through the vestibular branch of the auditory nerve. The utricle leads to the semicircular canals, three tubular loops that are

oriented in three different planes. Fluid movement in these structures gives information regarding the body when it is moving or when the head changes its angle or degree of rotation. The brain receives these impulses and converts them into information about body orientation based on the location of the stimulated hair cells within the semicircular canals. Receptors in neck joints provide additional information about body posture. The brain integrates this information with visual input to maintain equilibrium, explaining why it is easier to maintain balance when one's eyes are open.

Fish and aquatic amphibians have sensory organs similar to those in the middle ear of humans that are responsible for maintaining equilibrium. The structures responsible for hearing, however, do not include an eardrum or an opening that leads to the outside of the body. Because they live in an aquatic environment, sounds waves traveling in the water travel directly though the skeleton to the inner ears, where they stimulate the hair cells. The swim bladder, an organ that functions in maintaining buoyancy, may also aid in transferring sound waves to the inner ear. A lateral line system that runs along the animal's sides contains mechanoreceptors that receive and transmit information about direction or rate of the animal's movement through the water. This system also detects slight alterations in currents of the surrounding water, such as those caused by nearby moving objects, including possible predators or prey.

Terrestrial vertebrates other than mammals also utilize the inner ear for both hearing and balance functions. Frogs and toads have a tympanic membrane on the surface of their body and a single bone that transmits the energy of the sound waves to the inner ear. Frog lungs might also send auditory information. The saccule contains a side pocket that functions as the main hearing organ, similar to the cochlea in mammals. Birds have cochlea like mammals, but a single ear bone like amphibians.

TASTE AND SMELL

The senses of taste and smell (olfaction) both utilize chemoreceptors that detect the presence of specific chemicals. In aquatic animals, no distinct difference exists between taste and smell. In terrestrial animals, olfaction detects chemicals present in the air, whereas taste is the sensation of chemicals in a solution. Many animals rely on chemical signals to communicate information regarding mating, location of food sources, danger, or territory. In insects, the feet and mouthparts contain hairs called sensilla that have chemoreceptors for taste.

In mammals including humans, the mouth is the main taste organ. Taste buds are clusters of sensory cells associated with papillae, the visible bumps on the surface of the tongue. Saliva dissolves chemicals in ingested substances, and penetrates taste pores leading to the taste buds. Five primary perceptions of taste result from the stimulation of five classes of receptors on sensory cells:

- salty,
- sour,
- bitter,
- sweet,
- and umami receptors.

Each class of receptor recognizes one type of molecule most efficiently. Salt receptors recognize ions of salts such as sodium ions (Na^+). Sour receptors recognize hydrogen ions (H^+) that form when acids are present in the saliva. Sweet taste results from many different chemicals including but not limited to sugars. The bitter receptors bind a variety of alkaloids (bases). Amino acids such as glutamate stimulate umami receptors. The extensive range of different recognizable flavors is possible through variations of combinations of the input of many receptors stimulated simultaneously. The binding of a chemical substance to a chemoreceptor induces a series of chemical changes within the sensory cell ranging from a cascade of biochemical reactions to a change in membrane permeability to a specific ion. These cellular events ultimately lead to the sensory cell releasing a neurotransmitter into a chemical synapse. Binding of the neurotransmitter to specific receptors on a sensory neuron triggers an action potential.

Olfaction occurs by similar mechanisms except that the chemoreceptors are found on the millions of cilia that extend from olfactory neurons lining the roof of the nasal passage. Chemicals present in the air bind to their specific receptors, triggering action potentials that carry the signals to the brain. More than 1,000 human genes encode olfactory receptors that recognize different chemicals. The sense of smell enhances the perception of taste. Food in the mouth releases odors that diffuse into the nasal passage via the pharynx.

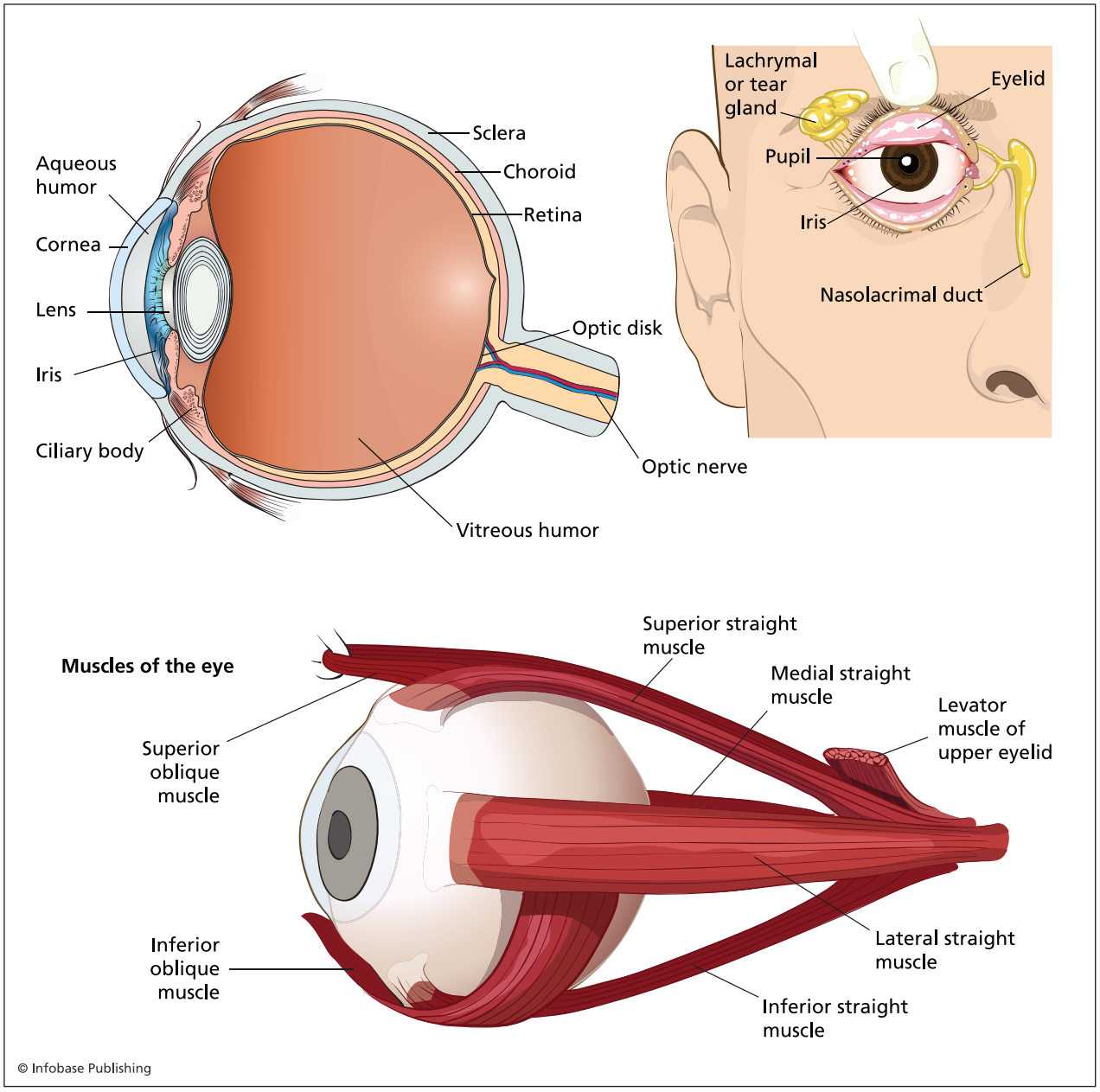
VISION

Photoreceptors contain pigments that absorb electromagnetic radiation at different wavelengths. Many animals possess sensory organs capable of detecting light. Primitive invertebrates such as planarians contain simple visual organs called eyespots that allow for the detection of light but not for the formation of images. The animal uses this information to move away from light to hide from predators. Many invertebrates possess eyes, visual organs that can transform the energy of electromagnetic waves into visual

images. Compound eyes, found in insects and crustaceans, contain thousands of individual light detection units (ommatidia), each having a lens to focus light and a retina that contains the photoreceptors. The individual units send information to the brain, which integrates the information from all the visual units to form an image. Compound eyes allow for the rapid detection of movement, which is why swatting a fly is so difficult, but have a very short depth of focus, making insects very nearsighted. Some insects have a second pair of visual organs called ocelli that can detect movement or the presence of light, but cannot form images.

Other invertebrates, such as some jellies, marine worms, spiders, octopuses, and squids, have single-lens eyes. The iris of a single-lens eye adjusts the diameter of its pupil (the opening through which light enters) depending on the amount of light available. Muscles that move the entire lens forward or backward help focus the light on a layer of photoreceptors that send the information to the brain.

Vertebrate eyes also have single lenses, but they evolved by a completely different lineage from the single-eye system in invertebrates. Occurring in pairs, vertebrate eyes can see in color and over a



The vertebrate eye is well adapted for color vision, depth perception, and movement.

wide range of distances. A layer of white connective tissue called the sclera surrounds the entire outer surface of the eyeball and acts as the attachment site for muscles that move the eyeballs. In front of the lens, the sclera is clear and is called the cornea. Light enters the roughly spherical eyeball through the pupil, the opening in the center of the eye. A thinner layer of tissue called the choroid surrounds most of the eye and forms the iris, the colored part of the eye that defines the pupil. The iris acts like a diaphragm that adjusts the diameter of the pupil to control the amount of light that enters. The ciliary body produces the liquid aqueous humor, a substance that fills the region between the lens and the cornea. Light passes through the lens, a flexible structure that focuses the light onto the retina, the light-sensitive layer that lines the interior of the back of the eyeball. Ciliary muscles help the lens focus by contracting and relaxing to adjust the thickness of the lens. The space behind the lens and in front of the retina is filled with a jellylike fluid called the vitreous humor that helps focus the light onto the retina. The retinas of humans contain about 125 million rods, photoreceptors that are better for seeing in the dark but do not distinguish colors, and 6 million cones, photoreceptors that can distinguish colors. Though representatives from all vertebrate classes have color vision, not all vertebrates do. Among mammals, color vision is mostly restricted to primates, but evidence suggests that a few other mammalian species have limited color vision. Animals that are nocturnal benefit from having as many rods as possible since they permit sharp night vision.

Both rods and cones have retinal, a derivative of vitamin A that absorbs visible light. Retinal rests in the center of membrane proteins called opsins. Formed by the combination of retinal with the particular opsin found in rods, rhodopsin is the visual pigment that functions in dim light. In the presence of light, rhodopsin changes its shape. Vertebrates contain three types of cones that function in bright light and are characterized by different opsins. Called photopsins, each pigment primarily absorbs one type of light, though overlap does exist. Blue cones respond to light with a wavelength of 430 nanometers (nm), green cones 535 nm, and red cones 575 nm. The perception of color depends on the combination and degree of stimulation of each of the three types of cones. The sex-linked condition of color-blindness, distinguished by the inability to perceive differences in some colors, occurs when one or more types of photopsin are absent. When a visual pigment is activated by the absorption of light, a complex series of events leads to the generation of an action potential that travels via the optic nerve to the thalamus, which then relays the visual input to the cerebral cortex in the occipital lobe for processing. The brain integrates sensory input from both eyes with other information to create a colorful, three-dimensional perceived image.

See also CELL COMMUNICATION; EUKARYOTIC CELLS; HOMEOSTASIS; NERVOUS SYSTEM.

FURTHER READING

Kahle, Werner, and Michael Frotscher. *Color Atlas of Human Anatomy: Nervous System and Sensory Organs*. Vol. 3. 5th rev. ed. New York: Thieme, 2003.

Nagel, Rob. *Body by Design: From the Digestive System to the Skeleton*. Vol. 2. Detroit, Mich.: U*X*L, 2000.

Purves, Dale, George J. Augustine, David Fitzpatrick, William C. Hall, Anthony-Samuel LaMantia, James O. McNamara, and S. Mark Williams, eds. *Neuroscience*. 3rd ed. Sunderland, Mass.: Sinauer, 2004.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

Silverthorn, Dee Unglaub. *Human Physiology: An Integrated Approach*. 4th ed. San Francisco: Benjamin Cummings, 2006.

Wolfe, Jeremy M., Keith R. Kluender, Dennis M. Levi, Linda M. Bartoshuk, Rachel S. Herz, Roberta L. Klatzky, and Susan J. Lederman. *Sensation and Perception*. Sunderland, Mass.: Sinauer, 2005.

sex determination Sexual reproduction in animals usually involves the production of offspring following the union of two gametes produced by two different parents. Almost all vertebrates and many invertebrates are dioecious, meaning separate sexes exist. Each sex has its own specialized reproductive organs and structures designed to produce gametes and facilitate the process of fertilization. In some species, hermaphroditism, the condition in which one individual has both male and female reproductive organs, can lead to the production of offspring through self-fertilization, the union of two gametes made by one parent. The gametes produced by the male are called spermatozoa, or sperm, and the gametes produced by the female are called ova, or eggs. The gametes only contain half of the genetic material. Fertilization, the union of an egg and a sperm cell, restores the full complement of genetic material in the resulting zygote, which subsequently develops into an adult of the same species.

Sex determination, whether the individual turns out to be male or female, results from a complicated series of precisely controlled processes that occur during development. Sex determination is different

from sex differentiation, the process by which undifferentiated gonads develop into either testes or ovaries. Environmental factors and genetic mechanisms both play a role in sex determination to varying degrees dependent on the species, and in some species, individuals have the ability to switch between genders in response to environmental triggers, a condition called sequential hermaphroditism.

ENVIRONMENTAL MECHANISMS

In some species, environmental mechanisms play the major role in the determination of sex. For example, the incubation temperature during a critical phase of embryogenesis determines the gender of the hatchling in many egg-laying reptiles such as turtles and crocodiles. Temperature-dependent sex determination has been found in at least one known species of lizard, *Eulamprus tympanum,* that gives live birth. Three different patterns of temperature-dependent sex determination have been observed in different species of reptiles: cooler nest temperatures result in females, and warmer temperatures result in males; males are produced at lower temperatures and females at higher temperatures; or females are produced at extreme temperatures, whether high or low, and intermediate temperatures produce males. Because studies have shown that treatment with different steroid hormones and with enzymes that affect the synthesis of steroid hormones affects sex determination in these reptiles, the temperature is thought to act on genes of enzymes that synthesize steroid hormones and steroid hormone receptors.

Other environmental cues that stimulate a change in gender are social or behavioral. Slipper limpets, a type of gastropod sea mollusk, exhibit sequential hermaphroditism. They are males when free-swimming but become female when they settle down and attach to a rock. If a free-swimming male passes by, he will join the female to copulate. If another male swims by and settles on top of the first male, the first male transforms into a female. The sex of individual slipper limpets is determined by their relative position in the stack, with all but the uppermost individual being female. Wrasse are brightly colored fish that live in harems consisting of numerous females and a single male who mates with all of them. If the male dies or is removed from the harem, the largest female starts behaving like a male, her body absorbs all of her eggs, and within one week the individual, now a male, produces enough testosterone to make sperm. The small tropical fish called hamlets are simultaneous hermaphrodites, meaning they can act as males and females at the same time. During copulation, two hamlets take turns acting as one sex, then the other.

CHROMOSOMAL DETERMINATION

Chromosomal factors determine the gender of most other animals. Chromosomes that carry genes responsible for determining sex are called sex chromosomes. All others are called autosomes and do not influence gender. Five different chromosomal methods for sex determination include XY, ZW, XO, haplodiploid, and compound chromosomal mechanisms. The alphabetical notation is completely symbolic. The letters signify nothing about the size or shape of the chromosomes they represent.

With the exception of mole voles and the spiny rat, all mammals follow the XY system. In this system, both sexes contain the same total number of chromosomes, but females exhibit homogamety, meaning they have two X chromosomes (XX), and males exhibit heterogamety, meaning they have one X and one Y chromosome (XY). Because the females have two sex chromosomes, all of the eggs they produce will contain an X chromosome. Males, however, can contribute either an X or a Y to each sperm cell. Thus, the type of sex chromosome that the sperm cell contains determines the sex of the offspring.

Flies also have an XY system, but sex determination is not based simply on the presence of a Y, as in mammals, but rather on the ratio of the total number of X chromosomes to sets of autosomes. *Drosophila* typically has four pairs of chromosomes, three different autosomal pairs and one pair of sex chromosomes. As in most mammals, if an individual fruit fly possesses two X chromosomes, the individual will be female, and a fly with one X and one Y chromosome will be male. This seems to resemble the chromosomal mechanism for sex determination in mammals, but the examination of flies with abnormal sets of chromosomes reveals a different underlying mechanism. Flies containing two X chromosomes and one Y chromosome (XXY) are female, whereas in humans, this combination of sex chromosomes results in a male with Klinefelter syndrome. A fly with a single X chromosome is male (but sterile), whereas in humans, an individual with one X chromosome (abbreviated XO) is a female with the condition called Turner syndrome. The genic balance, or the ratio of the number of X chromosomes to autosomal sets of chromosomes (X:A), determines the gender outcome. A normal diploid female fly has one pair of sex chromosomes and two sets of autosomes comprising three different pairs; so the X:A ratio of a normal female would be two X chromosomes to two sets of autosomes, or 2:2, which equals 1.0. If the X:A ratio is 1.0 or higher, then the outcome will be female. If the ratio is 0.5 or lower, the organism is male. A normal male fly would have one X chromosome, one Y chromosome, and two sets

of autosomes; the X:A ratio would equal 1:2. Based on this explanation, an abnormal XXY fly would have an X:A ratio of 1.0, and therefore be female. An abnormal XO fly would have a ratio of 1:2, and therefore be male. If the ratio is between 0.5 and 1.0, the organism is considered intersex.

Birds, moths, butterflies, and some fish follow the ZW mechanism. The Z and W chromosomes are not related to the human X and Y chromosomes. In the ZW system, the males are homogametic (ZZ), and the females are heterogametic (ZW). The males can contribute only a Z to the offspring, but females can contribute either a Z or a W, thus the type of sex chromosome that the egg contains determines the gender of the offspring in the ZW mechanism.

In the XO mechanism, only one type of sex chromosome, the X, exists. The presence of two X chromosomes usually results in a female, and a single X chromosome results in a male. Males produce sperm that have either an X chromosome or no sex chromosome. The gender of animals such as grasshoppers, cockroaches, and some other insects is determined by the XO mechanism.

In the haplodiploid system for sex determination, no sex chromosomes exist. Eggs that are fertilized develop as females, and eggs that are not fertilized develop as males. Females are diploid, and the fatherless males remain haploid. Bees, ants, and wasps develop by this mechanism.

Some species of beetles and bedbugs carry several X and Y chromosomes that collectively determine the sex. This is the compound chromosomal method of sex determination. While the genes carried by the sex chromosomes initiate the process of sexual development, there are still many other genetic and hormonal influences that ultimately may affect the outcome.

SEX DETERMINATION IN HUMANS

Normally human females are XX and thus can contribute only an X chromosome when making eggs. Males can contribute either an X or a Y chromosome to sperm cells. Sex determination occurs at the moment of fertilization. Under normal circumstances, if the sperm that fertilizes an egg carries an X chromosome, then the offspring will be female. If the sperm cell carries a Y chromosome, then the offspring will be male. Much research has focused on the process of differentiation that leads to the male or female phenotype from a sexually indifferent embryo.

By five weeks after fertilization, paired bulging structures called the genital ridges form at the back of the abdominal cavity of the embryo. These develop into indifferent gonads that remain indistinguishable between males and females until the seventh week. During week six, two pairs of sexually indifferent ducts, the wolffian ducts and the mullerian ducts, also are present. Indifferent external genitalia include the genital tubercle (phallus), paired urogenital folds, and a labioscrotal swelling. If a Y chromosome is not present, the indifferent gonads develop into ovaries. If a Y chromosome is present, the expression of the *SRY* gene, named after sex-determining region of the Y chromosome, initiates differentiation of the indifferent gonads into testes during week seven. Once the testes develop, they secrete testosterone, which stimulates the differentiation of the wolffian ducts into epididymis, vas deferens, seminal vesicles, and the ejaculatory duct. The testes also secrete a chemical called mullerian-inhibiting substance (MIS) that causes the mullerian ducts to degenerate. In males, the genital tubercle develops into most of the penis, the urogenital folds form part of the penis shaft, and the labioscrotal swelling becomes the scrotum, which holds the testes. In the absence of SRY, testes do not develop, and the wolffian ducts regress. Because no MIS is made, the mullerian ducts grow and differentiate into the oviducts, uterus, cervix, and the upper vagina. The genital tubercle develops into the clitoris, the urogenital folds and the labioscrotal swelling develop into the labia.

See also CHROMOSOMES; HUMAN REPRODUCTION; REPRODUCTION.

FURTHER READING

Berta, Phillippe, J. Ross Hawkins, Andrew H. Sinclair, Anne Taylor, Beatrice L. Griffiths, Peter N. Goodfellow, and Marc Fellous. "Genetic Evidence Equating *SRY* and the Testis-Determining Factor." *Nature* 348 (1990): 448–450.

Ellegren, Hans. "Hens, Cocks, and Avian Sex Determination: A Quest for Genes on Z or W?" *EMBO Reports* 2 (2001): 192–196.

Haqq, C. M., C.-Y. King, E. Ukiyama, S. Falsafi, T. N. Haqq, P. K. Donahoe, and M. A. Weiss. "Molecular Basis of Mammalian Sexual Determination: Activation of Mullerian Inhibiting Substance Gene Expression by SRY." *Science* 266, no. 5190 (1994): 1,494–1,500.

Kraak, S. B. M., and I. Pen. "Sex Determining Mechanisms in Vertebrates." In *Sex Ratios: Concepts and Research Methods,* edited by Ian C. W. Hardy, 158–177. Cambridge: Cambridge University Press, 2002.

Sinclair, Andrew H., Philippe Berta, Mark S. Palmer, J. Ross Hawkins, Beatrice L. Griffiths, Matthijs J. Smith, Jamie W. Foster, Anna-Maria Frischauf, Robin Lovell-Badge, and Peter N. Goodfellow. "A Gene from the Human Sex-Determining Region Encodes a Protein with Homology to a Conserved DNA-Binding Motif." *Nature* 346 (1990): 240–244.

sexual and reproductive health Knowledge about subjects related to sexual health is important for avoiding or preventing undesirable conditions

ranging from a bacterial infection of the urethra to an unplanned pregnancy in addition to recognizing symptoms of conditions that can cause severe health problems, irreversible damage to one's tissues or organs, or even potential death. Cancer can develop in organs of the reproductive system, but early detection greatly improves the prognosis. Sexually transmitted diseases affect millions of people each year. An awareness of the signs and symptoms can help prevent long-term damage from such infections. The numerous available methods of birth control target different steps in the process of conception.

CANCERS OF THE REPRODUCTIVE ORGANS

Physicians recommend gynecological exams for women starting within three years of when a woman begins sexual activity or at age 21, whichever comes first. During a pelvic examination, the health care professional will check the reproductive organs to make sure they are healthy and perform a pap test, also called a pap smear, by collecting a small sample of cells and mucus from the cervix. In a laboratory, the cells are examined for abnormalities or structural deformities that are characteristic of cancer or precancerous cells. Abnormal cells can develop into cancer, but, if detected early, the woman can be treated to prevent cervical cancer. The National Program of Cancer Registries (NPCR) of the Centers for Disease Control collects and reports on U.S. cancer statistics. According to NPCR, in 2004 (the most recent year for which data is readily available to the public) 11,892 women were diagnosed with cervical cancer, and 3,850 women died from it that year. One major risk factor for cervical cancer is infection with human papillomavirus (HPV). In 2006 the U.S. Food and Drug Administration approved for females age nine to 26 years a vaccine to prevent cervical cancer caused by HPV.

The NPCR also reported that more than 186,772 women were diagnosed with breast cancer in 2004, and 40,954 died from the disease. The most common sign is the appearance of a new lump or mass in the breast tissue. Most cancerous lumps feel hard, are painless, and have irregular edges. Breast cancers that are large enough to cause noticeable symptoms are likely already to have spread to other tissues; thus, screening and early detection are crucial for a positive prognosis. Women who are older, Caucasian, have a family history of breast cancer, started menstruating earlier than age 12, experienced menopause before the age of 55, underwent hormone replacement therapy for an extended period of time, currently take oral contraceptives, are obese, or consume alcohol have an increased risk, though anyone can develop breast cancer. The American Cancer Society recommends that women age 40 and older should have annual mammograms (X-ray of the breast) as long as they are in good health. Women who are in their 20s and 30s should have a clinical breast exam performed by a health care professional every three years, and anyone who notices or feels a change in their breast tissue should speak with their health care provider. Performing regular self-examinations by systematically palpating breast tissue for lumps aids in early detection and also helps a woman learn what her breast tissue normally feels like, so she will be more likely to recognize an abnormality if one occurs.

The prostate is an accessory sex gland that produces fluid that protects and nourishes sperm. The urethra runs through the center of the small, doughnut-shaped organ. In older men, the portion of the prostate gland that encircles the urethra continues to grow, resulting in a condition called benign prostatic hyperplasia (BPH) that can cause problems during urination. Though the cause is unknown, factors that increase a man's risk for developing prostatic cancer include aging, being African American, being from North America or northwestern Europe, having a family history of prostatic cancer, having a high fat diet or eating lots of red meat, and lack of exercise. Because the prostate rests against the rectum, a physician can detect abnormalities in shape or size by performing a digital rectal exam. High levels of prostate specific antigen (PSA) in the blood can also indicate early signs of prostatic cancer.

Testicular cancer is an uncommon form of cancer that usually occurs in younger men, but it is highly curable. Men who have had cryptorchidism, a condition in which the testes do not descend from the abdomen into the scrotal sac, are at greater risk for testicular cancer. Other risk factors include having a family history of testicular cancer, being white, having a tall and slim body frame, and having a certain type of moles on the skin of the back, chest, abdomen, and face. The most common sign of testicular cancer is the presence of a typically painless lump or swelling on the testicle. The American Cancer Society recommends men have testicular exams as part of regular checkups.

SEXUALLY TRANSMITTED DISEASES

Communicable diseases are diseases that can be spread from one person to another person. Sexually transmitted diseases (STDs) include the diseases or infections that are usually transmitted by direct sexual contact, though some can be contracted by other means as well. According to the U.S. Department of Health and Human Services, the United States has the highest rates for STDs among the developed countries. The Centers for Disease Control (CDC) estimates that 19 million new infections occur each

year in the United States, and that half of those occur in people 15 to 24 years old.

Because a person can have an infection without exhibiting observable symptoms, many STDs go undetected. Women who remain untreated suffer more serious complications than men. Pelvic inflammatory disease (PID) is a collective term for infection of the female pelvic organs. Most often associated with gonorrhea or chlamydia, PID can cause chronic pain and lead to the development of scar tissue in the reproductive tract. Blockage of the oviducts with scar tissue can cause infertility or result in an ectopic pregnancy, a dangerous condition in which an embryo begins to develop somewhere other than the uterus. STDs can cause premature labor in pregnant women; some can cross the placenta and infect the fetus or pass to the baby during childbirth or cause other harmful effects to the baby.

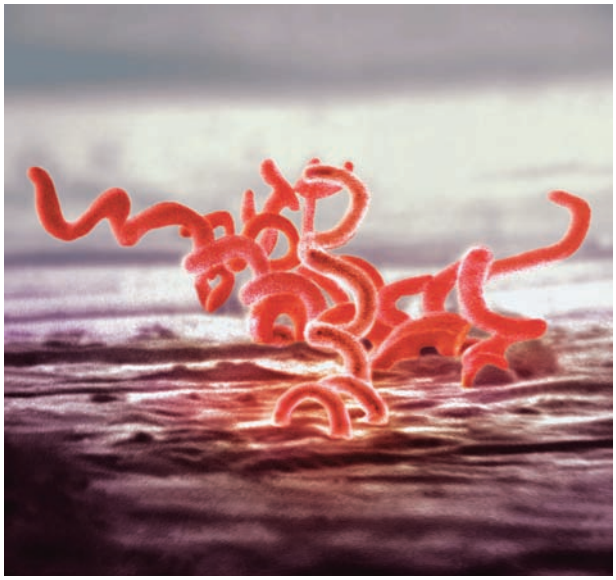
Abstaining from all sexual contact is the best method for avoiding any STD infection. Consistent proper use of latex condoms prevents the spread of many STDs, but since they do not cover the base of the penis, the scrotal sac, or the surrounding area, they are not foolproof. Most birth control methods do not offer any protection against STDs.

The CDC requires physicians to report all new diagnoses of certain STDs including syphilis, gonorrhea, and chlamydia. HPV and genital herpes are two other prevalent STDs, but the CDC does not mandate their reporting. The human immunodeficiency virus (HIV) is also spread by sexual contact as well as by coming into contact with infected blood or sharing needles with an infected individual. HIV causes the serious illness called acquired immunodeficiency syndrome (AIDS).

Chlamydia is the most common STD, especially among females, in particular, African-American females. More than 1 million new cases were reported to the CDC from the 50 states and the District of Columbia in 2006, the most recent year for which data is available, but because many people do not know they are infected, the estimated number of infected U.S. civilians ages 14–39 is approximately 2.3 million, based on the U.S. National Health and Nutrition Examination Survey. More than half of the males infected with the causative organism, *Chlamydia trachomatis,* exhibit no symptoms, and almost three-fourths of infected women have no symptoms. Early symptoms in women can include vaginal discharge and burning during urination. If the bacteria move up the reproductive tract, a woman might experience abdominal pain, back pain, nausea, fever, or bleeding in between menstrual periods. Laboratory tests can detect chlamydia in a urine sample or in a swab of cervical cells. The disease responds well to antibiotic treatment, but both partners must be treated or the male can reinfect the female.

The second most commonly reported STD in the United States is gonorrhea, with approximately 358,366 new cases reported in 2006. The organism that causes gonorrhea, *Neisseria gonorrhoeae,* can grow throughout the female reproductive tract, and in the mouth, throat, eyes, rectum, anus, and urethra of both males and females. Laboratory tests can detect the bacterium on a swabbed sample of the infected area or in a urine sample if the cervix or urethra is infected. Some medical offices prepare a bacterial stain to observe under the microscope for a rapid diagnosis. As with chlamydia, men with gonorrhea can be asymptomatic or they might experience a discharge from the penis, burning during urination, and painful or swollen testicles. Females might initially experience burning during urination, vaginal discharge, and bleeding between menstrual periods, but whether or not they exhibit any initial symptoms, they are at risk for developing PID if the gonorrhea is left untreated. Unfortunately, resistance of *N. gonorrhoeae* to traditional antibiotics is rising.

From 1990 until 2000, the incidence of syphilis steadily decreased, but between 2001 and 2006, the rate increased every year due to a disproportionate increase in male cases characterized by high rates of coinfection with HIV and high-risk sexual behavior. In 2006 the CDC received reports of 36,935 total cases of syphilis (at all stages). Syphilis is caused by the bacterium *Treponema pallidum* and is characterized by ulcers located mainly on or around the external genitalia. The organism is spread by contact with the sores during sex. The progression of a syphilis infection occurs in three stages. During the primary stage, a single, small, round sore called a chancre appears at the site where the bacteria entered the body on average 21 days after infection. After three to six weeks, the sore heals, even if untreated, but the infection progresses to the secondary stage, which can either immediately follow the primary stage or follow several weeks later. The second stage is characterized by a rough, red rash on the skin and mucous membranes, often on the palms of the hands and soles of the feet. Other symptoms that can occur during the second stage include fever, weight loss, hair loss, sore throat, muscle aches, fatigue, and headaches. A shot of the antibiotic penicillin during these early stages usually cures syphilis, but, if left untreated, it can cause serious damage to the internal organs and lead to dementia and even death. Some damage to the nerves, heart, and other organs that occurs during the late stage can be permanent. A health care professional can diagnose syphilis by



Treponema pallidum is a spirochete bacterium that causes syphilis. *(Science Source/Photo Researchers, Inc.)*

observing spiral-shaped bacteria in fluid from a chancre using a special microscope or by analyzing a blood sample.

The CDC estimates that half of all sexually active people eventually become infected with one of the 30 different sexually transmitted strains of HPV. Many individuals fight the infection naturally without ever developing genital warts. In others, HPV causes the growth of warts on the penis, anus, vulva, vaginal lining, or cervix. Some strains of HPV are associated with cancer, primarily of the cervix, but also of the vulva, vagina, anus, or penis. If warts develop internally, a person might not be aware they exist, thus regular gynecological exams are crucial for females. During a pap test, a heath care professional takes a small sample of cells from the cervix and sends them to a laboratory for analysis. There is no cure for HPV infection—it usually clears up on its own—but if abnormal or precancerous cells appear, they can be treated to prevent cancer from developing. The Food and Drug Administration has licensed a new vaccine for use in females ages 9–26 years to prevent HPV infection. Available since 2006, the vaccine, Gardasil® protects against four types of HPV that cause 70 percent of cervical cancers and 90 percent of genital warts.

Genital herpes can result from either herpes simplex virus (HSV) I or HSV II, but the latter is most common. The CDC estimates that at least 45 million people in the United States ages 12 and older have been infected with HSV. The virus causes periodic outbreaks of blisters around the genitals. The first outbreak is usually the worst, though in many people the sores are mild and often mistaken for an insect bite or other irritation. After the blisters break they leave sores or ulcers that can take up to four weeks to heal. Transmission of the virus occurs through contact with the sores, but an infected individual can also shed the virus in between outbreaks. The virus can remain in a person indefinitely causing recurrent outbreaks that usually decline in frequency and severity over time. During a typical outbreak, a physician might be able to diagnose herpes by visual inspection. Blood tests can also be performed, but the results are sometimes ambiguous. No cure exists for herpes, though antiviral medications can reduce the number and severity of outbreaks.

Several different microorganisms can cause vaginitis, inflammation of the vagina, but the yeast *Candida albicans,* the protozoan *Trichomonas vaginalis,* or the bacteria *Gardnerella vaginalis* is the most frequent culprit. Infection with *G. vaginalis* is sometimes referred to as vaginosis rather than vaginitis because it does not usually cause inflammation. Vaginitis can be sexually transmitted, or it can occur as a result of upsetting the delicate ecological balance of microorganisms that normally inhabit the female genitalia. Symptoms include a fishy odor and pronounced discharge. Antibiotic treatment usually allows the normal population of microorganisms to reestablish themselves, which benefits the woman by making the vaginal environment inhospitable to the organisms causing the bothersome symptoms.

BIRTH CONTROL

Birth control encompasses the methods used to reduce the number of children born by preventing pregnancy or reducing the number of pregnancies. Most types of birth control work by impeding the process of conception, the successful fertilization of an egg by a sperm and implantation of the embryo. Different mechanisms can impede the many different steps along the way, preventing the outcome of pregnancy. Methods targeting the female include inhibiting the production of eggs, the egg from traveling down the oviducts to the uterus, the sperm from entering the cervix, implantation of an early embryo in the uterus, or the maintenance of an early pregnancy. Because the male's only role in establishing a pregnancy is depositing sperm into the female reproductive tract, methods targeting the male are limited to blocking the transport of sperm from the testes to the penis or from the penis to the female vagina. Many practical and personal considerations influence a couple's decision concerning the type of birth control they prefer: effectiveness, convenience, cost, availability, risks, and other factors.

The failure rate of a birth control method is the number of women out of 100 total women expected to become pregnant while using that method of birth control for one year. For comparison, the pregnancy rate for women who do not use any means of birth control averages 85 percent.

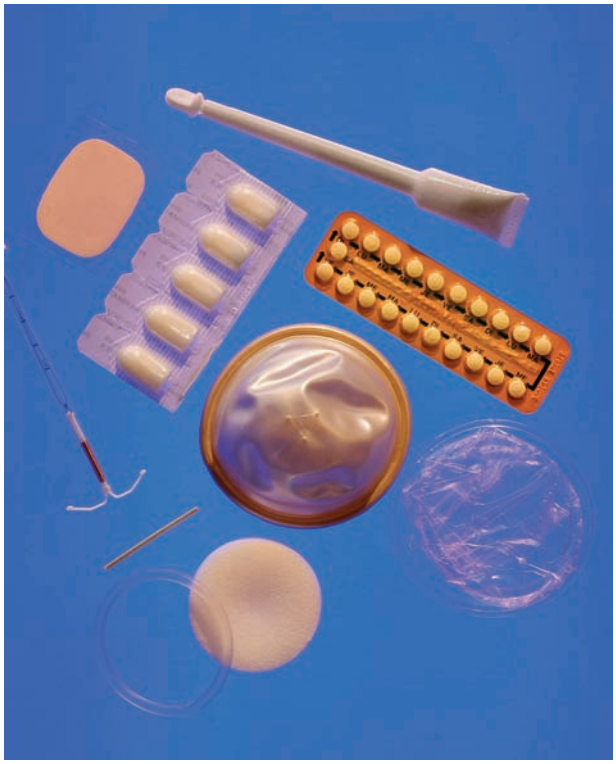
The convenience, low risk, low cost, effectiveness at preventing many sexually transmitted diseases (STDs), and availability without a prescription make barrier methods a popular choice for birth control. The male condom is a stretchable latex sheath that covers an erect penis and blocks sperm from entering the female reproductive tract during intercourse. Condoms made of other materials are also available; however, latex is most effective in preventing the transmission of STDs. When used correctly and consistently, the failure rate for condoms as a means of birth control approaches 3 percent. The actual failure rate is closer to 14 percent, due to inconsistent or improper use. The effectiveness increases when condoms are used in conjunction with spermicide, a substance that kills sperm when inserted into the vagina prior to intercourse. Nonoxynol-9 is a common spermicide that comes in the form of suppositories, gels, foams, or creams and has a 20 to 50 percent failure in preventing pregnancy when used alone. Spermicides do not protect against STDs.

A diaphragm, a dome-shaped, flexible rubber shield that covers the cervix, prevents the passage of sperm into the uterus. A woman must undergo a physical exam for proper fitting to obtain a prescription for a diaphragm and, when applied with spermicide before insertion, diaphragms have a failure rate of approximately 17 to 23 percent. Other contraceptive devices that physically block sperm from entering the cervix include the cervical cap, the FemCap, and Lea's shield, with the only significant difference in the devices being how the female body holds them in place. The failure rates are comparable, ranging from 15 to 23 percent for consistent and correct use. Contraceptive sponges also physically block the entrance to the cervix and contain spermicide that is activated by adding water prior to insertion. The failure rate ranges from 14 to 28 percent.

Intrauterine devices (IUDs) are T-shaped pieces of plastic that a physician inserts into the uterus through the vagina. Some IUDs have a copper wire wrapped around the stem, and others have the hormone progesterone embedded in the plastic. IUDs work by making the uterus inhospitable for an embryo and have a failure rate of less than 1 percent. An IUD can be used for up to 10 years and must be removed by a physician. Risks include cramping, bleeding, PID, infertility, or perforation of the uterus.

In 1960 the U.S. Food and Drug Administration first approved the use and marketing of oral contraceptives, specifically, the combination pill. Today many different types of available oral contraceptives contain various types and quantities of estrogen and/or progesterone. They prevent pregnancy by interfering with the woman's menstrual and ovarian cycles, which are normally regulated by hormones produced in the woman's body. Ingestion of hormones in birth control pills overrides the body's natural regulatory mechanisms that function to maximize the chance for reproductive success. The combination pill contains both estrogen and progesterone and inhibits ovulation by preventing the surge of luteinizing hormone and follicle-stimulating hormone that normally occurs near midcycle. If ovulation does occur, the combination of hormones also makes the cervical mucus unfavorable for sperm transport and impedes implantation. Because the combination pill inhibits ovulation, fertilization, and implantation, the failure rate for consistent use is less than 1 percent. Usually, a woman takes the pill for three weeks, and then either takes an inert pill or no pill for seven days, during which she menstruates. Other oral contraceptives include progesterone only pills (sometimes called the minipill) that are taken daily, even during menstruation, which can occur between every 25 and 45 days. A physician will often prescribe the minipill when the combination pill causes too many side effects in a patient or if she is breastfeeding, since estrogen can suppress milk production. The minipill makes the cervical mucus unfavorable to sperm transport and interferes with implantation, but it is not as effective at blocking ovulation and therefore has a slightly higher failure rate than the combination pill—approximately 2 percent. Birth control pills must be prescribed by a medical professional, and the side effects include headaches, nausea, weight gain, irritability, breast tenderness, spotty uterine bleeding, vision changes, high blood sugar and fat levels, increased blood pressure, and an increased risk for stroke. Because events leading up to ovulation may have already been initiated, a couple should use additional contraception during the first month of use. Fertility is usually restored within a few months of coming off the pill.

Other methods for delivering hormonal contraception include diffusion through patches applied to the skin, vaginal inserts, injections, and implants, all of which require prescriptions and have low failure rates. The side effects are similar to those of the oral contraceptives and vary from woman to woman. Post-coital contraceptives are pills that are taken within 72 hours after intercourse that reduce the risk for pregnancy. Because the failure rate is high and complications can occur, these are intended for use only as emergency contraception and not as a regular method of birth control.



Different types of contraceptives include condoms, implants, patches, pills, diaphragms, intrauterine devices, spermicide suppositories and creams, sponges, and vaginal rings. *(GARO/PHANIE/Photo Researchers, Inc.)*

Natural methods of birth control include complete abstinence, periodic abstinence, or withdrawal. Complete abstinence, not having sexual intercourse, is the only 100 percent effective method for preventing pregnancy. Periodic abstinence means deliberately refraining from having intercourse during the times in a woman's cycle when she is most likely to become pregnant. By keeping careful track of the dates of her menstrual periods and becoming aware of the subtle changes that occur to her body during different phases of her cycle, a woman can estimate when she is most likely to ovulate. Cyclical changes in basal body temperature can also indicate when a woman is likely to ovulate. Because sperm can live in the female reproductive tract for up to six days and an egg can be fertilized for two days after ovulation, even when a woman knows the exact day she ovulates, she must avoid intercourse for at least six days prior to ovulation and two days afterward to avoid a possible pregnancy. The regularity of a woman's cycles depends on her age, stress level, weight gain or loss, general health, and other physiological conditions. Because of this unpredictability, the failure rate for periodic abstinence is high. Using the withdrawal method requires the male partner to remove his penis from the vagina prior to ejaculation. This technique requires the male partner to be attuned to his body during sexual arousal and also highly motivated. Because sperm deposited before withdrawal or left on the vulva during withdrawal can potentially reach the cervix, this method is unreliable.

Surgical sterilization renders an individual incapable of reproduction. As a means for preventing pregnancy, sterilization is very effective, but it is considered permanent and, as with all surgical procedures, it carries some risk. In males, the procedure involves cauterizing, tying, or cutting the vas deferens in order to block sperm transport from the testes to the penis. A physician can perform this procedure, called a vasectomy, in an office under local anesthesia. Female sterilization, called tubal ligation or tubal sterilization, involves cauterizing, tying, or banding the oviducts to prevent the ova from coming into contact with sperm. A tubal sterilization can be performed laparoscopically through a small incision and is usually an outpatient day surgery. One serious side effect of a tubal ligation is ectopic pregnancy, when a fertilized egg begins development somewhere other than the uterus, most often within an oviduct. If diagnosed early, treatment for an ectopic pregnancy consists of hormonal treatment to terminate the pregnancy. Approximately one-fourth of all cases require surgical removal of the conceptus and sometimes the oviduct. Undiagnosed ectopic pregnancies result in rupture 12 to 16 weeks after the last menstrual period, resulting in abdominal hemorrhaging, shock, and sometimes death. While the reversal of sterilization procedures has resulted in successful pregnancy, reversal requires a second surgery, which involves risks and is expensive. Success rates for reversals vary from 60 to 75 percent in females and 30 to 75 percent in males.

See also ANATOMY; ASSISTED REPRODUCTIVE TECHNOLOGY; CANCER, THE BIOLOGY OF; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; ENDOCRINE SYSTEM; HUMAN REPRODUCTION; PHYSIOLOGY.

FURTHER READING

Jones, Richard E., and Kristin H. Lopez. *Human Reproductive Biology*. 3rd ed. San Diego, Calif.: Academic Press, 2006.

Mader, Sylvia S. *Human Reproductive Biology*. 3rd ed. New York: McGraw Hill, 2004.

National Institute of Allergy and Infectious Diseases, National Institutes of Health. "Sexually Transmitted Infections." Available online. URL: http://www3.niaid.nih.gov/healthscience/healthtopics/sti/default.htm. Updated August 28, 2007.

Seeley, Rod R., Trent D. Stephens, and Philip Tate. *Anatomy and Physiology*. 7th ed. Dubuque, Iowa: McGraw Hill, 2006.

U.S. Cancer Statistics Working Group. *United States Cancer Statistics: 2004 Incidence and Mortality*. Atlanta, Ga.: Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute, 2007. Also available online. URL: http://apps.nccd.cdc.gov/uscs/. Accessed January 16, 2008.

U.S. Food and Drug Administration. "Birth Control Guide." Available online. URL: http://www.fda.gov/fdac/features/1997/babytabl.html. Updated December 2003.

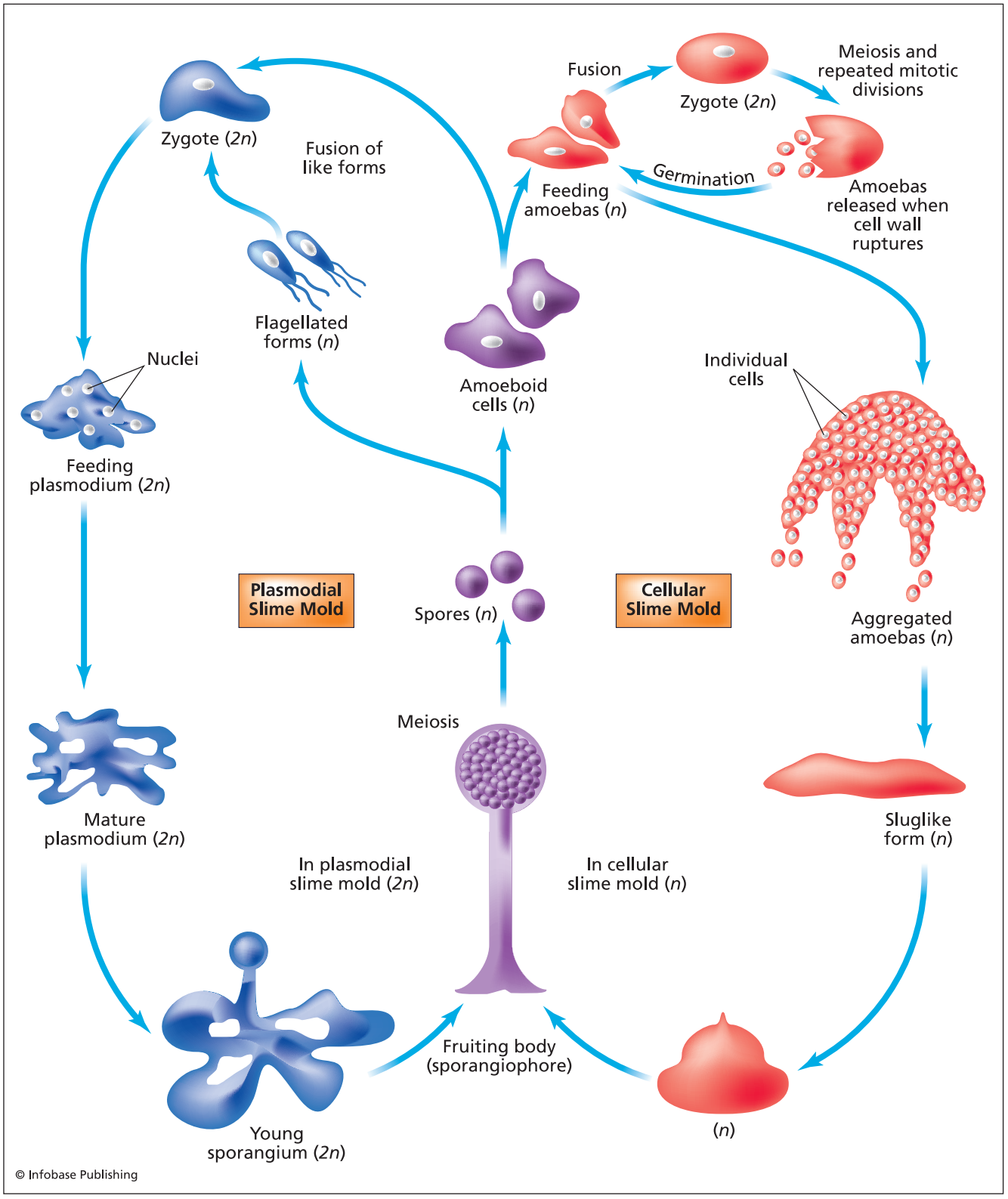
slime molds Traditionally included in the kingdom fungi because they produce spores for dispersal, slime molds also share similarities with protists, as they are eukaryotic and can exist in a unicellular form. Some modern proposed classification schemes separate organisms traditionally grouped together as slime molds (now considered an informal category) into different kingdoms. Slime molds live in moist terrestrial habitats such as damp mulch, decaying wood, or fresh cow manure. The two main branches of slime molds, the plasmodial slime molds and the cellular slime molds, share similar life cycles. The slime molds exhibit a sporangia stage, like fungi, and when growth conditions are unfavorable, the organisms produce spores that facilitate dispersal. The individual spores germinate into amoeboid cells.

Plasmodial slime molds, such as this one shown advancing over a wet log and leaves in Costa Rica, form when thousands of individual cells fuse to form one giant cell that moves by cytoplasmic streaming. *(Gregory G. Dimijian, M.D./Photo Researchers, Inc.)*

In plasmodial slime molds, or myxomycetes, the haploid spores germinate into either amoeboid or flagellated cells that can fuse with another like form to create a diploid individual. Repeated mitotic divisions without cytokinesis ensue, resulting in a coenocytic plasmodium, one enormous fused cell containing thousands of nuclei. A single membrane encloses the structure, which moves by cytoplasmic streaming to find decaying vegetation to engulf for food. To migrate in this manner, the cell first uses microfilaments to push outward against the cell membrane to form an extension called a pseudopod ("false foot") from the cell's surface, and then the cytoplasm flows, or streams, in that direction, pulling the cell in one direction. If the habitat begins to dry out or food becomes limited, conditions no longer favor growth. Fruiting bodies, stalks that terminate in spore-containing sacs, form and initiate sexual reproduction. Meiosis results in the formation of resistant haploid spores that disperse through the air and develop into amoeboid individuals, completing the life cycle. Examples of plasmodial slime molds include *Physarum* and *Echinostelium*.

Cellular slime molds are similar to plasmodial slime molds; however, their multicellular fruiting bodies are composed of individual cells with independent plasma membranes, rather than being a giant single cell. In cellular slime molds, such as *Dictyostelida* or *Acrasida,* unicellular amoebid cells aggregate when nutrients become low or environmental conditions become otherwise unfavorable. The aggregate of cells forms a multicellular, sluglike organism that moves to a suitable location and develops into a fruiting body that functions in asexual reproduction, in contrast to the fruiting bodies of plasmodial slime molds. Cells composing the stalk dry out, and cells that reach the tip develop into spores that are released from the fruiting body. If they settle in a favorable environment, they develop into vegetative amoeboid cells. Cellular slime molds typically complete their life cycle in the haploid form, but occasionally the amoeboid cells fuse, generating a diploid individual, necessitating meiosis to regenerate haploid individuals.

See also EUKARYA; EUKARYOTIC CELLS; FUNGI; MICROBIOLOGY; PROTOZOA; REPRODUCTION.



Both plasmodial and cellular slime molds produce fruiting bodies that release spores for dispersal, but plasmodial slime molds spend most of their time as diploid organisms and undergo meiosis to produce haploid spores, whereas cellular slime molds spend most of their lives as haploid organisms, though they are capable of fusing to form a diploid zygote. When this happens, the zygote consumes numerous haploid amoebas, forms a large tough cell, and undergoes meiosis followed by repeated mitotic divisions to form haploid amoeboid cells that are released when the cell wall ruptures.

FURTHER READING
Stephenson, Steven L., and Henry Stempen. *Myxomycetes: A Handbook of Slime Molds*. Reprint ed. Portland, Ore.: Timber Press, 2000.

social behavior of animals Social behaviors are behaviors directed toward or that take place between members of the same species. One can explore from an evolutionary standpoint the reasons why some animals are social, whereas the majority are solitary. Given that natural selection favors traits that increase an individual's reproductive success, with the exception of antagonistic behaviors (social does not imply gentle or kind), most social behaviors appear to contradict evolutionary theory. If the driving force of evolution is passing on genetic characteristics that confer higher fitness to the next generation, then understanding antagonistic or aggressive behaviors makes sense. For example, a male elephant seal will defend his territory against other males on a beach where female seals come to mate, resulting in the defender fertilizing numerous females. By preventing other males from accessing the females in his harem, his own reproductive success increases. A benefit to one individual often comes at the expense of other individuals, a phenomenon that makes sense in light of selection favoring increased relative fitness. The defender's fitness increases, while the unsuccessful intruders' fitness decreases. But examples of puzzling social behaviors abound. Scrub jays help feed and defend the offspring of others. Paper wasps live in societies in which only some of the members reproduce, and the rest work for the reproducing members' benefit. Why would some individuals not breed, but help raise their siblings at the nest instead? Ground squirrels whistle an alarm call when they spot a predator, even though it brings attention to themselves. Predators kill alarm-callers at a rate more than twice as high as noncallers, thus direct selection cannot be responsible for the evolution of this behavior. This leads to the ultimate question of why some animals exhibit altruistic behavior or participate in cooperative behaviors that benefit others even when it decreases their own opportunities to reproduce and pass on their own genes?

The concept of inclusive fitness provides some insight into the social behavior of animals. Inclusive fitness is the sum of an individual's direct and indirect fitness. While direct fitness describes fitness gained through an individual contributing genes to the next generation by producing descendant offspring, indirect fitness is a measure of the genes contributed to the next generation through an individual helping relatives who share many of the same genes to produce offspring. Inclusive fitness measures an individual's total contribution to the genes of the next generation and helps one quantitatively compare the genetic success of two or more behavioral strategies. One can determine inclusive fitness by dividing the sum of one's own contribution plus the contribution of relatives by the average contribution of the population.

ALTRUISM AND KIN SELECTION

In evolutionary biology, altruism is the apparent selfless, helpful behavior of one individual of a species that reduces its own individual fitness but that increases the direct fitness of other members of the same species. This is puzzling because evolutionary theory would predict natural selection to eliminate these behaviors. When individuals participate in cooperative behavior, such as when several females and their offspring live together in a group and the females help one another care for their young by taking turns seeking food or guarding the group, then all the participants benefit. The cooperative behavior increases the fitness of everyone in the group. In altruism, however, one individual acts in a way that improves another's chances of producing surviving offspring, but decreases its own probability of passing on its genes in the process. The evolutionary biologist William Hamilton suggested kin selection as an explanation to this seemingly puzzling altruistic behavior.

Kin selection acts on behaviors that improve the survival of offspring, including one's own offspring and the offspring of relatives. Though often summarized as the reproductive success of an individual, fitness is more precisely defined as the success in passing on one's genes. Because close relatives have many genes in common, behavior that increases the survival rate of the offspring of an individual's close relatives increases that individual's indirect fitness, thus selection favors these behaviors. Whereas direct selection acts on variations that increase one's individual fitness, indirect selection acts on variations in characteristics that improve the reproductive success of one's relatives. In both cases, the frequencies of the responsible alleles will increase in the next generation of the population at large. The term kin selection is sometimes used interchangeably with indirect selection, but kin selection includes indirect selection, which selects for behaviors that help nondescendant kin survive, and direct selection, which selects for behaviors that help descendant kin survive.

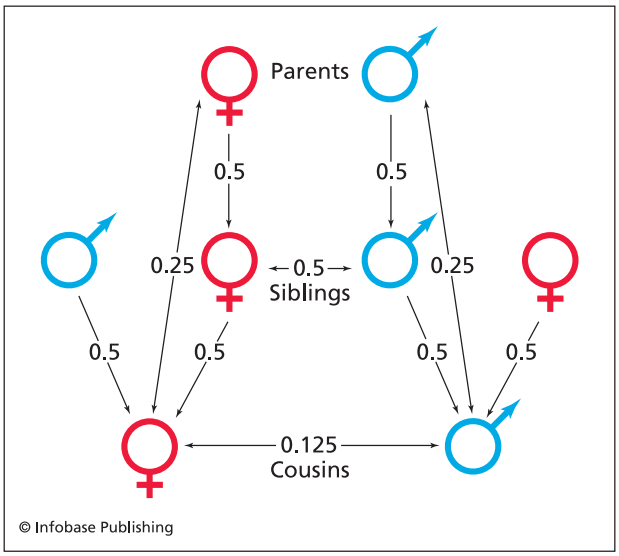
Hamilton suggested a method for determining whether an altruistic behavior increased inclusive fitness more so than an alternate strategy that increases direct fitness. Now called Hamilton's rule, the fol-

lowing inequality can be used to determine if natural selection would favor an altruistic behavior:

$$rB > C$$

where r is the coefficient of relatedness (the probability that two individuals who share a common parent or ancestor will also share the same gene), B (benefit) is the average number of extra offspring produced by the recipient as a result of the altruistic act, and C (cost) is the number of fewer offspring the altruist produces. If the value of the product of r and B is greater than C, then the individual performing the altruistic behavior will gain inclusive fitness. Natural selection will favor altruism when the benefit to the recipient of the helpful behavior, multiplied by the coefficient of relatedness, exceeds the cost to the altruist. On average, the genes of the altruist will be passed on via the relative as a result of the altruistic act. The benefit decreases with decreasing relatedness because the value of r decreases; in other words, indirect selection is stronger between siblings than between cousins or more distantly related individuals.

Occasionally, animals exhibit altruistic behavior toward individuals that are not closely related. One possible explanation for this is reciprocal altruism, in which individuals help nonkin, in return for future assistance. Simply put, the altruist is saving up favors for later. Only animals that live in stable social groups, such as humans and chimpanzees, could depend on reciprocal altruism as a means to increase inclusive fitness.



An individual shares 50 percent of its genetic material with each of its parents, but also about half with its siblings; 25 percent with its grandparents, uncles, aunts, and grandchildren; and 12.5 percent with its cousins. The coefficient of relatedness indicates the proportion of one's genotype in common with other individuals as a result of shared ancestry, or the probability that a specific allele will be present in two related individuals.

EUSOCIALITY

Animals that live in cooperative groups in which a single female and several male individuals reproduce and the nonbreeding individuals help to care for and protect the young are said to be eusocial. Termites, ants, wasps, and naked mole rats are examples of eusocial animals. Within a population, specialized castes that carry out one particular function exist. For example, honeybees exhibit complex social behaviors—most will readily give their lives to defend their hive. Within a hive, the only reproducing female is the queen; all the other females are the workers. Upon hatching, females immediately clean their brood enclosures, preparing them for the queen to lay a new egg there. Nurse bees care for the young, store pollen and nectar that the field bees gather, and defend the hive by stinging possible predators, though it kills them in the process. Field workers seek and gather honey and nectar to bring back to the nest. Drones are males whose sole job is to mate with the queen, an act that results in their death. Biologists do not completely understand the evolution for behaviors in which individuals give up reproducing in order to help care for the young of others, but indirect fitness likely plays a major role.

Sexual determination in many hymenopterans (members of the insect order Hymenoptera, which includes bees, wasps, and ants) occurs by a haplodiploid system. By this mechanism, males form from unfertilized eggs, and females arise from fertilized eggs; thus, males are haploid and females are diploid. Females produce genetically variable gametes by meiosis, but males produce gametes that are all genetically identical. Because sisters that share a father will have all the same genes from their father, and on average half the same genes from their mother, they share a relatively high coefficient of relatedness, 0.75. This could explain the development of eusociality, because females would gain indirect fitness by helping their sisters raise their offspring. Close relatedness cannot completely explain eusociality though, because, in some species, the queen mates with several males, leading to the creation of daughters that do not have high coefficients of relatedness. When the chance for personal reproductive success is low for individuals leaving a natal nest, then indirect selection favors individuals helping relatives to produce surviving offspring, forming eusocial societies.

See also ANIMAL BEHAVIOR; COMMUNITY ECOLOGY; ECOLOGY; ETHOLOGY; EVOLUTION, THEORY OF; SOCIOBIOLOGY.

FURTHER READING

Alcock, John. *Animal Behavior: An Evolutionary Approach*. 8th ed. Sunderland, Mass.: Sinauer, 2005.

Dugatkin, Lee Alan. *Principles of Animal Behavior*. New York: W. W. Norton, 2003.

McFarland, David. *Animal Behavior: Psychobiology, Ethology, and Evolution*. 3rd ed. Harlow, England: Longman, 1999.

sociobiology Sociobiology is a branch of biology and of sociology that attempts to explain the behavior of animals including humans, as a consequence of evolution. The degree to which genes control social behavior has not been established. The world-renowned, Pulitzer Prize–winning Harvard entomologist, Edward O. Wilson, first proposed that behaviors are a consequence of genes favored by natural selection in his book *Sociobiology: A New Synthesis,* published in 1975. While the book's introduction of sociobiology as a discipline of zoology has withstood the test of time, one segment of the book that addressed human behavior stirred up one of the most controversial scientific debates of the century, as many people did not like the notion that biology controlled social behavior. Some fear the contention that genetics restricts human nature and thus provides an excuse for deviant or aggressive behaviors and limits society's control over such actions.

Sociobiological theory purports that just as organisms pass on to offspring genes encoding physiological or anatomical adaptations that increase survival and reproductive fitness (ability to reproduce and transmit one's genotype to the offspring), natural selection also favors behaviors that increase a species' success at survival and reproduction. For example, sociobiology contends that the number of partners with which an animal mates is an adaptive trait. Males of most species generally can increase their reproductive fitness by inseminating numerous females. In contrast, male clown shrimp remain with a single female for several weeks. The rarity of females of this species, who are receptive to mating only for a short period of time every three weeks, makes it more cost beneficial for a male to stop hunting and stay with a female even if at the time she is not receptive to mating.

Sociobiologists study the evolution and ecology of many different behaviors, including instinctual behaviors, mate attraction, child care, altruism, aggression, social hierarchies, and others. Their research operates on the principle that animals should behave in a manner that increases their fitness. An example of a research topic in the field of sociobiology might be a comparison of the benefits (such as finding food and protection against predators) and disadvantages (such as having to share resources and compete for mates) of animals living in social groups. Or a sociobiologist might seek an explanation for why worker bumblebees initially try to steal and eat newly laid eggs, but then later guard and nourish them. Sociobiology as it relates to humans is also referred to as evolutionary psychology. An evolutionary psychologist might investigate an apparent association between a particular genotype and social class, something for which no evidence currently exists. Another avenue of research might be an examination of the division of labor and gender roles in different tribes. Why do men primarily gather food in some societies, while in others the task falls to females?

For any evolutionary explanation regarding a behavior, one can also provide a cultural explanation. Whereas biologically based behaviors are transmitted via genetics, cultural behaviors pass from one generation to the next via learning. Social behavior has a biological foundation but is also profoundly affected by environmental factors. The degree to which biology or environment influences a behavior is hard to determine and surely differs for different behaviors and between different species.

See also ANIMAL BEHAVIOR; ECOLOGY; ETHOLOGY; EVOLUTION, THEORY OF; SOCIAL BEHAVIOR OF ANIMALS.

The American biologist Edward O. Wilson founded a new scientific discipline when he published *Sociobiology: A New Synthesis* in 1975, proposing that genetic factors govern the social behavior of animals. *(AP Images)*

FURTHER READING

Alcock, John. *The Triumph of Sociobiology*. New York: Oxford University Press, 2001.

Wilson, Edward O. *Sociobiology: A New Synthesis*. Cambridge, Mass.: Harvard University Press, 2000.

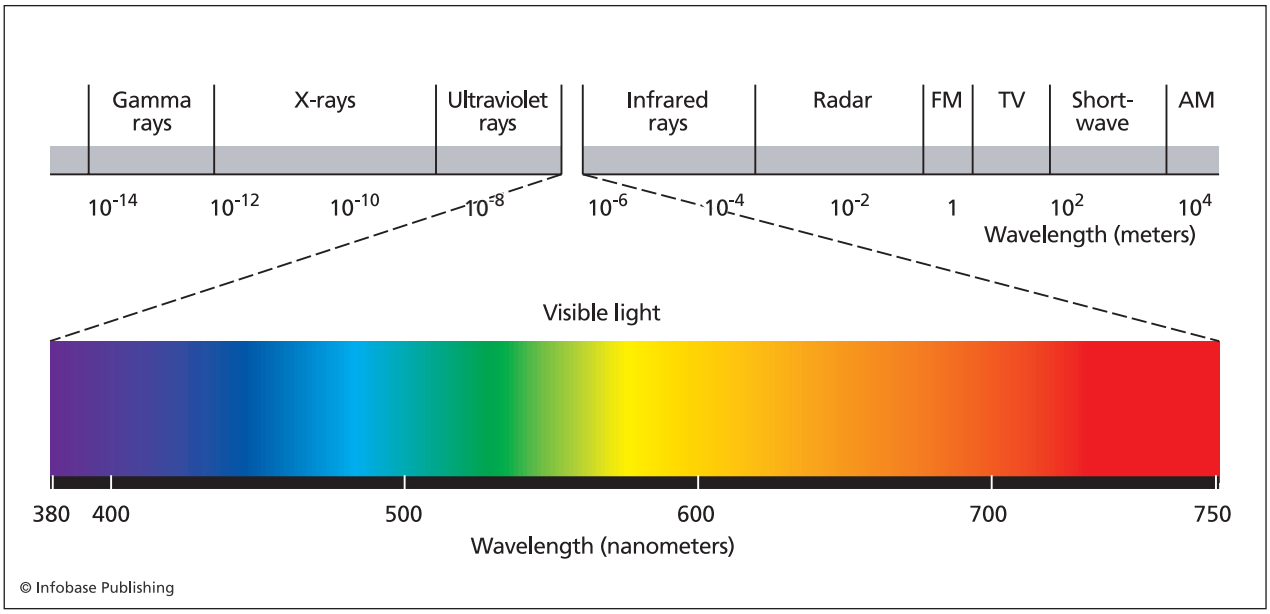
spectrophotometry Spectrophotometry is a technique used in life science research to measure relative intensities of light at different wavelengths using a piece of equipment called a spectrophotometer. Scientists use spectrophotometry for many purposes, including monitoring bacterial growth, measuring the amount and purity of a nucleic acid preparation, and determining the protein concentration of a solution. The technique operates on the principle that specific wavelengths of light pass through some objects, while others absorb the radiant energy. The amount of light that a solute absorbs is proportional to the concentration of the solute.

Light is a form of electromagnetic radiation, energy emitted as waves. The electromagnetic spectrum includes the entire range of electromagnetic radiation, from less than one nanometer (nm) to more than one kilometer (km) in wavelength. Shorter waves are more energetic than longer waves. Radiation with very short wavelengths includes gamma rays and X-rays. At the other end of the spectrum are microwaves and radio waves. Laboratory scientists commonly use spectrophotometry to measure light in the ultraviolet region (185 to 400 nm) and the visible region (400 to 700 nm) of the electromagnetic spectrum, but also the infrared region (700 to 15,000 nm). Visible light includes the region of the spectrum with wavelengths that the human eye is capable of detecting. Nearly all matter reflects and transmits some light. The molecular composition of a substance determines the specific wavelengths of light that an object absorbs and transmits. For example, the pigment chlorophyll, which harvests light energy for photosynthesis in plants and algae, gives leaves a green color. The chlorophyll molecules absorb violet-blue and red light (at the far ends of the visible light spectrum), but reflects green light, giving leaves a green appearance.

Spectrophotometers contain a light source, such as an incandescent light bulb, that sends a beam of white light through a sample, which is usually placed in a specially designed tube that fits in a holding chamber. The tubes are called cuvettes, and high quality cuvettes are constructed of quartz. Glass and plastic are also common, but they cannot be used when measuring ultraviolet light as the plastic and glass often absorb those wavelengths. White light is the presence of all colors, or all wavelengths of visible electromagnetic radiation. On the other side of the sample chamber, a diffraction grating or monochromator separates the different colors or wavelengths of light, and a detector measures the intensity of light at a specific wavelength after it has passed through the sample. The investigator chooses the particular wavelength of light he or she wants to measure, depending on the solute of interest in the sample. A meter reports the information as either percent of light transmitted (%T) or the absorbance (A), which is a logarithmic value. These two values are related by the following formula:

$$A = -\log\left(\frac{\%T}{100}\right)$$

If the amount of light absorbed by the sample is high, not as much light will be transmitted, so as the value

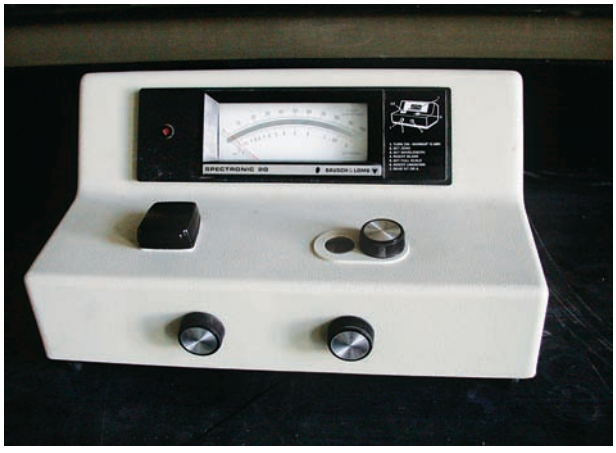


The electromagnetic spectrum includes radiation ranging from less than one nanometer (nm) in wavelength to more than one kilometer (10^3m).

for A increases, %T decreases, and vice versa. The value for %T equals I/I_0, where I_0 is the intensity of the incident light, the light before it passes through the sample, and I represents the light transmitted through the sample. Spectrophotometers can be single beam or double beam. In a single-beam spectrophotometer, all of the light is transmitted through the sample, so one must remove the sample from the chamber in order to measure I_0. In a double-beam spectrophotometer, the light splits into two paths before reaching the sample, so one beam of light can be used as a reference. In this manner, the spectrophotometer can measure both I_0 and I with the sample remaining in place. On some machines, one must switch back and forth to read I or I_0; in other machines, separate detectors measure both intensities simultaneously.

One of the most basic applications of spectrophotometry is to measure growth of a bacterial culture. Bacterial cells suspended in a liquid broth will scatter some light and allow some light to pass through. The turbidity, or degree of cloudiness, of a culture increases as bacterial number increases. The more bacterial cells that are present, the higher the absorbance reading. A spectrophotometer allows the scientist to quantitate or approximate the relative number of bacterial cells. While the data collected by spectrophotometry cannot give a concentration, the number of bacterial cells per unit volume can be estimated based on previous experimental samples as standards. Plotting the absorbance readings onto semilogarithmic paper reveals a growth curve, so the scientist can determine the stage of growth for the bacterial culture at a given time.

Ultraviolet-visible spectrophotometry can be used to quantitate solutions of biomolecules. For this application, the spectrophotometer must have a source of ultraviolet light, such as a deuterium arc lamp. The Beer-Lambert law relates the concentration of a solution to its absorbance.



A spectrophotometer is an instrument that measures the amount of light that passes through a specimen. *(Department of Biological Science, University of Manitoba)*

$$A = -\log_{10} (I/I_0) = \varepsilon \cdot c \cdot L$$

The letter A is the measured absorbance, L is the length or diameter of the path of light as it travels through a tube holding the sample, and c is the concentration of the solute. The constant ε represents the molar absorptivity or extinction coefficient, something that is a fundamental molecular property in a given solvent. Molar absorptivity is defined as the amount of light at a specific wavelength absorbed by a specific concentration of solute in moles per liter. One can use this information to determine the concentration of a specific type of molecule in a sample.

Ultraviolet spectroscopy measures the intensity of light at wavelengths between 190 and 400 nm, just below the visible light spectrum. Most biological molecules absorb ultraviolet light. Aromatic rings, chemical structures found in organic molecules such as the side chains of certain amino acids or the nitrogenous rings of nucleic acids, absorb ultraviolet radiation. This is why ultraviolet light can cause genetic mutations. Spectrophotometry provides a rapid, relatively easy means to assay for the presence and purity and to estimate the concentration of nucleic acids in a sample. Both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) absorb light with a wavelength of 260 nm, whereas light with wavelengths of 230 and 280 nm detects mostly proteins, salts, buffers, and solvents. Because the molar absorptivities of DNA and RNA are known, one can calculate the concentration by applying Beer's law. A sample of double-stranded DNA with an absorbance reading at 260 nm (A_{260}) of 1.0 has a concentration of approximately 50 µg/ml. If the DNA is single-stranded, an absorbance of 1.0 indicates a concentration of approximately 33 µg/ml. Pure RNA will give an A_{260} of 1.0 for 40 µg/ml. A rough estimate for pure protein samples is an A_{280} of 1.0 indicates 1 mg/ml. The ratio A_{260}/A_{280} should be between 1.8 and 2.0 for nucleic acids; a lower ratio indicates that contamination is present. For a pure RNA sample, the ratio should be 2.0, and for a pure DNA preparation, the ratio should be 1.8. An A_{260}/A_{280} ratio of 0.6 is characteristic of pure protein.

Investigators routinely use these approximations to estimate sample concentrations and purity. For example, a scientist might obtain the following data from a sample of double-stranded DNA:

$$A_{260} = 0.720$$
$$A_{280} = 0.380$$

Since an A_{260} of 1.0 indicates a concentration of approximately 50 µg/ml of double-stranded DNA, plugging the data into the following formula allows one to calculate the approximate concentration:

$$\begin{aligned}\text{Concentration of DNA} &= 50\ \mu g/ml \times A_{260} \\ &= 50\ \mu g/ml \times 0.720 \\ &= 36\ \mu g/ml\end{aligned}$$

Examining the ratio of A_{260}/A_{280} gives an indication of purity. For this sample data,

$$\begin{aligned}A_{260}/A_{280} &= 0.720/0.380 \\ &= 1.895\end{aligned}$$

For double-stranded DNA, pure samples have a ratio approaching 1.8, so this DNA sample could benefit from further purification.

If the molar absorptivity is not known for a specific solute, the concentration can be determined from a standard curve, a graph relating solute concentration to absorbance readings obtained from samples of known concentration. Without knowing the molar absorptivity or having access to a standard curve, only relative concentrations can be determined.

FURTHER READING

Gore, Michael G. *Spectrophotometry and Spectrofluorimetry: A Practical Approach*. Oxford: Oxford University Press, 2000.

spontaneous generation Until the 19th century, people believed that life arose from the action of mysterious vital forces on nonliving matter, a belief called spontaneous generation. Many observations seemed to demonstrate this phenomenon. Maggots commonly appeared on decaying meat, rats were found around garbage piles, and mice appeared in grain storage bins. More than 2,300 years ago, Aristotle thought that aphids arose from dew on plants and fleas from decaying matter.

During the 1600s, natural philosophers, people who studied the world around them and all that was in it, began to develop procedures for establishing truths about natural phenomena. Following the Scientific Revolution of the 1600s and the Age of Enlightenment in the 1700s, a set of guidelines for distinguishing facts from beliefs and for establishing something as a scientific truth emerged. Scientists began to apply the new scientific method to notions previously accepted but never demonstrated to be fact, such as spontaneous generation. An alternative hypothesis, biogenesis, claimed that life arose only from life.

In 1668 Francesco Redi, an Italian physician and poet, performed the first in a series of experiments that led to the dismissal of spontaneous generation. Many people believed that maggots spontaneously arose from decaying meat, but Redi thought they arose from eggs laid on the meat by flies. He set out two dishes of decaying meat and covered one of them with gauze. Using fine gauze would allow any air and any vital forces present in the air to access the meat and the nutrients it would provide. Though flies gathered around both dishes, they could not gain access to the covered meat, thus they laid eggs only on the uncovered meat. Maggots developed only on the uncovered meat, suggesting that they came from the flies that had laid eggs on the meat, rather than from the meat itself.

When Antoni van Leeuwenhoek discovered microorganisms in the late 1600s, he demonstrated that life existed beyond the easily observable level. Scientists soon established a correlation between putrefaction and the presence of microorganisms. Some interpreted this to mean that very simple life forms could arise from nonliving or decaying matter, but others used microscopic life to dispel spontaneous generation. Louis Jablot, in France, examined the possibility of abiogenesis, the generation of life from nonliving matter, of microorganisms using hay infusions (dried hay soaked in water). He boiled an infusion to kill any viable microorganisms, divided the sample into two heated containers, and covered one of them. After a period of time, the container with the infusion open to the air became cloudy, indicating growth had occurred. The covered container remained clear; no growth. In 1745 an English clergyman named John Needham conducted a similar experiment using boiled chicken broth instead of a hay infusion. In contrast to Jablot's results, both the covered and the uncovered containers became cloudy. An Italian priest, Lazzaro Spallanzani, suspected that microorganisms entered Needham's broth after boiling but before sealing. To test this, Spallanzani sealed the flask, removed the excess air in the sealed container, then boiled it, and nothing grew. Those who believed in spontaneous generation countered that the absence of air prevented vital forces from acting on the broth. At the time, scientists did not know that some bacteria form endospores, dormant derivatives of bacterial cells that are resistant to boiling. The unexpected growth in Needham's chicken broth can also be explained by the unfortunate presence of endospores, a setback for the proponents of biogenesis, the development of life from preexisting life.

Franz Shultze and Theodor Schwann of Germany suspected that dust particles carried microorganisms through the air. To test this, they passed air through potent chemicals and heated tubes designed

to kill any passing microorganisms. After treatment, they exposed the air to flasks of boiled infusions, but the infusions remained free of microbial growth, supporting their hypothesis and biogenesis. Opponents argued that the harsh treatments inactivated vital forces present in the air.

Spontaneous generation received its final blow from French chemist Louis Pasteur, who responded to a challenge by the French Academy of Sciences in 1859 to prove or disprove spontaneous generation. Pasteur had been studying microorganisms and their role in fermentation and believed that life arose only from similar life-forms. To show that microbial life arose only from previously existing microbial life, he designed some unique flasks with curved necks like swans. He filled the flasks with broth containing nutrients for the bacteria, heated the neck of the flasks so he could mold them into the shape of an S, and boiled them to kill any living organisms that might already be present. The curved necks allowed air (and any vital forces it might contain) to enter the flasks, but gravity would cause dust particles and microorganisms riding on them to collect in the lower curvature of the neck. After long periods of incubation, the flasks remained free of microbial growth. When he either broke the flask so dust could fall into the broth or tipped the flasks so that the broth contacted the region of the neck where dust had settled and then allowed it to incubate longer, growth occurred. These simple experiments left little for the few remaining proponents of spontaneous generation to doubt.

Though abiogenesis technically encompasses the concept of spontaneous generation, today, the term generally refers to the chemical origin of life—how atoms and inorganic compounds first formed organic compounds capable of self-replication.

See also Leeuwenhoek, Antoni van; microbiology; origin of life; Pasteur, Louis; scientific investigation; scientific theory.

Stevens, Nettie (1861–1912) American *Cytologist* Nettie Stevens was a highly respected cytologist who discovered the chromosomal basis of sex determination. (Edmund Beecher Wilson independently made the same discovery at the same time.) She was also one of the first American women scientists recognized by her peers for her contributions to scientific research.

Nettie Maria Stevens was born on July 7, 1861, in Cavendish, Vermont, to Ephraim Stevens, a carpenter and handyman, and the former Julia Adams. Nettie had one surviving sibling, a younger sister named Emma, and her mother died while Nettie was still a child. Nettie attended the Westford public elementary school and then Westford Academy, where she earned excellent grades. After graduation from high school in 1880, she taught high school Latin, English, math, and science in Lebanon, New Hampshire, for three terms.

In 1881 Stevens entered a four-year program at Westfield Normal School, a teacher's training school, in Massachusetts, but she completed the requirements necessary for a teaching certificate in only two. From 1883 to 1896 she taught at her former high school, worked as a librarian, and also as an assistant principal elsewhere, all the while saving money so she could continue her education. Stanford University in California accepted Stevens in 1896, and she decided to major in physiology. During her second year she began working with Professor Frank Mace MacFarland, a renowned microscopic anatomist who taught her histological methodology. Stevens spent the summers of 1897 to 1901 at Stanford's Hopkins Seaside Laboratory, where she worked with marine organisms. She earned a bachelor of arts degree in 1899 but remained at Stanford to study experimental physiology with Oliver Peebles Jenkins.

In 1900 Stevens received a master's degree in biology with a thesis titled "Studies on Ciliate Infusoria," published in 1901 in the *Proceedings of the California Academy of Sciences, Zoology*. The next step in her career involved a move back east to Bryn Mawr College in Pennsylvania, where she became a doctoral candidate and worked with Joseph Weatherland Warren on the neuromuscular system of frogs. She then began collaborating with Thomas Hunt Morgan, who at the time was researching the process of regeneration. In 1901 Stevens went to Woods Hole, Massachussets, to study cell division and regeneration in turbellaria, aquatic flatworms. Morgan helped her obtain a fellowship to study in Europe, and she spent six months spanning the winter of 1901–02 at the Naples Zoological Station in Italy, where she began focusing on chromosomes.

A second fellowship and her interest in chromosomes, particularly those in germ cells (i.e., eggs and sperm) brought her to the laboratory of the famous cytologist Theodor Boveri, in Würzburg, Germany. Here she immersed herself in learning the processes of oogenesis and spermatogenesis, the making of eggs and sperm. She completed her dissertation and obtained a Ph.D. from Bryn Mawr in 1903, at 42 years of age. She remained as a research fellow until 1904, a reader in experimental morphology with the financial support of the Carnegie Institution of Washington until 1905, and an associate in experimental morphology until she died in 1912.

Around 1904 Stevens began studying aphid chromosomes with Morgan. Aphids are small insects

that feed off the juices of plants. She was particularly interested in chromosomes from parthenogenic eggs, those that developed without being fertilized. One 1905 paper reporting her findings, "A Study of the Germ Cells of *Aphis rosae* and *Aphis oenotherae,*" won the $1,000 Ellen Richards Prize for the best scientific publication by a woman.

Around the same time she published a booklet titled *Studies in Spermatogenesis*. Clarence Erwin McClung, a zoologist from the University of Kansas, first proposed the presence of an accessory chromosome in the male as the sex determining factor in 1901, but few supported his notion. Stevens and a cytologist from Columbia University named Edmund Beecher Wilson thought it a valid hypothesis, however. By examining several diverse species of insects, Stevens found that aphid species had a unique set of chromosomes with characteristic forms and sizes. She also noticed that in *Tenebrio molitor* (mealworms), though both males and females had 20 total chromosomes, males had 19 large chromosomes and one smaller one, whereas females had 20 large chromosomes. The unequally paired chromosomes are referred to as heterochromosomes or more commonly as X and Y chromosomes, with the Y chromosome being smaller than the others. Stevens correctly claimed that sperm cells that contained nine large chromosomes and the one smaller chromosome led to a male offspring, whereas sperm cells containing 10 large chromosomes led to a female offspring. She deduced that the presence of the Y chromosome made the offspring male, and the absence of the Y chromosome resulted in a female. Until this time, scientists thought factors such as the mother's diet, temperature fluctuations, or other external factors determined the sex of offspring.

The mechanism of chromosomal sex determination was not immediately apparent. Scientists thought it had something to do with the total amount of chromatin present or the quality of the chromatin, but today scientists know that the expression of certain genes whose loci are on the heterochromosomes determine the sex of the offspring. The discovery of heterochromosomes led to a series of papers, published between 1905 and 1908, concerning germ cells, the differently sized chromosomes, the chromosomal complement of additional species, and how the different chromosomes behaved during meiosis, when homologous chromosomes paired up during synapsis before separating into sister cells.

It should be noted that Wilson arrived at the same conclusion as Stevens, namely, that a specific chromosome was responsible for sex determination. The two worked completely independently, but Wilson served on the committee that reviewed Stevens's paper for the Carnegie Institution. Wilson's reputation was already well established, and his paper was dated a few weeks before Stevens's paper, so he usually receives credit, but Stevens provided more details from which she drew her conclusions. The chromosomal basis of sex determination was the first physical characteristic to be associated with an observable difference in chromosomes.

At the time of her death from breast cancer on May 4, 1912, at Johns Hopkins Hospital in Baltimore, Maryland, Stevens was still investigating the behavior of the heterochromosomes during meiosis of spermatogenesis and how they related to regeneration and reproduction.

The chromosome studies conducted by Stevens provided strong support for the Mendelian basis of heredity and for the chromosomal theory of inheritance. Her determination and keen observation skills also led to the elucidation of one of the world's oldest questions, "What determines sex?" Though her life was short, her contribution to cytology was considerable. In her 11 year career, she published 38 papers. Stevens also made a lesser known contribution to life science research. She was responsible for introducing Morgan to *Drosophila melanogaster* (the fruit fly), the model organism he used to make many scientific breakthroughs in classical genetics.

See also CHROMOSOMES; MORGAN, THOMAS HUNT; REPRODUCTION; SEX DETERMINATION.

FURTHER READING

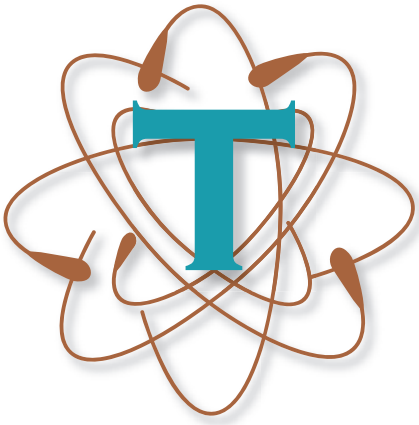
Ogilvie, Marilyn Bailey, and Clifford J. Choquette. "Nettie Maria Stevens (1861–1912): Her Life and Contributions to Cytogenetics." *Proceedings of the American Philosophical Society* 125 (August 1981): 292–311.

Stevens, Nettie M. "A Study of the Germ Cells of *Aphis rosae* and *Aphis oenotherae*." *Journal of Experimental Zoology* 2, no. 3 (1905): 313–333.

———. *Studies in Spermatogenesis, with Especial Reference to the "Accessory Chromosome."* Publication no. 36, Part I. Washington, D.C.: Carnegie Institution of Washington, 1905.

———. *Studies in Spermatogenesis. Part II. A Comparative Study of the Heterochromosomes in Certain Species of Coleoptera, Hemiptera, and Lepidoptera, with Especial Reference to Sex Determination.* Publication no. 36, Part II. Washington, D.C.: Carnegie Institution of Washington, 1906.

———. *Studies on the Germ Cells of Aphids.* Publication no. 51. Washington, D.C.: Carnegie Institution of Washington, 1906.



Thomson, Sir C. Wyville (1830–1882) Scottish *Naturalist* In the mid-19th century, not much was known concerning the deep ocean. Although shallow waters had been explored and animal life along the coasts had been surveyed, practically nothing was known of the deep oceans that cover 70 percent of the Earth's surface. A Scottish naturalist, Sir C. Wyville Thomson, was the scientific director on the world's first scientific oceanographic expedition on HMS *Challenger*. He debunked the azoic theory, which stated that no life existed in the deep ocean. Under his direction, thousands of new species of marine life were discovered, and a vast amount of oceanographic data was amassed, enough to give birth to the field of oceanography, the scientific study of the sea and all aspects of its contents (biological, physical, and chemical).

A POPULAR TEACHER

Charles Wyville Thomson was born in Bonsyde, Linlithgow, Scotland, on March 5, 1830, to Andrew Thomson, a surgeon, and his wife. He enrolled in medical school when he was only 16 years old, but he had to quit after three years due to health problems. He married Jane Ramage Dawson in 1853, and they had one son, Frank Wyville Thomson, who became a surgeon.

Thomson held a series of academic posts. He was first hired as a botany lecturer at the University of Aberdeen in Scotland (1851), but left there to serve as professor of natural history at Queen's College in Cork, Ireland (1853). The following year he moved to Belfast to become professor of geology at Queen's College. At Belfast he became well known as an invertebrate marine biologist, and, in 1860, he was named professor of zoology and botany. In 1865 he published a landmark paper in the *Philosophical Transactions of the Royal Society* titled "On the Embryogeny of *Antedon rosaceus*," which dealt with the development of a feathery echinoderm. In 1868 he moved once again, accepting a position as professor of botany at the Royal College of Science in Dublin. Finally, in 1870 he landed at the University of Edinburgh, where he held a post as professor of natural history. As a teacher, Thomson was popular. He lectured enthusiastically without notes and always brought props to share with his students.

BECOMES INTERESTED IN OCEAN LIFE

In the mid 1800s, not much was known about the deep ocean. Scientists knew that the environment was cold, dark, and under extreme pressure, and, based on this, the Scottish naturalist Edward Forbes declared that the ocean had an azoic zone, meaning no life existed below a depth of 300 fathoms. (One fathom is approximately six feet or 1.8 m.) On a trip to Christiania (present-day Oslo), Norway, in 1866, Thomson saw animals that were supposedly brought up from depths below 300 fathoms. He wondered: Was there life deep down? Where did they get their food? What sorts of adaptations were necessary to support life in what seemed like such an inhospitable environment? Those interested in evolutionary theory wondered if living fossils were to be found hidden under the sea.

Thomson decided to search for life in the azoic zone and garnered the support of another interested individual, Professor William Benjamin Carpenter from the University of London. Carpenter was the vice president of the Royal Society of London, a highly regarded premier academic organization.

Together the men asked the Royal Society for help convincing the Admiralty (the British navy) to provide them support for a deep-sea dredging expedition. The Admiralty donated the use of a paddle-steamer, HMS *Lightning*. In 1868 Thomson and Carpenter successfully collected specimens of marine life while dredging below 300 fathoms. Dredging is a process by which samples from the bottom of the ocean are obtained using a framed net or scooping device. They also discovered that the temperature of the deep ocean was not consistently 39.2°F (4°C), as was previously assumed.

With findings that contested two prevailing theories, the men easily obtained further support from the Admiralty for a second expedition. On HMS *Porcupine,* with John Gwyn Jeffreys, they performed temperature assays, dredged, and analyzed seawater samples from the waters off the west coast of Ireland and off the Shetlands. Amazingly, they obtained evidence of abundant life at 2,435 fathoms (14,610 feet, or 4,453 m). Most of the life-forms belonged to unknown species, and many resembled fossils of animals believed to be extinct.

Following the *Porcupine* cruise, the Royal Society elected Thomson a fellow. He published the results from the *Lightning* and *Porcupine* expeditions in *The Depths of the Sea* in 1873. The findings that life existed in the deep sea and that temperature varied despite similar depths in different regions sparked a renewed general interest in ocean exploration. Thomson took advantage of this by presenting yet another application to the Royal Navy and the Royal Society, proposing to study the biology, chemistry, geology, and physics of the oceans systematically. He planned to determine the depths of the sea at hundreds of locations, record temperatures at different levels, chart currents, take bottom mud samples, and, most important to Thomson, collect and study the marine life that appeared to exist in the deep ocean.

THE *CHALLENGER* EXPEDITION

The proposal was accepted. The British government failed to anticipate that, upon the conclusion of the expedition and publication of all the reports on the findings, the bill would exceed £200,000, equivalent to over $10 million today. The Admiralty provided a wooden steam corvette (a small, speedy, lightly-armed warship) and crew of approximately 225 men. The ship's captain was George Strong Nares, an experienced survey officer who was later knighted. Within 18 months HMS *Challenger* had been refurbished. Arms were removed, and laboratories and civilian quarters were added. A special platform for dredging had been installed. Much storage space was needed for the equipment as well as for the specimens and samples to be collected.

Sir C. Wyville Thomson discovered thousands of new marine species during an expedition aboard the HMS *Challenger* from 1872 to 1876. *(SPL/Photo Researchers, Inc.)*

The *Challenger* expedition departed Portsmouth, England, in December 1872, and it would not return for 41 months. The ship carried Thomson and his staff of five: Scottish chemist John Young Buchanan, English zoologist Henry Nottidge Moseley, Scottish-Canadian zoologist and future oceanographer John Murray, German zoologist Rudolf von Willimöes-Suhm, and Swiss artist Jean Jacques Wild. By the time the corvette returned to Spithead in May 1876, it had traveled over 68,890 nautical miles and gathered information from 362 stations. The crew performed 492 deep soundings and 133 dredgings. Crates of samples were sent back to Edinburgh from locations such as Bermuda, Halifax, the Cape of Good Hope, Sydney, Hong Kong, and Japan. In all, the *Challenger* crew collected 563 cases containing 2,270 large glass bottles, 1,794 smaller glass bottles, 1,860 glass tubes, and 176 tin cases of marine specimens preserved in wine spirits. In addition, they had assembled 180 tin cases with dried specimens and 22 casks with specimens in salt water. Amazingly, only four bottles were reported broken, and none of the samples rotted. In this prodigious endeavor, 10 men gave up their lives.

Every two or three days the *Challenger* stopped at a new station and gathered a bounty of information.

The naval staff recorded magnetic, navigational, and meteorological data. They also observed and noted the direction and speed of surface currents and attempted to collect subsurface current information. Sounding was performed to measure the depth of the ocean floor. A weighted line, with a bucket attached, was dropped until it hit bottom, then the rope let out was measured to indicate the depth of the sea. When the sounding apparatus hit bottom, a cup-shaped device attached at the end of it would grab a handful of the sediment from the floor for later analysis. A registering thermometer recorded the temperature at the ocean floor. The surface temperature was also noted. Water samples from different depths at each station were taken for chemical analysis. Dredging was performed to obtain samples of marine life from the ocean depths. Plankton nets were also used to take samples of marine life from mid-level depths in order to obtain a vertical distribution of life-forms.

In the beginning, the crew, the scientific staff, and Thomson himself grew excited each time a haul was brought to the surface, but the work was extremely tedious. Letting down the equipment into the deep took over an hour, and winding the dredge rope back up took several hours. Many hauls were lost to sea. Men became tired and bored, and, without a vested interest, the physical labor grew monotonous. Over the course of the three and one-half years, 61 men deserted the expedition while in harbor at locations in South America, Australia, New Zealand, Hong Kong, Japan, South Africa, and many islands of the Atlantic and Pacific Oceans. Though the labor was wearisome, the quality and the vast quantity of the scientific information gathered from this voyage yielded valuable results. Virtually all the oceans, with the exception of the Arctic Ocean, were explored and analyzed. Comparisons were made, and the results were astounding.

Most remarkably, over 4,717 new species and 715 new genera were discovered. The researchers found a great variety of forms and structures of all invertebrate classes at all depths. The dredging at its deepest was from an astonishing depth of 18,701 feet (5,700 m). The crew performed 25 successful dredgings at depths greater than 14,765 feet (4,500 m). Other information proved useful to oceanographers interested in oceanic circulation. Sediment composition analysis revealed hints of how the ocean floor formed as well as the direction of the subsurface currents. Thomson's staff discovered that a red clay bottom was common at depths below 2,000 fathoms (12,000 feet or 3,656 m). Above that depth, they found a distribution of pelagic oozes formed from the calcareous and siliceous shells and spicules from organisms such as foraminiferans and radiolarians.

Sounding data allowed for the drafting of the first contour map of the ocean basins. The deepest sounding—at 26,904 feet (8,200 m)—was found in the Marianas Trench in the southwest Pacific. They named it the Challenger Deep. To their surprise, the crew discovered the existence of an underwater mountain range that splits the Atlantic Ocean from north to south, which was later called the mid-Atlantic Ridge.

The members of the expedition returned with biological specimens and geological samples and with zoological and botanical specimens collected from faraway lands and their offshore waters. They also learned about other races and the cultures of more primitive societies.

After returning from the expedition, Thomson set up an office in Edinburgh for use in sorting through the collected materials, distributing them for analysis, and organizing the reporting of the results. Thomson established the format for the *Challenger* reports, but unfortunately his health did not permit him to see this work to completion. He did publish a preliminary account of the voyage, *The Voyage of the Challenger: The Atlantic* (1877), that contained beautiful figures of very ornate life-forms, including echinoderms such as starfish and sponges. Thomson summarized data concerning the contour of the Atlantic bed, the composition of the ocean bottom, the temperature variations, the distribution of deep sea fauna, the density of sea water, and the amount of carbonic acid contained in sea water. The Pacific complement of this report was never written. In honor of his service to science, Queen Victoria knighted Thomson in 1876. He was also awarded a gold medal by the Royal Society.

Controversy emerged in the immediate aftermath of the expedition. The British government registered distress at the total bill, and disputes arose over the manner in which the samples and specimens should be distributed. The British Museum claimed sole rights to receive all the samples, organize their examination, and coordinate the reporting of the results. Some expressed the belief that British nationals alone should be in charge of the actual research on the samples and specimens. Thomson believed otherwise. He thought that the research materials should be sent to the most qualified experts for analysis. In the end, over 100 scientists from several nations, including France, Germany, Italy, Belgium, the Scandinavia countries, and the United States, as well as from the United Kingdom, participated in the investigations.

STRESS CAUSES EARLY DEATH

The bickering and stress probably contributed to the early demise of Thomson at age 52. He suffered a paralytic attack in 1879, then another in 1881, when

the original five-year Treasury grant expired. That same year he resigned his professorship at the University of Edinburgh and his directorship of the *Challenger* Commission. Sir C. Wyville Thomson died on March 10, 1882.

One of the junior naturalists from the expedition who later became a famous oceanographer, John Murray, assumed responsibility for coordinating the completion of the reports. Though it was originally estimated this task would take about five years and compose 15 volumes, in the end it took 19 years and consisted of 50 volumes, totalling 29,552 pages. The *Report on the Scientific Results of the Voyage of the H.M.S. Challenger* has been a source for study for over 100 years. The vast amount of information it contains as well as the beautiful artwork and photography make it a valuable resource even today. Specimens collected by Thomson's staff are stored at the Natural History Museum in London. The *Challenger* expedition is considered to mark the dawn of the field of oceanography. Marine biologists, geographers, and hydrographers are indebted to Thomson, a man whose relentless pursuit of knowledge of the sea opened up new fields in marine science.

See also MARINE BIOLOGY.

FURTHER READING

Challenger Oceanic. "The Voyage of the H.M.S. Challenger (1872–1876)." Available online. URL: http://www.challengeroceanic.com/chal.htm. Accessed January 22, 2008.

Herdman, William A. *Founders of Oceanography and Their Work: An Introduction to the Science of the Sea.* London: Edward Arnold and Company, 1923.

Linklater, Eric. *The Voyage of the Challenger.* London: John Murray Publishers, 1972.

Thomson, C. Wyville. *The Depths of the Sea. An account of the general results of the dredging of H.M.S. Porcupine and Lightning during the summers of 1868, 1869, and 1870, under the scientific direction of Dr. Carpenter, F.R.S., J. Gwyn Jeffreys, F.R.S., and Dr. Wyville Thomson, F.R.S.* London: Macmillan, 1873.

———. *"The Voyage of the Challenger." The Atlantic; a preliminary account of the general results of the exploring voyage of H.M.S. "Challenger" during the year 1873 and the early part of the year 1876.* London: Macmillan, 1877.

Yount, Lisa. *Modern Marine Science: Exploring the Deep.* New York: Chelsea House, 2006.

Tinbergen, Nikolaas (1907–1988) Dutch-British *Ethologist* Nikolaas Tinbergen is considered one of the founders of the field of animal behavior. He is well known for his ethological research, including studies on the social behavior of herring gulls, homing mechanisms in digger wasps, and territoriality in sticklebacks.

Nikolaas Tinbergen was born on April 15, 1907, in The Hague, the Netherlands. His parents, Dirk C. Tinbergen, who taught grammar school, and Jeanette van Eek had four other children. Nikolaas's older brother Jan received the Bank of Sweden Prize in Economic Sciences in Memory of Alfred Nobel in 1969. As a child, school did not interest Nikolaas, who preferred to spend his time camping, bird-watching, skating, playing grass hockey, and watching sticklebacks in his two backyard aquaria. In high school, a natural history teacher put Nikolaas in charge of the classroom seawater aquaria. He also joined a club that studied wildlife.

DIGGER WASPS

After graduation from high school, Tinbergen did not plan to go to college, claiming he was intimidated by the academics. Through the encouragement of a family friend, Nikolaas visited Königsberg, where he spent time with the famous experimental biologist Johannes Thienmann, who directed a bird migration institute. During his two months in Germany, he observed birds, photographed bull elk, and watched the migration of wild moose.

Tinbergen returned to Holland in November 1925 and enrolled at Leiden University, where he developed a more formal interest in animal behavior. After completing his undergraduate studies in biology, he remained at Leiden to pursue a doctorate degree. Tinbergen had developed a passion for birds, in particular, birds of prey such as owls. Birds were not considered a suitable project for a doctoral student, however, so for his dissertation he chose a subject that he had studied as part of an undergraduate research project, digger wasps. The species he used was *Philanthus triangulum,* also called the beewolf or bee-hunting wasp because it preys on honey bees.

Tinbergen performed famous field experiments in the Dutch sand dunes exploring the mechanism by which digger wasps found their nests. He performed some rather simple but meaningful experiments to examine the hypothesis that the immediate environment of a digger wasp's nest helps it find and return to the nest later. Knowing that female wasps visit their nests daily to bring food to their larvae, he set up a ring of pinecones around the entrance to a ground nest. After the female wasp departed the nest, he moved the circle of pinecones a few feet over. When the wasp returned, she immediately flew to the center of the ring of pinecones instead of to the nest's actual entrance that was just a few feet away. In another experiment, after the wasp departed the nest, he arranged the pinecones into a triangle around the nest entrance rather than in a circle, and he set up

a circle of stones a few feet away. When the wasp returned, it flew to the center of the circle of stones rather than the triangle of pinecones that actually surrounded the nest. He carried out the same experiment 17 times, always with the same results. These experiments suggested that wasps find their nests by recognizing the arrangement of visual landmarks near the hole.

He next tested the role of scent in the wasp's recognition of the nesting site by placing cardboard dipped in pine oil near the nest in the presence and absence of pinecones. Moving the pinecones caused the wasps to become disoriented, but the cardboard seemed to have no effect when compared to results obtained using unscented cardboard. Colored paper had no effect either. He also examined the bee-hunting behavior of the wasps. Normally, a wasp approached a honey bee and quickly killed it by insertion of its stinger under the bee's chin. When Tinbergen cut off the wasp's antennae, wasps did not respond to bees even though they were trapped together in close quarters. The digger wasps specifically hunted honey bees and no other insects. Tinbergen wondered if scent played a role in the recognition of honey bees by the wasps. To test this, he crushed up a honey bee and rubbed the extract onto a bluebottle (a blowfly), which wasps then attacked, demonstrating that scent is important.

Tinbergen's dissertation was a reportedly embarrassingly short 29-page paper that did not impress the faculty. Hildebrand Boschma, Tinbergen's mentor and new head of the department of zoology, convinced the other faculty to award him a doctorate, promising that the young man had a bright future. Tinbergen's Ph.D. research eventually brought him scientific renown.

GREENLAND AND LEIDEN

Tinbergen rushed to complete his graduate studies in part because of an invitation to join a meterological expedition for the International Polar Year (1932–33). In 1929 he had became engaged to Elisabeth (Lies) Rutten, and in 1932 the two were married. Tinbergen and his wife traveled with the group to Angmagssalik, a town in Greenland, where Tinbergen made numerous observations and carried out comparative studies on various types of birds, including snow buntings, phalaropes, glaucous gulls, and others. Some of his findings contradicted those of the ornithologist Max Nicholson, who had previously visited Greenland and claimed that the snow buntings there were nonterritorial. The fact that the female phalatrope, a shorebird related to the sandpiper, was brightly colored and the male was drab intrigued Tinbergen, since this was the opposite of the norm. His studies examining how the coloring of the female affected territorial and courtship behavior took some time away from his snow bunting research, but he felt it was worth it. Tinbergen produced one book, two scientific papers, and several popular articles from this Arctic exploration, all of which established his reputation as an authority in ornithology.

They returned to Holland in September 1933, and Nikolaas and Lies had their first son, Jack, in 1934. They later had a daughter, Catrina, in 1937, another son, Dirk, in 1939, a second daughter, Janet, in 1945, and a third daughter, Gerry, in 1950.

In 1934 Tinbergen took an instructor's position at Leiden. He taught comparative anatomy and developed an undergraduate course in animal behavior. The department head, C. J. van der Klaauw, gave him special permission to extend his 12-day annual vacation to two months each year so he could introduce students to field research. During his early years working at Leiden, Tinbergen began pulling ethology into the realm of experimental sciences. He posed fundamental questions and sought a theoretical basis for his scientific explorations. With his students, he carried out courtship and territoriality experiments on the three-spined stickleback, a common, scaleless, bony freshwater fish in Holland. Their research showed that the red belly of the males stimulated other males to attack, and the large-bellied females were courted more frequently than those with smaller bellies that contained fewer eggs. Territoriality became a major focus of his research, and he began publishing numerous papers related to this subject and to fighting and the use of song in birds. He also became interested in instinct, an innate phenomenon involving an initial awareness in the animal, followed by a feeling and striving that led to a specific overt behavior.

Tinbergen met his future Nobel corecipient and intellectual giant, the Austrian naturalist Konrad Lorenz, at a workshop concerning instinct in 1936. Tinbergen was familiar with Lorenz's work describing releasing mechanisms responsible for eliciting instinctive behaviors. The Lorenzes invited Tinbergen, his wife, and their small son Jack to Altenberg, near Vienna. During their four-month stay, Tinbergen and Lorenz became collaborators and established a lifelong friendship. The single paper coauthored by the two described egg-rolling behavior in greylag geese. When an incubating goose sees an egg outside of her nest, she reaches out and rolls it into the nest using the underside of her beak. Lorenz and Tinbergen found that once set in motion, the goose continues the behavior even when someone removes the egg during the retrieval process. A separate behavioral component with its own stimulus was responsible for maintaining the direction of the rolling.

The herring gull chicks peck at the red spot on the mother's bill to solicit regurgitation. *(© Duncan Usher/Alamy)*

Tinbergen became a lecturer in 1939. His students held him in high regard and enjoyed his lecture style. He continued to pursue research involving digger wasps but also started other projects with his animal behavior students. One study related to natural selection on camouflage and courtship behavior in grayling butterflies.

World War II interrupted Tinbergen's work. The Nazis overran Holland in May 1940, and Tinbergen spent two years in a German hostage camp.

Within a year of the university reopening after the war, in 1947, Tinbergen was appointed full professor and the chair in experimental zoology at Leiden, even though he was only 39 years old. One study that an undergraduate student carried out around this time at one of Tinbergen's field research camps brought later recognition—an examination of the pecking behavior exhibited by herring gull chicks. A hungry nestling will peck at a red spot on its parent's bill, stimulating the parent to regurgitate food. Experiments demonstrated that the chicks pecked at red-colored spots more frequently than at black, blue, white, or colorless spots. Also, chicks pecked more often at long bills than at shorter ones. Although some aspects of these experiments were later criticized, they demonstrated Tinbergen's unique approach to studying animals in their own environments rather than in a laboratory.

Another famous Leiden study measured how the strength of a stimulus affected the elicited response in the corresponding behavior. Tinbergen and his students found that "supernormal" stimuli elicited a stronger behavioral response. For example, oystercatchers (a type of wading bird belonging to the family Haematopodidae) showed a preference for incubating eggs that were four times the average size of their own eggs, even though they had difficulty incubating it. The same was true for a nest that contained five eggs rather than three; they chose the nest with more eggs even though they could not cover the extra eggs sufficiently.

OTHER ETHOLOGICAL PURSUITS

Tinbergen grew increasingly frustrated with the politics and society of postwar Holland and sought an escape. The head of the department of zoology at Oxford University, Sir Alister Hardy, had offered Tinbergen a position at the new Edward Grey Institute of Field Ornithology in 1949. He accepted and rarely returned to Leiden thereafter. While at Oxford, he established a center for teaching and research in the field of animal behavior and in 1948 founded a journal of ethology called *Behavior*.

In 1951 Tinbergen published his first book, *The Study of Instinct,* based on a series of lectures he gave on animal behavior in the United States and in Britain. Eliot Steller, one of the founders of the field of behavioral neuroscience called it "an important contribution to the study of behavior." The book, which presented many new facts and concepts, was remarkably successful. In it, Tinbergen explained how ethology related to physiology, animal psychology, and other biological sciences. Using straightforward vocabulary, he provided simple explanations for complex animal behaviors, and he described all aspects of such behaviors: the releasing stimuli, central nervous system function, hormones, the development of behavior, the biological function of behaviors, and the evolution of behaviors. Ethologists sometimes refer to this book as the book of "four whys" because it showcases Tinbergen's characteristic way of exploring a behavior—by asking why an animal does something from four different perspectives: physiologically, developmentally (related to learning and conditioning), evolutionarily, and functionally (related to adaptiveness).

Tinbergen's other major work was the classic field study *The Herring Gull's World,* published in 1953. This book was a shorter, more popular version of *The Study of Instinct* that focused on the social behavior of animals. Tinbergen had observed these beachcombing birds with interest since his childhood. Their intricate social structure impressed him, and he was curious to explore questions such as whether or not they recognized their own young and why the individuals fought with one another. The behaviors he described in *The Herring Gull's World* were based on his own research and the findings of others. The book consisted of five sections:

- an introduction explaining the purpose of research on bird behavior, some general characteristics of herring gulls such as different calls and sense organs, and a summary of nonreproductive behaviors,
- a section on colonies, fighting, and territories,
- a description of pairing behaviors,
- a section on incubation of eggs,
- and a summary of family life.

In 1958 Tinbergen published *Curious Naturalists*. Throughout his career, he had nurtured a love for experimental research on animal behaviors in his students. This substantial volume summarized many of the research projects his students had performed while Tinbergen taught them how to question nature. Topics covered included insect camouflage, bee-hunting wasps, kittiwakes (a type of gull), hobbies (a type of falcon), and aspects of his Greenland research. The book did not sell as well as *The Study of Instinct* and *The Herring Gull's World,* perhaps due to its broad coverage, but the more sophisticated, updated revision, published in 1974, did.

Tinbergen's major scientific article of the 1950s was "Comparative Studies of the Behavior of Gulls," published in *Behavior* in 1959. The 70-page paper, which was Tinbergen's main contribution on the behavior of gulls, focused on 14 different gull displays, which are exaggerated movements or postures. For each he summarized what caused the birds to perform the display, what effect the display had on other birds, and how the display evolved. Although this paper was merely descriptive rather than experimental, Hans Kruuk (Tinbergen's biographer) described it as a turning point in Tinbergen's career, after which he focused on biological effects of behavior rather than causes.

In 1963 Tinbergen published "Aims and Methods of Ethlogy," a paper that defined the biological study of animal behavior and that he dedicated to Konrad Lorenz. Later, ethologists would call this paper the best of Tinbergen's career. It was also his last scientific paper on ethology.

Hans Kruuk, one of Tinbergen's students who later became his biographer, went to the Serengeti, a large savannah that overlies Tanzania and Kenya in East Africa, to study the carnivores and their effect on the local ecosystems. During the summers of 1964 to 1967, and again in 1969, Tinbergen visited him. The reputation of the ecological research from Serengeti attracted more scientists and funding to continue the research. The Serengeti Research Institute was established in 1966, with Tinbergen initially serving as chairman of its international supervisory body, the Scientific Council. Due to his efforts, Oxford University finally awarded him a full professorship in 1966, and an honorary doctorate of science.

AUTISM RESEARCH

In the late 1960s Tinbergen switched his research focus and began applying ethological research methods to the study of human behavior. In collaboration with his wife, he studied early childhood autism, a disorder characterized by withdrawal from the environment and from social interaction, a regression in overt speech, obsessive preoccupation with a limited number of objects, performance of repetitive movements, and other developmental delays. Experts in child development still know little about this mysterious disorder, which was first described in 1943. Tinbergen believed that autism resulted from environmentally induced emotional stress, most likely something in the behavior of the parents, in particular the mother. He specifically stated that he was not blaming the parents, but that they were perhaps inexperienced or under considerable stress themselves. He maintained that autism could be cured by establishing a secure mother-child bond. This conclusion offended many, and those in the medical profession claimed he was extending himself beyond his expertise.

AWARDS AND HONORS

In 1973 the Karolinsla Institutet awarded the Nobel Prize in physiology or medicine jointly to Nikolaas Tinbergen, Karl von Frisch, and Konrad Lorenz "for their discoveries concerning organization and elicitation of individual and social behavior patterns." Tinbergen was recognized for his general contribution of applying scientific methodology to the study of behavior in animals. Frisch elucidated the mechanisms by which bees communicate information regarding the direction and distance of food sources. Lorenz described imprinting in geese, the process by which young learn to recognize and bond with a parent during a specified time period. Together these three men are considered founders of the life science concerned with comparative animal behavior. Also known as ethology, this discipline focuses on the biological aspects of behavior—the anatomical and physiological adaptations related to the elicitation of a certain behavior, how an animal executes a behavioral response, the genetic programming responsible for a behavior, and the evolutionary implications of the behavior.

The Royal Society of London elected Tinbergen a member in 1962, an honor that Tinbergen felt was the ultimate British accolade in science. He became a foreign member of the Royal Netherlands Academy of Sciences and Arts, the U.S. National Academy of Sciences, the German Academy of Natural Sciences, and an honorary member of the American Academy

of Arts and Sciences. In addition to his honorary degree from Oxford, he received an honorary doctorate degree from Edinburgh University in 1973, one from the University of Leicester in 1974, and the Jan Swammerdam Medal, which is awarded by the Dutch Academy of Sciences to a natural scientist only once every decade, in 1973. He also received the Godman Salvin Medal of the British Ornithological Union. Oxford named the building that houses the departments of zoology and experimental psychology the Niko Tinbergen Building.

Beginning in the late 1960s, Tinbergen suffered from depression, hypochondria, and poor physical health due to his many years of heavy smoking. He retired from Oxford in September 1967, though he received an emeritus fellowship from Wolfson College. He continued his work on autism, something many, including his biographer, felt is best forgotten. He suffered a series of strokes in 1983, and he often required a wheelchair thereafter, though, interestingly, his depression disappeared. Nikolaas Tinbergen died on December 21, 1988.

Though later in life Tinbergen faced ridicule from the scientific community for his research into autism, his earlier research was important in establishing animal behavior as a science, at a time when many thought of such "animal-watching" as more of a hobby. His scientific record boasted 16 books and 336 scientific and popular articles. He was known for carefully designed, comprehensive experiments. His research on herring gulls, wasps, and sticklebacks has stimulated others to explore behavior in other animals, including mammals. This research has had far-reaching effects on fields such as evolution, social organization, individual development, psychology of abnormal behavior, and psychiatry. His legacy also lives on in the work of the numerous students he trained.

See also ANIMAL BEHAVIOR; ETHOLOGY; FRISCH, KARL VON; LORENZ, KONRAD; SOCIAL BEHAVIOR OF ANIMALS.

FURTHER READING

Kruuk, Hans. *Niko's Nature: A Life of Niko Tinbergen and His Science of Animal Behavior*. New York: Oxford University Press, 2003.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1973." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1973/index.html. Accessed January 25, 2008.

Tinbergen, Nikolaas. *Curious Naturalists*. London: Country Life, 1958.

———. *The Herring Gull's World: A Study of the Social Behavior of Birds*. London: Collins, 1953.

———. *The Study of Instinct*. Oxford: Clarendon Press, 1951.

Turner, Charles Henry (1867–1923) American *Entomologist, Ethologist*

Charles Henry Turner was a devoted African-American entomologist who overcame obstacles including lack of funding and equipment and racial discrimination to perform pioneering research in the field of animal behavior. He studied a wide variety of bugs, including ants, bees, cockroaches, moths, and spiders, and he discovered that insects can hear, see colors, and learn by trial and error.

TRAINING IN ZOOLOGY

Charles Henry Turner was born in Cincinnati, Ohio, on February 3, 1867, just two years after the Civil War ended. His father, Thomas Turner, was a church janitor from Canada who amassed an impressive literary collection that he shared with his son. His mother, Adeline (Addie) Campbell Turner, was a nurse who had been born in the slave state of Kentucky.

After graduating as valedictorian of his high school, Turner enrolled at the University of Cincinnati, where he struggled academically during his first year. He eventually came under the tutelage of biology professor and pioneering psychobiologist Clarence Luther Herrick. Herrick was impressed by Turner and published his undergraduate thesis, "Morphology of the Avian Brain" in the first issue of *Journal of Comparative Neurology* in 1891. Turner also studied gallery spiders, today called funnel weavers because they weave funnels in their webs in which they hide while waiting for prey. He observed that the spiders built webs of different shapes depending on the environmental conditions rather than simply out of instinct. For example, Turner repeatedly knocked down a web that one spider kept building by a windowsill. After being destroyed four times, the spider built the fifth web in a more discrete location underneath the windowsill. Turner also noticed different custom-built shapes in a variety of hunting ground locations and concluded that spiders were more intelligent than previously assumed.

While he was still an undergraduate, he took a short leave of absence (1888–89) and taught fifth grade at the Governor Street School in Evansville, Indiana and also substituted in the Cincinnati public grammar schools. Turner earned a bachelor of science degree in 1891 and a master's in zoology the following year from the University of Cincinnati. After graduating, he worked as an assistant instructor in the biology laboratory (1891–93).

In 1887 Turner married Leontine Troy. They had three children together before she died following a period of mental illness in 1895. He married Lillian Porter of Augusta, Georgia, in 1907 or 1908. She survived Turner and died in 1946.

TEACHES BIOLOGY

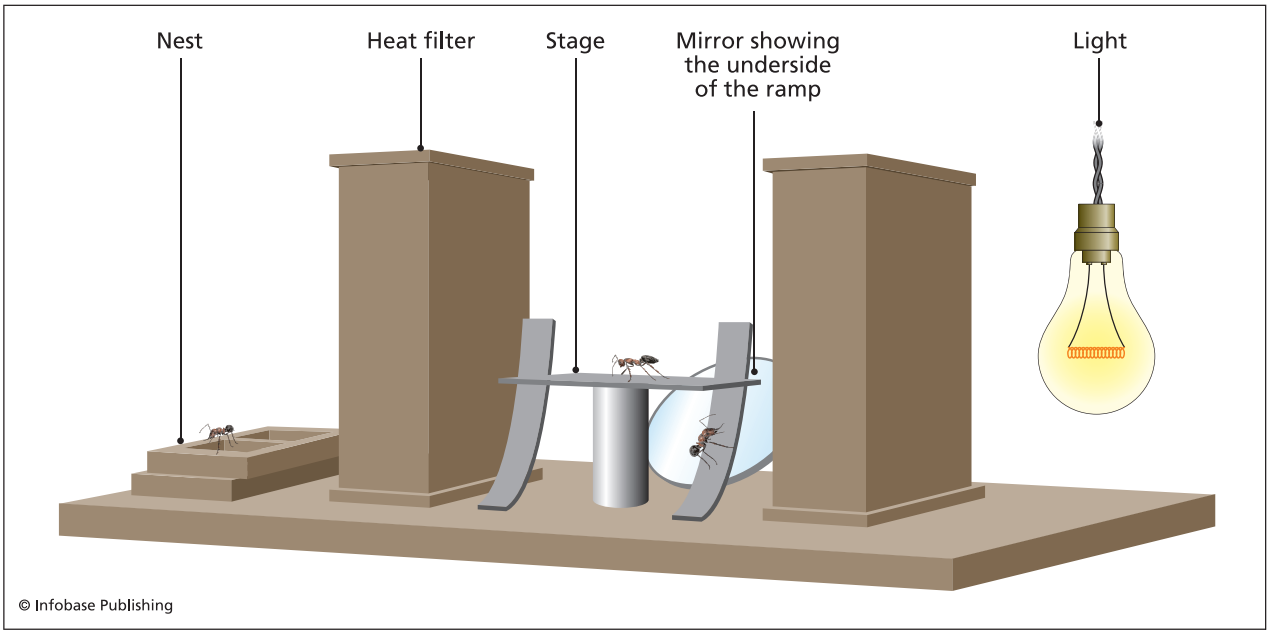
After obtaining his master's degree, Turner was hired as a biology professor at Clark University (today called Clark Atlanta University) in Atlanta, Georgia. He taught at Clark from 1893 until 1905, and then he took a job as principal of College Hill High School in Cleveland, Tennessee, for one year. From 1907 to 1908, he taught biology and chemistry at the Haines Normal and Industrial Institute in Augusta, and in 1908 he moved to St. Louis, Missouri, where he taught biology and psychology for the remainder of his career at the all-black Sumner High School and Teacher's College. He believed that he would be more valuable teaching other African Americans than in a profession where he could devote more time to scientific research. He used progressive teaching methods, often bringing in live animal and plant specimens for direct observation, and he encouraged his students to explore them using microscopes. On evenings and during vacations from school, Turner performed research on insect behavior. His experiments were many and varied in content, but they were always distinguishable and valuable.

While teaching, he pursued his doctorate degree. He went to the University of Chicago in 1898, but he returned to Clark the following year, planning to continue his graduate studies in absentia. In 1907 Turner became one of the first African Americans to earn a doctorate in the biological sciences when he received his doctorate in zoology from the University of Chicago. His notable dissertation, "The Homing of Ants: An Experimental Study in Ant Behavior," was published in the *Journal of Comparative Neurology and Psychology*. This analysis, which he presented at the International Zoological Conference in Boston, marked the turning point in his research from classical studies on structure and function to animal behavior.

ANT BEHAVIOR

Turner's first noteworthy independent research study examined ant behavior. Ants have the ability to find their way back home after traveling some distance in search of food. Turner wondered if this behavior was the result of instinct, scent, landmark recognition, or sunlight. While observing ants crawling on a vine on a brick wall by his home, he ripped off a leaf that had an ant on it and stuck it in a hole two feet away from its original location. The ant crawled all over the place in a seemingly random fashion until it finally found its home. After several repeated attempts, Turner found no evidence that ants were able to find their way home by instinct.

To see if smell played a significant role in guiding ants home, he set up a cardboard platform with an inclined bridge leading to a nest. He placed ants and immature young on the platform, knowing the adult ants would try to get them back to the safety of the nest as quickly as possible, and allowed them time to learn the pathway to get to the nest. After they did so, he added another inclined bridge leading from the opposite side of the platform to the nest, but none of the ants used the new route. To see if the ants left a scent trail that guided other ants, he then switched the ramps, so the one that ants had been using (that might contain an odor) was in the position of the



Turner used a raised platform with ramps on each side to study homing mechanisms in ants.

ramp that no ants had used and the unused ramp was on the side of the platform that the ants had been using. The ants continued to use the incline placed in the same pathway as they had been taking previously, indicating that scent was not a major factor in pathfinding.

To test directly whether light played a role in homing or pathfinding, Turner designed an experiment in which a nest was placed near two lighted ramps. The light bulbs were heat-filtered to ensure temperature would not interfere with the interpretation of the results. He placed pupae, larvae, eggs, and some ants on a platform in between the ramps. Turner alternated which ramp was lighted and kept track of which ramp the ants used to carry the larvae and eggs back to their nest. Though they appeared slightly confused initially after Turner switched which ramp was lighted, the ants always traveled the lighted ramp, in over one hundred trials. This experiment showed that light was one factor for ants in choosing a route to the nest. He also tested ramps with different textures and odors, and concluded that light, touch, and smells other than from ants themselves all played a role.

Tropisms are involuntary movements that occur in living organisms in response to external stimuli, such as light or touch. Turner demonstrated that some invertebrates demonstrated a circling movement when excited. He described a unique gyrating motion pattern performed by ants; they circled as they returned to their nest. Because Turner was the first to describe this characteristic action, French zoologists named it Turner's circling.

BEE RESEARCH

Several of Turner's honey bee experiments are his most famous. A former pupil described one experiment in which Turner set dishes of jam on a picnic table three times a day, and bees visited all three times. After a while, he stopped setting out the jam at lunch time and dinner time. Bees initially continued to appear at those times, but then only came at breakfast time, demonstrating that bees can learn and also have a sense of time.

A particular species of bee burrows in ground nests, and Turner wondered how they found their nest. According to one of his pupils, on the way to school Turner observed a bee entering a hole in the ground, then reappearing and flying away, presumably to collect more pollen for storage. While the bee was gone, Turner used a stick to make a second hole in the ground and then placed a bottle cap that had been next to the original hole near the new hole. When the bee returned, it entered the new hole, the one with the bottle cap nearby. The bee immediately came back out, appearing confused, and then found its original hole. After it left again, Turner made several more holes and proceeded to place the bottle cap in different positions. The bee had trouble finding the correct hole and had to fly in and out of each one randomly to locate the correct one.

To further explore the means by which bees recognized their ground nests, Turner next arranged a piece of white paper with a hole in the center so the hole was directly over the bee's nest entrance. When a bee appeared, it hesitated and hovered for a few minutes before entering the hole. As it was preparing to depart again, Turner noted that the bee hovered over the entrance for a bit, as if taking a memory picture of the opening. When the bee returned, it entered the nest without hesitation, as if it remembered the hole's surroundings. Turner then used a piece of watermelon rind with a circular region cut out and also a tented piece of white paper situated over the hole. In all instances when Turner varied the features surrounding the nest entrance, the bee seemed to take memory pictures in order to recognize the area. Changes to the topography (topography is the physical form surrounding a region) surrounding the hole appeared to confuse the bee.

Biologists knew that bees were attracted to particular types of flowers by their scent. Turner wanted to learn whether sight also played a role. Previous experiments designed to determine whether bees could recognize colors gave ambiguous and sometimes contradictory results. To determine whether bees could distinguish colors, Turner attached red circular discs to wooden sticks to simulate flowers. He dripped some honey on each one, and, after a few hours, bees began to visit the makeshift flowers and lap up the honey from them. Then Turner set out blue discs among the red ones, but he did not dribble them with honey. Bees ignored them. When he did put some honey on a blue disc, it took a while before the bees fed from it. Turner suspected they had learned that red meant honey-bearing whereas blue meant not-honey-bearing and that color vision played a role in drawing bees from a distance, whereas odor may be more significant when nearby. After demonstrating that bees had color vision, he extended his studies by using different geometric patterns instead of different colors and showed that bees could recognize patterns as well. He concluded that bees may be attracted by flower color and shape in addition to scent.

ANT LIONS AND COCKROACHES

The pit-making ant lion reportedly was Turner's favorite insect. An ant lion or doodlebug is a plump, hairy insect larva that has protruding jaws and can only walk backward. They exhibit an interesting behavior in which they back into a mound of sand while using their tails as shovels to push sand aside

and a back-and-forth head motion to further displace the sand. In this manner, they excavate a pit. The insect hides in the bottom of the pit, and if an ant crawls over the pitted area, the weakened sand gives way and the victim plunges into the pit, where the ant lion kills it and sucks out its body juices for nourishment. While observing this behavior, Turner witnessed the ant lions striking a death pose, a motionless posture that others might mistake for death. After much careful observation, he concluded that the insects were not actually playing dead, but rather were temporarily paralyzed out of terror. Turner became known as an expert on ant lions.

In order to determine if cockroaches had the ability to learn, Turner designed a flat metal maze with four blind alleys. When he placed a cockroach at one end, it proceeded to select random routes until it found the jelly jar that it considered home. A bath of water was placed under the maze, so if the roaches departed from the maze, they landed in the water. Turner counted the mistakes, meaning turns into blind alleys or drops into the water, and wiped the maze with rubbing alcohol to remove any scent trail in between trials. The first few attempts resulted in several falls into the water. A roach initially took between 15 and 60 minutes to complete the maze, but after many runs, it could complete the maze in one to four minutes. Turner concluded that if a roach completed the maze three times in a row without any mistakes, it had learned the route. Within one day, a roach could learn the pathway through the maze to the jar, proving roaches could learn by trial and error. He also discovered that if they were kept off the maze for 12 hours, the roaches forgot the path.

Cockroaches are nocturnal animals, meaning they are most active at night, and they naturally avoid light. Turner attempted to train the insects to avoid dark instead. He did this by setting up a device that administered an electric shock to the roaches when they entered a dark area. Over time, they learned to avoid the dark.

Turner also was able to successfully teach moths to relate low pitched sounds from an organ pipe using food as a reward, demonstrating not only that moths could learn from experience, but that they could also distinguish pitch. (He used a whistle to create higher pitched sounds.)

ACCOMPLISHMENTS

Charles Henry Turner died on February 14, 1923, in Chicago, Illinois. Though he never held a faculty position at a university, he conducted independent research for his entire career. He worked without assistance, financial support, or fancy equipment, yet he published over 50 research articles and several reviews on the behavior of insects and other invertebrate animals. He was the first African American elected to the St. Louis Academy of Sciences and was an honorary member of the Illinois Academy of Science and the Entomological Society of America.

Though Turner was dedicated to his scientific research, he was also active in black organizations and served as director of the Colored Branch of the St. Louis YMCA. He strongly believed that education was of paramount importance in the advancement of African Americans within the community and wrote articles on the subject. At the time of his death, Turner was composing a novel. He had also found time to author a children's book on nature studies and a book of poems.

Many biologists emulated Turner's groundbreaking research into the behavior of insects. Unfortunately, because he was ahead of his time in the not yet recognized field of animal behavior, many ethologists who followed were unaware of Turner's findings that insects could learn, his descriptions of honeybee feeding habits, and observations on homing in ants. Though he may not have received the due recognition for his scientific research, as a highly educated black man who spoke out for civil rights, he inspired many. In Turner's honor, a school in St. Louis for physically disabled children was named for him in 1925, the Charles Henry Turner Open Air School for Crippled Children. In 1954 it became the Turner Middle Branch School. In 1999 the Charles Henry Turner MEGA (Multimedia Electronic Graphic Arts) Magnet Middle School was founded.

See also ANIMAL BEHAVIOR; ANIMAL COGNITION AND LEARNING; ETHOLOGY; FRISCH, KARL VON; LORENZ, KONRAD; TINBERGEN, NIKOLAAS.

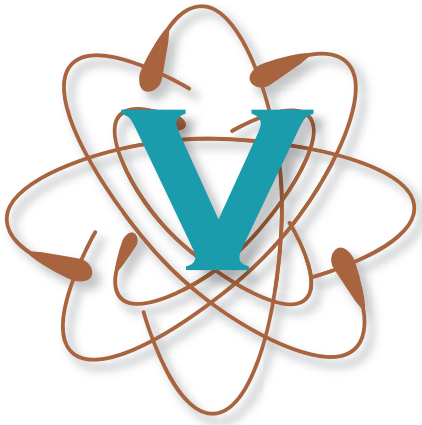
FURTHER READING

Abramson, Charles I. "Charles Henry Turner: Contributions of a Forgotten African-American to Scientific Research." Available online. URL: http://psychology.okstate.edu/museum/turner/turnermain.html. Accessed January 16, 2008.

———, ed. *Selected Papers and Biography of Charles Henry Turner (1867–1923); Pioneer in the Comparative Animal Behavior Movement*. Lewiston, N.Y.: Edwin Mellen Press, 2003.

Garraty, John A., and Mark C. Carnes, eds. *American National Biography*, Vol. 22. New York: Oxford University Press, 1999.

Gates, Henry Louis, Jr., and Cornel West. *The African-American Century: How Blacks Have Shaped Our Country*. New York: Touchstone, 2002.



vaccines Two hallmarks of specific immunity are specificity and memory. Following the first exposure to a specific antigen, the host develops active specific immunity to that antigen. Because activation of lymphocytes involves the production of memory cells that are specific for a particular antigen, during subsequent exposures, the immune system responds much more quickly and vigorously to fight the microorganism associated with that antigen. Specific memory is the basis for vaccines used in artificial active immunization.

The goal of vaccination is to confer active immunity to an individual in order to prevent that person from becoming ill from a particular disease. Most vaccines consist of either living organisms, killed microorganisms, parts of microorganisms that serve as antigens, or purified and altered toxins. Each method has its advantages and disadvantages.

Whole bacterial cells and viruses elicit a strong immune response. To prepare vaccines containing killed whole cells or inactivated viral particles, cultures are grown, and then treated with the chemical formalin, radiation, or heat. Though it is imperative that the culture be completely killed, preserving the natural antigenicity of the pathogen is also important. Otherwise, the immune system will not recognize the infectious agent during future exposures. Since the microbe is killed, it will not multiply inside the host; thus, larger doses of vaccines made from killed microorganisms are necessary, as are boosters, supplementary doses that increase the effectiveness of the immunization.

Live vaccines contain organisms that have been attenuated, or weakened, so they are no longer pathogenic. Altering the growth conditions for the microorganisms in a specific way can lead to the loss of virulence factors. Sometimes simply cultivating the microbes in the laboratory for a long time or selecting for mutants that prefer colder growth temperatures is sufficient to cause them to lose their ability to cause a clinical infection. Culturing a virus in a foreign host such as embryonic eggs or in tissue culture can also lead to the selection for a mutation that causes a preference of the virus for the artificial foreign host. When this happens, the mutated virus might not grow as well in the original host, making it less virulent. The advantages of using a live culture are that the microbe grows and multiplies in the host as it would in a real infection, the protection lasts longer, antibodies against multiple antigens from the organism are produced, and fewer or smaller doses of the vaccines are required. One disadvantage of using live vaccines is the organism can be transmitted to others and could be dangerous for immunocompromised individuals. Also, the attenuated strain potentially could revert back to the original virulent strain.

When a specific component of an infectious microorganism is known to elicit an immune response, vaccines can be synthesized using just that antigenic molecule. These acellular vaccines, also called subcellular vaccines or subunit vaccines in the case of viruses, are produced by purifying the antigenic portion of the microorganism from a culture, synthesizing it artificially in the laboratory, or by using genetic engineering and recombinant DNA technology. Though very safe, this type of vaccine does not elicit a strong immune response.

Many diseases cause harm to the host because of toxins they produce and secrete. For example, tetanus is caused by tetanospasmin, a toxin secreted by the anaerobic bacteria *Clostridium tetani*. The neurotoxin binds to nerve cells and blocks the release

of a neurotransmitter that inhibits muscle contraction, leading to uncontrolled contraction, a condition known as spastic paralysis. A vaccine that prevents tetanus contains a toxoid, a toxin that has been chemically denatured, making it nontoxic but still capable of eliciting a specific immune response. Immunization with the toxoid stimulates the production of antibodies that will remain in the body and neutralize tetanospasmin during possible future exposure.

Newer vaccine technology includes the "Trojan horse" vaccine, in which genes that encode antigens from pathogenic microorganisms are inserted into nonpathogenic microorganisms. The engineered microorganisms are introduced to the host, where they multiply and express the foreign antigen, leading to an immune response. Another promising approach is DNA vaccines. Plasmid DNA encoding microbial antigens is introduced directly into host tissues such as muscle. Some of the host cells uptake the plasmid and start synthesizing the proteins it encodes, but the plasmid itself is not replicated in the host tissue. B and T cells recognize the foreign antigens during immune surveillance and develop a specific response to them.

See also Bacteria (Eubacteria); genetic engineering; host defenses; infectious diseases; recombinant DNA technology; viruses and other infectious particles.

FURTHER READING

Allen, Arthur. *Vaccine: The Controversial Story of Medicine's Greatest Lifesaver*. New York: W. W. Norton, 2007.

Bowers, William, Abdul Ghaffar, and Gene Mayer. Edited by Richard Hunt. "Immunology." In *Microbiology and Immunology Online*. Department of Microbiology and Immunology, University of South Carolina School of Medicine. Available online. URL: http://pathmicro.med.sc.edu/book/immunol-sta.htm. Last modified October 28, 2008.

Centers for Disease Control and Prevention, Department of Health and Human Services. Vaccines & Immunizations. Available online. URL: http://www.cdc.gov/vaccines/. Last modified January 18, 2008.

Hunt, Margaret, Richard Hunt, N. Narayan, and Wayne Duffus. Edited by Richard Hunt. "Virology." In *Microbiology and Immunology Online*. Department of Microbiology and Immunology, University of South Carolina School of Medicine. Available online. URL: http://pathmicro.med.sc.edu/book/virol-sta.htm. Accessed January 22, 2008.

variation, genetic variation

Variation refers to a divergence in the structural or functional characteristics of an organism from other members of the species. For example, a rabbit may have white fur or brown fur. A bacterium may or may not be able to ferment the carbohydrate lactose. A fruit fly may have red eyes or white eyes. Variations in these traits are due to genetic variation, which specifically refers to differences in the genes between members of the same species. Some genetic changes are neutral, that is, they do not affect the structure or function of an organism, whereas other changes may impact the characteristics of an organism to a small degree or even tremendously. Whatever the immediate result, variation serves as the raw material for biological evolution, the changes that have transformed life on Earth for more than 3.6 billion years.

A population evolves as a result of the different reproductive success of its genetically variable members. The individuals that are best suited to their particular environment will produce the most offspring, thus natural selection will maintain the genetic combinations that bring about reproductive success in the population. Just as life evolves, so does the Earth, and thus the physical environment is constantly changing. A genetic combination that works in one environment might not work as well under the altered selection pressures. Because organisms in a community all interact with one another in different ways, changes in one population may also affect the reproductive success of another species in the community.

ORIGINS OF VARIATION

Deoxyribonucleic acid (DNA), consisting of long polymers of deoxyribonucleotides, carries the genetic information for all organisms. The information is embedded within the sequence of the four different nucleotides in a molecule of DNA, and organisms must duplicate this information to pass it on to their offspring. Specialized proteins called enzymes perform this task, which is monumental considering that organisms can have from 5 million to 5 billion nucleotides in their DNA. When the enzymes are copying the DNA, mistakes occur, just as if a student were to try to copy, letter for letter, several pages from the dictionary. These errors are called spontaneous mutations, and usually the enzymes spot and fix them soon after making them, but sometimes the mistake becomes a permanent part of the DNA sequence. In this case, the information can pass to the next generation, and the next, and so on. Natural physical processes and chemicals present in the environment can also cause the DNA to mutate. Though cells have mechanisms for repairing certain types of damage to the DNA, some induced mutations also become permanent. In organisms that reproduce asexually, that is, with only one individual involved in the creation of offspring, spontaneous and induced mutations are the major source of genetic variation.

Sexual reproduction provides more opportunities for variation beyond the molecular level—at the cellular and organismal levels. During meiosis, the production of haploid cells from diploid cells, homologous chromosomes participate in an event called crossing over or recombination. As a result, chromosomes exchange corresponding segments of DNA, changing the linkage relationship of alleles (forms of a gene) along the length of a chromosome. Another source of genetic variation is independent assortment, the random positioning and sorting of the maternally and paternally derived members of homologous chromosome pairs that results in numerous possible combinations of chromosomes, or associations of genes linked together on the same molecule of DNA. Crossing over and independent assortment allow for the creation of countless genetic combinations in the gametes made by an individual. When gametes of two individuals randomly unite during fertilization, the genetic variation increases even more.

Genetic variation can also originate by other less common mechanisms. When a virus infects a host cell, it directs the host cell to synthesize new viruses. During assembly of the new viruses, the viral genetic material gets packaged into the viral protein coat, but sometimes by accident, a segment of the host's DNA also gets picked up and packaged in the new viral particle. After the new viral particle goes out and infects another host cell, it deposits the DNA from the former host cell into the new host cell. If the host cell incorporates the new DNA into its own genome, that genetic material becomes part of the host's DNA and is replicated and passed on to daughter cells. Another source of genetic variation is the presence of transposable elements. Also called jumping genes, these sequences of DNA exist in all organisms, and they can move from one site to another on their own. Depending on the particular transposable element, the DNA either copies itself or cuts itself out of one region and insets itself elsewhere. Though this process of transposition does not introduce new genes into an individual, it does shuffle the order of genes. Transformation, the assimilation of foreign DNA into a cell, happens naturally and can lead to an alteration in the genotype and phenotype of an organism, which might affect its ability to survive and pass on its genes through reproduction. In transformation, a cell uptakes DNA from the environment. An example of how this happens is when intestinal bacteria die and their cells lyse, releasing DNA into the surroundings. Other bacterial cells, of the same or even different species, may pick up this DNA, and, through a process called recombination, incorporate it into their own chromosome. Plasmids are small circular extrachromosomal pieces of DNA that some bacteria have and some do not have. They often contain genes that encode for valuable proteins, such as for resistance to specific antibiotics, and can be taken in by transformation as well. Some species of bacteria can also exchange genetic information through conjugation, a process that requires possession of a specific type of plasmid called the F plasmid.

POLYMORPHISMS

Mutations can give rise to new alleles, or different forms of a gene. For example, the gene that encodes seed shape in pea plants has a round allele and a wrinkled allele. A gene locus, the position on a chromosome where a gene is located, is said to be polymorphic if several allelic forms persist in a population. If the new allele or genetic combination confers a distinct advantage in the survival or reproduction of a species, then the frequency of the new allele or genetic combination will increase, eventually replacing the old, less favorable form. The polymorphism in this case is transient or temporary during the process of selection. If the new allele is associated with a decreased fitness relative to the previous form, the new one will not become established—the polymorphism will not be maintained. In many cases, the different alleles are neutral, or the advantage differs depending on the circumstances. A population maintains such polymorphisms by a few different mechanisms.

The heterozygote advantage is one model for the maintenance of a polymorphism. Homozygous genotypes possess two identical alleles at a gene locus. In this model, the increased fitness, or the relative reproductive success of a genotype, of the heterozygous genotype ensures that a polymorphism is maintained. If natural selection favors the heterozygous condition in comparison to the phenotypes, or the observable characteristics, associated with both of the homozygous genotypes, then both of the alleles will persist in the population—the gene locus will remain polymorphic. If the mutant allele caused a disadvantage only in one genotype, then over time, that allele would disappear from the population. A classic study performed in fruit flies illustrates the heterozygote advantage. At a gene locus for body color, the homozygous dominant genotype resulted in flies that were dark ebony and also very weak. The homozygous wild-type genotype caused no apparent disadvantage, but the heterozygous genotype produced flies that had improved viability. Thus the heterozygote had increased fitness relative to either type of homozygote.

Frequency-dependent selection is another model that attempts to explain the maintenance of polymorphisms in a population. Also called dynamic selection, in this process, the fitness associated with

an allele depends on its frequency. To illustrate this concept, consider viruses. Viruses are not considered living entities, but they do contain nucleic acid and mutate rapidly, forming new strains. When a strain of virus is frequent in a population, many people will have developed immunity against that particular strain, leaving few available hosts for the virus to infect successfully in order to replicate itself. If the virus mutates to form a new genetic strain, people will be susceptible to that strain, conferring a reproductive advantage to the new, infrequent genetic strain of virus.

Different circumstances can also balance polymorphisms. A specific trait may confer a reproductive advantage to an organism in one season but a disadvantage during other seasons, or an advantage in one habitat but a disadvantage in another habitat. For example, animals living in northern climates may benefit from having larger bodies in order to conserve heat. Selection would favor variations resulting in greater body weight in colder climates. Moving south, the advantage may cost more in terms of the required energy input to achieve the larger size, and thus not confer the same advantage. Without distinct boundaries between populations, however, the genetic differences are maintained. Another factor to consider when attempting to explain how polymorphisms are maintained is that natural selection could act on an entire genome, the combination of all the alleles of an individual rather than an isolated gene locus. These models get rather complicated. One must also consider the possibility that a gene locus might be completely neutral, meaning no selection occurs at that locus, but the variation persists due to random chance.

VARIATION AND MOLECULAR EVOLUTION

Advances in molecular biology and biochemistry allow researchers to determine the nucleotide sequence of DNA molecules and the amino acid sequence of protein molecules. Within a gene on the DNA, each triplet codon, sequence of three adjacent nucleotides, designates one of the 20 possible amino acids. Because DNA contains four different nucleotides, there are 64 possible triplet codons, thus several amino acids are encoded by more than one triplet (a phenomenon known as redundancy), often with the nucleotide at the third position being irrelevant (a phenomenon known as wobble). Because mutations accumulate over time and can be measured within and between species, scientists can use this molecular variation as a molecular clock for keeping evolutionary time (determined by the number of nucleotide substitutions over millions of years). Scientists who believe that most of the molecular variation in nature is neutral suggest that the relative constancy of the molecular evolutionary clock demonstrates that selection does not act on these variations.

Some proteins perform functions that are so important to cell function that natural selection tolerates very little variation, as the effects might be deleterious. Examination of the amino acid sequences of these proteins and of the DNA sequences that encode them provide useful information to evolutionary biologists. Species that are closely related exhibit very few variations, and the variations that are present occur most often at the third position of redundant triplet codons where their effects are neutral. Distantly related species show much greater variation. The amount of variation that exists between species can be used to construct phylogenetic trees that reflect the same conclusions drawn from fossil evidence, anatomical, and physiological data. Regions of DNA on the chromosomes that do not encode for amino acids (or for RNA genes or direct the regulation of gene expression) show the most variation, as expected. While evolution depends on variation, at the molecular level, many changes have no effects. In other words, the variation is neutral.

See also EVOLUTION, THEORY OF; INHERITANCE; POINT MUTATIONS.

FURTHER READING

Futuyma, Douglas. *Evolution.* Sunderland, Mass.: Sinauer, 2005.

Golding, Brian, ed. *Non-neutral Evolution: Theories and Molecular Data.* New York: Chapman & Hall, 1994.

Maxwell, Kenneth. *The Sex Imperative: An Evolutionary Tale of Sexual Survival.* New York: Plenum Publishing, 1994.

vertebrates Vertebrata is the largest and most familiar subphylum of the phylum Chordata of the kingdom Animalia. All animals share several characteristics: they lack cell walls; they cannot photosynthesize; they require complex food materials such as other organisms or materials derived from other organisms; they can move spontaneously; and they can respond rapidly to stimulation. Animals are generally divided into two large groups, the protostomes, in which the mouth develops from or near the blastopore, and the deuterostomes, in which the mouth does not develop from the blastopore. Approximately 3 percent of the estimated 1.5 million known animal species exhibit the hallmarks of organisms belonging to the deuterostome phylum Chordata: dorsal, tubular nerve cord; flexible, supportive notochord that runs the length of the body; pharyngeal slits, endostyle, or thyroid gland; and a postanal tail. In many cases, some of these structures are present only during development and are absent from the adult stage,

but their presence at any point is characteristic of chordates. Many people think of vertebrate animals when they hear the term chordate, but the two words are not interchangeable. The phylum Chordata also includes the invertebrate subphyla Urochordata, the tunicates, and Cephalochordata, the lancelets.

The presence of a backbone distinguishes animals belonging to the subphylum Vertebrata. Vertebrae replace the notochord during development. Vertebrates also have skeletons consisting of either bones or cartilage, a cranium protecting their three-part brain, chambered hearts, and closed circulatory systems. Nine main classes of vertebrates include Myxini, Cephalaspidomorphi, Chondrichthyes, Actinopterygii, Sarcopterygii, Amphibia, Mammalia, Reptilia, and Aves. The fishes comprise Myxini, Cephalaspidomorphi, Chondrichthyes, Actinopterygii, and Sarcopterygii. The remaining vertebrates are called tetrapods, because they have four appendages.

MYXINI AND CEPHALASPIDOMORPHI

The uniqueness of fishes is due to their physical design, adapted to life in water. The monophyletic group of fishes includes more than 48,000 vertebrate species that breathe with gills. (Monophyletic groups contain all the descendants of a recent common ancestor.) Jawless agnathan fishes were the earliest vertebrates; now biologists recognize agnathans to be a paraphyletic group (meaning that the group does not contain all of the descendants that share a recent common ancestor), but the term *agnathan* still informally refers to jawless fishes. The jawed fishes, called gnathostomes, arose from one group of extinct agnathans, the ostracoderms, that developed paired pectoral fins, an adaptation that improved swimming efficiency.

Extant jawless fishes belong to the classes Myxini and Cephalaspidomorphi, which have a combined total of approximately 106 species. Both groups lack a bony skeleton, scales, and paired fins, and both exhibit an eel-like body shape and porelike gill openings.

Myxini, or hagfishes, are long, slender, marine fish that have no paired appendages, just a tail fin that extends along the dorsal surface. The notochord persists into the adult stage, and the skeleton is fibrous or cartilaginous. They are blind, but they have keen senses of smell and touch. They have no stomach and no cerebellum, and their bodily fluids have a similar osmolarity to seawater. Hagfish have no jaws but can hold food to their mouth using two keratinized pincers on the sides of the tongue and two rows of teeth on either side of the tongue. The hagfish use their teeth to pull apart food, which consists mostly of polychaetes (marine segmented worms) and dead or dying fish. One main heart and three accessory hearts pump blood throughout the body. Myxini undergo no larva stage, but instead develop directly into adults. They begin life as hermaphrodites, but later become male or female, though they retain the ability to switch back and forth between mating seasons. Hagfishes are well known for their ability to produce and secrete enormous quantities of slime that makes them slippery and difficult to grasp.

Lampreys are jawless fishes with mouths specialized for sucking. *(Tom McHugh/Photo Researchers, Inc.)*

The 30 or so species of Cephalaspidomorphi, or lampreys, are also jawless, and like Myxini, they have eel-like bodies, no scales, no paired appendages, fibrous or cartilaginous skeletons, and persistent notochords. They possess a differentiated brain with a small cerebellum and 10 pairs of cranial nerves. Lampreys in the Northern Hemisphere belong to the family Petromyzontidae, a name derived from the Greek words for stone and sucking, because they often grasp a stone to maintain their position in a current. Both parasitic and free-living lampreys exist, as do marine and freshwater forms, though they all must return to freshwater to breed. Males fertilize eggs as the female sheds them, and two weeks later the eggs hatch. The larvae, called ammocoetes, do not resemble the adult form but exhibit typical vertebrate characteristics. They remain nested in the sand until their yolk supply diminishes, then filter feed for three to seven years before becoming adults. They develop eyes, a suckerlike oral disc, keratinized teeth, one or two dorsal fins, and mature gonads. Parasitic lampreys attach to a host by suction, use a rasping tongue and their teeth to tear off flesh, and secrete an anticoagulant into the wound to prevent the blood from clotting. When full, the lamprey detaches. The wounds left behind can be fatal, thus lampreys are a potential threat to commercial and recreational fishing areas. After one or two years as an adult, the lamprey spawns, then dies. Nonparasitic lampreys never feed as adults; their digestive tract simply degenerates, they spawn, and die.

CHONDRICHTHYES

The class Chondrichthyes includes about 850 living species of mostly marine, cartilaginous fishes. Though calcification often occurs, true bone is absent in their skeletons, which consist of appendicular, girdle, and visceral parts. These animals are large, averaging about 6.5 feet (2 m) in length, having a fusiform (spindle-shaped) body, a heterocercal caudal fin (meaning the upper lobe is larger than the lower and the vertebral column extends into the upper lobe), pairs of pectoral and pelvic fins, two dorsal fins, and, in males, pelvic fins modified for clasping. The sharks, skates, rays, and chimeras all belong to this class. The evolution of jaws increased the variety of foods that these fish could eat. Some types of chondrichthyes have placoid scales, with a plate of dentin embedded in the skin, and a backward pointing spine, and others have no scales. An oil-filled liver, rather than a swim bladder or lung, provides buoyancy. Four-chambered hearts and extensive circulatory systems are present. Gas exchange occurs through five to seven pairs of gills. High concentrations of urea and trimethylamine oxide in the blood and extracellular fluids make the bodily fluids either isosmotic or hyperosmotic to the seawater. The brain of a chondrichthyes is more highly developed than in hagfishes and lampreys, as it contains two olfactory lobes, two cerebral hemispheres, two optic lobes, a cerebellum, a medulla oblongata, and 10 pairs of cranial nerves. These vertebrates have acute senses of smell, vision, reception of vibrations in the surrounding waters, and electroreception. The sexes are distinct, and they reproduce by internal fertilization.

The subclass Elasmobranchii contains sharks, skates, and rays. Sharks are the largest living vertebrates with the exception of whales, which are mammals. Some sharks reach almost 40 feet (12 m) long. Their streamlined body form is ideal for gliding through the water. Sharp, triangular teeth grow in rows and replace worn out teeth in a conveyor belt fashion. Vertebrae are complete and separate. Five gill slits are located in front of the pectoral fins. Their highly developed senses of smell and sight make sharks successful predators. They also have a lateral line system, specialized receptors located on their sides that can detect vibrations due to movement in the water far away and electroreceptors that perceive electric fields generated by other animals. Some elasmobranchs are oviparous and lay eggs immediately following fertilization, but many sharks are ovoviviparous and retain the fertilized eggs internally until birth (termed ovoviviparous). A few species of elasmobranchs are viviparous, meaning they nourish embryos in utero through their blood or through a placenta.

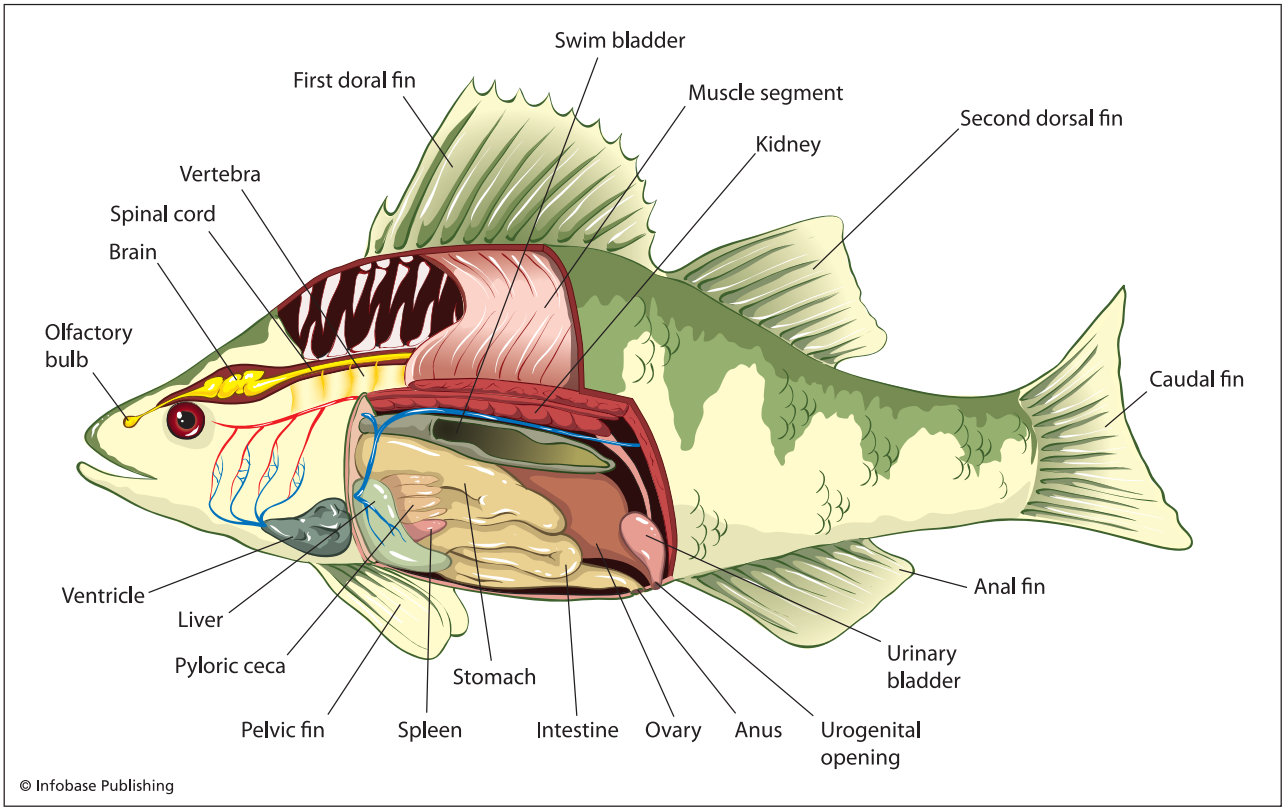
Rays are a group of elasmobranchs that include vertebrates such as skates, stingrays, and manta rays. Their bodies are typically flattened, and they have large dorsal fins that are continuous with the head. Because they dwell on the seafloor, they have openings called spiracles on the top to bring in water for breathing, and the water exits through gills underneath the head. Skates and rays look similar, but skates have more muscular tails, two dorsal fins, and sometimes a caudal fin, while rays do not. Stingrays are unique in that they have sharp-edged spines and venom glands at the base of their tails. Electric rays can discharge a current into the surrounding water to protect themselves from predators.

The subclass Holocephali includes the chimaeras, fish that live in subarctic and Antarctic waters. Chimaeras have vertebrae and four gill slits covered by opercula, but no scales and no stomach. They have a tapered tail and a diphycercal caudal fin. Their upper jaw is fused with the cranium, and they have no teeth, but instead have jaws with large flat toothplates suited for grinding. Chimaeras eat a variety of foods, including seaweed, mollusks, echinoderms, crustaceans, and fish. Their coloring is iridescent.

The chimaera are cartilaginous fishes whose upper jaw is fused with the cranium. *(Tom McHugh/Photo Researchers, Inc.)*

ACTINOPTERYGII AND SARCOPTERYGII

The sister classes Actinopterygii and Sarcopterygii consist of organisms traditionally known as Osteichthyes, the bony fishes, the most abundant of all living fishes. Like tetrapods, vertebrates with four limbs, endochondral bone replaces cartilage in the skeleton of bony fishes. Bony fishes also share the presence of lungs or a swim bladder, derived from the gut and craniopharyngeal features, but Osteichthyes is no longer considered a valid taxon because all of the bony fish are not derived from a single common ancestor. An operculum made from bony plates covers the gills and helps the fish draw in water across the gills.



Ray-finned fish make up the largest vertebrate class, Actinopterygii.

More than 23,600 fish species belong to Actinopterygii, the ray-finned fishes, the largest vertebrate class, which contains many diverse species. Ancestral forms usually have heterocercal caudal fins, but the descendant forms usually have homocercal fins, in which the upper and lower lobes are symmetrical and the vertebral column ends at the base of the fin. Long dermal rays called lepidotrichia control the movement of the fins, which include paired and median fins. Ancestral fishes have ganoid scales (thick, nonoverlapping rhombus-shaped scales), whereas teleost (modern) species have thin, overlapping, embedded, dermal versions—either cycloid (thin, overlapping with smooth edges) or ctenoid (thin, overlapping with rough edges) scales. A few species, including eels and catfishes, have no scales at all. The lighter, more flexible scales in combination with the homocercal tail increase swimming efficiency. The evolution of the swim bladder from a respiratory organ to a structure used to control buoyancy also improved the fish's locomotion. The development of jaws that could protrude, grind, and crush made fish better predators. Separate sexes exist, and fertilization occurs externally.

The class Sarcopterygii, or the lobe-finned fishes, includes only eight species, six lungfish species and two coelacanth species. These fishes have diphycercal tails, in which one continuous fin forms around the tail. The pectoral and pelvic fins are strong and fleshy. Lungfish are predators and have a unique capability of being able to survive dry seasons by aestivation, a process in which they burrow in the mud, curl into a ball, and secrete a sac of mucous that hardens into a cocoon. Coelacanths belong to the oldest living lineage of jawed fishes and are the only known living species to have a functional joint that separates the front and the back portions of the cranium. Biologists believed they were extinct until observing one in 1938. Since then many more have been spotted. They average 176 pounds (80 kg) and can reach 6.5 feet (2 m) long. They are opportunistic feeders and eat scuttlefish, squid, sharks, and other fish. Coelacanths can slow down their metabolisms in a process similar to hibernation.

TETRAPODS

One lineage of bony fishes gave rise to the tetrapods, vertebrates that have two pairs of limbs. The monophyletic unit of tetrapods contains two main groups: amphibians and amniotes (birds, reptiles, mammals). The development of limbs and lungs, adapted sensory systems, and a modified skeleton led to the successful invasion of land by amphibians, but they still required a nearby body of water to reproduce.

Early reptiles evolved from a group of amphibianlike tetrapods and produced shelled eggs that eliminated their dependence on aquatic habitats by enclosing water inside of a hardened shell. The modern amniotes emerged from three lineages, two of which are reptilian, and the third mammalian. Reptiles and birds share a common evolutionary ancestor, and despite their distinctive appearances, they maintain many common features. The skulls of both reptiles and birds meet the vertebral column at the neck with a single condyle, or process on a bone, whereas mammals have two. Reptiles and birds have only one bone in the middle ear, a lower jawbone consisting of five or six bones, and excrete nitrogenous waste as uric acid, whereas mammals have three bones in the middle ear, a single bone composing the lower jaw, and excrete nitrogenous waste as urea. Crocodiles are the closest living relative of birds.

AMPHIBIA

Frogs, salamanders, toads, and newts belong to the class Amphibia, which includes the only extant vertebrates that include a transition from water to land in both their ontogeny (the course of development of an organism) and phylogeny (the evolutionary history of a related group of organisms). Modern amphibians are descendants of the first vertebrates capable of breathing air and living outside of water, though they still must spend part of their lives in water or at least in very moist conditions. Strong skeletons provide the necessary support for maintaining structure against the force of gravity and enable movement on land. The skeletons are bony and may include ribs. Skeletons remodeled to form distinct limbs with stronger pectoral and pelvic girdles that could support walking on land. The forelimbs are smaller than the hindlimbs and usually contain four digits. The skin is moist and does not contain scales. The notochord does not persist. Some amphibians have gills but most possess lungs and breathe through a nose with paired nostrils. Respiration also occurs through the skin. The circulatory system consists of a three-chambered heart and double circulation, or a separate pulmonary circuit that carries blood to and from the lungs and a systemic circuit to supply the rest of the body. Olfactory and auditory organs evolved to detect odors and sounds in air rather than water.

More than 4,200 amphibian species currently exist. The development of an individual mirrors their evolutionary history. For most amphibians, life begins in an aquatic environment, where females lay eggs. The larval form respires using gills. As metamorphosis takes place, the gills disappear, lungs form, and the animal moves onto land. Exceptions exist; for example, some salamanders remain aquatic throughout life, and others live entirely on land. Certain frogs also live completely on land and have no aquatic larval stage. Even those amphibians that are terrestrial must, however, remain in a moist environment. Thin skin allows for some gas exchange with the atmosphere, but it is also more prone to desiccation. Amphibians are ectotherms (cold-blooded) and require moderate climates.

Three major orders of amphibians include Gymnophiona, Caudata, and Anura. Gymnophiona includes approximately 200 species of limbless, elongate, burrowing wormlike creatures called caecilians. They reside underground, where they hunt worms and other small invertebrates.

Caudata are tailed amphibians more commonly known as salamanders. Their bodies consist of a head, trunk, and tail and have no scales covering them. They are small, usually less than six inches (15 cm) long, and their limbs are generally positioned at right angles to their bodies, though some burrowing species do not have them at all. Salamanders eat worms, arthropods, and small mollusks. Most have an aquatic larval form with gills and a tail and move onto land as adults, but some do not pass through an aquatic larval stage, and others remain in the water their entire life. Mud puppies are a type of salamander that retains its gills permanently and lives on the bottom of ponds. Newts are small salamanders that are semiaquatic as adults. Fertilization is commonly internal, and the female lays eggs in clusters.

Frogs and toads belong to the order Anura, composed of about 3,500 species of amphibians that have no tail, as the name suggests. Species vary in length from less than 0.4 inches (1 cm) to 12 inches (30 cm), and their heads are fused to their trunks. Though they live in a variety of habitats, the climate must be moderate, and anurans must be near water to reproduce. Eggs laid in the water hatch into legless tadpoles with long-finned tails, internal and external gills, mouthparts made for eating vegetation, and an internal anatomy that differs greatly from the adult form. Unless it is mating season, frogs are usually quiet and solitary. During the winter, frogs in temperate climates often hibernate in the muddy bottoms of streams and ponds or underneath the decomposing material on the forest floor. Some can tolerate freezing temperatures by accumulating glucose and glycerol in their extracellular fluids to prevent damage from ice formation. Except for jumping away, frogs are generally defenseless against predators, though some will act dead to fool predators or jump toward them and bite.

Composed of two layers, a dermis and an epidermis, the skin of frogs is thin and moist. The epidermis contains keratin, and the dermis contains glands that secrete a waterproof mucous and poison to ward off potential predators. Chromatophores,

The red hills salamander, which can grow to about 10 inches (25.5 cm) long, spends most of its time in its burrow and has a geographic range limited to a narrow belt in Alabama. *(Emmett Blankenship/U.S. Fish and Wildlife Service)*

cells containing pigments, color the skin, and can adjust to match the surroundings in many frogs. The vertebral column of most frogs has between six to 10 vertebrae and is not as flexible as in fish, since frogs move by using their limbs to jump rather than swim. Frogs breathe through their skin, mouth, and lungs. They use positive pressure to push air into their lungs, which have a separate circulatory circuit from the rest of the body. Insects, spiders, worms, slugs, millipedes, and snails serve as food for frogs. A protrusible tongue jets out and sticks to the prey and pulls it into the mouth. The auditory sense has adapted for detecting airborne sound waves rather than waves traveling through the water, and vision is keen. Males use their more pronounced vocal cords to attract females for mating. When their eggs are mature, a male grabs onto a female in a process called amplexus, and he releases sperm into the water as she discharges her eggs. A fertilized egg begins cleavage, a series of cell divisions, followed by the development of an embryo. After six to nine days, a tadpole hatches and feeds on vegetation in the water. During metamorphosis, the external gills are covered, limbs emerge, the tail disappears, and the tadpole matures into an adult frog.

REPTILIA

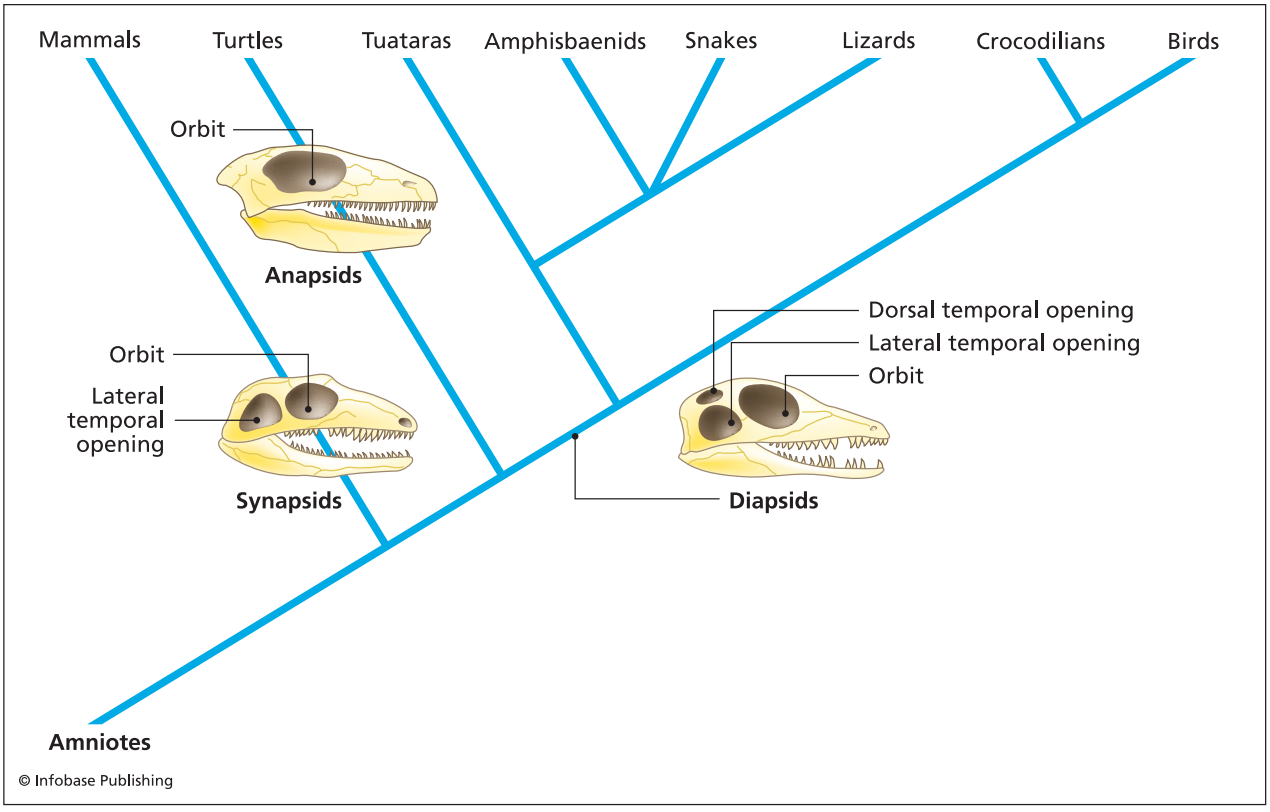
Reptilia is no longer considered a valid taxon, since it is not monophyletic. In other words, it does not contain the most recent common ancestor and all of its descendants. Traditionally, Reptilia excluded birds, but biologists now recognize that birds have a common ancestor shared with all other reptiles. The common term *reptiles* still refers to reptiles with heavily keratinized skin that are not birds, however.

Shelled eggs containing fluid encased in a series of membrane-enclosed sacs allowed vertebrates to move farther onto land by lessening the dependence on water. The innermost sac encloses a fluid-filled cavity in which the embryo develops. Because the sac is called the amnion, this lineage of vertebrates is known as the amniotes. Other extraembryonic membranes include the allantois, which serves respiratory and excretory functions, and the chorion, through which oxygen and carbon dioxide readily diffuse. A large, nutrient-rich yolk supplies the developing embryo with all the energy and nutritional requirements. A hardened, porous, fibrous or calcareous shell surrounds and protects the embryo during its development.

Reptiles have dry, heavily keratinized skin that prevents them from dehydrating in dry habitats and also against injury. Because of this, their lungs are more developed than amphibians', which rely on the skin as a major organ for gas exchange. Reptilian limbs are better suited for movement on land than amphibians, and reptiles have more complex nervous systems and therefore more complex behaviors than amphibians. They have strong, large jaws with developed musculature designed for quick closure and for maintaining a crushing grip, making them good predators and vicious carnivores. Fertilization occurs internally via a copulatory organ, and eggs are usually laid, but some reptiles are viviparous—the eggs remain inside the female reproductive tract and the mother gives birth to live young. Reptiles excrete nitrogenous waste in the form of uric acid or urea, a strategy that helps them to conserve water.

The amniote lineage that gave rise to the reptiles, birds, and mammals comprises three main groups. The anapsids have no temporal opening in the skull behind the orbits, and today consists entirely of turtles. The diapsids have two pairs of openings, one low in the cheeks, and the other pair above that. Four subgroups include lepidosaurs (all extant reptiles except turtles and crocodiles), archosaurs (dinosaurs and their living descendants, crocodiles, and birds), sauropterygians (extinct aquatic reptiles), and ichthyosaurs (extinct dolphinlike forms). The third group of amniotes, the synapsids, includes the mammals, and it is characterized by a single pair of temporal openings low on the cheeks.

The anapsid reptiles, turtles, are unique in that rigid shells made of a dorsal carapace and a ventral plastron enclose their bodies. The inner layer of the shell is made of bone, while the outer layer is composed of horny keratin. The ribs and vertebrae are fused with the carapace. Because the shell does not allow for expansion, the animal breathes by working the abdominal and pectoral muscles to expand and contract the thoracic cavity. The limbs and limb girdles are located within the ribs, and, though turtles do not have teeth, they have keratinized plates in their jaws for gripping food. The neck is long and flexible, and the turtle can withdraw its head as well as its limbs into the shell for protection. In general, turtles have poor hearing and do not make sounds except during mating. Fertilization is internal, and they are oviparous, meaning they lay eggs that develop outside of the body. In some types of turtles, the temperature of the nest during embryonic development determines the sex. They have low metabolisms and move slowly, and some types grow



The clade amniotes consist of the monophyletic groups mammals and reptiles, which include birds.

The lack of hinges on the lower jaws of snakes allows for the ingestion of large food items, such as whole chicken eggs. The snake will later eject the shell. *(Karl H. Switak/Photo Researchers, Inc.)*

very large—up to 6.5 feet (2 m) and 1,600 pounds (725 kg) in some marine species.

Diapsid reptiles include vertebrates such as lizards, snakes, tuataras, and crocodiles. The order Squamata comprises more than 95 percent of all known living reptiles and includes lizards, snakes, and worm lizards. Squamates are viviparous, often because of an advantage in keeping the eggs in the oviduct for a longer period of time in colder climates. Their heads exhibit greater flexibility than did their ancestors due to modifications in the skull, improving their ability to grasp prey in their jaws.

Members of the suborder Sauria, lizards such as geckos, skinks, and chameleons have diverse habitats, living in the ground, in trees, or in water. Most lizards have four limbs, no glands in their skin, and a slender, short body. They can open and close their eyelids, have good vision, and external ears. Some use vocalizations for mating and defense. Lizards are ectotherms, and many live in hot, dry environments where they can warm themselves. They produce semisolid nitrogenous waste containing uric acid.

Worm lizards, of the suborder Amphisbaenia, are not really worms, but they are not lizards either. They have limbs and can move forward and backward. Modified skulls assist in burrowing, and their eyes and ears are located underneath their skin.

Snakes belong to the suborder Serpentes. Limbless, and usually lacking both pelvic and pectoral girdles, snakes are very agile. Most move by undulating in the shape of an S, but some move in a rectilinear fashion, in which portions remain on the ground and the body lifts the intervening portion and pulls it forward. Side-winding is another mechanism that minimizes contact with the ground while the body throws portions forward. The lower jaws are not hinged, and therefore can open very wide, allowing the consumption of large masses of food. To minimize damage to self during feeding, snakes usually eat smaller organisms such as worms, insects, and frogs, but some species first immobilize their prey by suffocation from constriction or injection with venom. Most snakes have poor vision and can barely move their eyeballs but can detect slight vibrations in the ground from a distance. Though they have a nose, snakes detect scent molecules brought to the mouth by a forked tongue with Jacobson's organs, pitlike organs in the upper mouth that have olfactory receptors. Most snakes are oviparous, but some are ovoviviparous and give birth to developed young.

The order Sphenodonta, more commonly known as tuataras, contains only two known living species, belonging to the genus *Sphenodon*. Tuataras are large, slow-growing, spiny reptiles with four legs that live off the coast of New Zealand. They reach up to 26 inches (66 cm) in length. A unique feature is a "third eye" that is covered with scales but sensitive to light.

Crocodiles and alligators belong to the order Crocodilia. Modern crocodiles have not changed much over the past 200 million years. Crocodilians have elongate skulls with large, strong jaws and teeth set in sockets. They are semiaquatic and carnivorous and have complex social behaviors. One unique characteristic is the presence of a second palate that allows breathing even when the mouth is full of water or food. A completely divided four-chambered heart keeps oxygenated and deoxygenated blood separated, allowing for more efficient gas exchange and delivery. Some crocodilian species grow up to 2,200

Tuataras (*Sphenodon guntheri*) are reptiles that belong to the order Sphenodonta and live off the coast of New Zealand. *(Norma Cornes, 2007, used under license from Shutterstock, Inc.)*

pounds (1,000 kg), but they can move rapidly. One way to distinguish a crocodile from an alligator is that in crocodiles, the fourth tooth of the lower jaw fits outside of the upper jaw, and is therefore visible. Crocodiles also have a narrower snout.

AVES

The class Aves, commonly known as birds, includes feathered vertebrates that have forelimbs modified for flight, though some have lost the ability to fly, and high metabolisms. Consisting of approximately 9,700 species, Aves is a monophyletic class, meaning all types of birds evolved from a common ancestor. Flight provides many advantages: a means to escape predators, the ability to chase and eat flying insects and hunt other food, and the ability to migrate long distances for finding food or for seasonal breeding. Other characteristics of birds are endothermy (the generation of body heat internally), keratinized beaks, absence of teeth, large, muscular stomachs, strong but light skeletons, and large-yolked, hard-shelled eggs.

Living birds consist of two main groups, or superorders: Paleognathae and Neognathae. The Paleognathae are the flightless birds such as ostriches, kiwis, rheas, emus, and tinamous, though evidence strongly suggests that some evolved from birds capable of flight. Also called ratite birds, they have a flat sternum and underdeveloped pectoral muscles compared to other birds. Without having to restrict body weight for flight, the ratite birds are much larger than the Neognathae. The Paleognathae can reach incredible ground speeds, up to 60 miles (96 km) per hour, thus are still able to escape most predators. Neognathae encompasses all the flying birds, including doves, owls, herons, cranes, swans, ducks, eagles, quail, domestic fowl, loons, pelicans, kingfishers, toucans, woodpeckers, whippoorwills, hummingbirds, perching songbirds, and penguins (which use their wings for swimming rather than flight). They have a sternum with a thin keel to which powerful breast muscles attach.

Birds have common structures due to the fact that their anatomy is designed around their ability to fly. The major adaptations for flight are a reduction in body weight and modifications that promote power for flight. Feathers, made of keratin and derived from the scales of reptiles, are lightweight but tough. The shaft is hollow, and barbs branch from the vanes that extend from the shaft. Parallel barbules branch from the main barbs and have tiny hooks that hold neighboring barbs together in a precise, aerodynamic shape in contour feathers. Pectoral muscles attach to the wings, which contain extended contour feathers called flight feathers. Wings can be adapted to soaring, high-speeds, maneuvering, and high lifting for carrying heavy prey. Downy feathers are fluffy, lack hooks, and function to trap air close to the body for insulation, though metabolism provides most of the body's warmth. Feathers shed, or molt, at least once a year, gradually in most species. Many birds have brightly colored feathers, though marked differences between genders are common.

The internal structure of the bones is pneumatized, meaning it has a honeycombed, lacy appearance and contains air-filled cavities, making the bones strong while keeping them light. The absence of teeth reduces the weight of the head, birds have no bladder, and female birds have only one ovary. Bird skulls consist of one fused piece with large openings to accommodate large eyes. The leg bones are denser than the skull and wings in birds, lowering the center of gravity and facilitating flight. Most of the vertebrae are fused, providing rigidity necessary for efficient flight.

Birds have a variety of shapes, colors, and sizes, as exhibited by this preening sandhill crane (*Grus canadensis*), which is among the world's tallest birds and can have a wingspan of up to seven feet (2.1 m). *(Millard H. Sharp/Photo Researchers, Inc.)*

Flying demands a lot of energy, and many body systems possess unique adaptations to accommodate this need. The respiratory system must be very efficient to bring in oxygen used in aerobic respiration and eliminate carbon dioxide produced as a by-product. Rather than end in saclike alveoli, the bronchi of birds branch into parabronchi, which always contain flowing air. The thorax and abdomen also contain air sacs that serve as reservoirs of air. During inhalation, air flows into the posterior air sacs, then upon exhalation, the air flows into the lungs. During a second breath, the air then leaves the body, allowing air to continuously flow in one direction, making the most efficient respiratory mechanism of terrestrial animals. The circulatory system is powered by a four-chambered heart that beats rapidly to carry oxygen and nutrients around the body efficiently. Chickadee hearts can beat up to 1,000 times per minute during exercise.

The pectoral muscles, the largest muscles in birds, power flight. Of the leg muscles, the thighs are the largest, and the feet contain very little muscle. Tendons tighten automatically when a bird perches on a branch, thus the feet remain clenched even when the bird sleeps.

Birds also have keen eyesight, with large but relatively immobile eyes. They turn their heads rather than their eyes to see around them. Birds that hunt prey have eyes positioned in the front of their heads, whereas birds that eat mostly plants have eyes positioned more toward the sides, enabling them to watch for predators. Beak shape varies tremendously with diet, depending on whether the bird has a generalized diet, eats mostly seeds, burrows for worms, cracks open nuts, sifts through mud, spears fish, or tears flesh off prey. Foot structure also varies based on its main use. Feet can be adapted for perching, grasping, defense, swimming, or walking.

By excreting waste as uric acid, which is relatively insoluble, birds do not need to carry around excess water to eliminate nitrogenous wastes. Also, in a developing embryo, the uric acid crystallizes out of solution and remains in the eggshell with the embryo until hatching. Bird kidneys are not very efficient, however, and birds such as seagulls that drink marine water must have adaptations for removing excess salts from their bodies. Salt glands, located above the eyes, secrete concentrated solutions of sodium chloride that leave the body through the nostrils.

Birds reproduce by internal fertilization, even though the males of most species do not have a penis. Instead, the male and female birds simply bring their cloacal regions into contact with one another. Eggs released by the ovary into the oviduct pass by the cloacal region, and fertilization occurs before special glands secrete albumin (egg white), and the shell membranes and the eggshell are added. Birds lay their eggs in a nest or on the ground, and one parent, usually the female, incubates them. Some birds hatch as precocial young that are covered with down and can swim or run as soon as their feathers are dry. In contrast, altricial young are naked, blind, and unable to walk for up to one week after hatching. Parents must bring food to altricial young, who consume more than their own weight in food each day as they complete their development.

MAMMALIA

Members of the class Mammalia share three distinguishing characteristics: hair; the production of milk from modified sweat glands called mammary glands; and the presence of three bones in the middle ear. Most mammals also have developed placenta for nourishing developing embryos in utero, advanced nervous systems, and exhibit complex social behaviors. Like birds, mammals are endotherms (meaning they generate their body heat internally) and homeotherms (meaning they maintain a relatively constant body temperature), allowing them to inhabit colder environments. Some, such as collard lemmings, live in tunnels one foot (30.5 cm) underneath the surface of a snow field in the Arctic. The more than 4,675 species of mammals evolved from the synapsid amniote lineage, characterized by one pair of temporal openings low on the cheeks. Mammals are extremely diverse; ranging in size from a few grams to more than one hundred tons; they live in land, in water, and some are capable of flight; they exhibit all types of diets; they include diurnal and nocturnal species; and they tolerate a wide range of temperatures.

Compared to other vertebrates, mammals have thicker skin consisting of a dermis and an epidermis. Areas covered by hair are thinner, whereas areas that do not have hair, such as the palms, are thickened from keratin. Hair is composed of dead epidermal cells packed with keratin. Some mammals, such as bears or cats, are completely hair-covered, and others such as whales have only a small amount on their snouts. The adaptation of hair helps mammals preserve some of the body heat they generate internally. Two types of hair make up a fur coat—the softer underhair that functions to insulate and trap warm air near to the body, and denser, longer guard hair that protects and colors the animal. Most mammals shed their coats periodically. Some mammals have specialized hairs such as the sensory vibrissae on snouts, protective spines of porcupines, or other related specialized structures such as horns and antlers. Mammalian epidermal glands consist of sweat, scent, sebaceous, and mammary glands. Sweat glands aid in cooling. Scent glands secrete substances

that play a role in communication, defense, mating, recognition, and marking territories. Sebaceous glands, associated with hair follicles, secrete sebum that keeps the skin pliable. Mammary glands, from which the class name is derived, produce milk to nourish young.

Mammals eat a variety of foods, demonstrated by their different dentitions, the number, type, and arrangement of teeth. Incisors have sharp edges for biting, canines are pointed for puncturing, and premolars and molars are compressed for crushing and grinding. Mammals typically have a set of deciduous teeth (also called milk teeth) as young, then a permanent set as adults.

Insectivores, carnivores, herbivores, and omnivores have adaptations suited to their particular diets. Warm bodies are expensive to maintain but give mammals the energy to hunt for prey and allow them to do so during day or night. Small mammals such as shrews, moles, armadillos, anteaters, and some bats are called insectivores, though they also eat other small invertebrates. They have pointed teeth that can pierce through an insect's exoskeleton and short digestive tracts. Herbivores have broad, flattened molars for grinding plant tissue. Because mammals do not produce cellulase, the enzyme that breaks down cellulose from plant cell walls, symbiotic protozoa and bacteria live in their digestive tracts and break down the cellulose into molecules that the mammals can further digest and absorb. Herbivores often eat continuously. Ruminants are grazing herbivores that have four-chambered stomachs. Ground food first enters the rumen, where microorganisms start to digest it and small balls of food called cuds form. The cud returns to the mouth, where the ruminant chews it up more, and then swallows it back to the rumen. After microorganisms further digest the cellulose, the food material travels to the reticulum and the omasum, where substances are absorbed. After reaching the abomasum, digestive enzymes continue hydrolyzing the foodstuffs for absorption along the extended digestive tracts. Some animals, such as rabbits and zebras, exhibit other strategies for digesting grass. Rabbits eat their own droppings for a second pass through their digestive system, allowing for the extraction of even more of the nutritional substances. Herbivores such as zebras have long caecums, blind pouches found near the junction of the small and large intestines that increase the time the foodstuff spends in the digestive tract. Carnivores, such as lions, tigers, wolves, hyenas, and weasels, eat mostly herbivores. They have teeth specialized for biting into and tearing flesh and shorter digestive tracts. Because they must chase and capture their prey, they have other adaptations such as strong limbs with claws, keen senses, intelligence for strategic hunting, and musculature for speed and agility. Omnivorous mammals include animals, such as raccoons, bears, rodents, and primates (including humans), that eat plants, fruits, fish, and other animals to fulfill their nutritional needs. Many store foods like nuts, seeds, or fungi for winter feeding. Like birds, smaller mammals have higher metabolisms and must eat often to maintain their body weights. This is because their surface area to body volume is greater. Larger carnivorous mammals might eat only one large meal once a week, whereas smaller mammals must spend all their waking hours hunting and feeding.

Most mammals do not migrate seasonally, but some do. In East Africa, herds of one-half million wildebeest and other grazing species migrate with the changing wet and dry seasons. Whales travel to warmer waters to give birth. Caribou and elk migrate in the winter to find food. Some mammals, like bears and chipmunks, hibernate in the winter; their body temperatures drop significantly, and their heartbeats and breathing slow down to conserve energy.

Mating in mammals is often timed so that birth will occur in the most favorable conditions. Fertility in the females often cycles, and they are receptive to copulation only during estrus, also known as heat. Different species have different lengths and numbers of cycles during a breeding season. Old World monkeys and humans have menstrual rather than estrus cycles, during which they lose their uterine lining following ovulation in the absence of pregnancy. One pattern of reproduction is exhibited by monotremes, a group that includes the duck-billed platypus and echidnas. The monotremes are the only oviparous mammals. Echidnas lay their eggs directly into a pouch on the belly, and platypuses lay eggs into a nesting burrow. Since the females have no nipples, the hatched young lap up milk from their mother's belly fur. Marsupials are viviparous mammals, meaning embryos develop inside the mother and their young are born as juveniles. Primitive placentas called choriovitelline placentas nourish the embryos during a very brief gestation. In general, the young are born early and crawl across the mother's belly into a pouch until they are fully developed. In red kangaroos, after one joey (baby kangaroo) is born, the mother becomes pregnant again, but the embryo arrests development until the first joey is out of the pouch, then it resumes development, moves to the pouch, and the mother can become pregnant a third time. The last embryo also pauses mid-development until the second joey has left the pouch. Viviparous placental mammals, called eutherians, exhibit a third type of reproduction pattern. In these mammals, gestation is prolonged rather than lactation, as in marsupials. A

A mother kangaroo carries her joey in her pouch. *(Sandra Caldwell, 2007, used under license from Shutterstock, Inc.)*

well-developed placenta allows for the exchange of oxygen, carbon dioxide, nutrients, and waste products between the embryo or fetus and the mother during the gestation, which can last from 21 days in mice to 22 months in elephants. Larger mammals usually have fewer young.

Many mammals have territories, an area from which one member of a species restricts entry by other members, especially of the same sex during breeding season. Animals mark their territory by urinating or defecating around the boundaries, and animals who enter must prepare to defend themselves. Home ranges are foraging areas and are generally larger than territories.

See also ANIMAL BEHAVIOR; ANIMAL FORM; BIODIVERSITY; BIOLOGICAL CLASSIFICATION; CIRCULATORY SYSTEM; DIGESTIVE SYSTEM; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT; EUKARYA; EVOLUTION, THEORY OF; HOMEOSTASIS; HUMAN EVOLUTION; INTEGUMENTARY SYSTEM; INVERTEBRATES; MUSCULOSKELETAL SYSTEM; NERVOUS SYSTEM; NUTRITION; REPRODUCTION; RESPIRATION AND GAS EXCHANGE; SOCIAL BEHAVIOR OF ANIMALS; ZOOLOGY.

FURTHER READING

Attenborough, David. *The Life of Mammals*. Princeton, N.J.: Princeton University Press, 2002.

Cobb, Alan, ed. *Animal Sciences: Macmillan Science Library*. 4 vols. New York: Macmillan Reference USA, 2002.

Hickman, Cleveland P., Larry S. Roberts, Allan Larson, Helen I'Anson, and David Eisenhour. *Integrated Principles of Zoology*. 13th ed. New York: McGraw Hill, 2006.

Miller, Stephen A., and John P. Harley. *Zoology*. 7th ed. New York: McGraw Hill, 2007.

Tudge, Colin. *The Variety of Life: A Survey and a Celebration of All the Creatures That Have Ever Lived*. Oxford: Oxford University Press, 2000.

Virchow, Rudolf (1821–1902) German *Pathologist* Rudolf Virchow was one of the 19th century's most prominent physicians. He was a skilled pathologist who emphasized the importance of studying disease at the cellular level. Among cell biologists, he is well known for popularizing the concept that all cells come from preexisting cells, an important tenet of the cell theory.

Rudolf Carl Virchow was born on October 13, 1821, in the rural town of Schivelbein, Pomerania, Germany. His father was a merchant of modest means. Having shown an interest in the natural sciences and in classical languages, Rudolf enrolled in the Gymnasium in Köslin in 1835. His excellent academic performance earned him a military scholarship in medicine at the Friedrich-Wilhelms Institut in Berlin in 1839.

After completing a dissertation on the effects of rheumatic disease on the cornea and obtaining his medical degree in 1843, Virchow was appointed medical house officer at the Charité Hospital in Berlin. While there, he studied vascular inflammation, thrombosis, and embolisms and became proficient in microscopy. He earned a reputation as a good speaker and developed his perception of life as the mere sum of physical and chemical activities of cells. This affected his view of medicine—as the ultimate science of man—and how he believed research should be conducted. His model was based on the following:

- Diseases originate from disturbances of individual living cells, as opposed to tissues or organs.
- Cells arise from other cells.
- Cellular function depends on chemical and physical activities, which one can partly assess by morphological appearances.
- Anatomical indications of the pathological states are due to degenerations, transformations, or repetitions of normal structures.

Virchow criticized the field of medical research for drawing wide-ranging conclusions based on weak or sparse evidence. Medical research should follow the scientific method, beginning with the formulation of a hypothesis based on scientific laws, and involve experimentation to obtain answers. Many colleagues took offense when in 1846 Virchow opposed the renowned pathological anatomist Carl Rokitansky and the Viennese Medical School for upholding the false notion that imbalances in the four humors (blood, bile, phlegm, and black vile) caused most diseases, a concept that dated back to the ancient Greeks. Some of Virchow's older peers did not like his innovative approaches, yet he passed his licensure examination in 1846 and began teaching pathological anatomy.

In 1847 he completed his habilitation, a more advanced degree, and the University of Berlin appointed him instructor. He became a prosector, a person who performs anatomical dissections, at the Charité Hospital. He also launched a new medical journal, *Archiv für pathologische Anatomie und Physiologie, und für die klinische Medizin* (Archives for pathological anatomy and physiology, and for clinical medicine, commonly known as *Virchows Archiv*), for which he served as editor until his death.

After visiting the Prussian province of Upper Silesia in 1848 in order to survey the extent of a typhus epidemic, Virchow passionately supported the economic and educational reforms and political freedom for the people there. His political activity led to a membership in the Berlin Democratic Congress and to the loss of his job at Charité Hospital. Although the hospital partially reinstated him after his students and supporters protested, the climate was hostile, so he quit.

In 1849 Virchow moved to the University of Würzburg as the first chair of pathological anatomy. At Würzburg, Virchow was able to concentrate on his teaching and his research. In seven years there, he nearly quadrupled the medical student enrollment. The University of Berlin hired him back in 1856 as professor of pathological anatomy and as director of the newly opened Pathological Institute, which grew into a respected training facility for medical scientists under Virchow's guidance. He also directed a clinical section at the hospital.

Virchow used the microscope to follow the pathological progress of disease and described sequential cellular changes in diseased tissue. He attributed all aspects of a diseased state to chemical and physiological actions occurring at the cellular level and emphasized that these abnormal disturbances manifested in observable alterations under the microscope. He was aware of the limitations of a purely anatomical approach, however, in particular with respect to distinguishing cause and effect. In 1858 he published *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebenlehre* (Cellular pathology as based upon physiological and pathological histology), the most influential work on disease of the 19th century. Though he also believed that disease was never purely biological, he asserted that the biology of the cell must be studied in conjunction with its anatomy, as the cell was both the fundamental unit of life as well as the fundamental unit of disease.

In 1859 he became a member of the Berlin City Council, and his political career focused on improving the social aspects of diseases, such as poverty, living conditions, water treatment and sewer systems, that marked the beginnings of what is referred to as social medicine.

Other contributions he made to medicine include describing one of the first two earliest reported cases of leukemia (1845) and identifying the enlargement of a specific lymph node as a sign of malignant gastrointestinal cancer. He also determined that part of a thrombus (a clot that forms within and remains attached to a blood vessel) could detach and form an embolism, a term he coined to mean an obstruction of a blood vessel, such as by a blood clot or other abnormal particle circulating in the blood. In recognition of his demonstration that pathological states in dead bodies correlated with symptoms and illnesses in living people, a standard autopsy procedure that he introduced in 1874 is named after him. Medical science also credits Virchow with founding the disciplines of cellular pathology and comparative pathology.

Though a distinguished pathologist, Virchow's fame in biology relates to the phrase "*omnis cellula e cellula,*" translated from Latin to mean all cells arise from preexisting cells. The French chemist and physiologist François-Vincent Raspail coined the phrase in 1825, but Virchow popularized it and demonstrated its importance. This statement opposed the then common belief in spontaneous generation, the emergence of living organisms from nonliving matter. The French chemist Louis Pasteur provided the final evidence disproving spontaneous generation in 1862. This principle comprises one of the components of the cell theory, originally formulated by Matthias Schleiden and Theodor Schwann in 1838–39. The modern cell theory states:

- Cells are the fundamental structural and functional unit of life.
- All organisms consist of one or more cells. (Viruses are not considered cells.)
- All cells come from preexisting cells (with the exception of the first cells formed when life originated).

- All cells are composed of the same basic substances.
- Metabolism is a cellular function.

Later in his career Virchow developed another interest—anthropology. One of his studies in this subject area involved a nationwide racial survey of 7 million school children. In 1876, based on physical characteristics, he concluded that no pure German race existed but that German people exhibited a variety of morphological types. In 1869 he cofounded the German Anthropological Society and founded the Berlin Society for Anthropology, Ethnology, and Prehistory, for which he served as president and editor of its *Zeitschrift für Ethnologie* (Journal of ethnology) until his death. He also assisted in securing construction of the Berlin Ethnological Museum and the Museum of German folklore.

The Prussian Academy of Sciences elected Virchow to membership in 1873. The Royal Society of London awarded Virchow their highest honor, the Copley Medal, in 1892. In 1894 he was made privy councillor.

In 1850 Virchow married Rose Mayer, with whom he had three sons and three daughters. After breaking his hip in 1901 Virchow's health declined, and he died of a heart attack on September 5, 1902. He is remembered for his theory of the cellular nature of disease and his insistence on applying scientific methodology to the field of medicine.

See also CELL BIOLOGY; SCHLEIDEN, MATTHIAS; SCHWANN, THEODOR.

FURTHER READING

Ackerknecht, Erwin H. *Rudolf Virchow: Doctor, Statesman, Anthropologist*. Madison: University of Wisconsin Press, 1953.

Virchow, Rudolf. *Disease, Life, and Man: Selected Essays by Rudolf Virchow*. Translated and with an introduction by Lelland J. Rather. Stanford, Calif.: Stanford University Press, 1958.

viruses and other infectious particles

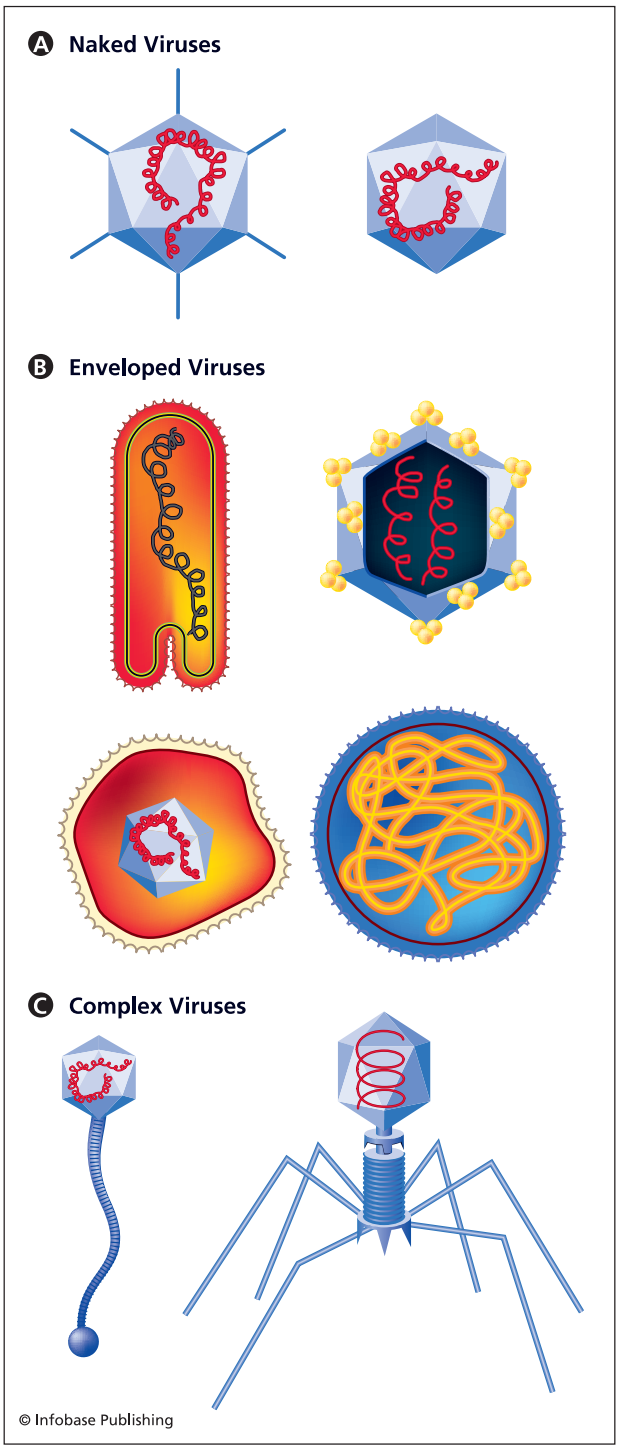
Simply put, viruses are tiny packages of genetic material. Though they consist of biomolecules, they are acellular, and thus are not considered living organisms. Cells are enclosed within a biological membrane composed of a phospholipid bilayer, a structure that viruses do not possess. The simplest viruses contain only a fragment of genetic material surrounded by a coat of protein, and viral particles can be observed only with the assistance of an electron microscope. As obligate intracellular parasites, viruses can replicate only within a host cell. They lack the necessary molecular machinery and metabolites to reproduce on their own. Viruses are specific for the type of host they infect, and viruses are known that infect organisms across all the domains and kingdoms: animals, plants, fungi, protists, bacteria, and archaea. Some viruses harm their hosts or cause serious damage. Human viruses are responsible for numerous diseases including chicken pox, mumps, influenza, poliomyelitis, the common cold, and acquired immunodeficiency syndrome, to name a few. Certain types of viruses cause cancer in humans and other animals.

VIRAL STRUCTURE AND CLASSIFICATION

Most viruses are less than 2,000 angstroms wide, and some are 10 times smaller than that (one angstrom is 10^{-10} meter), barely larger than a small protein. All viruses possess some form of nucleic acid enclosed by a proteinaceous coating. Called a capsid, this outer covering protects the viral nucleic acid from harmful chemicals and enzymes and consists of regular repeating subunits called capsomers that often assemble into intricate geometric arrangements. Cylindrical viral capsids have rod-shaped capsomers that form a hollow helix. Another common arrangement is the icosahedron, a three-dimensional, 20-sided structure with 12 corners. Viruses that have only a nucleocapsid (the nucleic acid and the surrounding protein capsid) are referred to as naked viruses. As the nucleocapsid of some viruses emerges from its host cell, it takes with it part of the host cell membrane, which persists as an envelope. Specific glycoprotein molecules embedded in the envelope, also called spikes because they protrude, often play an important role in host cell recognition. Some viral particles have more complex structures, but all still possess a capsid with a nucleic acid core.

Viral genomes consist of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), can be double-stranded (ds) or single-stranded (ss), linear or circular, and may or may not be segmented into several pieces containing different viral genes. RNA viruses can be either positive-sense (+) or negative-sense (-), depending on whether the RNA genome directly encodes a polypeptide chain, or if the genome is complementary to the sequence that a ribosome reads. The smallest viral genome, that of hepatitis B, encodes only four genes, though many are much larger. (In comparison, the human genome encodes 30,000–40,000 genes.) Because viruses do not use their own molecular machinery to replicate, they only require the genes necessary to build and assemble new viral particles, or virions. Viral genomes may also encode certain proteins or enzymes that regulate the host cell's activity or that are required if the virus has a unique mode of replication.

Numerous classification schemes exist for viruses, depending on various purposes. For exam-



Viral particles have many forms and may or may not be covered by an envelope.

ple, a biologist might categorize viruses based on what type of organism they infect. A physician might group them according to the sort of disease they cause. In 2000 the International Committee on the Taxonomy of Viruses devised a scheme dependent on the configuration of the viral genomes. Though viruses do not belong in any kingdom, virologists grouped them into three main groups called orders, then into families (see the table Human Virus Families), genera, and species. One must remember, however, that these classifications are not based on the same criteria as living organisms, but rather on slight variations in different properties such as host range or antigenicity.

VIRAL LIFE CYCLES

Viruses cannot replicate unless they are inside of a host cell, thus the life cycle of a virus must begin with a virus seeking and entering a suitable host cell. Viruses are specific for the type of cell they can infect. Not only are they host specific, but some are tissue specific as well, meaning one that infects humans might be able to infect cells that line the respiratory tract but not liver cells. The first step of the viral life cycle is adsorption, during which a virion attaches to receptors on the exterior of the host cell membrane. This is the step that defines the host range and specificity of the virus because the interactions between the virus and molecules exposed on the host cell surface, usually glycoproteins, are specific. On naked viruses, components of the capsid perform this role, whereas in enveloped viruses, spikes embedded in the envelope bind to the host cell receptors.

After adsorption, either the entire viral particle or its nucleic acid must penetrate into the host cell. In some cases, the complete viral particle enters by endocytosis, an invagination of the cell membrane that results in the virion being enclosed in a membrane-bound vesicle within the host cell cytoplasm. Digestive enzymes break down the membrane and the viral envelope, leaving the nucleic acid floating freely in the cytoplasm. Another mechanism for penetration involves fusion of a viral envelope with the host cell membrane, so that only the nucleocapsid enters into the host cell. An uncoating step follows the entry of the nucleocapsid, and also results in free nucleic acid.

The next step—the synthesis of new nucleic acid, capsomers, and spikes—is the most complex and variable between viruses. The specific sequence of steps largely depends on the form of the viral nucleic acid. In general, viruses with a DNA genome must travel to the nucleus for transcription of the viral genes and replication of the nucleic acid, whereas RNA viruses complete these processes in the cytoplasm. If the virus contains a (+) strand of RNA, then a ribosome can immediately dock onto the RNA transcript and initiate translation, or the synthesis of viral proteins. Viral RNA that is (-) must first direct the synthesis of a complementary strand, which serves as the transcript for translation by ribosomes. Some viruses package enzymes that can synthesize a (+)

RNA strand from a (-) RNA strand in their capsid, and the enzyme is released into the host cell along with the RNA. In either case, synthesis can occur only if a supply of ribonucleotides is available. The host cell provides these. When (+) RNA is present, host ribosomes will begin translation using amino acids and energy in the form of ATP, also supplied by the host cell. Polypeptides necessary for building capsomers, spikes, and other unique viral protein accumulate.

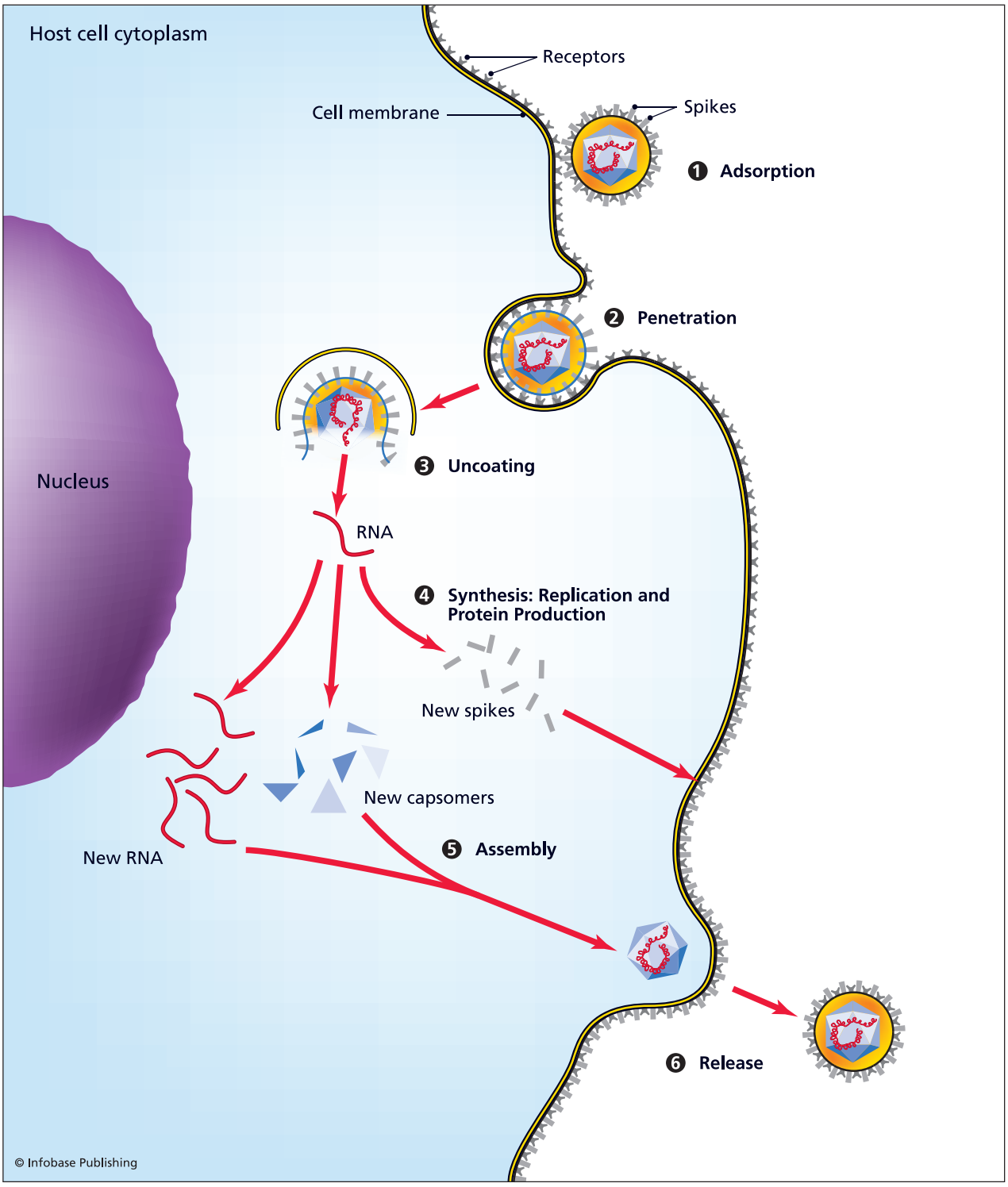
Following the synthesis stage, assembly of the viral capsids occurs, and then nucleic acid is packaged into the protein shells. For enveloped viruses, spikes are inserted into the cell membrane as assembly takes place. The nucleocapsids become coated as they push their way through the membrane to the cell's exterior during budding, or exocytosis. Nonenveloped viruses are released into the environment when the cell bursts open, or lyses. The host cell dies immediately when it ruptures, but host cells infected with enveloped viruses eventually die as well from the virus interfering with cellular processes or physical damage to cellular structures. Thousands of newly released virions seek new host cells to infect, and the cycle begins again.

Bacteriophages, viruses that infect bacterial cells, exhibit a slightly different life cycle. Scientists know most about the T-even bacteriophages (phages) that infect *Escherichia coli*. Their structure is complex, consisting of an icosahedral head that contains the DNA, a collar, a cylindrical sheath ending in a base plate surrounded by tail pins, and long tail fibers. Like animal viruses, the first step of the bacteriophage life cycle is adsorption, mediated by specific interactions between the phage and receptors on the bacterial cell surface. The sheath contracts, bringing

HUMAN VIRUS FAMILIES

Family	Nucleic Acid	Enveloped	Important Genera
Parvoviridae	ss DNA	no	*Dependovirus*
Adenoviridae	linear ds DNA	no	*Mastadenovirus*
Papovaviridae	circular ds DNA	no	*Papillomavirus* (causes warts), *Polyomavirus*
Poxviridae	ds DNA	yes	*Orthopoxvirus* (causes smallpox), *Molluscipoxvirus*
Herpesviridae	ds DNA	yes	*Simplexvirus* (HHV-1 and 2), *Varicellovirus* (HHV-3), *Lymphocryptovirus* (HHV-4), *Cytomegalovirus* (HHV-5), *Roseolovirus* (HHV-6), HHV-7, Kaposi's sarcoma (HHV-8)
Hepadnaviridae	ds DNA	yes	*Hepadnavirus* (causes hepatitis B)
Picornaviridae	ss (+) RNA	no	*Enterovirus, Rhinovirus,* Hepatitis A
Calciviridae	ss (+) RNA	no	Hepatitis E, Norwalk agent
Flaviviridae	ss (+) RNA	yes	*Flavivirus, Pestivirus,* Hepatitis C virus
Nidovirales	ss (+) RNA	yes	*Coronavirus*
Rhabdoviridae	ss (–) RNA	yes	*Vesiculovirus, Lyssavirus* (causes rabies)
Filoviridae	ss (–) RNA	yes	*Filovirus* (Ebola and Marburg)
Paramyxoviridae	ss (–) RNA	yes	*Paramyxovirus* (causes mumps)
Orthomyxoviridae	ss (–) RNA, segmented	yes	*Influenzavirus* (causes the flu)
Bunyaviridae	ss (–) RNA, segmented	yes	*Bunyavirus, Hantavirus*
Retroviridae	ss RNA, encodes reverse transcriptase	yes	*Lentivirus* (Human immunodeficiency virus, causes AIDS), Oncoviruses
Reoviridae	ds RNA	no	*Reovirus*

the head closer to the bacterial cell body, and the virus injects the DNA through the cell membrane. After penetration, the virus can enter the lytic phase or the lysogenic phase. If the former occurs, the virus replicates using the bacterial enzymes and metabolites and then assembles new viral particles. A single cell can hold up to 200 new viral particles before it lyses—hence the name "lytic cycle." The released phage can then infect other nearby host cells. Temperate phages enter lysogeny rather than the synthe-



Enveloped animal viruses typically enter a host cell by fusing with the cell membrane, and the viral progeny obtain their envelope as they exit the host cell.

sis stage following penetration. During lysogeny, the viral DNA becomes incorporated into the bacterial genome and can remain latent, or inactive, meaning viral replication does not occur. While the virus exists as a prophage, the bacterial cell replicates the viral DNA along with its own, making numerous copies that end up in all the daughter cells produced by binary fission. Induction is the conversion of a lysogenic cell into a virus-replicating factory. In a process that is not completely understood, the viral DNA pops out of the bacterial chromosome and enters the lytic phase.

OTHER INFECTIOUS PARTICLES

Prions and viroids are other acellular, subviral forms of infectious agents. Prions are small, proteinaceous, infectious particles that are not associated with any nucleic acid and cause scrapie in sheep and other spongiform encephalopathies of animals and humans. Spongiform encephalopathies are rare, progressive, fatal diseases that cause the brain tissue to become porous and spongy. One example, Creutzfeldt-Jakob disease, causes premature dementia and loss of muscular coordination. Bovine spongiform encephalopathy, also known as mad cow disease, is similar in nature. Also caused by a prion, this disease can be transmitted from animals to human by eating contaminated meat. Though prions are obviously medically important, they also pose an interesting biological problem—how can a piece of protein replicate itself without any nucleic acid component? All life-forms possess DNA that encodes all the information necessary for that organism to carry out the functions of life. Prions conspicuously lack this macromolecule. After a prion enters a host cell, the protein converts normal proteins that are already present into prions by causing them to refold in a different way. As more of the proteins refold in the prion fashion, conversion of normal to abnormal proteins occurs more rapidly until the cell becomes clogged with the prions, a situation that interferes with normal cell function and eventually cell death. When a cell dies, the prions it contained are released and can infect neighboring cells, causing the infection to spread.

Viroids are viruslike agents composed only of circular ss RNA, less than 400 nucleotides long, that is not encapsidated by protein or other type of coating. They are known to infect agriculturally important plants such as tomatoes, cucumbers, coconuts, potatoes, and citrus trees.

Satellites are subviral agents that require coinfection with another virus, called a helper virus, to replicate. When the satellite encodes for the capsid proteins, it is called a satellite virus.

See also DEOXYRIBONUCLEIC ACID (DNA); EUKARYOTIC CELLS; GENE EXPRESSION; INFECTIOUS DISEASES; IVANOVSKY, DMITRI; PROKARYOTIC CELLS; PRUSINER, STANLEY.

FURTHER READING

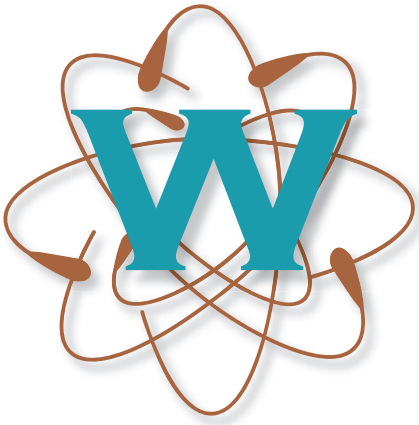
American Society for Microbiology. MicrobeWorld. "Meet the Microbes: Viruses—and Some Virus-like Agents." Available online. URL: http://www.microbeworld.org/microbes/virus/. Accessed January 16, 2008.

Crawford, Dorothy H. *The Invisible Enemy: A Natural History of Viruses*. Oxford: Oxford University Press, 2002.

Dimmock, Nigel, Andrew Easton, and Keith Leppard. *Introduction to Modern Virology*. 6th ed. Malden, Mass.: Blackwell, 2006.

Sander, David. "All the Virology on the WWW." Available online. URL: http://www.virology.net/garryfavweb.html. Accessed January 16, 2008.

The Universal Virus Database of the International Committee on Taxonomy of Viruses. Available online. URL: http://phene.cpmc.columbia.edu/. Last modified August 7, 2008.



Wallace, Alfred Russel (1823–1913) English *Naturalist* Though he was one of the 19th century's most brilliant biologists, Alfred Russel Wallace is seldom mentioned in the history of science except as the man who spurred Charles Darwin to complete his seminal book, *On the Origin of Species*. Wallace independently devised the theory of natural selection while exploring the natural history of Indonesia and Malaysia. He drafted a short manuscript that he sent to Darwin, whom he held in high regard, asking for his opinion and feedback. The work of both men was presented jointly to the Linnean Society in 1858, but Darwin receives priority credit, as he was able to demonstrate that he had been developing his ideas for decades and because he authored what has become possibly the most influential piece of biological literature. Wallace also authored several successful books, helped establish evolutionary biogeography as a new subdiscipline, and contributed to the fields of geology, geography, ethnography, and others.

FORMULATION OF THE THEORY OF NATURAL SELECTION

Alfred Russel Wallace was born on January 8, 1823, in Usk, Monmouthshire, Wales. His parents, Thomas Vere Wallace and Mary Anne Greenell, also had eight other children. At age five, the family moved to Hertford, where Alfred attended grammar school. To supplement his education, he read a great deal at home. When he was 14 years old, Alfred moved in with one of his brothers in London. The following year he began an apprenticeship with a different brother who was a surveyor.

During his five years of surveying, Wallace developed a love of nature. He bought a botany book to start a herbarium. When his brother's surveying business failed, Wallace took a job teaching at the Collegiate School in Leicester, but he continued reading about natural history. He found a friend who shared his interests, Henry Walter Bates, and they explored a nearby forest together. In 1845 his brother died and Wallace worked for the surveying business until 1848, at which point he set off on a four-year voyage to the Amazon basin with Bates.

Wallace established his reputation as a naturalist from his research in South America. The two naturalists explored a large portion of the Amazon River basin and collected numerous insect and bird specimens for later analysis. Unfortunately, on the way back to England, Wallace's ship caught fire and sank, and Wallace lost almost all of the specimens he had collected. A few notes and drawings on fishes and Amazon palm trees survived, and he compiled these into a small book. He also published an account of the expedition, *A Narrative of Travels on the Amazon and Rio Negro, With an Account of the Native Tribes, and Observations on the Climate, Geology, and Natural History of the Amazon Valley* (1853), based on some sparse notes and letters he had written during his expedition, since everything else had been destroyed.

Beginning as a teenager in London, Wallace had developed an agnostic viewpoint toward religion. The notion of evolution was not new, but no one had proposed a likely mechanism by which organisms could evolve. Wallace believed natural laws rather than divine intervention was responsible, and during his first voyage to South America he sought to collect evidence of species mutability and search for a possible mechanism. The loss of his collections prevented him from sharing his ideas on species mutability publicly, but he did mention specific adaptations to food sources and habitats displayed by species unique to

the region. He also described the geographic distribution of different monkeys, birds, and insects.

Wallace next traveled to the Malay Archipelago, now Indonesia, Malaysia, and Singapore, where from 1854 to 1862 he traveled a total of almost 15,000 miles (24,140 km). He collected more than 125,000 biological specimens, including mammals, reptiles, birds, shells, butterflies, beetles, and other insects. It was during this trip that he wrote the fateful letter to Darwin. Wallace was struck by the differences between species that lived on the eastern islands and those on the western islands. He examined their variations and geologic distribution. These observations led him to develop the theory of the origin of species by natural selection, though it was Darwin who coined the phrase. As it did for Darwin, reading *Essay on the Principle of Population,* written by the English political economist Thomas Malthus stimulated the development of Wallace's ideas. Malthus stated that organisms produced more offspring than can survive, and he warned that society may suffer if the human population were allowed to increase without restriction, as poverty would also increase and many would die from famine. Wallace and Darwin recognized a similar struggle for existence in the natural world, and both proposed that nature would favor certain variations already present in plants and animals in this survival competition. New species, then, may eventually emerge through the process of natural selection of these favorable characteristics in specific environments.

On this second voyage, Wallace sought to collect information about the distribution of animals in hopes of shedding light on the process of "organic progression," or evolution. One of the first publications from this trip was "On the Law Which Has Regulated the Introduction of New Species," dated February 1855 and published later that year, in which he defended the fact that organic evolution occurred. He concluded that the geological distribution of life on Earth resulted from changes to the surface of the Earth through geological phenomena and to its inhabitants. The paper also included Wallace's deduction of the following law: "Every species has come into existence coincident both in space and time with a preexisting closely allied species." Natural selection was not discussed in this paper, which received no public response, though Darwin, the British geologist Charles Lyell, and the English zoologist and chemist Edward Blyth all read and took to heart what Wallace proposed. On the basis of this paper, Lyell urged Darwin to cease hesitating to publish his own ideas on the evolution of species.

Wallace contracted malaria while on the Malay island of Gilolo. While laid up, he formulated his principle of natural selection. In contrast to Darwin, who took more than two decades to put his theory on paper, Wallace wasted no time in writing out his ideas. Oblivious to the fact that Darwin was working on the exact same concepts, Wallace sent a draft of his paper, "On the Tendency of Varieties to Depart Indefinitely From the Original Type," dated February 1858, to Darwin, whom he respected, seeking his approval. Darwin was amazed at the striking resemblance of Wallace's formulation on the evolution of species to his own. He felt as if he were reading an abstract of his own theory. Lyell and another friend of Darwin, botanist Sir Joseph Hooker, suggested that this paper, often referred to as "the Ternate essay" since Ternate served as Wallace's home base for this leg of his trip, be read to the Linnean Society in conjunction with one unpublished essay written by Darwin on the same topic in 1844 and a letter, dated September 1857, from Darwin to the American botanist Asa Gray. All three of these writings focused on the process of natural selection and divergence rather than on a defense of the more encompassing process of evolution. The Ternate essay was read on July 1, 1858, and published in volume three of the Society's *Proceedings* in August. Wallace was unaware his paper was read publicly until after the fact. Both men end up sharing credit for the theory of evolution by natural selection, but because Darwin had established priority and because he wrote the masterpiece, *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* (1859), he is better known. No complaints from Wallace regarding his second-rate stature in the development of the theory of natural selection have ever been recorded. In fact, in his autobiography, Wallace wrote that Darwin and Hooker had given him more honor and credit than he deserved.

Wallace published several more original articles on the geographical distribution of animals and on the topic of evolution by natural selection. He returned to England in 1864, and in 1869 he published a two-volume narrative, *The Malay Archipelago; The Land of the Orang-utan and the Bird of Paradise; A Narrative of Travel With Studies of Man and Nature,* with the following dedication to Charles Darwin: "as a token of personal esteem and friendship but also to express my deep admiration for his genius and his works." Wallace also published a collection of his own essays regarding evolution—*Contributions to the Theory of Natural Selection. A Series of Essays,* published in 1870. During the 1880s he presented several lectures on the subject, including several in the United States in 1886–87.

One recurring phenomenon that Wallace used in support of evolution was Batesian mimicry, named after his colleague who discovered it. Organisms exhibit mimicry when they outwardly resemble

another species in a manner that protects against predators by a bad taste or smell. Predators avoid species that display characteristics of species with these characteristics, thus mimicry confers some protection to the species in their struggle for survival.

EVOLUTIONARY BIOGEOGRAPHY

Another monumental two-volume treatise written by Wallace, *The Geographical Distribution of Animals; With a Study of the Relations of Living and Extinct Fauna as Elucidating the Past Changes of the Earth's Surface* appeared in 1876. Some consider this to be the most influential work on the subject of zoogeography. In it, Wallace summarized the existing information on the distribution of land animals, particularly of terrestrial vertebrates, past and present, and he related it to geological and paleontological data. For example, he noticed that species on different sides of a river were similar but not identical; thus, the river served as a geological barrier for reproduction between organisms on either side. Before either of his scientific expeditions, Wallace had already accepted the notion of evolution; thus, his observations on geographical distribution and variation served to confirm his belief.

Following the publication of *The Geographical Distribution of Animals,* more scientists relied on biogeographical data to support evolution. Naturalists also started paying more attention to recording more precise locations from which specimens were collected. Wallace later extended his biogeography research to include island flora and other fauna such as insects. The seminal book *Island Life: Or, The Phenomena and Causes of Insular Fauna and Floras, Including a Revision and Attempted Solution of the Problem of Geological Climates* appeared in 1880. The interaction between living and nonliving components of an organism's environment constituted a major theme in Wallace's biogeographical research. In particular he felt the ice ages played a significant role in the history of species.

In 1863 Wallace proposed an unseen boundary dividing the Indo-Malayan and Australian zoological regions based on his studies of bird populations. In recognition for his pioneering contributions to the field of evolutionary biogeography, this imaginary line now bears his name. Modern geophysicists have confirmed that the Wallace Line corresponds with the boundaries of tectonic plates.

Encouraged by his publishers, Wallace wrote the autobiographical *My Life: A Record of Events and Opinions* in 1905. *The World of Life; A Manifestation of Creative Power, Directive Mind and Ultimate Purpose* (1910) was Wallace's last great scientific work. The book summarizes five decades worth of his studies on Darwinian evolution. He began the book by discussing the biogeography of plants and animals, and then he introduced relevant topics of geology. The text outlined natural selection, mentioned examples of coevolution, and proceeded to a description of the role of recognition marks, such as insect-coloration, in evolution. The last portion of the book examined the nature and cause of life and claimed man to be the crowning product of evolution.

In 1866 Wallace married Annie Mitten, with whom he had three children: Herbert (who died at age four), Violet, and William.

The Royal Society of London elected Wallace to membership in 1893. He received many awards and honors for his work: the Royal Medal of the Royal Society (1868), the Gold Medal of the Geographical Society (1870), the Darwin Medal of the Royal Society (1890), the Founder's Medal of the Royal Geographical Society (1892), the Gold Medal of the Linnean Society of London (1892), the Copley Medal of the Royal Society (1908), the Order of Merit (1908), the first Darwin-Wallace Medal of the Linnean Society of London (1908), and honorary degrees from Dublin (1882) and Oxford (1889).

Wallace died in his sleep on November 7, 1913, and he was buried in Broadstone, Dorset, England. A memorial plaque in his honor was unveiled at Westminster Abbey in 1915. Wallace left behind a legacy that comprised at least 764 publications, including 21 books. His interests spanned many fields—certainly evolution and biogeography, but also sociopolitics, railway systems, vaccinations, the possibility of life on Mars, and Shakespeare. He published many written works on the evolution of man and his moral and intellectual capabilities, a topic that later forced him to modify his earlier views on the origin of species. He thought that natural selection and evolution could not explain these unique characteristics, traded his agnosticism for spiritualism, and introduced the existence of a supreme deity into the equation. Wallace also contributed to the history of science by urging governments and scientific institutions to establish decent natural history collections for scientific research and analysis. For his numerous contributions, this intelligent and modest interdisciplinary scholar deserves more than a footnote in the history of life science.

See also BIOGEOGRAPHY; DARWIN, CHARLES; EVOLUTION, THEORY OF.

FURTHER READING

Berry, Andrew, ed. *Infinite Tropics: An Alfred Russel Wallace Anthology*. New York: Verso, 2002.

Camerini, Jane R., ed. *The Alfred Russel Wallace Reader: A Selection of Writings from the Field*. Baltimore, Md.: The Johns Hopkins University Press, 2002.

Fichman, Martin. *An Elusive Victorian: The Evolution of Alfred Russel Wallace*. Chicago: University of Chicago Press, 2004.

Raby, Peter. *Alfred Russel Wallace: A Life*. Princeton, N.J.: Princeton University Press, 2001.

Smith, Charles H. The Alfred Russel Wallace Page. Available online. URL: http://www.wku.edu/~smithch/home.htm. Last modified October 18, 2008.

Wallace, Alfred Russel. *Contributions to the Theory of Natural Selection. A Series of Essays*. 2nd ed. London: Macmillan, 1871.

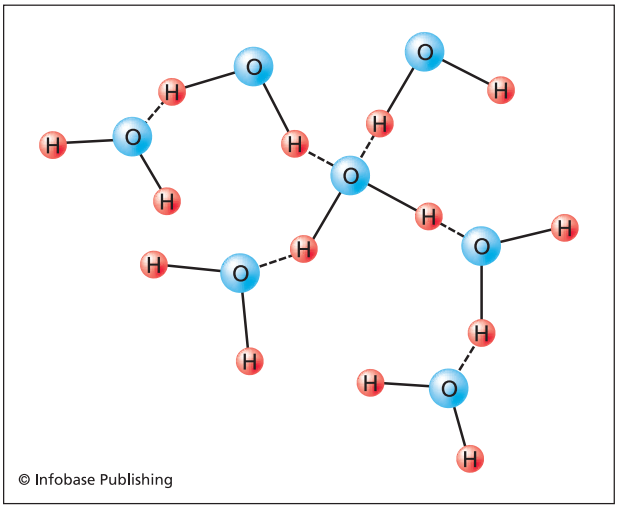
———. *My Life: A Record of Events and Opinions*. 2 vols. London: Chapman & Hall, 1905.

———. *The Geographical Distribution of Animals; With a Study of the Relations of Living and Extinct Faunas as Elucidating the Past Changes of the Earth's Surface*. 2 vols. London: Macmillan, 1876.

water, its biological importance Water is the most abundant component of cells, living organisms, and the planet Earth. More than 71 percent of Earth is covered by water, and life on this planet is possible only because water can exist on it as a liquid. Water makes up 75 to 85 percent of the weight of a cell, and most cells or organisms either live in aquatic environments or are bathed in water-based bodily fluids. Organisms that do not reside in aquatic habitats have unique mechanisms for obtaining and transporting water into their tissues or have adapted special means to survive in conditions of low water availability. The importance of water to living systems is due to its polarity, which is related to its cohesiveness, high heat capacity, characteristic of expanding when frozen, and versatility as a solvent.

POLARITY OF WATER

A molecule of water consists of two hydrogen atoms covalently linked to one oxygen atom. The bond angle is 109.5°, thus the molecule is triangular rather than linear. Oxygen is very electronegative, meaning it has a great tendency to attract electrons. Because of this, the electrons associated with the hydrogen atoms spend more time closer to the oxygen atom than to the hydrogen nuclei. This uneven distribution of electrons confers a partially negative charge on the oxygen end of the molecule and a partially positive charge on the side containing the hydrogen atoms. The separation of charges gives water its polarity, or the difference in the properties at opposite ends. Electrical interactions (when a positive atom from one molecule interacts with a negative atom from another molecule) result, giving water many properties relevant and necessary to life.



The polarity of water is due to the great electronegativity of oxygen, conferring a partially negative charge on the oxygen atom and partially positive charges on the hydrogen atoms. These opposite charges then attract one another, forming extensive hydrogen bonds (dashed lines) between water molecules.

COHESIVENESS OF WATER

The polarity of water allows extensive hydrogen bonds to form between atoms of different water molecules. An oxygen atom will be attracted to hydrogen atoms of other molecules, creating hydrogen bonds that form a three-dimensional network of molecules. Hydrogen bonds are constantly forming and breaking between water molecules. The ability to form so many hydrogen bonds gives water cohesiveness, the property of exhibiting a molecular attraction by which the particles of a body are united throughout the mass. More simply, the molecules of water stick together tightly, explaining why a droplet of water can hang suspended from an object until its weight becomes too great and gravity pulls it downward. Capillary action, the force that acts to pull water up from the roots of a plant, also depends on cohesiveness. One molecule of water attracts another due to hydrogen bonding, effectively pulling others along with it up through the vessels. Adhesion, the attachment or joining of different substances, holds the molecules of water to the sides of the vessels, and cohesion moves them upward.

Cohesiveness also gives water a high surface tension, a characteristic that allows some bugs to walk over the top of water without their legs penetrating through the surface or that prevents a slightly overfilled glass of water from spilling over the rim. Because water has a high surface tension, organisms must produce compounds called surfactants to lower that surface tension to ease certain physiologi-

Owing to its cohesiveness, water has a high surface tension, allowing this water strider to walk on the surface. *(Hermann Eisenbeiss/Photo Researchers, Inc.)*

cal functions. For example, a surfactant produced by epithelial cells of the alveolar walls lowers the surface tension to allow the opening of alveolar spaces in the lungs to promote gas exchange during respiration.

HEAT CAPACITY OF WATER

Another chemical property of water that makes it so crucial to life is its ability to stabilize temperature. Water has a high heat capacity, meaning that a lot of energy is required to raise the temperature of an aqueous solution one degree Celsius. Like cohesiveness, this property also is related to the extensive hydrogen bonding found in water. Much of the heat absorbed by water breaks hydrogen bonds rather than simply increases molecular motion. A high heat capacity means that large bodies of water such as lakes and oceans warm and cool very gradually. During the daytime, a lake can absorb enormous amounts of heat energy from the sun, yet its temperature rises only a few degrees. Then, during the nighttime, as the lake slowly cools, it releases heat that warms the surrounding air. Because significant quantities of heat can be absorbed with minimal effect on the temperature of the water, bodies of water act to moderate the temperatures of the surrounding areas, giving coastal areas milder climates.

In addition to moderating the Earth's climate, the high heat capacity of water stabilizes the temperature of individual cells and organisms. Metabolic processes that occur within a cell release heat energy, but the aqueous environment prevents the cell from becoming too hot and the proteins from becoming denatured. Sweating is a physiological mechanism that reduces body temperature. As sweat evaporates from the skin, it changes from the liquid to the gaseous state, a physical change that requires the input of energy in the form of heat. Heat generated from the body's metabolic processes vaporizes the water in the sweat, cooling the body in the process.

EXPANDS WHEN FROZEN

Another unique quality of water is the fact that as it freezes, it becomes less dense. Most substances are more dense as solids, but again, the prevalent hydrogen bonds come into play. In the liquid state, water expands as it warms and contracts as it cools, as do most substances. In the solid state, however, each water molecule stably participates in hydrogen bonds with four other water molecules. The result is a three-dimensional ice crystal that has channels or spaces in between molecules, making ice less dense than water. As an ice crystal absorbs heat and its temperature surpasses 0°Celsius, the hydrogen bonds are broken, and the structure collapses.

The low density of ice has great ecological significance. Because ice is less dense or lighter than water, it floats. If it sank, then all the lakes, ponds, and oceans would freeze solid from the bottom up, and only a few inches at the top would thaw during the summers. A layer of frozen ice on the surface of a body of water, however, insulates the water below, allowing life to persist there even during the winter months.

VERSATILE SOLVENT

A solvent is a fluid that dissolves other substances called solutes. Because water is chemically inert and polar, most biomolecules can easily dissolve in it. Solutes that are ionic or polar easily dissolve in water, and are therefore termed hydrophilic (water-loving). Most of the organic solutes within a cell are hydrophilic due to the presence of polar functional groups such as hydroxyl groups (as found in carbohydrates), carboxyl groups (as in fatty acids), or amino groups (found in amino acids). Ionic compounds easily dissolve because the partial negative charge of the water's oxygen atoms induces interaction with the cations (the positively charged ion) and the partial positive charge of the water's hydrogen atoms allows interaction with the anions (the negatively charged ion). These ionic interactions lead to the formation of a sphere of hydration around the ions, preventing them from reassociating and forming an ionic bond with the oppositely charged ion. Many biological molecules have side chains that are ionized at a neutral pH of the cell, thus the same principles apply, and the biological molecules dissolve easily. Biochemical reactions occur in aqueous environments.

Hydrophobic interactions, influences that cause nonpolar substances to cluster together, are biologically relevant in the formation of membranes and in the folding of proteins. Meaning "water-fearing,"

hydrophobic interactions are not really an attraction between nonpolar substances, but are due to the common exclusion by water molecules. The polar water molecules form hydrogen bonds at the surface of the hydrophobic substance, excluding any molecules that might disrupt the hydrogen bonding, causing the hydrophobic substances to group or stick together. Proteins, which generally have hydrophobic interiors, dissolve in the aqueous environment of the cell due to the hydrophilic regions on their surface. Cell membranes have significant hydrophobic regions that not only act in selective transport, but also prevent the membranes from dissolving. Cellulose, the carbohydrate used to construct cell walls of plants, is an example of a hydrophilic substance that absorbs water, but does not dissolve in it.

PH

Hydrogen atoms from one water molecule occasionally leave that molecule and become part of another. An electron is left behind, associated with the oxygen atom and the remaining hydrogen atom to form an ion called a hydroxide ion (OH^-) that has a charge of −1. Only a hydrogen ion (H^+), a positively charged single proton, actually transferred to the other water molecule, making a hydronium ion (H_3O^+). This dissociation event is reversible and rare and, in equilibrium, occurs at the same rate as the reassociation event. At any time, only a single molecule of water in 554 million is dissociated, making the concentration of H^+ ions and OH^- ions in pure water 10^{-7} molar (M). (Molarity is a unit of measure for concentration and indicates the number of moles of solute per liter).

The addition of acids or bases to an aqueous solution upsets the balance of H^+ and OH^- ions. In most circumstances, an acid is a substance that increases the concentration of H^+ ions to a solution. For example, in water hydrochloric acid dissociates as follows:

$$HCl \rightarrow H^+ + Cl^-$$

A base is a substance that decreases the concentration of H^+ ions, usually by the contribution of OH^- ions to the solution. Sodium hydroxide, a base, also dissociates in water, but then can absorb the excess H^+ ions present by forming a molecule of water in a neutralization reaction:

$$NaOH \rightarrow Na^+ + OH^-$$

$$H^+ + OH^- \rightleftharpoons H_2O$$

Some bases accept the excess hydrogen ions rather than contribute OH^- ions, but, in both cases, the hydrogen ion concentration is reduced.

$$NH_3 + H^+ \rightleftharpoons NH_4^+$$

The pH of a solution is the negative log of the H^+ concentration.

$$pH = -\log[H^+]$$

Scientists measure pH as an indicator of the acidity of a solution. The more acidic a solution is the higher the H^+ concentration, the lower the pH. The pH scale ranges from zero to 14 and the product of the H^+ and the OH^- concentrations is always 10^{14}. A solution with a pH of 7 is considered neutral. A pH lower than 7 is acidic, and a pH higher than 7 is basic. The cytoplasm of most cells and of the extracellular fluids of tissues is neutral. Environments that are too acidic or too basic can denature proteins or inhibit biochemical reactions. Substances called buffers maintain an optimal pH by preventing large fluctuations in pH. Buffers have the ability to accept H^+ ions from a solution or to donate them, depending on whether the concentration is too high or depleted.

One example of a biological buffering system is the carbonic acid-bicarbonate system in blood. Carbonic acid (H_2CO_3) can release an H^+ ion to yield a bicarbonate ion (HCO_3^-). If the pH of the blood rises, or becomes more basic, then more carbonic acid dissociates to release H^+ into the blood and raise the pH. If the opposite occurs and the pH becomes too acidic, then bicarbonate accepts excess H^+ ions to form carbonic acid.

See also BIOMOLECULES; CHEMICAL BASIS OF LIFE; EUKARYOTIC CELLS; PROKARYOTIC CELLS.

FURTHER READING

Chang, Raymond. *Physical Chemistry for the Biosciences*. Sausalito, Calif.: University Science Books, 2005.

Murray, Robert K., Daryl K. Granner, Peter A. Mayes, and Victor W. Rodwell. *Harper's Illustrated Biochemistry*. 26th ed. New York: McGraw Hill, 2003.

Myers, Richard. *The Basics of Chemistry*. Westport, Conn.: Greenwood Press, 2003.

water and sewage treatment Humans depend on water for a variety of uses: drinking and food preparation, household uses such as showering and laundry, recreational purposes such as boating and swimming, and for industrial purposes. Wastewater refers to water from domestic or industrial uses that cannot be directly released into lakes or streams. Sewage specifically refers to wastewater that contains fecal material. Wastewaters often contain chemicals or microorganisms that threaten public health or cause environmental damage. Depending

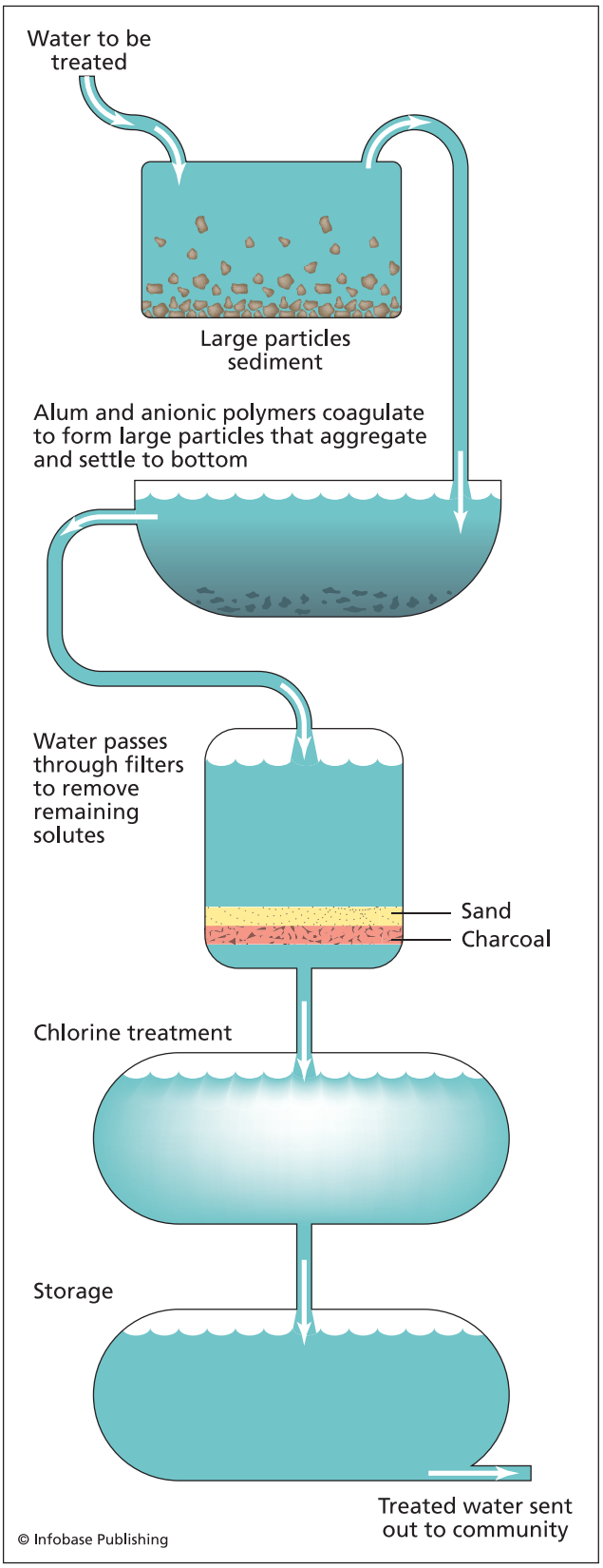
on the industrial facility, the Environmental Protection Agency may require pretreatment of wastewater to remove toxic chemicals or large debris that could clog treatment facilities. Publicly owned treatment facilities treat wastewaters and sewage to remove the dangerous pollutants before returning the water to the environment.

Contaminated water is the most common source of infectious diseases worldwide. Because human and animal excrement contains microorganisms shed from the digestive tracts, improperly treated wastewater can spread the pathogens quickly. Bacteria, viruses, and parasites, such as *Salmonella* (causes typhoid fever), *Vibrio* (causes cholera), hepatitis A virus, *Giardia,* and *Cryptosporidium,* can all be transmitted through a water source contaminated with feces or urine.

WATER PURIFICATION

Most drinking water comes from rivers, aquifers, or springs. As water penetrates into the ground, it undergoes a natural filtering process that removes most microbes, so sources such as deep wells or springs that are far removed from populated areas may be used directly as a source of drinking water. Water sources that supply municipalities are often contaminated by substances released into the environment by activities of that population. In particular, pathogens transmitted by the fecal-oral route may enter the water supply through feces. In rural areas, runoff often contains pesticides or excess nitrates from fertilizers, both of which are dangerous to humans; thus, water purification is necessary to ensure its safety before being sent to consumers. Water that is safe for human consumption is referred to as potable water.

Modern municipal water treatment plants begin the process by collecting water in a catch basin that both stores water and allows for the sedimentation of larger particulates such as soil and mineral particles. The addition of copper sulfate inhibits the growth of algae and cyanobacteria, which would only increase the amount of organic matter in the water. Other substances such as aluminum sulfate (alum) and chlorine may also be added at this step. The chlorine acts as a disinfectant, and the alum helps smaller suspended solids aggregate into larger masses so they can trap microorganisms and settle out. The water is pumped to another area where it settles further. Passage through sand or diatomaceous earth removes microorganisms, and filtration through activated charcoal removes potentially hazardous organic compounds. An ion filtration step may also be employed to remove compounds based on their charge. Chlorine gas chemically disinfects the filtered water, which is stored in tanks until sup-



Before water is distributed to the community, it is purified by a series of mostly physical and chemical processes, including sedimentation, coagulation, filtration, and chlorination.

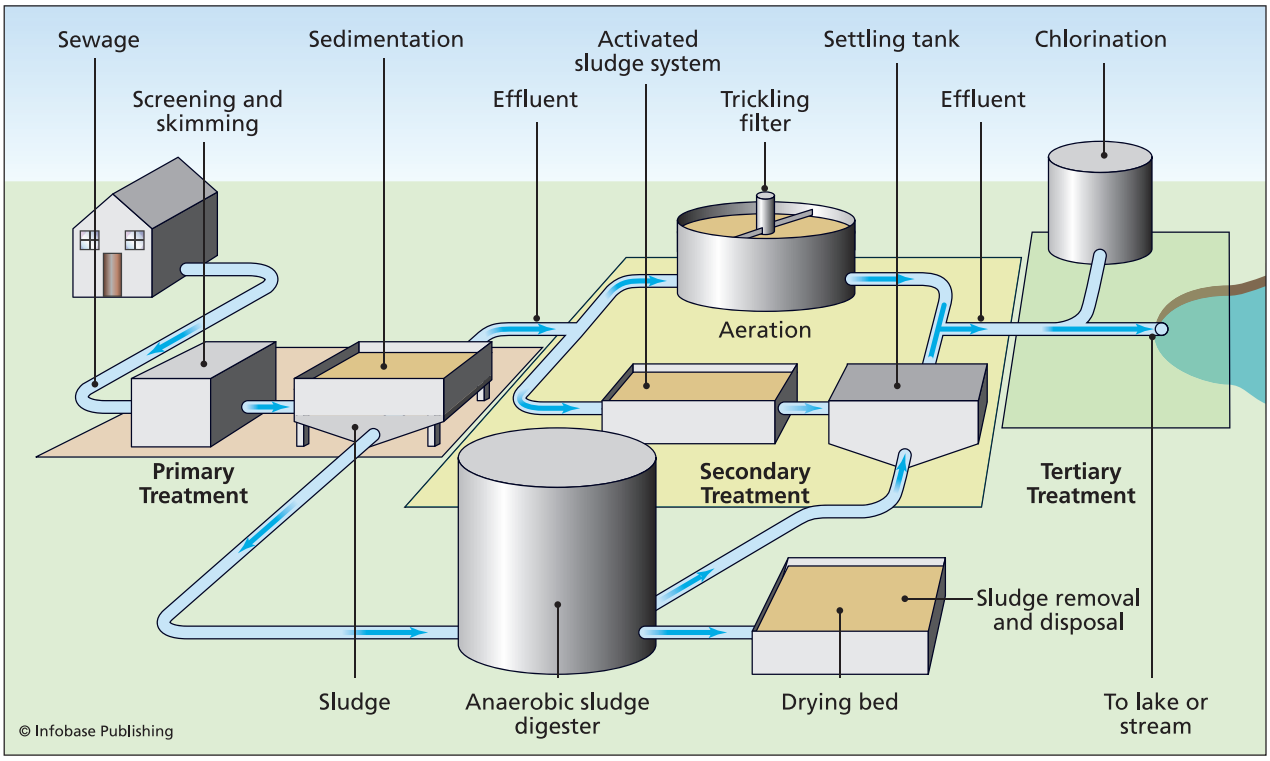
plied to the consumers. The chlorine also neutralizes any remaining organic compounds to improve the taste and smell of the water.

WASTEWATER TREATMENT PROCESS

Historically, filtration was the first water purification technique widely used. Its goal was to reduce turbidity, or to clarify the water. Removing suspended material from the water also removed much of the microbial contamination, but many pathogens persisted. In the early 1900s chlorine treatment became standard, further reducing the levels of viable microbes in the water supply. Modern wastewater treatments include a variety of physical, chemical, and biological mechanisms for purifying water. The illustration below depicts a typical wastewater and sewage treatment and purification procedure carried out by a modern facility.

The goal of treatment is to remove dangerous chemicals and compounds so that the water no longer contains toxic substances and cannot support the growth of microorganisms. The first level of treatment, called primary wastewater treatment, uses only physical methods to separate solids and particles from the wastewater. A series of grates or screens removes the floating and the largest objects. The wastewater that makes it through the screening is called the effluent, and it is allowed to sit for several hours, during which time particulate matter that was too small to be removed initially settles to the bottom of a large tank or reservoir, forming sludge.

Pumps send the effluent to a secondary treatment facility, where both aerobic and anoxic treatments further reduce the levels of organic nutrients. Secondary treatment is mostly biological. A community of diverse microorganisms carries out aerobic digestion of much of the organic material. One common method for aerobic digestion is the trickling filter method, which involves spraying the wastewater over a bed of crushed rocks. A biofilm of various microbes forms on the surface of the tiny rocks, and the communities of microbes with the biofilm mineralize the organic matter into carbon dioxide, ammonia, nitrate, sulfate, and phosphate, components that are useful in fertilizers. In the activated sludge method, the wastewater is mixed in a large tank to stir in oxygen. This aerated effluent is allowed to settle in a large holding tank, where some aerobic metabolism occurs, removing most of the soluble organic material in the liquid effluent. Some of the flocculent material that settles to the bottom may be returned to the aerator, where it is mixed into the effluent as an inoculum. For industrial wastewater containing lots of insoluble organic material such as cellulose or fiber, anaerobic sludge digesters, or bioreactors, are used to help digest the remainder of the



Wastewater and sewage undergo a series of primary (mostly physical), secondary (mostly biological), and tertiary (mostly chemical) treatments before being released into the environment.

Sewage and wastewater undergo extensive treatment before being discharged back into the environment. The round tanks in the lower left of this aerial view are settling tanks. The rectangular tanks in the right center portion of the photo are aeration tanks, into which air is pumped to encourage aerobic metabolism of the organic pollutants. The round and rectangular tanks near the top are used for further settling and filtration. The aboveground cylindrical tanks in the upper left are gas tanks in which anaerobic microorganisms digest the remaining sludge into methane that is burned to produce electricity. *(Chris Knapton/Photo Researchers, Inc.)*

flocculent material. Anaerobic sludge digestors also break down the sludge resulting from the primary treatment. The chambers remain sealed, and other microorganisms anoxically decompose the remaining material into products including methane gas, carbon dioxide, hydrogen gas, and other volatile compounds. The mixture of these gases is called swamp gas, due to its resemblance of the gases produced by microorganisms that reside in and anerobically digest organic matter in swamps. Some facilities harvest this gas and utilize it as a source of fuel for the sewage processing plant.

The treated water may undergo tertiary treatment, which may include physical, chemical, or biological means to further reduce inorganic nutrient levels that might encourage the growth of micro-

organisms in the water supply. This typically also involves chlorination to disinfect the effluent, though some wastewater treatment plants in the United States use ultraviolet radiation or ozone (O_3) to kill viable bacteria or viruses. Due to the prohibitive expense of tertiary treatment, its use is not widespread.

After treatment, the wastewater plants discharge the treated water into lakes or streams in the environment.

MONITORING FOR CONTAMINATION

Monitoring is an important step in the process of water treatment. Just because water looks clear does not mean it is not contaminated. In order to be sure that water from a certain source is safe, one must assay samples for the presence of microorganisms. Coliform bacteria serve as indicator organisms for such assays because bacteria in this group commonly inhabit the intestines of animals. Their presence indicates probable fecal contamination and makes the water unsafe for human consumption. Because many pathogens are shed from the intestines in feces, if coliforms are present, intestinal pathogens might be also. Treatment that destroys coliforms will also destroy most pathogenic bacteria. Coliform bacteria are facultatively aerobic, gram negative, nonspore-forming, rod-shaped, lactose-fermenting, gas-producing bacteria. Assays include methods or media that test for these characteristics.

Two common tests for coliforms include the most-probable number (MPN) and membrane filter techniques. The MPN test involves broth cultures in test tubes to which different volumes of inoculum are added. This test allows for estimation of the number of bacteria in a sample based on statistical analysis and is especially useful when the microorganisms being counted do not grow on solid medium or when liquid media reveals a metabolic activity useful in the identification of the microbes. In the membrane filter test, a sample of liquid (100 ml or more) is passed through a sterile filter that contains pores too small to allow the passage of bacteria, thus the bacteria remain trapped by the filter. Placement of the filter on an agar plate of a special medium called eosin-methylene blue (EMB agar) transfers the trapped bacteria onto the agar. EMB inhibits the growth of noncoliform bacteria and allows one to distinguish between bacteria that do and do not ferment lactose. The number of lactose-fermenting colonies present after incubation allows for the determination of the concentration of coliforms in the original sample.

In the United States, the Safe Drinking Water Act, originally passed in 1974 with amendments adopted in 1986 and 1996, authorizes the U.S. Environmental Protection Agency to set the national standards for safe tap water with respect to chemical and biological pollutants. Permissable levels differ based on the type and size of the water system. Samples are considered coliform positive if they contain between one and five coliforms within a 100-ml sample. The water utility must notify the public and take steps to resolve the problem when a certain number of samples give positive results for a certain number of tests. The Safe Drinking Water Act also includes preventative measures.

Another way to monitor the effectiveness of water treatment is by measuring the biological oxygen demand (BOD), an indicator of the relative amount of degradable organic material present in a water sample. The more organic material that is present, the more oxygen required for microorganism to oxidize it. Primary treatment typically removes about 25–35 percent of the BOD of sewage, and more efficient secondary treatment removes about 75–95 percent of the BOD from sewage.

In developed countries, the incidence of waterborne diseases is very low, demonstrating the effectiveness of rigid quality standards and water treatment procedures. In underdeveloped countries, however, the water and sewage treatment facilities are often inadequate. The same water body may serve multiple purposes in addition to use as a source of drinking water, including washing clothes, bathing, and dumping wastes. Because of this, waterborne diseases such as cholera and typhoid fever are worldwide public health concerns.

See also GERM THEORY OF DISEASE; INFECTIOUS DISEASES; MICROBIOLOGY.

FURTHER READING

Centers for Disease Control and Prevention. U.S. Department of Health and Human Services. Water-Related Environmental Public Health. Available online. URL: http://www.cdc.gov/nceh/ehhe/water/. Accessed January 25, 2008.

Madigan, Michael T., and John M. Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, N.J.: Pearson/Prentice Hall, 2006.

Watson, James D. (1928–) American *Molecular Biologist* James D. Watson received the Nobel Prize in physiology or medicine in 1962, shared with Maurice Wilkins and Francis Crick, for the elucidation of the double helical structure of DNA. The monumental discovery, made in 1953, immediately suggested a mechanism for self-replication of the genetic material and, eventually, a means of encoding for the synthesis of proteins. This accomplishment gave rise to the molecular biological revolution.

TRAINED IN ZOOLOGY

James Dewey Watson was born on April 6, 1928, in Chicago, Illinois, and graduated from the public schools when he was only 15 years old. After earning a scholarship, he entered an experimental four-year college at the University of Chicago in the summer of 1943. As an undergraduate, bird watching occupied much of his time. After receiving a bachelor of science degree in zoology in 1947, Watson enrolled in the graduate program at Indiana University in Bloomington, where his thesis research centered on the X-ray inactivation of bacteriophage (a virus that infects bacteria). He obtained a doctorate in zoology in 1950, then moved to Copenhagen to continue his studies on viral nucleic acid as a Merck postdoctoral fellow of the National Research Council for one year under the supervision of the biochemist Herman Kalckar and the microbiologist Ole Maaløe. In the fall of 1951, Watson moved to the University of Cambridge, in England, where he joined the Cavendish Laboratory. Watson went to Cambridge to assist the English chemist John Kendrew with X-ray diffraction studies on the oxygen-carrying muscle protein myoglobin, but once he arrived and met Francis Crick, he became sidetracked. Though they worked in a lab filled with protein chemists, Watson and Crick were both fascinated by DNA.

SOLVES STRUCTURE OF DNA

At the time, the most challenging problem in biology was how the genetic material was replicated and how it coded for the synthesis of proteins. In February 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty, researchers from the Rockefeller Institute Hospital, published biological evidence demonstrating that DNA was the genetic material, a shocking discovery since DNA consisted of only four different subunits. Each nucleotide building block contained the same sugar portion called deoxyribose, a negatively charged phosphate group, and one of four different nitrogenous bases. Two of the bases, adenine and guanine, were purines and contained two fused ringed structures made of mostly carbon and nitrogen. The other two, cytosine and thymine, were pyrimidines and contained a single ring. Biologists had trouble understanding how such a seemingly simple molecule could perform the complex tasks of self-replication and encoding all of a cell's genetic information. Solving the structure of DNA would facilitate the elucidation of these complicated molecular processes.

Earlier in 1951, while attending a symposium lecture at the Zoological Station in Naples given by Maurice Wilkins, a biophysicist from King's College in London, Watson had the chance to observe X-ray diffraction photographs of crystalline DNA. X-ray crystallography is a technique often used by biochemists to gather information on the structure of molecules. Diffraction is the bending of waves as they pass by an obstacle, in this case, atoms of the molecules of DNA. Because atoms are much smaller than the wavelengths of visible light, they cannot diffract the light rays or be seen, even with microscopes. The wavelengths of X-rays are even smaller, and thus can be used to "see" atoms and molecules. The substance to be x-rayed must first be crystallized, a procedure in which the molecules become highly ordered, with regular spacing occurring between atoms in the arranged molecules. When bombarded with X-rays, the crystallized molecules diffract the waves, which pass through the created spaces as through a grating, forming a unique pattern of blurry circles on photographic film. Wilkins's X-ray diffraction photographs excited Watson, because the fact that DNA could be crystallized meant that the structure was a regular, organized arrangement and was therefore solvable. Distinguished scientists from many different laboratories were working hard to determine the structure of DNA. Wilkins and Rosalind Franklin, a skilled crystallographer who joined Wilkins's lab to assist him with X-ray diffraction studies of DNA, were making some progress, as was the American biochemist Linus Pauling from the California Institute of Technology, who had recently described the alpha-helix, a common structure found in proteins. Some scientists, including Watson, believed that DNA might be helical.

Crick was working on his Ph.D. thesis on the structure of hemoglobin under the supervision of Max Perutz, but, like Watson, he thought DNA was as interesting as proteins. The two spent much time discussing their opinions on scientific matters. Watson served as a good sounding board for Crick's frequent flashes of brilliant but short-lived ideas, and Crick helped Watson understand crystallographic theory. Wilkins had previously told Crick that the diameter of DNA indicated it was a compound molecule, consisting of more than one chain, likely twisted about one another. Watson and Crick spent endless hours trying to figure out how the chains were held together. Though a collaboration between Wilkins and Franklin at King's College and Watson and Crick at Cambridge might have been productive, Franklin was not willing to work together or to share her X-ray diffraction data. She even hesitated to share it with Wilkins, with whom she did not get along. Wilkins invited Watson to come hear Franklin present her recent findings at an upcoming lab meeting in November 1951. In preparation, Watson taught himself as much about X-ray diffraction techniques as possible, and he enjoyed her presentation despite his limited knowledge. He annoyed Crick, however,

by not taking lecture notes and not clearly remembering the details of her research—in particular, the water content of the DNA samples she had photographed. What he did remember, however, seemed to justify the possibility that DNA was helical.

Pauling's physical model-building approach to solving molecular structures impressed Watson. Upon returning to Cambridge, he had new phosphate group models built to help him figure out the exact structure. Watson and Crick constructed one arrangement that they believed was the answer. The model consisted of a triple helix held together by magnesium ions linking the phosphate groups of the nucleotides. The sugar-phosphate backbone ran down the center and the nitrogenous bases pointed outward. Excitedly, they invited Wilkins and Franklin to come see their model, but when they arrived, it became apparent that Watson had incorrectly remembered or misunderstood Franklin's data. Their model had at least tenfold less water than it should have. Franklin chided them for their mistake, and Sir Lawrence Bragg, head of the Cavendish Laboratory, directed Watson and Crick to focus their efforts on their own formal scientific investigations. Bragg's order did not accomplish the desired effect. The pair continued to pursue the structure, though they did so less openly.

During the next year, Watson made progress toward solving the structure of tobacco mosaic virus (a virus that infects tobacco plants). Ironically, he believed the protein subunits formed a helical arrangement, a theme he seemingly could not escape. He also improved his understanding of chemistry by studying Pauling's book *The Nature of the Chemical Bond*. He decided that the sugar-phosphate backbone could not possibly lie in the center of the helical DNA, as the situation would force atoms closer together than the laws of chemistry allowed. Coincidentally, Pauling's son Peter joined the Cavendish Laboratory that summer to work on his Ph.D. under the supervision of Kendrew.

In 1949 the Austrian-American biochemist Erwin Chargaff determined that, within a species, the percent composition of adenine equaled the percent composition of thymine and the percent composition of cytosine equaled the percent composition of guanine. Chargaff explained his crucial findings, now referred to as Chargaff's rules, to Watson and Crick at a meeting in 1952. This biochemical information suggested that the nucleotide bases must exist in regular pairs and was a key factor in their ultimate successful development of a real model.

Meanwhile, in the United States, Pauling, who was on track to win a Nobel Prize in chemistry (1954) for his research on the nature of chemical bonds, struggled fervently to solve the structure of DNA. All thought the race was over in February 1953, when Pauling forwarded a preprint of a manuscript soon to be published in *Proceedings of the National Academy of Sciences* to his son Peter. His paper claimed that DNA consisted of a triple helix with a sugar-phosphate backbone running down its center. The model completely ignored the acidic nature of DNA and was reminiscent of the model that brought Watson and Crick ridicule 15 months prior. After realizing how close they were, and not wanting Pauling to determine the correct structure before his own lab, Bragg gave Watson and Crick permission to work full-time on solving the structure of DNA.

Once again, Watson rushed to King's College to inform Wilkins and Franklin of Pauling's paper and to try to convince them to collaborate with him and Crick in order to beat Pauling, whom Watson believed would have recognized his error and would have been working to correct it. Franklin was not only uninterested, but also she disapproved of the model-building approach altogether and denied the existence of any evidence that DNA was helical. Undeterred, Watson returned to Cambridge, and, while impatiently awaiting new metal nucleotide and phosphate group models from the Cavendish machine shop, he used cardboard cutouts. Within two weeks, Watson and Crick developed an elegant three-dimensional model for the molecular structure of DNA that both incorporated Chargaff's base-pairing rules and followed the rules for the formation of chemical bonds. After Wilkins came to inspect the new model, he confirmed that it agreed with his and Franklin's X-ray diffraction data. After agreeing to report their research simultaneously, Watson and Crick published "A Structure for Deoxyribose Nucleic Acid," their seminal paper describing a simple yet attractive model for double helical DNA in the British journal *Nature* in April 1953. Wilkins and Franklin published their supporting physical evidence in two separate papers that appeared in the same journal issue.

Watson and Crick's model consisted of a double-stranded arrangement with the sugar-phosphate backbones of the strands running in opposite directions, a characteristic termed anti-parallel. The bases faced inward, with adenine always pairing with thymine and cytosine always pairing with guanine. Hydrogen bonds held the nucleotides of the complementary strands together. By pairing the bases in this manner, the distance between the two strands would remain relatively constant along the length of the molecule. Finally, the two strands wound around one another, resembling a twisted ladder, with the paired bases as the rungs and the sugar-phosphate backbones forming the sides.

One month after the publication of their first landmark paper, Watson and Crick published

another classic paper, "Genetical Implications of the Structure of DNA," in which they speculated on the possible mechanism for replication of DNA.

In 1962 Watson, Crick, and Wilkins were awarded the Nobel Prize in physiology or medicine "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material." Though Franklin's experimental data was crucial to the structure's determination, she was ineligible for a nomination. Nobel recipients must be living at the time of the nomination, and tragically she had passed away in 1958.

LATER CAREER

Following his famous discovery of the double helical configuration for DNA, Watson switched his focus to ribonucleic acid (RNA) structure as a senior research fellow in biology at the California Institute of Technology. He returned to Cambridge in 1955 to collaborate with Crick on viral structure, and one year later he joined the faculty in the biology department at Harvard University, where he examined the role of RNA in protein synthesis until 1976. While a professor at Harvard, Watson authored the extremely successful *Molecular Biology of the Gene,* the first comprehensive molecular biology text, now in its sixth edition.

In 1968 Watson published *The Double Helix,* his personal account of the events leading up to the discovery of the molecular structure of DNA. The controversial narrative became a bestseller, but angered many scientists who believed the story portrayed their colleagues negatively. During that same year, Watson married Elizabeth Lewis, with whom he has two children, Rufus and Duncan.

Cold Spring Harbor Laboratory (CSHL) on Long Island appointed Watson director in 1968, and under his leadership the laboratory has become a world-class institution known especially for its research and educational programs in molecular biology. Watson served as president of CSHL from 1994 to 2003 and became chancellor one decade later. He retired as chancellor in October 2007 after causing controversy by making remarks about the intelligence of people of African descent. He later apologized.

Watson served as associate director, then director of the Human Genome Project for the National Institutes of Health from 1988 until 1992. This goal of this undertaking, to identify and sequence the 30,000 genes that comprise the entire human genome, was accomplished in 2003. Watson was involved with the scientific research in addition to advocating the confrontation of ethical issues it raised.

Watson has been outspoken in support of scientific research and is recognized as a brilliant scientist despite his reputation for challenging scientific doctrine and his provocative offhand remarks. He has received numerous awards to accompany his Nobel Prize, including the Albert Lasker Prize with Crick and Wilkins in 1960, the Presidential Medal of Freedom (1977) and the National Medal of Science (1997). He belongs to many premier academic organizations, including the American Academy of Arts and Sciences, the National Academy of Sciences, and the Royal Society of London.

See also Avery, Oswald; Chargaff, Erwin; Crick, Francis; deoxyribonucleic acid (DNA); Franklin, Rosalind; MacLeod, Colin Munro; McCarty, Maclyn; Pauling, Linus; Wilkins, Maurice H. F.; X-ray crystallography.

FURTHER READING

McElheny, Victor K. *Watson and DNA, Making a Scientific Revolution.* Cambridge, Mass.: Perseus, 2003.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1962." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1962/. Accessed January 16, 2008.

Watson, James D. *Avoid Boring People: Lessons from a Life in Science.* New York: Oxford University Press, 2007.

———. *The Double Helix.* New York: Touchstone, 2001.

———. *Genes, Girls, and Gamow.* New York: Oxford University Press, 2001.

Watson, J. D., and F. H. C. Crick. "A Structure for Deoxyribose Nucleic Acid." *Nature* 171 (1953): 737–738.

———. "Genetical Implications of the Structure of DNA." *Nature* 171 (1953): 964–967.

Whittaker, Robert (1920–1980) American *Ecologist* Robert Whittaker was one of the 20th century's most prominent plant community ecologists. He developed concepts and methods of gradient analysis for relating environmental variables such as temperature, light, and water availability to the number and distribution of plants in a community. A respected researcher, Whittaker established methods for documenting data regarding field composition, productivity, and species diversity in plant communities. His name is also associated with a taxonomic classification system that groups organisms into five kingdoms including Animalia, Plantae, Fungi, Protista, and Monera.

DISSERTATION SHIFTS PLANT COMMUNITY ECOLOGY PARADIGM

Robert Harding Whittaker was born in Wichita, Kansas, on December 27, 1920. He spent his childhood surrounded by the Kansas prairies and enjoyed collecting butterflies. He received his bachelor's degree in biology and languages from Washburn Munici-

pal College (now Washburn University) in Topeka, Kansas, in 1942. After graduation he joined the U.S. Army Air Force and was stationed in England where he worked as a weather observer and forecaster. The department of botany at the University of Illinois rejected his application for admission to graduate school due to an insufficient course background, but the department of zoology there accepted him in 1946.

In the 1940s, two competing schools of thought dominated plant community ecology: the discrete community hypothesis and the continuum community hypothesis. A community comprises all of the species inhabiting a given place at a given time. Frederic Clements proposed the community theory, stating that environmental gradients formed defined boundaries that separated communities of plant species and that species within a community coadapted as the community matured. The climax community was a predictable endpoint of associated, interacting species. Tight linkages existed between species, and all the species cooperated for the benefit of the entire community. The Clementsian viewpoint dominated plant community ecology into the 1960s. In 1926 Henry Gleason published the first of a series pf papers that challenged the idea that gradients formed boundaries between groups of associated species and suggested that species developed in a community based on their individual responses to the environment as a consequence of their own genetic makeup, physiology, and life cycle. The continuum theory supported by Gleason stated that the distribution of species along gradients resulted from individual tolerances to different environmental conditions. Any overlap of species resulted from similar responses to the environmental conditions, independent of any gradients. No empirical data supported or disproved either hypothesis, thus Whittaker set out to examine the validity of the opposing schools of thought for his dissertation research.

Whittaker performed a statistical analysis to examine the relationship between the distribution patterns of the different plant species and gradients of environmental factors in a region of the Great Smoky Mountains in Tennessee. His data showed significant variation in the distribution of plant species across environmental gradients. He found absolutely no evidence for segregated communities or distinct boundaries as Clements hypothesized. Though he planned to complete a manuscript on insect populations of the same area, the dissertation that earned him a doctoral degree in zoology in 1948 was strictly on plant ecology. Because his original dissertation was too lengthy and theoretical, his results were not published until 1956, but Walter Westman later referred to the paper as "probably the most important ecological paper of the present century" in his memoir of Whittaker. The paper brought about a new paradigm in plant community ecology, a shift from idealistic, strongly interacting, Clementsian communities to Gleason's individualistic groups of species that simply coexisted.

OTHER CONTRIBUTIONS

Whittaker accepted a position as an instructor in the department of zoology of Washington State College (now Washington University) in Pullman, Washington. For a while he studied and compared the vegetation growing in different soils, serpentine and quartz-diorite. Serpentine is a mineral that consists of a hydrous magnesium silicate. Quartz is a mineral that consists of silicon dioxide that occurs in crystals, and diorite is a granular, crystalline, igneous rock commonly of acid plagioclase and hornblende, pyroxene, or biotite. He returned to his dissertation promise and analyzed his data in the insects living on the foliage in the Great Smoky Mountains, and also did a study on communities of copepods, which are tiny crustaceans. Washington State promoted him to assistant professor in 1950 but did not renew his teaching contract the following year.

After losing his academic position, Whittaker joined the Aquatic Biology Unit of the Department of Radiological Sciences of the Hanford Laboratories of the General Electric Company in Richland, Washington, as a senior scientist. He followed a radioactive phosphorus tracer in aquatic communities. His research was important for understanding the flow of nutrients through ecosystems as well as the impact of radioactive elements in the environment.

Whittaker rejoined academics when the department of biology of Brooklyn College, at the City University of New York, hired him as an instructor in 1954. During the summers, he visited the Great Smoky Mountains, where he continued his gradient studies of forest communities at high elevations. He was interested in productivity, the rate per unit area or per unit volume at which photosynthetic organisms produce biomass consumable as food by other organisms. While performing these studies, he developed the concept of dimension analysis, a means to estimate the biomass of a tree using its diameter at breast height and total height, and for shrubs and herbs based on weight samples from all plant parts. He performed an exhaustive study that compiled measurements of production for the major plant communities of the area, a project that required much patience and attention to detail.

The classification of organisms into kingdoms also intrigued Whittaker. From an ecological viewpoint, he divided Earth's biota into three categories based on how they obtained their nutrition: primary

producers (photosynthetic organisms), consumers (animals), and decomposers (fungi and bacteria). After giving prokaryotic organisms (monerans) and unicellular eukaryotic organisms (protists) their own kingdoms, he instituted a five-kingdom classification system in 1969. The kingdoms included Animalia, Plantae, Fungi, Protista, and Monera, and though it has been modified based on new knowledge regarding evolutionary relationships, his system still appears in most biology textbooks with the addition of a new domain consisting of prokaryotic organisms that live in external or harsh environments, the Archaea.

During his time at Brooklyn, Whittaker began collaborating with William A. Niering to study the productivity gradients of a desert community of Arizona Saguaro cacti in the Santa Catalina Mountains. By 1964 Whittaker had become an associate professor at Brooklyn College and had developed a reputation as a prominent field ecologist. He was known for challenging ideas of the established ecologists.

Whittaker teamed up with George M. Woodell during much of the 1960s. He took a leave of absence from Brooklyn College in 1964 to join Woodell at the Brookhaven National Laboratory and Forest on Long Island, New York. Together they researched several aspects of plant ecology related to the Brookhaven oak-pine forest including surface area, biomass and production, nutrient flow, and the effects of gamma radiation on forest ecosystems. Other work he initiated at this time with Gene E. Likens and Herbert Bormann led to two monographs, published in 1970 and 1974, about the Hubbard Brook Experimental Forest, a hardwood forest ecosystem in New Hampshire.

Whittaker never returned to Brooklyn. In 1966 he became a professor at the University of California at Irvine, but he only stayed two years due to his disappointment at the urbanization of the area. He moved to Cornell University in 1968 as a professor of biology in the Section of Ecology and Systematics. With a graduate student, Walter Westman, Whittaker continued a study of production and nutrient cycling along a gradient from the pygmy forest region to the redwoods in the Mendocino County area of California. Though earlier in his career he had alienated many European ecologists by creating a rift between the discrete community and continuum schools of ecological thought, in 1970 he presented a paper at a symposium in Rinteln, Germany, "Convergences of Ordination and Classification," that emphasized the mutual theories of the two schools rather than their differences. As a result of his attempt at international scientific peacekeeping, he was named editor for the European ecology journal *Vegetatio* in 1973, a position he held until his death. Whittaker authored the first edition of his landmark undergraduate textbook, *Communities and Ecosystems*, in 1970, and a second edition five years later.

During his time at Cornell, Whittaker's contributions to vegetation analysis were widely recognized. Fellow ecologists cited his scientific works frequently and relied on *Communities and Ecosystems* as a source for factual information and methodology. In 1966 the Ecological Society of America (ESA) gave Whittaker the Mercer Award, shared with Niering for their work on the Arizona Saguaro cactus desert. He was elected vice president of the ESA in 1971. The National Academy of Sciences elected Whittaker to membership in 1974 and the American Academy of Arts and Sciences did so in 1979. Cornell University named him the Charles A. Alexander Professor of Biological Sciences in 1976. The ESA honored him with their highest award, that of Eminent Ecologist "in recognition of an outstanding body of ecological work or of sustained ecological contributions of extraordinary merit" in 1980.

Whittaker married a biologist named Clara Caroline Buehl on New Year's Day in 1953, and the couple had three sons: John Charles, Paul Louis, and Carl Robert. Clara died of cancer in 1977, and Whittaker married his doctoral student, Linda Olsvig, in 1979. He died from cancer on October 20, 1980, in Ithaca, New York.

See also BIOGEOGRAPHY; BIOLOGICAL CLASSIFICATION; BOTANY; COMMUNITY ECOLOGY; ECOLOGY.

FURTHER READING

Peet, R. K., ed. *Plant Community Ecology: Papers in Honor of Robert H. Whittaker*. Dordrecht, The Netherlands: Dr. W. Junk Publishers, 1985.

Westman, Walter E., Robert K. Peet, and Gene E. Likens. "Robert H. Whittaker (December 27, 1920–October 29, 1980)." In *Biographical Memoirs. National Academy of Sciences*. Vol. 59. Washington, D.C.: National Academy Press, 1990.

Whittaker, Robert H. "Allelochemies: Chemical Interactions between Species." *Science* 171 (1971): 757–790.

———. *Communities and Ecosystems*. 2nd ed. New York: Macmillan, 1975.

———. "Dominance and Diversity in Land Plant Communities." *Science* 147 (1965): 250–260.

———. "Evolution and Measurement of Species Diversity." *Taxon* 21 (1972): 213–251.

———. "Gradient Analysis of Vegetation." *Biological Review* 42 (1967): 207–264.

———. "New Concepts of Kingdoms of Organisms." *Science* 163, no. 863 (1969): 150–160.

Wilkins, Maurice H. F. (1916–2004) New Zealand-born British *Biophysicist* Maurice Wilkins won the Nobel Prize in physiology or medicine in 1962, shared with James Watson and Francis

Crick, for his research leading to the discovery of the molecular structure of deoxyribonucleic acid.

Maurice Hugh Frederick Wilkins was born on December 15, 1916, at Pongaroa, New Zealand, and moved to England when he was six years old. Wilkins earned a degree in physics from St. John's College, Cambridge University in 1938, and then entered Birmingham University. As a research assistant in the laboratory of Dr. J. T. Randall, he examined luminescence in crystals. After obtaining a doctorate degree in physics in 1940, with a thesis on phosphorescence, he worked on improving cathode-ray tube screens for radar. Then Wilkins moved to Berkeley, California, where he joined the Manhattan Project and worked with M. L. E. Oliphant on the separation of uranium isotopes for nuclear bombs.

After World War II ended, Wilkins became interested in the molecular structures of biomolecules and believed that his physics background could contribute in a meaningful way to the field of biology. Randall had shifted his research focus to biophysics and invited Wilkins to join him in the physics department at St. Andrews University in Scotland. In 1946 the biophysics group became part of the newly formed Medical Research Council Biophysics Research Unit, and moved to King's College at the University of London. He started studying the genetic effects of ultrasonics, but soon switched to ultraviolet microscope studies of nucleic acids in cells and then to the arrangement of crystalline tobacco mosaic virus particles. One technique he used was circular dichroism (CD), a form of spectroscopy that measures differential absorption of polarized light due to structural asymmetries in protein molecules. While preparing samples for CD studies of calf thymus DNA, Wilkins first noticed the presence of thin, delicate filamentlike fibers. Their uniformity suggested the fibers would be suitable subjects for X-ray diffraction studies. X-ray crystallography is a technique widely used by structural biologists to determine the relative positions and arrangement of atoms within a molecule. Shooting X-rays through crystals of a purified substance produces distinct patterns on a piece of film, and the patterns reveal the molecular structure. In 1950 Wilkins's graduate student, Raymond Gosling, was able to obtain detailed X-ray diffraction patterns from the DNA fibers by keeping them moist, since biological molecules naturally exist in an aqueous environment. Having limited experience with X-ray diffraction techniques, Wilkins sought additional help from a trained crystallographer. In 1952 Rosalind Franklin joined the lab to assist on the project, though she and Wilkins later came to disagree as to whether she was his assistant or an independent researcher.

Though Oswald Avery, Colin MacLeod, and Maclyn McCarty, researchers from the Rockefeller Institute Hospital, published evidence demonstrating that DNA was the genetic material in 1944, their work was not yet widely known, and many, including Wilkins, still believed the genetic material was protein. Chromosomes, which contained a cell's DNA, were believed to play an accessory role in replication of the protein. Other chemical analysis demonstrated that nucleic acid had a well-defined structure, even if unknown. For example, DNA was a polymer of repeating nucleotide subunits, the percent chemical composition of the nucleotide adenine equaled the composition of thymine, and the percent composition of cytosine equaled that of guanine. Electron microscopy showed that DNA was a long threadlike molecule with a regular width, and other structural studies revealed that the nitrogenous bases rested parallel to one another, yet were perpendicular to the length of the molecule. These collective studies demonstrating that DNA was a pure chemical substance convinced scientists, including Wilkins, that determination of its three-dimensional configuration was a worthwhile endeavor.

At King's College, Wilkins's colleague Alex Stokes worked on the mathematical interpretation of diffraction data for a helical structure, while Rosalind Franklin concentrated on obtaining even better X-ray diffraction photographs. One puzzle to solving the structure of DNA was reconciling the knowledge that the purines and the pyrimidines had different dimensions and seemed to appear in an irregular sequence along the DNA molecule with the fact that the width of the molecule remained constant. Franklin's diffraction patterns suggested that DNA was helical, but she was unwilling to discuss her results or to collaborate with Wilkins, Watson, or Crick at the time. Wilkins shared Franklin's data with Watson without first asking her permission. Watson and Crick, working in the Cavendish Laboratory at Cambridge, figured out that the overall width would be preserved if DNA were double-stranded, and adenine always formed hydrogen bonds with thymine, and cytosine always formed hydrogen bonds with guanine. The focused X-ray diffraction studies from Wilkins's laboratory, in combination with the molecular modeling at Cambridge, led to the successful discovery of the double helical structure of DNA in early 1953. After Watson and Crick shared their discovery with Wilkins, all the parties involved, including Franklin, copublished their research findings.

A collection of three papers appeared in the British journal *Nature* dated April 25, 1953. The first paper contained Watson and Crick's description of their double helical model for DNA. The second constituted Wilkins's paper, coauthored by

Stokes and Herbert R. Wilson, describing the X-ray crystallography evidence for Watson and Crick's model and showing that this was the form that existed in biological systems, namely, bacteriophage T2 particles and sperm heads. The third paper, by Franklin and Gosling, provided further X-ray diffraction evidence for the helical nature of DNA and provided evidence that the phosphate backbone laid on the outside of the structure. This work earned Wilkins the 1962 Nobel Prize in physiology or medicine, shared with Watson and Crick. Unfortunately, Franklin passed away in 1958 and thus was ineligible for a Nobel Prize.

Wilkins's subsequent research further established the essential features of the double helix and described alternate configurations assumed by DNA in conditions with different water and salt content and in the presence of various cations (positively charged particles). All three configurations were basically the same, further demonstrating the Watson-Crick model was correct. Wilkins also made significant contributions to RNA structural analyses, particularly the structure of transfer RNA. He later studied aspects of how the nervous system operates.

In 1950 Wilkins became the assistant director of the Medical Research Council Unit, and the deputy director five years later. The Royal Society elected Wilkins a fellow in 1959, and that same year he married Patricia Ann Chidgey, with whom he had two children, Sarah and George.

See also AVERY, OSWALD; CHROMOSOMES; CRICK, FRANCIS; DEOXYRIBONUCLEIC ACID (DNA); FRANKLIN, ROSALIND; MACLEOD, COLIN MUNRO; MCCARTY, MACLYN; WATSON, JAMES D.; X-RAY CRYSTALLOGRAPHY.

FURTHER READING

Langridge, R., H. R. Wilson, C. W. Hoper, M. H. F. Wilkins, and L. D. Hamilton. "The Molecular Configuration of Deoxyribonucleic Acid. I. X-ray Diffraction Study of a Crystalline Form on the Lithium Salt." *Journal of Molecular Biology* 2 (1960): 19.

The Nobel Foundation. "The Nobel Prize in Physiology or Medicine 1962." Available online. URL: http://nobelprize.org/nobel_prizes/medicine/laureates/1962/index.html. Accessed January 16, 2008.

Spencer, M., W. Fuller, M. H. F. Wilkins, and G. L. Brown. "Determination of the Helical Configuration of Ribonucleic Acid Molecules by X-ray Diffraction Study of Crystalline Amino-acid-transfer Ribonucleic Acid." *Nature* 194 (1962): 1014.

Wilkins, Maurice. *The Third Man of the Double Helix*. New York: Oxford University Press, 2003.

Wilkins, M. H. F., A. R. Stokes, and H. R. Wilson. "Molecular Structure of Deoxypentose Nucleic Acids." *Nature* 171 (1953): 738–740.

Wilmut, Sir Ian (1944–) Scottish *Embryologist* In 1996 a team of researchers led by Sir Ian Wilmut from the Roslin Institute in Edinburgh, Scotland, witnessed the live birth of a sheep named Dolly, the first mammal cloned from an adult cell. Cloning is the creation of an organism from the deoxyribonucleic acid (DNA) of one parent resulting in an offspring genetically identical to the parent. The team's success marked a remarkable biotechnological breakthrough, but, just as significantly, the event brought the potential of human cloning to light and sparked heated controversial ethical debates that continue today.

FIRST TO SUCCEED IN CALF-EMBRYO CRYOPRESERVATION

Ian Wilmut was born on July 7, 1944, in Hampton Lucey, England. As a child growing up in Coventry, Ian wanted to enter the navy, but he was colorblind, a condition that would have prevented his being able to read signals from other boats. He was interested in the outdoors, and though he lived in an industrial area, as a teenager, he worked on farms during the weekends. He especially enjoyed working with the animals; performing tasks such as milking cows and assisting during births instilled in Ian a desire to study farming.

Wilmut attended the University of Nottingham and spent the summer of 1966 as an intern in the laboratory of Professor E. J. Chris Polge in the Unit of Reproductive Physiology and Biochemistry at the University of Cambridge. At the time, Polge was trying to understand how an animal knew whether or not she was pregnant, so her body could make the physiological decision of preparing to mate again or to focus on maintaining the pregnancy. Wilmut became fascinated with embryos and the fact that entire organisms developed from a single cell, an experience that caused Wilmut's interests to shift from practical agriculture to embryological research.

Sir Ian Wilmut led the team that cloned the first mammal from adult differentiated cells, a sheep named Dolly. *(Gusto/Photo Researchers, Inc.)*

He received a bachelor of science degree in animal physiology from Nottingham in 1967.

After marrying his high school sweetheart, Vivienne, with whom he shares three grown children, Wilmut entered graduate school at the University of Cambridge. Back in Polge's laboratory, Wilmut examined the reasons bull semen could survive freezing and thawing, but boar semen could not. In 1971 Wilmut obtained his doctorate degree from Darwin College at Cambridge University. His dissertation was titled "Deep Freeze Preservation of Boar Semen." After receiving a doctorate degree in animal genetic engineering, Wilmut pursued a postdoctoral fellowship to stay at Cambridge and continue his research on freezing. His goal was to develop deep-freeze storage methods for mammalian embryos, and he was the first scientist to freeze a calf embryo, thaw it, and grow it inside a surrogate mother. A live birth resulted in a healthy calf named Frostie, an exciting event because until then, cryopreservation, the procedure of freezing cells or embryos for later use, had been applied only to single cells. The development of this technique led to better quality herds, since farmers could implant embryos from genetically superior parents into inferior cows.

GENETIC MODIFICATION OF ANIMALS

In 1973 the Animal Breeding Organization (ABRO) in Scotland hired Wilmut, who was by then considered an expert in reproductive physiology, as an embryologist. Later renamed Roslin Institute, the animal genetics research center that was funded by a combination of government and private funds expanded, and Wilmut became one of its leading scientists. In 1981 he was promoted to principal investigator in animal physiology and genetics, in 1993, to principal investigator and joint head of the department of gene expression and development, and then in 2000, head of the department of gene expression and development. His research initially focused on the identification of developmental and physiological causes of embryo death in farm animals, but the interests of ABRO shifted toward molecular biology in the early 1980s. At the time, Wilmut resented being told to discontinue his research in progress and to redirect his efforts to the genetic modification of sheep, but he obliged his director, mostly because he and his wife did not want to uproot their family.

Wilmut spent much of the next decade preparing zygotes (the single-celled fusion products of eggs and sperm) for injection with human genes that encoded pharmaceutically important proteins, a project supported by a collaborating biotech company called PPL. After injection, he placed the zygotes back into a ewe and hoped the offspring would express the special gene. A major disadvantage of this method for genetically modifying animals is each attempt with a single zygote demanded an entire animal. He did succeed in producing Tracy, born in 1990, a sheep that carried the gene encoding alpha-1-antitrypsin, a protein used to treat cystic fibrosis and emphysema, and secreted the human protein in her milk.

CLONES SHEEP FROM ADULT CELLS

Many plants can reproduce asexually by vegetative propagation, a procedure in which a horticulturist trims a section of a root or stem from a plant, and it grows into a mature plant that is genetically identical to the donor. This characteristic is beneficial to farmers, who have an economic interest in propagating potatoes that have a particularly smooth texture or flowers that have uniquely decorated petals. The ability to create replicates of farm animals with favorable characteristics was a practical goal of agriculture in the 1980s. For example, creating multiple animals from a genetically superior dairy cow that produced a high yield of milk would be economically desirable. One often cannot measure the success of a selective breeding until the offspring reach adulthood, an expensive venture when unsuccessful. Previous failed attempts convinced many scientists that cloning large farm animals was an unfeasible goal.

In the early 1980s, Steen Willadsen (1944–), a Danish researcher from Polge's laboratory, had the best track record for attempts leading to cloning a mammal from differentiated cells, cells that have begun the processes leading to specialization. He developed methods for separating cells from cattle and sheep embryos at the eight-cell stage and nurturing them separately to adulthood. During the 1983–84 breeding season, he succeeded in fusing a sheep cell from an eight-cell embryo with an enucleated egg cell (a cell whose nucleus has been removed), producing the first cloned farm animal. Willadsen did not publish these results until 1986 ("Nuclear Transplantation of Sheep Embryos," in *Nature*). In 1985 Willadsen left Cambridge and took a job with Grenada Genetics in Texas, where he proceeded to clone a cow from a one-week-old differentiated embryonic cell using nuclear transfer (NT), the process of removing the nucleus from an unfertilized egg and replacing it with the nucleus from a differentiated (specialized) cell. At a pub in Dublin, Ireland, in January 1987, Wilmut heard about Willadsen's progress in developing the method of NT using early embryonic cells, and he became convinced that he could genetically modify sheep using transformed embryonic stem (ES) cells. ES cells are the cells from which an entire organism develops and that have the capacity to develop into any different tissue type, such as bone, nerve, or muscle.

(continues on page 752)

STEM CELLS: THE NEXT MAJOR MEDICAL BREAKTHROUGH?

by Jacob P. Harney, Ph.D.
University of Hartford

What is the one thing type 1 diabetes, Parkinson's disease, spinal cord injury, cardiomyopathy, and cancer all have in common? They are all diseases in which specific cell types lose their ability to function normally. If one could simply replace cells that no longer function properly with new cells, millions of people could live longer, healthier lives. While that sounds easy, like taking a car into the shop to replace the air and oil filters, such simple repairs are not possible when dealing with the human body.

The beauty of the human body is in the elaborate distribution of labor that occurs among cells, tissues, organs, and organ systems. Fully differentiated cells take on one of the thousands of very specific jobs necessary for the body to function effectively. When the cells that carry out a specific job incur damage or die, the body must replace them to continue functioning successfully. Without replacing the cells, their job is not completed, and the body is at risk of injury or loss of life. As a result, one of the greatest challenges to medical research right now is to find ways to replace cells in the body with new ones that can work for years into the future without loss of function or attack by the immune system.

Stem cell science is a fascinating field that promises to surprise and captivate the field of medical research as scientists learn more about how bodies replace damaged cells and continue to function in the face of age, injury, and fatigue. Stem cells are unspecialized cells that have the ability to self-renew and divide to replenish lost cells without limit. When a stem cell replicates, it produces two stem cells that can each either continue to divide (serving themselves as stem cells) or receive stimulation to differentiate into a more specialized cell. The specialized or differentiated cell "stems" from the unspecialized precursor cell, hence the name stem cell. As long as one of the daughters from the initial division remains an unspecialized "stem" cell there is no limit to the number of ultimately specialized cells that can be produced from a given stem cell.

Scientists identified two sources of stem cells in the body long ago—the bone marrow and the testes. Stem cells in the bone marrow continue to divide throughout a person's life as they replenish lost red and white blood cells. Red blood cells carry life-giving oxygen to all the tissues in the body while white blood cells help protect the body from injury and invasion of foreign pathogens. Both types of blood cells are ultimately highly specialized to carry out very different functions, yet both arise from the same unspecialized stem cells. In the testes of postpubertal males, stem cells continue to divide and their daughter cells ultimately differentiate into sperm cells for reproduction of the species.

One cannot overemphasize the importance to the species of the aforementioned stem cells. Without bone marrow stem cells, an individual would not be able to deliver oxygen throughout the body or protect itself from infection, both of which would result in death to the individual. Lack of stem cells in the testes of mature males would eliminate the ability of that individual to reproduce and thus could ultimately result in the demise of the species.

A good place to start when trying to understand the sources and classifications of stem cells is with reproduction. When a human sperm cell fertilizes an egg (ovum), the result is a one-cell embryo or zygote. That one cell represents the ultimate stem cell of the resultant human being as every cell in the body will "stem" from that cell. The one-cell embryo, however, is not termed a true stem cell because it cannot self-renew in an unlimited manner. Because the one-cell embryo has the potential to become every cell in the body as well as the placenta, it has "total potential" developmentally and is, therefore, referred to as being totipotent. As the one-cell embryo continues to divide into two, four, eight, 16, 32, 64, 128 cells and beyond, it eventually reaches a point when some differentiation or specialization begins to occur. The resultant cells, while still fairly unspecialized, may not have the capacity to become every cell in the body so they are no longer totipotent but instead are referred to as pluripotent. Totipotent and pluripotent cells are the sources of all stem cells and understanding their capacity to divide and differentiate is crucial to our ability to ultimately know and harvest the potential of stem cell biology.

Developmental biologists classify stem cells by the source of their origin. The inner cell mass of the five- to six-day-old human embryo is the source of pluripotent human embryonic stem cells (hESCs). Most biologists acknowledge that the nature of embryological development suggests that embryonic stem cells should have the greatest potential to develop into and potentially replace any cells, based on the fact that they appear so early in the development of the individual. However, the ability to harvest their ultimate potential to replace any cell in the body is probably the most challenging as those cells are also the farthest developmental distance from any fully differentiated cells. So while hESCs may have the greatest promise as a single cell source capable of replacing the greatest number of differentiated cells, the road to that end remains the longest and most difficult to travel.

The challenge of hESC biology extends beyond the science itself into the moral and ethical questions that society faces with this emerging science. In order to harvest hESCs from five- to six-day-old human embryos, the viability of the embryo to produce an offspring must be completely compromised or at least put at significant risk. Is it appropriate to sacrifice the potential life that an embryo rep-

resents (acknowledging that there is no way of knowing if any embryo will result in the birth of a normal healthy human being) for the promise of scientific discovery and the hope for potential therapies? If that decision is affirmative there are presently hundreds of thousands of excess embryos in reproductive fertility clinics that could be made available for hESC research if the owners of such embryos agree to it. The reality is that many people are in complete support of biomedical research if it can help people presently suffering from diseases, and many would donate their excess embryos. The current political issue in the United States revolves around whether or not the federal government should fund research that requires the destruction of embryos. These are some of the fundamental issues that are being debated each day in the United States as other countries (with more liberal political agendas) move forward and in some cases take American scientists with them.

Adult stem cells, like embryonic ones, can self-renew to produce more stem cells, or undergo differentiation into particular types of specialized tissues. A critical feature of any stem cell is its developmental potential, in other words, the range of different mature cell types the stem cell can produce in a given contextual situation. Generally speaking, adult stem cells are isolated from mature or differentiated tissues, whether those tissues are found in a child or an adult. In fact some "adult" stem cells can be essentially identical to cells found in fetal, neonatal, or pediatric tissues.

The list of adult stem cells, which include those found in bone marrow and in the testes, has grown significantly in the last several years. Locations of adult stem cells include the brain, blood, cornea, retina, heart, fat, skin, dental pulp, bone marrow, blood vessels, skeletal muscle, and intestines. Unlike embryonic stem cells, adult stem cells appear more limited in their range of differentiation; they are generally limited to the cell types found within their tissue of origin. For instance, an adult brain stem cell can become a neuron or glial cell, both found in the brain, but not a muscle or liver cell.

From a developmental perspective, one might predict that adult stem cells should have a shorter path to their differentiated destination simply because they are farther along the pathway. That developmental head-start, however, can be a detriment as the more differentiated a cell is, the harder it is (in general) to proliferate and renew in culture. For stem cells to become a reliable and replicable therapy they must exist in large numbers, and generation of those large numbers requires an understanding of what stimulates them to divide in the first place. Many tissues and organs in the adult body contain stem cells that lie dormant until stimulated to divide due to injury or illness. The greatest challenge regarding adult stem cells is and will continue to be our ability to identify, isolate, and expand them in vitro (outside the body).

In research to date, adult stem cells have a much more successful track record than embryonic stem cells as far as potential therapies are concerned. Adult stem cells have somewhat successfully treated spinal cord injury in animals and humans, in addition to Parkinson's disease in animal models and in humans. In some of these studies, scientists have used proteins to stimulate patients' own brain stem cells with significant improvement in symptoms. Adult stem cells also have been used successfully to treat diabetes in mice. Work in humans has focused on islet cells transplants, which have successfully treated humans with diabetes but which may or may not contain stem cells. The most effective and well-known adult stem cell therapies involve bone marrow transplantation.

While hESCs have not been credited with curing any diseases, they have received only a small fraction of the funding and have been the target of that limited research for less than a decade. Researchers have been investigating adult stem cells for more than five decades; bone marrow transplantation has been practiced since 1968.

In addition to embryonic and adult, fetal and umbilical cord stem cells and embryonic germ cells are recognized as pluripotent cells with therapeutic potential. Fetal stem cells are primitive cell types found in the organs of fetuses. Umbilical cord stem cells seem to be distinct from bone marrow cells yet similar in their hematopoietic (blood cell) replacement potential. Hospitals routinely collect and store umbilical cord blood due to the presence of highly proliferative stem cells. Embryonic germ cells are the precursors to the gametes (eggs and sperm) and can be isolated from the primordial gonad of five- to nine-week-old fetuses. While these cells are pluripotent and can develop into most cell types, similar reservations as for hESC and fetal stem cells accompany their isolation and use. In the midst of the recent debate over the use of hESCs scientists have isolated and characterized cells from amniotic fluid. These cells appear to have excellent therapeutic potential and may become routinely collected and stored in the future. A very recent source of stem cells that might have the broadest appeal due to ease and frequency of collection is menstrual fluid, which does not require pregnancy as a prerequisite to collection.

To appreciate the challenges faced with cellular therapies of any kind one must understand the nature of the immune system and how it protects from disease. All cells in the body have marker proteins on their surface that distinguish them as "self," or originating within the individual. As the immune system patrols the body, it looks for the "self" markers, and if it does not see them it assumes the cell is an invader and mounts an attack. For this reason standard protocol in any tissue transplantation includes avoidance or suppression of the immune attack that might destroy the transplanted tissue, while not compromising the immune function so much as to put the individual's health at risk. The most effective way, therefore, to ensure that a stem cell therapy has the greatest potential to succeed is to utilize stem cells that possess the same self-markers, in other words, genetically identical to the recipient. For that to occur the stem cell must have originated within the individual or their identical twin. Since most people do

(continues)

(continued)

not have an identical twin, scientists are working on ways to generate "individualized" stem cell populations through two procedures, somatic cell nuclear transfer (SCNT) and induced-pluripotency.

SCNT has brought the debate over embryonic stem research to a whole new level. The procedure is simple in theory but quite complex in reality: Remove the nucleus from an oocyte and replace it with the nucleus from a mature, differentiated somatic (body) cell. Following nuclear transfer, stimulation of the engineered cell induces division as if it were a one-cell embryo. Once the "embryo" reaches the five- to six-day-old stage, scientists harvest the stem cells from the inner cell mass and generate embryonic stem cell lines that are genetically identical to the donor nucleus (with the exception of the mitochondrial DNA). Because these individualized cells would have great therapeutic potential, the process is also referred to as therapeutic cloning. One can imagine generating stem cell lines for every person on the planet, making them available when necessary to replace cells that have been damaged due to trauma, injury, or age.

While therapeutic cloning has great promise in theory, it also generates fear related to a more controversial application of cloning. If SCNT can be used to generate genetically identical cells than it can also, in theory, be used to generate genetically identical individuals, also called delayed twins. This is referred to as reproductive cloning and would result if the SCNT-generated embryo were transplanted to the uterus of a woman and resulted in a successful birth. The concerns over human reproductive cloning are real, understandable, and must be addressed if therapeutic cloning is ever to have a chance to help cure diseases.

Induced-pluripotent cells (IPCs) have successfully been generated from human skin cells injected with a few specific genes. The IPCs reportedly behave like stem cells and represent "self" tissue, which would likely not be subject to immune attack. The ability to get cells to essentially dedifferentiate and reprogram for another cellular function could revolutionize cellular medicine in the years to come.

The most accepted truth about human stem cell research is that it is an area of exploration that will continue to fascinate the entire community as it directly addresses the issue of what is "life" and how much control over "life" is acceptable. Currently, the Ethics Working Party (of which the author has been a participating representative), a committee associated with the International Stem Cell Forum, is charged with evaluating the current and potential future ethical considerations that need to be addressed to allow for meaningful, productive international collaboration in the area of human stem cell research. The issues are challenging as individual political administrations are in a constant state of flux worldwide and so, therefore, are the prevailing philosophies and mandates. As time passes the direction of stem cell research will become more defined while the results remain anyone's guess.

FURTHER READING

Bellomo, Michael. *The Stem Cell Divide: The Facts, the Fiction, and the Fear Driving the Greatest Scientific, Political, and Religious Debate of Our Time.* New York: AMACOM, 2006.

Bongso, Ariff, and Eng Hin Lee, eds. *Stems Cells: From Bench to Bedside.* Hackensack, N. J.: World Scientific, 2005.

Lanza, Robert, and Nadia Rosenthal. "The Stem Cell Challenge." *Scientific American* 290, no. 6 (June 2004): 92–99.

Panno, Joseph. *Stem Cell Research: Medical Applications and Controversy.* New York: Facts On File, 2004.

Weiss, Rick. "The Power to Divide." *National Geographic* 208, no. 1 (July 2005): 2–27.

(continued from page 749)

Wilmut thought their success rate in genetically modifying sheep would increase if they added DNA to a whole plate of cultured cells and only created embryos from ones that incorporated the DNA, but cultured cells did not develop into whole animals. Researchers were able to culture mouse ES cells, genetically modify them, and put them back into embryos that developed into live births. Providence sometimes directed the altered ES cells to differentiate into gamete-producing cells, creating new genetic strains. Wilmut wanted to create ES cell lines from sheep, but culturing the cells destroyed their ability to develop into different types of specialized tissues.

Keith Campbell (1954–), a cell biologist with expertise in the cell cycle, joined Wilmut in 1991 at ABRO to assist in the quest to produce genetically modified sheep. Preliminary efforts to make undifferentiated sheep cell lines from cultured ES cells were futile. The cells grew in vitro, but they continued to differentiate. Despite this, Wilmut and Campbell attempted to force the cells into a state of resting or inactivity, called quiescence, by starving them. Active embryonic cells normally cycle between growing and dividing, but nutrient deprivation can induce quiescence. They outlined a method of starving cells in order to coordinate the cycle of the cell containing the donor nucleus and the egg cell to which the donor cell is fused. After synchronizing the cultured ES cell nuclear donor and unfertilized recipient sheep egg cells, they attempted to transfer nuclei by fusing the enucleated cell and the egg cell using blasts of electrical current.

Of 244 nuclear transfers that Wilmut and Campbell performed using nine-day-old cultured embryo cells, 34 developed enough to be transferred to the uteri of surrogate mother sheep. In 1995 five lambs were born, including Megan and Morag, the first two mammals cloned from cultured differentiated cells that survived to become healthy, fertile adults. Wilmut views these sheep as the most important of all their clones, since they were the first clones created from frozen cultured cells.

In 1996 they repeated their experiments using nine-day-old embryonic cells that they transferred into eggs from which the genetic material had been removed. Feeling more propitious this time, they also used cultured 26-day-old fetal fibroblast cells and mammary gland cells that had been removed from a six-year-old ewe, cultured in vitro, and frozen in liquid nitrogen. Of 835 total attempts, ultrasound revealed 21 single fetuses that led to eight live births. Of the 277 attempts using mammary cells, Wilmut and Campbell transferred 29 embryos into surrogate mother sheep, resulting in a single live birth. The lamb, named Dolly (after country music singer Dolly Parton), born on July 5, 1996, resulted from the nuclear transfer of adult cells. Though Dolly was clearly a Finn Dorset ewe, as opposed to progeny of the Scottish blackface ewe that supplied the enucleated egg, DNA testing definitively proved Dolly was indeed a clone. Wilmut and Campbell attributed their success to the exact attention they paid to synchronizing the cell cycles of the donor and recipient cells.

Roslin Institute did not announce their results until 1997 in order to be sure that Dolly was healthy and developed normally. Their *Nature* article, "Viable Offspring Derived from Fetal and Adult Mammalian Cells," grabbed the attention of the entire world. The media had a field day, emphasizing the closeness to human cloning represented by Dolly, as the first mammal cloned from adult cells. The number of requests for television, radio, and print media interviews far exceeded the expectations of Wilmut, who had no idea of the controversy the announcement would cause. Though Dolly marked the culmination of years of gradually increasingly successful experiments performed by numerous researchers, the publicity that Dolly generated made it seem as if scientists secretly had been performing cloning experiments and that this success came unexpectedly. This was far from the truth; every year scientific journals contained numerous articles reporting the successes in developmental biology that led to cloning. Society had been largely apathetic about these achievements, however, until a large mammal was cloned. Suddenly society saw the potential reality of human cloning, an ethical issue no one was prepared to face.

CLONES GENETICALLY MODIFIED SHEEP

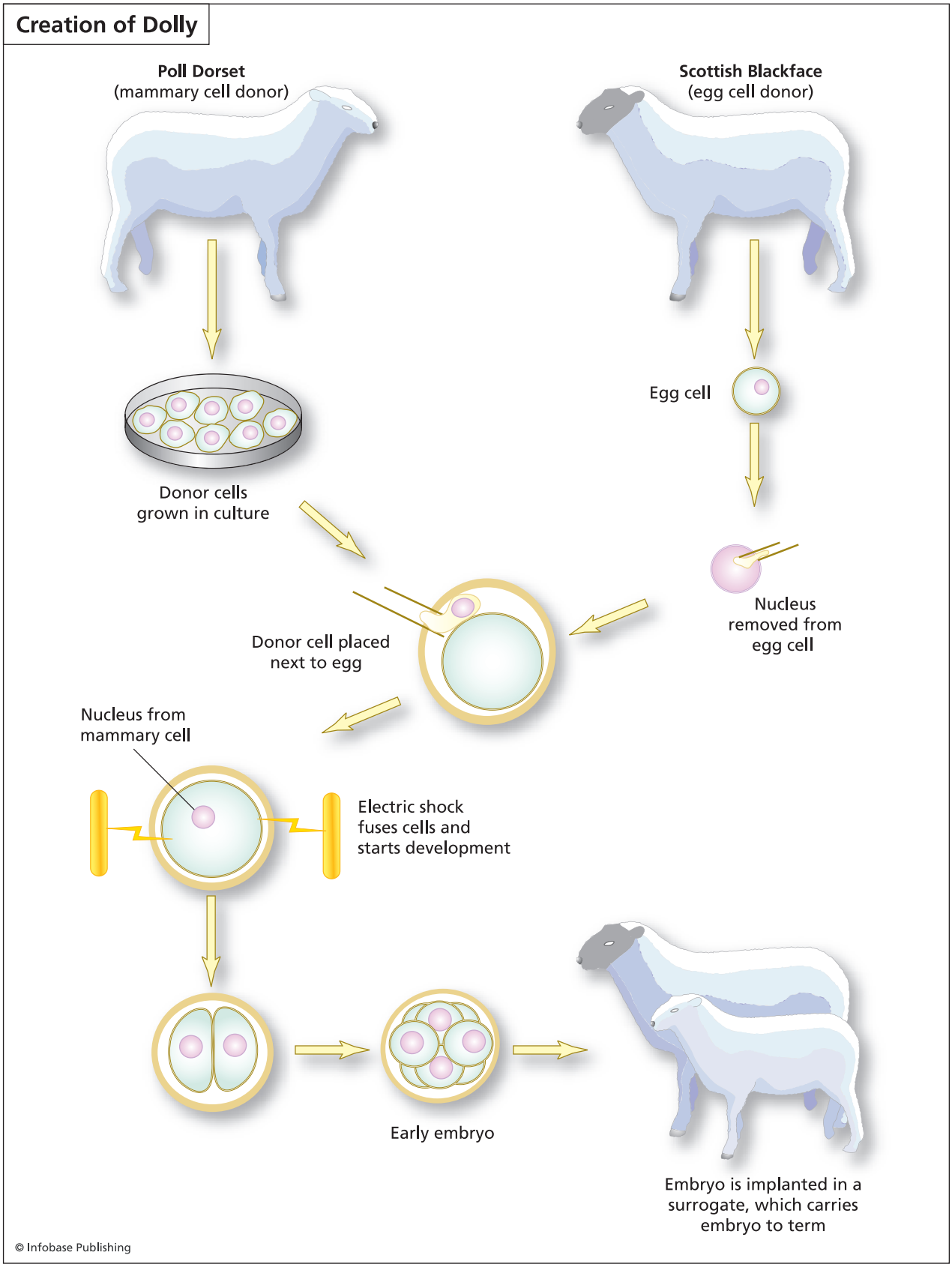
The year after Dolly was born, Roslin achieved another breakthrough, cloning a genetically modified sheep. Wilmut had inserted a gene for the production of a blood clotting factor, protein factor IX, into the nuclear genome of the donor nucleus while it was in culture. The protein was secreted in milk produced by the new sheep, named Polly. Wilmut hopes that similar cloning technology will advance progress in xenotransplantation, the process of transplanting into humans the organs from other species such as pigs. In 2002 two teams created cloned piglets from which the gene that stimulates the human immune system to reject transplantation had been eliminated, but many questions remain unanswered.

Dolly gave birth to six offspring, all bred conventionally and apparently healthy. In 2003 examinations showed that the six-year-old Dolly had developed a progressive lung disease, and the decision was made to euthanize her. Sheep normally live 11 or 12 years, and lung disease is common as they age. The year before, veterinarians had diagnosed Dolly with arthritis, sparking a debate on the true age of the first cloned mammal, further fueled by the diagnosis of lung disease at such a young age. The cell from which Dolly was cloned was already six years old before Dolly was born. Examination of her body after death showed no abnormalities other than arthritis and lung disease.

Since Dolly, others have achieved success cloning cattle, goats, pigs, cats, rabbits, deer, mules, and mice, but 10 years later, the mortality rate remains very high. Only 2 to 5 percent of egg clones develop into live births. Of those, many suffer severe developmental defects, and only a fraction of a percent survive the first few weeks. Cloned animals also exhibit abnormal gene expression patterns. Cloning technology has been used in a variety of ways, some more controversial than others. Objections to therapeutic cloning are fewer than to reproductive cloning. Therapeutic cloning is the production of human embryos for research purposes. Wilmut strongly objects to the reproductive cloning of humans and is steadfast in claiming that his use of cloning technology is purely for research purposes in hopes of preventing disease and reducing suffering.

OTHER ACCOMPLISHMENTS

In March 2005 Wilmut joined the Research Institute for Medical Cell Biology at the University of Edinburgh as a professor of reproductive biology and later that year became the head of the new Scottish Centre for Regenerative Medicine. Wilmut's current research goal is to better understand the regulatory mechanisms of early embryonic development and the reprogramming of somatic cells in order to establish new ways to study inherited human diseases. Though reproductive cloning is illegal in Britain, therapeutic cloning for research has been legal since 2001. In 2005 the Human Fertilisation and Embryology Authority granted Wilmut a license to use cloned human embryos to study motor neuron disease (MND). MND is an incurable progressive neurodegenerative disease that causes the death of nerve cells in the brain and spinal cord that control movement. In collaboration with Christopher Shaw from the Institute of Psychiatry in London, Wilmut's original research plan was to create cloned embryonic cells by removing the nucleus from a cell of someone with



To create Dolly, Wilmut removed the nucleus from an unfertilized recipient egg, fused the donor cell with the enucleated egg by electrical stimulation, cultured the cloned embryo in the laboratory (this can also be done in the oviducts of a ewe), and transferred the developing embryo into the reproductive tract of a surrogate mother. The cloned animal is genetically identical to the mammary cell donor.

MND and transferring it to a human egg cell from which the nucleus has been removed. Growth could then be stimulated by using a variety of chemical and physical manipulations. Because stem cells have the ability to develop into any type of cell, they planned to induce the stem cells derived from a cloned embryo to differentiate into nerve cells. This would allow for examination of the stage in development when the cells change from normal to abnormal nerve cells in the case of MND. Recently, however, Wilmut has switched his efforts from using cloned human embryonic stem cells to modifying adult cells at the genetic level in order to give them the flexibility of stem cells. In November 2007 two groups reported achieving success at reprogramming human skin cells into cells that resembled pluripotent cells by inserting genes using a virus: one group led by Shinya Yamanaka at Kyoto University in Japan and the other group by James Thomson from the University of Wisconsin-Madison. The degree to which the so-called induced pluripotent cells resemble embryonic cells is still unclear and the safety of such cells is under examination, but Wilmut believes the newer technique shows more potential for success.

Wilmut has served as an editor for the *Journal of Reproduction and Fertility* and is currently the editor in chief for *Cloning and Stem Cells*. In 2000 he copublished *The Second Creation: Dolly and the Age of Biological Control,* with Keith Campbell and Colin Tudge. The authors described the work leading up to the birth of Dolly, and the initial impressions afterward, to try to dispel some of the myths surrounding Dolly's cloning. In 2006, with Roger Highfield, he coauthored a second book, *After Dolly: The Uses and Misuses of Human Cloning,* in which the authors discuss the social, medical, and scientific implications of human cloning. On a personal note, Wilmut remains a committed family man who enjoys curling and has run two marathons.

Wilmut's awards and honors include at least three honorary doctorate degrees, the Sir John Hammond Memorial Prize from the Society for the Study of Fertility (1998), the Research Medal from the Royal Agricultural Society of England (1999), the Sir William Young Award from the Royal Highland and Agricultural Society of Scotland (1999), and the Scotsman Innovator of the Year Award, Edinburgh (2001). The Royal Society of Edinburgh elected Wilmut to membership in 2000 and the Royal Society of London did so in 2002. In 2005 he received the prestigious Paul Ehrlich prize, Germany's top medical research award. Most recently, Queen Elizabeth II bestowed the honor of knighthood on Wilmut in the 2008 New Year Honors list.

Wilmut's accomplishment of cloning a mammal from an adult body cell resulted from a combination of breakthrough technologies in genetic engineering and in cloning methods from cultured cells. The procedure itself is simple to describe, yet it took considerable effort to achieve success and required scientific expertise in embryology and cell biology. Scientists had firmly asserted that mammalian cloning was farfetched, calling it science fiction, but Wilmut shattered that fallacy. Society reacted strongly because, for the first time, people recognized the eventual possibility of human cloning and were forced to confront the accompanying ethical issues. By cloning a mammal from fully differentiated adult cells, Wilmut and his team answered a basic question of developmental biology, demonstrating that genetic material is not altered irreversibly during differentiation and development. The DNA from specialized cells can be reprogrammed to create an entire new being.

See also CLONING OF ORGANISMS; EMBRYOLOGY AND EARLY ANIMAL DEVELOPMENT.

FURTHER READING

Academy of Achievement. "Ian Wilmut, Ph.D., Pioneer of Cloning." The Hall of Science and Exploration of the Gallery of Achievement, Available online. URL: http://www.achievement.org/autodoc/page/wil0bio-1. Last revised January 9, 2008.

Campbell, K. H. S., J. McWhir, W. A. Ritchie, and I. Wilmut. "Sheep Cloned by Nuclear Transfer from a Cultured Cell Line." *Nature* 380 (1996): 64–66.

Encyclopedia of World Biography, Supplement. Vol. 20. 2nd ed. Farmington Hills, Mich.: Gale Group, 2000.

Panno, Joseph. *Animal Cloning: The Science of Nuclear Transfer.* New York: Facts On File, 2004.

Schneike, A. E., A. J. Kind, W. A. Ritchie, K. Mycock, A. R. Scott, M. Ritchie, I. Wilmut, A. Colman, and K. H. S. Campbell. "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Fetal Fibroblasts." *Science* 278 (1997): 2,130–2,133.

U.S. Department of Energy Office of Science. Office of Biological and Environmental Research, and Human Genome Program. "Human Genome Project Information: Cloning Fact Sheet." Available online. URL: http://www.ornl.gov/sci/techresources/Human_Genome/elsi/cloning.shtml. Last modified September 16, 2008.

Wilmut, I., A. E. Schnieke, J. McWhir, A. J. Kind, and K. H. S. Campbell. "Viable Offspring Derived from Fetal and Adult Mammalian Cells." *Nature* 385 (1997): 810–813.

Wilmut, Ian, and Roger Highfield. *After Dolly: The Uses and Misuses of Human Cloning.* New York: W. W. Norton, 2006.

Wilmut, Ian, Keith Campbell, and Colin Tudge. *The Second Creation: Dolly and the Age of Biological Control.* New York: Farrar, Straus & Giroux, 2000.

Wilmut, I., N. Beaujean, P. A. de Sousa, A. Dinnyes, T. J. King, L. A. Paterson, D. N. Wells, L. E. Young. "Somatic Cell Nuclear Transfer." *Nature* 419 (2002): 583–586.

Woese, Carl (1928–) American *Microbiologist*

Carl Woese initiated the Woesian revolution by creating a new paradigm for understanding life, evolution, and biological diversity. He proposed the existence of a third domain of living organisms, Archaea, after discovering that differences between some prokaryotic organisms were too great to justify their placement into the single kingdom Monera. His findings overturned the accepted dogma of microbiology and evolutionary history.

INTEREST SHIFTS FROM BIOPHYSICS TO EVOLUTIONARY RELATIONSHIPS

Carl Woese was born on July 15, 1928, in Syracuse, New York. He earned his bachelor's degree in mathematics and physics from Amherst College in 1950 and his doctorate degree in biophysics from Yale University in 1953. He remained at Yale as a postdoctoral scholar and studied the development of ribosomes, the molecular machinery responsible for synthesizing proteins. The ribosomes translate a sequence of nucleotides into a sequence of amino acids based on the genetic code. Each combination of three nucleotides specifies a particular amino acid that the ribosome links to the growing polypeptide chain. Woese developed an interest in the origin of the genetic code, of which one characteristic is universality. This means that the same trinucleotide sequences encode for the same amino acids in the bacteria *Escherichia coli,* in the pea plant *Pisum sativum,* in human beings *Homo sapiens,* and in every other organism with very few exceptions. Between 1960 and 1963 Woese briefly worked as a biophysicist for the General Electric Research Laboratory and at the Pasteur Institute in Paris. In 1964 he joined the faculty at the University of Illinois at Urbana-Champaign, where he became interested in primitive cells and the evolution of the molecule of heredity, deoxyribonucleic acid (DNA).

In the 1970s Woese developed a new method for investigating evolutionary relationships between organisms based on DNA sequences. Genes for molecules that play central roles in cells are highly conserved between organisms, meaning that organisms whose lineages diverged a very long time ago will share similar sequences for those genes. The gene encoding the 16S ribosomal ribonucleic acid (rRNA) subunit is one such example. Most importantly, the gene is universal because all living organisms must be able to synthesize proteins. This subunit is a major component of ribosomes, cellular machinery that carries out one of the most fundamental and crucial functions in a living cell—protein synthesis. Because of its importance, a cell cannot tolerate mutations to this gene, as its function, which is crucial to cell survival, might be impaired; thus, fewer differences exist in the DNA sequences of this gene between organisms, even organisms that are distantly related, than in other genes. The number of mutations between organisms indicates the evolutionary distance, or how long ago the two lineages diverged. The more differences between the DNA sequences of genes from two organisms, the less related they are evolutionarily.

Woese chose to utilize rRNA for his evolutionary investigations because, in his opinion, the process of protein synthesis defines the origin of cells. The group of primitive organisms that first evolved this capability, referred to as the progenote, is a common ancestor for members of all forms of life. He embarked on these studies in the late 1960s, before automated DNA sequencing was available. He performed the sequencing using a now-outdated, labor-intensive technique called oligonucleotide cataloging. After extracting RNA from cells, he first cut it into smaller pieces by slicing it at every guanine residue. (Guanine is one of the four ribonucleotide building blocks that make up RNA.) Treatment with different enzymes then cut the fragments into even smaller pieces by selectively cutting at adenine, cytosine, or uracil residues, yielding segments of RNA ranging from six to 20 nucleotides long. Woese spent countless hours each day for years arranging the fragments like pieces of a puzzle. He then compared each of those fragments with fragments from other species to determine how many they shared in common. Though the fragments were short, enough variation existed for comparisons to yield information about molecular relatedness. Cataloging 60 bacterial species took a decade to complete.

A NEW DOMAIN OF LIFE

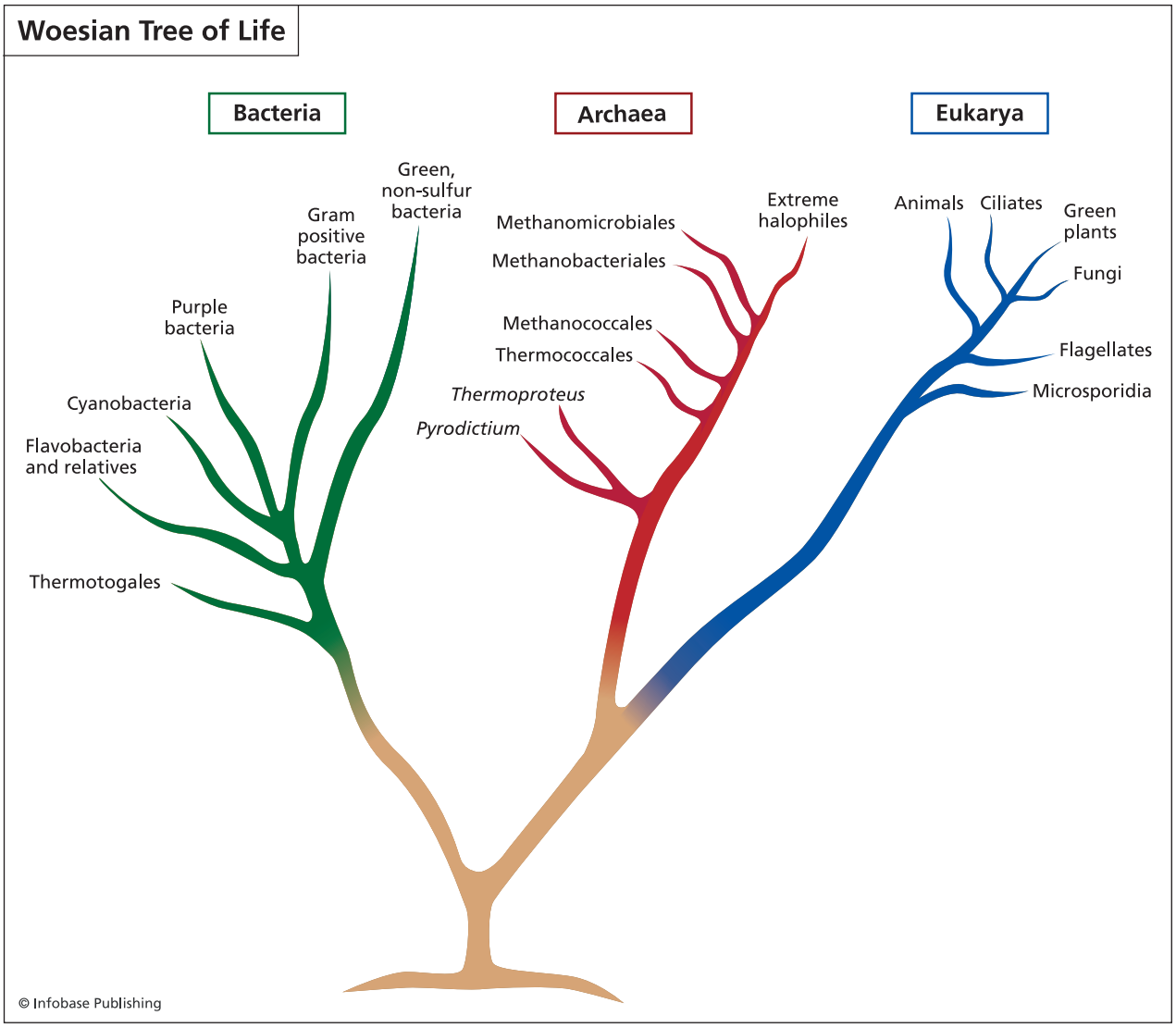
In 1976 a colleague from the University of Illinois, Ralph Wolfe, gave some methanogenic bacteria to Woese. At the time, about eight species of methanogens were known. Under the microscope, methanogens look similar to bacteria in size and shape; however, they produce methane gas, a biochemical characteristic shared by no other known organism. Like all microbiologists at the time, Wolfe wondered where this group of prokaryotic organisms fit in with other bacteria, so he sought Woese's help in hopes of gaining a better understanding of its biological relationships. Woese compared its rRNA to the many others he had amassed. His shocking results placed it in its own family tree. None of the oligonucleotides that he had come to recognize as specific for bacteria were present in the methanogens. The differences were so great, it belonged in a completely separate kingdom that he tentatively called Archaebacteria (now just Archaea). He announced, "These 'bacteria' appear to be no more related to typical bacteria than they are to eukaryotic cytoplasms" (Woese and Fox, 1977). His findings were not well received.

Biologists long held that all life-forms belonged to one of two lineages; they were either prokary-

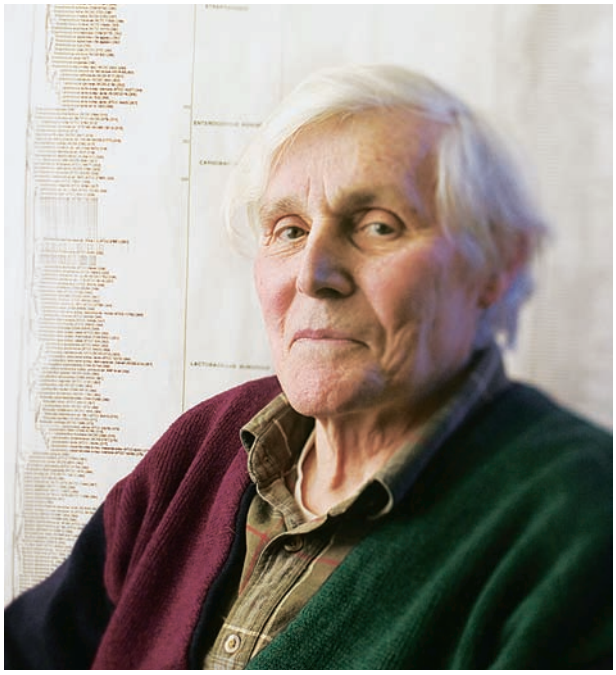
otic or eukaryotic. Many biologists believed Woese's announcement was preposterous, perhaps because Woese did not have the training of a typical microbiologist, but rather came to the field of microbiology via a detour through the field of physics. Others simply chose to ignore it rather than deal with its implications. Accepting the existence of a third lineage of life would force biologists to admit ignorance about their supposed area of expertise having completely missed out on one-third of all living things, overthrowing the established dogma, and rewriting all the textbooks. In retrospect, their lack of understanding of his technique could have been one major factor contributing to their lack of warm reception. The rejection hurt Woese, who was already shy, and for a long time, he remained a loner. Some thought his ideas were too eccentric to associate with him; others feared risking their own professional reputations. Very few stood by him.

In Germany, one respected microbiologist, Otto Kandler, found unique qualities to methanogen cell wall structure and lent his support to Woese. Kandler even organized the first scientific conference on Archaea in 1981. Over time, more molecular data that supported Woese's findings accumulated, and microbiologists discovered more archaean groups including the halophiles ("salt-lovers") and the thermoacidophiles (sulfur-metabolizing methanogens). Members of the domain Archaea often inhabit extreme environments, such as in hot sulfur springs or in volcanic vents. The conditions were once thought too harsh to support any type of life-forms, but these newly discovered organisms proved otherwise. By the mid-1980s, Woese finally began receiving recognition and praise for his contributions.

In 1990 Woese, Kandler, and Mark Wheelis (from the University of California at Davis) published



The tree of life Woese presented in 1990 shows that the prokaryotic domains Bacteria and Archaea dominate life on Earth.



Carl Woese proposed a taxonomic level higher than kingdom, the domain, composed of the Archaea, Bacteria, and the Eukarya. *(Courtesy Institute for Genomic Biology—Don Hamerman photo)*

"Towards a Natural System of Organisms: Proposal for the Domains Archaea, Bacteria, and Eucarya," formally outlining and justifying the taxon of domain, consisting of three listed groups, as his data suggested 13 years before.

In 1996 a team including Woese, University of Illinois professor Gary Olsen, and a team from the Institute for Genomic Research completed sequencing of the first archaean genome, that of *Methanococcus jannaschii*. Analysis of the genome structure confirmed Woese's conclusions from two decades before; the data revealed that archaea resembled eukaryotes as closely as they resembled prokaryotes from the domain Bacteria. This led to Woese's proposal of the theory of the universal ancestor, stating that the universal ancestor was not a single organism, but rather a group of loosely structured cells that existed together during a time when genetic mutation rates were high and the transfer of genes between cells occurred frequently, as the cells were not the structurally constrained entities that they have become. These groups of primitive cells, called progenotes, evolved together and eventually formed the three ancestral lineages. He published this idea in 1998 in the *Proceedings of the National Academy of Sciences,* and built upon it in 2002, when he claimed that life originated from three different types of simple cellular organizations that evolved into the three basic cell types—archaean, bacterial, and eukaryotic.

CONTRIBUTIONS RECOGNIZED

Woese's contributions to the life sciences have earned him numerous prestigious distinctions and honors. The John D. and Catherine T. MacArthur Foundation awarded him the "genius" award in 1984, and the National Academy of Sciences elected him to membership in 1988. In 1992 the Dutch Royal Academy of Science gave him the highest honor bestowed upon any microbiologist, the Leeuwenhoek Medal, awarded only once every 10 years. He was given the National Medal of Science in 2000 "for his brilliant and original insights, through molecular studies of RNA sequences, to explore the history of life on Earth." In 2003 the Royal Swedish Academy of Sciences awarded Woese the Crafoord Prize in Biosciences for his discovery of the third domain of life. The Crafoord award honors scientists whose work does not fall into any of the categories covered by Nobel Prizes. The Royal Society, the world's oldest continuously active scientific organization, elected Woese as a foreign member in May 2006.

Woese became associated with the prestigious Center for Advanced Study at the Urbana-Champaign campus in 1989. He currently holds the Stanley O. Ikenberry Endowed Chair. His recent work focuses on genomic analysis, in particular, the evolutionary significance of horizontal gene transfer. He hopes to outline the development of the three main ancestral cell types. Alongside his own studies, aimed at attaining a better understanding of the evolution of life, Woese has examined the evolution of the study of life. Sensing the inevitable end of the molecular era, he feels the life sciences need a new guiding paradigm. As a true scientist, Woese does not seek an easy path but remains more concerned with continued progress in his field.

See also ARCHAEA; BACTERIA (EUBACTERIA); BIOLOGICAL CLASSIFICATION; BROCK, THOMAS; EUKARYA; MARGULIS, LYNN; ORIGIN OF LIFE.

FURTHER READING

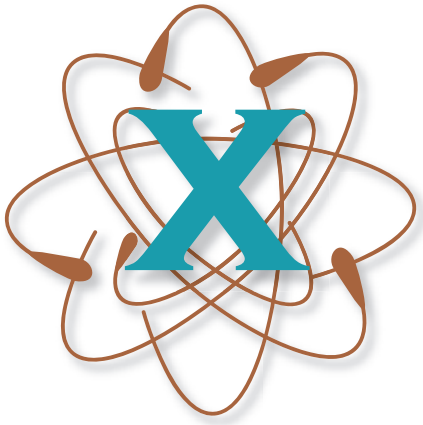
Anton, Ted. *Bold Science. Seven Scientists Who Are Changing the World.* New York: W. H. Freeman, 2000.

Morell, Virginia. "Microbiology's Scarred Revolutionary." *Science* 276 (1997): 699–702.

Woese, Carl R. "A New Biology for a New Century." *Microbiology and Molecular Biology Reviews* 68 (2004): 173–186.

Woese, Carl R., and George E. Fox. "Phylogenetic Structure of the Prokaryotic Domain: The Primary Kingdoms." *Proceedings of the National Academy of Sciences USA* 74 (1977): 5,088–5,090.

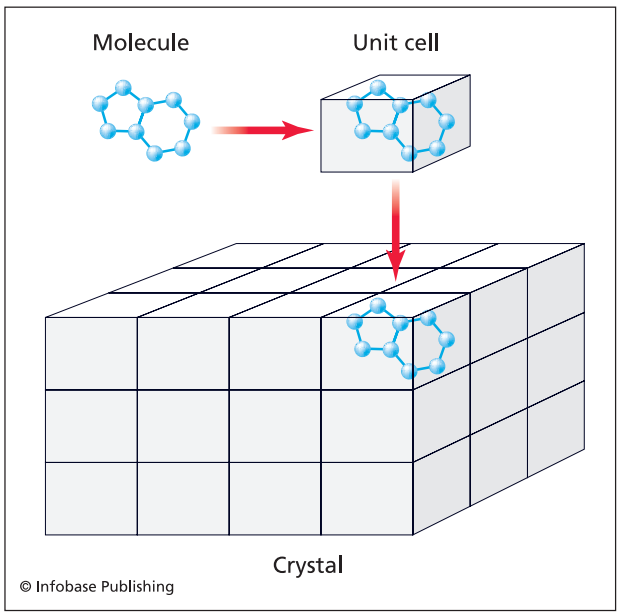
Woese, Carl R., Otto Kandler, and Mark. L. Wheelis. "Towards a Natural System of Organisms: Proposal for the Domains Archaea, Bacteria, and Eucarya." *Proceedings of the National Academy of Sciences USA* 87 (1990): 4,576–4,579.



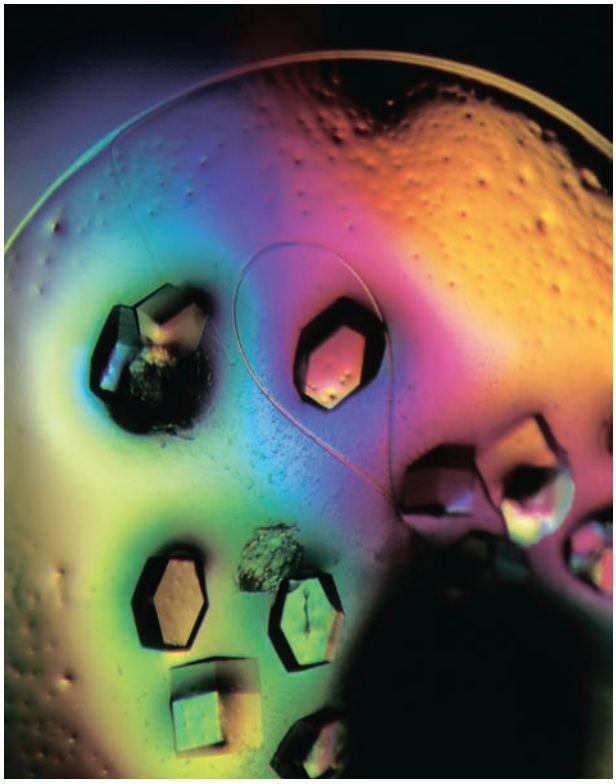
X-ray crystallography X-ray crystallography is a method for probing the molecular structure of a three-dimensional crystal using X-rays. The diffraction pattern of the X-rays allows for the determination of spacing of the atoms that compose the lattice of the crystal. X-ray diffraction of crystals is useful in inorganic chemistry for determining lattice structures, chemical formulas, bond lengths, and bond angles. In life science research, this method provides one of the highest resolutions for structural determinations of biological molecules such as proteins, deoxyribonucleic acid, ribonucleic acid, and macromolecular assemblies such as viral particles. X-rays are a form of electromagnetic radiation, just like visible light; however, the wavelength of X-rays is similar to the length of chemical bonds, thus X-rays can be used to probe structures at the molecular level. Humans cannot "see" molecular structures because vertebrate eyes have evolved to detect electromagnetic radiation with wavelengths in the visible range, which is much longer than the wavelength of X-rays.

The utility of this technique is evident by the number of Nobel Prizes awarded for research related to the ultimate development of methodology for probing the structures of biomolecules. The German physicist Max von Laue was the first to discover the phenomenon of X-ray diffraction by crystals, an accomplishment for which he won the Nobel Prize in physics in 1914. The following year, the British father-and-son team of Sir William Henry Bragg and William Lawrence Bragg won the Nobel Prize in physics for developing and simplifying the methodology used to apply X-ray diffraction to the study of structures. Two more British researchers, Max Perutz and Sir John Kendrew, won the Nobel Prize in chemistry in 1962 for their elucidation of the molecular structures of hemoglobin and myoglobin, the first protein structures to be solved using X-ray crystallography, a remarkable feat considering the complexity of proteins and the time-consuming, tedious mathematical calculations. Hemoglobin, a relatively small protein, binds and transports oxygen inside red blood cells, and myoglobin, one-fourth the size of hemoglobin, stores oxygen in muscle cells.

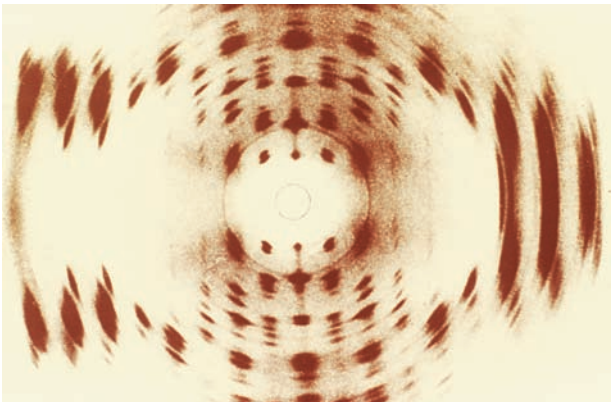
The first step is to crystallize the protein in order to increase the scattering power of the molecule, or to amplify the scattered waves. A crystal forms when the protein molecules in a very concentrated solution



Crystals are solids in which the constituent molecules pack into regular, repeated arrangements, forming a three-dimensional lattice.



Crystals, such as those made of the protein lysozyme shown here, can be used to probe the molecular structure of biomolecules by X-ray diffraction. *(Volker Steger/Photo Researchers, Inc.)*



Interaction of the photons from an X-ray beam with electrons from the atoms of the crystal creates a distinct pattern of spots that reveal information about the distribution of the atoms in the crystal. This is an X-ray diffraction image of a DNA molecule. *(Omikron/Photo Researchers, Inc.)*

solidify into an ordered, repeated arrangement of identical unit cells. To use for X-ray diffraction, the crystals must be high quality, thus the solution must be very pure, and the process must occur slowly, to ensure that the atoms throughout the crystal have the chance to settle precisely into position, so that the arrangement is exactly repeated throughout the entire crystal. This often takes numerous attempts using buffers of different pHs, containing different salts, at different temperatures. Biochemists use a variety of methods for growing crystals, each having advantages and disadvantages. Examples include vapor diffusion, batch crystallization, microbatch crystallization, macroseeding, microseeding, free interface diffusion, and dialysis. The procedure must be gentle enough to preserve the native conformation of the structure, as understanding the structure gives insight into the protein's function. The prepared crystal must then be mounted onto a glass fiber. Grease, an epoxy resin, or superglue will hold the crystal to the fiber, but the combination of oil and liquid nitrogen will also minimize radiation damage to the crystal, allowing one to use the same crystal for several measurements. Also, cooling the crystal decreases thermal motion, motion of the molecules in the crystal due to heat, so the image is higher in quality.

After being placed on the diffractometer, an instrument for measuring diffraction, an X-ray beam is directed at the crystal. Electrons from the atoms within the crystal diffract or bend the rays, the paths taken by the photons. Multiple detectors arranged around the crystal record the scattering pattern, which a computer interprets using mathematics. The crystal is rotated and data is collected from each angle of rotation, allowing for calculation of coordinates in three dimensions by comparison of the series of images. Since electrons generally surround atomic nuclei uniformly, electron density maps created from this information reveal the position of the atoms with respect to the distribution of atoms in space, the distance and bond angles, and overall structure.

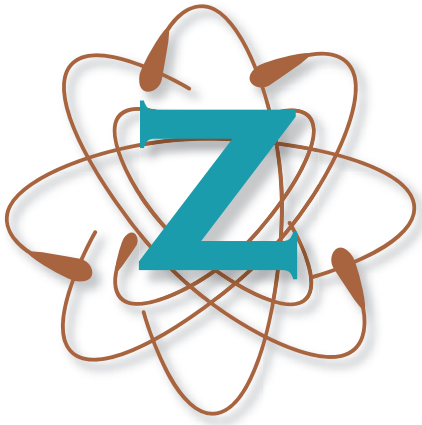
See also BIOMOLECULES.

FURTHER READING

Massa, Werner. *Crystal Structure Determination*. 2nd ed. Translated by Robert O. Gould. New York: Springer, 2004.

McPherson, Alexander. *Introduction to Macromolecular Crystallography*. Hoboken, N.J.: Wiley-Liss, 2003.

Hammond, Christopher. *The Basics of Crystallography and Diffraction*. 2nd ed. New York: Oxford University Press, 2001.



zoology Zoology is the branch of life sciences concerned with animals, including their classification, characteristics, distribution, and evolution. From the dawn of the human species, people have been intrigued by animal life, at first because survival depended on their knowledge of animal behavior for successful hunting and avoiding dangerous situations. Thus zoology is considered one of the oldest branches of the natural sciences. As society domesticated more animals and the issues of hunting and safety became only part-time considerations, people maintained a keen interest in animals; they recognized many similarities and began to appreciate differences between themselves and other animal forms. Modern zoological research is fueled by a desire to better understand the phenomenon of life, the mechanisms that sustain life, and the evolutionary history and relationships between different types of animals.

Because it includes the human species, the kingdom Animalia is the most familiar eukaryotic limb of the evolutionary tree of life. One characteristic that distinguishes animals from organisms belonging to the other eukaryotic kingdoms (traditionally Protista, Fungi, and Plantae) is their means for obtaining nutrition—animals eat other living organisms. Some animals ingest only plants and plant products while others eat only animals, but many animals consume a combination of the two. Smaller animals often depend on microscopic life-forms such as those composing plankton—bacteria, algae, protozoa, and tiny invertebrates. But all animals must take in their main nutrients as part of preformed organic compounds such as sugars or amino acids.

Two theories that guide research in the discipline of zoology are the theory of evolution and the chromosomal theory of inheritance. The theory of evolution unifies all of the biological sciences by providing a central theme or focus. In summary, all living organisms share common ancestry. Over millions and billions of years life-forms have evolved, and they continue to do so, through alterations that occur at the genetic level. This process results in the origin of new and diverse species. The chromosomal theory of inheritance explains the mechanisms behind the amazing diversity of animal life, while at the same time it demonstrates that at the cellular and molecular levels, all animals from sea cucumbers to elephants are remarkably similar, providing a commonality between all living things.

Modern zoology encompasses a broad range of diverse topics that can be grouped in many different ways. Animals exhibit all the qualities typically associated with life. They are chemically unique, possess complex hierarchical organizations with new properties emerging at each level, have mechanisms for perpetuating their species, carry genetic information that passes from generation to generation, undergo metabolism, have characteristic life cycles, and interact with and respond to their environments. Zoology covers all aspects of these defining characteristics of life as they relate to animals. Research in zoology often focuses on one particular aspect of animal life, such as physiology (the study of the functions and activities of an organism), genetics (the study of heredity and variation between organisms), or ecology (the study of the interactions between organisms or between organisms and their environment). Zoology can also be divided into different subdisciplines based on different species: for example, herpetology is the study of reptiles; entomology is the study of

insects; and ornithology is the study of birds. The categories can be broader; invertebrate zoology is the study of invertebrate animals and marine zoology focuses on animals that live in the sea. A field of research can also encompass numerous different types of animals while focusing on a specific level of hierarchical organization: molecular, cellular, organismal, population, community, or ecosystem.

The applications of zoological research affect numerous aspects of society ranging from health care and industry to education and politics. Questions addressed include: how do organisms regulate their internal temperatures? What factors influence the differentiation of cells during embryogenesis? Can closely related species occupy the same habitat? What types of drugs can selectively treat a helminth (parasitic worm) infection while causing minimal harm to the host? What effect does global warming have on coral reef communities? What can zebrafish teach scientists about cancer?

An interest in zoology sometimes leads people into veterinary medical careers. Many zoologists perform basic research, research directed toward a greater understanding of a subject or process, while other zoologists carry out research with an immediate direct application. Zoologists can work in a laboratory, where they are able to control many aspects of their studies, or in the field, where animals can be observed in their natural environments. Zoos, parks, fisheries, wildlife refuges, and museums hire zoologists to work with, care for, study, and document animal health, behavior, changes, and interactions. Biomedical research institutions and industries also employ zoologists, as these facilties often depend on animals for testing new medical treatments or commercial products for effectiveness and safety, a process that, while highly controversial, saves countless lives. Governmental agencies seek, from zoologists, advice regarding policies that have the potential to affect wildlife. People who have expert knowledge in zoology and good written communication skills are needed to author science texts and articles for the general public to educate them about the life-forms with which society shares its resources.

See also ANIMAL FORM; BIOLOGICAL CLASSIFICATION; BIOLOGY; CHROMOSOMES; EUKARYA; EVOLUTION, THEORY OF; INVERTEBRATES; VERTEBRATES.

FURTHER READING

Cobb, Alan, ed. *Animal Sciences: Macmillan Science Library*. 4 vols. New York: Macmillan Reference USA, 2002.

Miller, Stephen A., and John P. Harley. *Zoology*. 7th ed. New York: McGraw Hill, 2007.

APPENDIX I
CHRONOLOGY

ca. 350 B.C.E. Aristotle attempts to classify all animals. Theophrastus describes about 500 species of plants

ca. 50–70 Pliny the Elder publishes *Historia naturalis* in 37 volumes

ca. 200 Galen describes human anatomy (based on his dissections of other animals)

ca. 1235 Roger Bacon emphasizes the importance of experimentation

1539 Heironymus Bock (Jerome Bock) attempts the first natural classification of plants

1543 Andreas Vesalius writes a textbook on human anatomy, *Fabrica*

1546 Hieronymus Fracastorius (Girolamo Fracastoro) publishes the first work on contagious infection

1590–95 Zacharias Janssen makes the first microscope

1626 Francesco Redi proposes that living organisms do not arise by spontaneous generation

1628 William Harvey publishes *On the Motion of the Heart and Blood in Animals*, summarizing his revolutionary ideas, including the theory that blood circulates throughout the body

1648 Jean Baptiste Van Helmont publishes *Ortus medicinae*, concluding that plants derive nutrition from water, coins the word *gas*, and describes the properties of carbon dioxide

1658 Jan Swammerdam observes red blood cells under the microscope

1661 Marcello Malpighi performs extensive studies of anatomy and embryology and discovers capillaries in frog lungs

1665 Robert Hooke publishes drawings of cells from cork and other biological specimens in *Micrographia*

1668 Francesco Redi publishes *Observations on the Generation of Insects*, in which he concludes flies hatch from eggs laid on decaying meat rather than arise spontaneously from it

1669 Swammerdam describes the metamorphosis of insects as support for the doctrine of preformation

1674 Antoni van Leeuwenhoek discovers microorganisms while observing pond water under a microscope

1682 John Ray defines the concept of a species and describes monocots and dicots in *Methodus plantarum novae*

1686–1704 Ray publishes *Historia plantarum* in three volumes

1736 Carl Linnaeus publishes the first of 12 editions of *Systema naturae*, a book that outlined a system for classification of plants, animals, and minerals

1738 Swammerdam posthumously publishes his description of cleavage in a frog egg

1749–1804 Georges Louis-Leclerc, comte de Buffon, states in *Histoire naturelle* that species are mutable

1753 Carl Linnaeus publishes *Species plantarum*, introducing bionomial nomenclature

1754 Joseph Black discovers "fixed air" (carbon dioxide)

1769 Lazzaro Spallanzani performs experiments that show microorganisms do not arise by spontaneous generation in solutions that have been boiled and placed in sealed bottles

1771 Joseph Priestley discovers that plants use carbon dioxide and create oxygen

1778 Antoine Lavoisier describes respiration in animals. Spallanzani demonstrates that semen is necessary for fertilization

1779 Jan Ingenhousz publishes *Experiments upon Vegetables,* establishing that leaves are the primary site of photosynthesis

1796 Edward Jenner introduces a vaccine against smallpox. Ingenhousz publishes *An Essay on the Food of Plants and the Renovation of Soils,* concluding that plants use carbon dioxide for nutrition and that they carry out both respiration and photosynthesis

1798 Thomas Malthus publishes *Essay on the Principle of Population,* which later stimulates the development of the theory of natural selection by Charles Darwin and Alfred Russel Wallace

1800–02 Jean-Baptiste Lamarck elaborates his theory of evolution based on the inheritance of modified traits

1802–05 John Dalton develops the atomic theory

1805 Georges Cuvier founds the study of comparative anatomy

ca. 1815 Robert Brown distinguishes angiosperms from gymnosperms

1822 Étienne Geoffroy Saint-Hilaire suggests that arthropods are simply upside-down chordates. Lamarck distinguishes between vertebrates and invertebrates in his *Natural History of Invertebrates*

1824 Rene Dutrochet claims that the cell is the fundamental element in the structure of living bodies

1828 Karl Ernst von Baer publishes *The Embryology of Animals,* opposing the doctrine of preformation, after discovering that mammals develop from eggs. Robert Brown describes Brownian motion

1830 Cuvier and Geoffroy Saint-Hilaire debate the existence and nature of homology. Charles Lyell publishes *Principles of Geology,* establishing uniformitarianism as a major theory of geology

1831–33 Brown discovers nuclei while studying orchid cells

1831–36 Charles Darwin collects evidence supporting his theory of evolution while traveling around the globe on HMS *Beagle*

1835–39 Hugo von Mohl describes aspects of mitosis in plants

1836 Theodor Schwann discovers pepsin, the first enzyme to be isolated

1838 Matthias Schleiden observes that cells occur throughout plants, rather than simply in the stems and roots

1839 Schwann claims that animals are composed of cells and formalizes his and Schleiden's ideas into the cell theory

1843 Richard Owen distinguishes homology and analogy

1847–49 Ignaz Semmelweis demonstrates that hand washing reduces the incidence of puerperal fever

1856 Louis Pasteur describes fermentation by microorganisms

1857 Rudolf Albcht von Körlliker describes "sarcosomes" (mitochondria) in muscle cells

1858 Rudolf Virchow states that cells arise only from other cells. Darwin and Alfred Russel Wallace jointly propose the theory of natural selection

1859 Charles Darwin publishes *On the Origin of Species by Means of Natural Selection, or the Preservation of Favored Races in the Struggle for Life*. Louis Pasteur disproves the notion of spontaneous generation

1860 Famous debate on evolution by Thomas Henry Huxley and Bishop Samuel Wilberforce takes place at Oxford

1864 Ernst Haeckel lays the foundation for modern zoological classification in his two-volume work, *Generelle Morphologie der Organismen,* in which he proposes a new kingdom, Protista

1865 Gregor Mendel presents landmark studies of the inheritance patterns of *Pisum* characteristics

1865–69 Pasteur links certain microorganisms to specific diseases in silkworms

1866 Haeckel coins the term *ecology* to mean the study of living organisms and their interactions with the environment and states his famous biogenetic law, that ontogeny recapitulates phylogeny

1867 Joseph Lister publishes a book that initiates the widespread use of aseptic techniques during surgeries

1869 Dmitry Mendeleyev's discovery of periodic law is presented to the Russian Chemical Society. Johann Friedrich Miescher discovers acids that he calls nuclein in the nucleus of cells while studying pus

1871 Johann Friedrich Miescher isolates nuclein from the nuclei of white blood cells. Darwin publishes *Descent of Man,* describing the role of sexual selection

1872 Ferdinand Julius Cohn coins the term *bacterium*

1872–76 Sir C. Wyville Thomson directs the *Challenger* expedition, during which thousands of new marine organisms are discovered

1873 Camillo Golgi discovers the Golgi complex. Anton Schneider describes mitosis in animal cells

1876 Koch provides evidence that supports *Bacillus anthracis* as the causative agent for anthrax. Oscar Hertwig observes and describes the fusion of two germ cell nuclei in sea urchin eggs

1877 Pasteur provides definitive evidence that *Bacillus anthracis* causes anthrax

1879–82 Walther Flemming observes and describes the behavior of chromosomes during mitosis

1881 Pasteur develops the first vaccine, directed against anthrax in animals. Koch introduces the use of pure culture techniques for handling cultures in the laboratory and demonstrates *Mycobacterium tuberculosis* causes tuberculosis

1882 Élie Metchnikoff discovers phagocytosis while observing starfish larvae. Koch demonstrates that *Vibrio cholera* causes cholera and describes his methodology for isolating bacteria in pure culture

1883 Edouard Van Beneden clearly describes the actions of chromosomes during meiosis

1884 Robert Koch summarizes his postulates for determining the causative agent of a disease. Christian Joachim Gram invents a staining method that differentiates between types of bacteria based on structural differences in the cell wall. Metchnikoff proposes the cellular theory of immunity

1886–90 Theodor Boveri publishes idea that chromosomes persist throughout the cell cycle and that sperm and egg contribute equal numbers of chromosomes during fertilization

1892 Dmitri Ivanovsky discovers the first virus, tobacco mosaic virus, and calls it a "filterable pathogen." August Weismann observes meiosis and proposes the germ-plasm theory of heredity

1894 Emil Hermann Fischer proposes the lock-and-key model for enzyme action. William Bateson publishes *Materials for the Study of Variation,* which states that many traits arise as discontinuities

1895 Boveri shows that the nucleus, and not the cytoplasm, determines the hereditary potential of an organism

1897 Sir J. J. Thomson discovers the electron and proposes the plum pudding model for the atom. Henry C. Cowles develops his concept of plant succession. Eduard Buchner discovers that extracts from yeast can carry out fermentation

1898 Camillo Golgi describes the Golgi apparatus

1899 Martinus Beijerinck independently confirms Ivanovsky's finding that tobacco mosaic disease is caused by something smaller than a bacterium and calls it a virus

1900 Hugo de Vries, Carl Correns, and Erich von Tschermak independently rediscover Mendel's 1865 findings on patterns of inheritance

1902 William Bateson publishes *Mendel's Principles of Heredity: A Defense.* Emil Fischer determines that proteins consist of amino acids. Ivan Pavlov

formulates his law of learning by conditioning

1902–03 Walter Sutton and Theodor Boveri independently suggest that the behavior of chromosomes during meiosis explains Mendel's rules of inheritance

1905 Bateson coins the term *genetics*. Nettie Stevens identifies male and female sex chromosomes in insects

1908 Thomas Hunt Morgan initiates experiments using fruit flies to study the transmission of heritable traits. Paul Ehrlich uses salvarsan to treat syphilis

1910 Francis Rous discovers viruses that can cause cancer

1911 Morgan proposes that genes lie on chromosomes in a linear manner

1913 Alfred Sturtevant creates the first genetic map containing six genes from *Drosophila*

1914–30s Karl von Frisch pioneers the practice of field experiments for studying animals while studying honeybees

1916 Morgan, Sturtevant, Hermann Muller, and Calvin Bridges publish *The Mechanism of Mendelian Inheritance*

1917 Joseph Grinnell coins the term ecological niche

1918 Influenza kills 20 million people across the world

1921 Sir Frederick Banting and Charles Best discover the antidiabetic properties of insulin

1922 J. B. S. Haldane describes the law of gene linkage (later referred to as Haldane's law)

ca. 1924 Theodor Svedberg develops the first analytical ultracentrifuge

1925 John Scopes is arrested for teaching evolution in school, stimulating national debate in the United States

1926 Muller shows that X-rays cause mutations in the DNA of *Drosophila*

1928 Frederick Griffith publishes his studies on the transformation of the bacteria *Streptococcus pneumoniae*

1929 Sir Alexander Fleming publishes his research on the antibacterial properties of penicillin. Hans Fischer solves the structure of heme

1931 Ernst Ruska and Max Knoll make the first electron microscope

1931–43 Carl and Gerty Cori elucidate the mechanisms by which glucose is converted to muscle glycogen and vice versa

1935 Gerhard Domagk publishes his discovery of the first sulfa drug. Arthur George Tansley coins the term *ecosystem*, bridging the fields of ecology, physics, chemistry, and others. Konrad Lorenz publishes the first of several major studies that result in the founding of the study of animal behavior

1937 Theodosius Dobzhansky links evolution by natural selection and genetic mutation in *Genetics and the Origin of Species*. Sir Hans Krebs elucidates the tricarboxylic acid cycle (also known as the Kreb's cycle)

1939 Linus Pauling publishes *The Nature of the Chemical Bond and the Structure of Molecules and Crystals: An Introduction to Modern Structural Chemistry*

1940 Fritz Lipmann proposes that ATP carries energy in cells. Sir Howard Florey and Ernst Chain perform first clinical trials with penicillin

1941 George Beadle and Edward Tatum propose "one gene, one enzyme" hypothesis

1942 Raymond Lindeman analyzes trophic relationships

1944 Oswald Avery, Colin Macleod, and Maclyn McCarty demonstrate that DNA is the carrier of genetic information. Barbara McClintock discovers that genes can move from one location on a chromosome to another location on the same chromosome or on a separate chromosome

1945 Dorothy Hodgkin solves the structure of penicillin. Erwin Schrödinger publishes *What Is Life?*, a book that inspires many biologists

1946 Melvin Calvin begins studies that lead to the elucidation of the biochemical process of photosynthesis. Joshua Lederberg and Edward Tatum demonstrate genetic recombination in bacteria

1949 Erwin Chargaff describes the base composition of DNA, stating that, in DNA, the number of adenines equals the number of thymines and the number of cytosines equals the number of guanines. Pauling describes sickle-cell anemia as a molecular disease

1951 Nikolaas Tinbergen proposes an important role for instinct in animal behavior

1952 Alfred Hershey and Martha Chase demonstrate that DNA is the hereditary material in bacteriophage. Rosalind Franklin produces high quality X-ray diffraction images of DNA that play a key role in the discovery of the structure of DNA

1953 James D. Watson and Francis Crick announce the discovery of the double helical structure of DNA. Max Perutz and John Kendrew solve the structure of hemoglobin using X-ray diffraction studies. Stanley Miller shows that amino acids form from water, methane, ammonia, and hydrogen when struck with simulated lightning

1954 Jonas Salk develops the first polio vaccine

1955 Severo Ochoa discovers RNA polymerase. Arthur Kornberg discovers DNA polymerase. Frederick Sanger sequences the first protein, insulin

1957 Melvin Calvin outlines the carbon fixation reactions of photosynthesis

1958 Matthew Mesehlson and Franklin Stahl demonstrate that DNA replication is semiconservative

1960s A number of scientists discover ribonucleic acid (RNA) and determine its role in protein synthesis

1961 François Jacob and Jacques Monod publish a paper describing the repressor model of genetic regulation. Mary Lyon publishes a paper suggesting one X chromosome becomes inactivated in females

1962 Max Perutz solves the structure of the protein hemoglobin. Rachel Carson publishes *Silent Spring*, a book that stimulates the environmental movement

1964 Keith Porter and Thomas Roth discover the first cell receptors. W. D. Hamilton formalizes the theory of kin selection, which explains certain social behaviors of animals

1965 Cambridge Instruments makes the first commercial scanning electron microscope

1966 Marshall Nirenberg, Robert Holley, and Har Gobind Khorana finish deciphering the genetic code

1967 John Gurden clones the first vertebrate, a frog

1968 Frederick Sanger uses radioactive phosphorus to determine the sequence of a piece of RNA 120 nucleotides long

1969 Dorothy Hodgkin discovers the structure of insulin. Robert Whittaker institutes a five-kingdom classification system

1970 Hamilton Smith and Daniel Nathans discover restriction enzymes. Howard Temin and David Baltimore independently discover the retroviral enzyme reverse transcriptase. Lynn Margulis proposes the endosymbiotic theory for the origin of cellular organelles

1971 Günter Blobel proposes the signal hypothesis to explain how cells sort proteins

1972 Stephen Jay Gould and Niles Elderidge propose the theory of punctuated equilibrium as a method for evolutionary events. Paul Berg and colleagues combine DNA from different species, making the first recombinant DNA, and insert it into a host cell. S. J. Singer and G. L. Nicholson describe the fluid mosaic model for cell membranes

1973 Herbert Boyer and Stanley Cohen clone the first DNA from a frog into bacteria using plasmids. The bacteria reproduce the DNA, marking the beginning of genetic engineering

1975 César Milstein, Georges Kohler, and Niels Kai Jerne develop a technique for making monoclonal antibodies. Participants of the Asilomar conference urge the government to develop guidelines for working with recombinant DNA. Edward O. Wilson publishes the influential text *Sociobiology*

1977 Robert Ballard and his team discover chemosynthetic communities surrounding hydrothermal vents. Carl Woese proposes a third domain of life, Archaea. Walter Gilbert and Allan Maxam describe a method for determining the sequence of DNA molecules. Frederick Sanger describes an alternative technique for sequencing DNA using dideoxynucleotides

1978 Sanger sequences the first complete genome, the 5,386-base sequence for the PhiX174 virus. Recombinant human insulin is produced

1981 The first transgenic mice and fruit flies are produced

1982 Stanley Prusiner describes infectious particles that he calls prions. A new syndrome characterized by a severely impaired immune system is identified and named acquired immunodeficiency syndrome (AIDS)

1983 Kary Mullis first conceptualizes the idea of polymerase chain reaction. Luc Montagnier and Robert Gallo isolate and characterize the human immunodeficiency virus

1984 Alec Jeffreys describes the technique of genetic fingerprinting

1989 J. Michael Bishop, Robert Huber, Hartmut Michel, and Harold Varmus characterize oncogenes, genes that cause cancer. The Human Genome Project commences

1990 W. French Anderson performs the first trial of human gene therapy to treat a child with an immune disorder

1993 First human cloned embryos are produced

1994 A gene that causes human breast cancer is isolated

1995 Craig Venter and colleagues at the Institute for Genomic Research sequence the first whole bacterial genome (*Haemophilus influenzae*)

1996 The complete *Escherichia coli* genome is sequenced

1997 Ian Wilmut and colleagues clone the first mammal, a sheep named Dolly, from adult somatic cells

1998 First mice cloned from somatic cells is reported. The first complete genome sequence of a multicellular organism, the roundworm *Caenorhabditis elegans,* is published. Andrew Fire and Craig Mello discover RNA interference

1999 The *Drosophila* genome sequence is published

2001 First rough map of the human genome is 90 percent completed

2002 Mouse genome sequence is completed. Eckard Wimmer and colleagues create a live polio virus from scratch

2003 The Human Genome Project is completed

2004 Scientists complete a more refined analysis of human genome sequences

2007 J. Craig Venter publishes the sequence of his genome, the first complete diploid human genome sequence

2008 Venter synthesizes an entire genome from the bacterium *Mycoplasma genitalium* from scratch

Appendix II
Glossary

abiotic not involving or not produced by a living organism

absorption the stage of digestion during which cells uptake small molecules from the digestive compartment

abyssal relating to the broad, flat expanse of ocean floor at depths between 10,000 and 20,000 feet (3,000–6,000 m)

acid a substance that releases hydrogen ions into a solution

acoelomate an animal with no body cavity

acquired immunodeficiency syndrome (AIDS) a life-threatening disease of the human immune system, caused by infection with the human immunodeficiency virus and characterized by a reduction in the numbers of CD4 T helper cells

actin a contractile protein that forms microfilaments in muscle cells

Actinopterygii ray-finned fishes, the dominant aquatic vertebrates today

action potential a temporary reversal in the membrane potential across the membrane of an excitable cell caused by the opening of voltage-gated sodium and potassium channels

activation energy the energy barrier that separates the reactants from the products of a chemical reaction. Enzymes lower the activation energy in order for a reaction to proceed, resulting in an increase in the reaction velocity

active site on an enzyme, the site to which the substrate specifically binds

active transport the transport of a substance across a biological membrane against its concentration gradient with the expenditure of energy

adaptation a modification of an organism that increases its fitness in a particular environment

addiction the compulsive need for a habit-forming substance, often accompanied by tolerance and physical dependence

adenine a nitrogenous base that is a component of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)

adenosine triphosphate (ATP) the most useable form of energy inside the cell consisting of a ribose sugar, an adenine, and three phosphate groups linked together by two high-energy phosphoanhydride bonds

affinity the attractive force of one molecule, atom, or electron for another

affinity chromatography a type of chromatography that separates molecules in a solution based on their different degrees of chemical attraction for a specific substrate, ligand, or other molecule that has been linked to the resin of the column

agar a component that is extracted from the cell walls of some red algae and is used as a solidifying agent in microbiological culture media

agarose a polysaccharide purified from agar that is used as a semisolid matrix for gel electrophoresis and as a chromatography resin

aging the period of deterioration of the physical condition of a living organism that leads to death

agnathan informal term meaning jawless fishes

agricultural science the multidisciplinary field involving aspects of the natural, social, and economic sciences that are used in agriculture

agriculture the practice of cultivating the soil, producing crops, and raising livestock for market

agronomy a branch of agriculture concerned with crop production and soil management

algae a group of chlorophyll-containing, photosynthetic protists that exhibit a wide variety of forms and complexity

allantois one of four extraembryonic membranes in reptiles, birds, and mammals that serves as a receptacle for the embryo's nitrogenous waste

allele an alternate form of a gene that produces a different phenotype

allergen an antigen that induces an allergic response (a type I hypersensitivity)

allopatric speciation the origin of new species while geographically separated

allostery the characteristic of a protein (usually an enzyme) in which a change in the conformation

and therefore activity results from the binding of a molecule to the protein at a site other than the catalytic site

alternation of generations a reproductive process in which two forms, diploid and haploid, occur alternately

altruism behavior by an animal that appears to reduce their individual fitness, but that improves the benefits of other individuals of its species

alveolus a small sac at the terminus of a bronchiole that fills with air during inhalation and is surrounded by numerous capillaries across which gas exchange occurs with the inhaled air

amino acid the basic building block of proteins. There are 20 naturally occurring amino acids

amnion the innermost of four extraembryonic membranes forming a fluid-filled sac that encloses the embryo

amniote an animal that develops an amnion during its embryonic stage, including birds, reptiles, and mammals

amoeba a type of protozoan characterized by pseudopodia; member of the phylum Sarcodina

Amphibia a class of the phylum Vertebrata consisting of cold-blooded animals that often spend their larval stage in freshwater and, after metamorphosis, live on land

amplexus the mating embrace of a frog or toad during which the female sheds eggs into the water, where they are fertilized

anabolism the metabolic processes involved in the synthesis of new molecules, usually requiring the input of energy

analogous structures parts of different organisms that have similar structure and function but that evolved independently

anaphase the stage of mitosis after metaphase and before telophase during which sister chromatids of chromosomes separate and are pulled toward opposite ends of the cell

anapsid an amniote of the lineage consisting of turtles and that is characterized by the absence of temporal openings in the skull

anatomy the study of the shape and structure of an organism at both the visible level and the microscopic level

angiosperm flowering plant

animal any member of the kingdom Animalia

animal behavior a branch of zoology concerned with answering the how and why of behavioral traits; ethology

Animalia a kingdom consisting of organisms that are multicellular, lack cell walls, lack chlorophyll, require more complex food materials, and have the capacity for spontaneous movement and rapid motor responses to stimulation

anion a negatively charged ion or molecule

annelid a member of the invertebrate phylum that includes elongated, segmented, coelomate worms

anorexia nervosa a dangerous eating disorder characterized by an irrational fear of weight gain, obsessive eating habits, and usually extreme weight loss

anther the terminus of a stamen's filament, divided into pollen sacs in which pollen grains form

antheridia the male-gamete-producing organs of a plant

anthropology the scientific study of humankind and culture around the world and throughout time

antibiotic a chemical that kills bacteria or inhibits their growth, produced by or derived from another living organism

antibody a large protein molecule produced by B lymphocytes in response to activation by recognition of a specific antigen. Antibodies, also called immunoglobulins, are specific for the antigen they recognize

antigen a foreign molecule that elicits an immune response

antioxidant a substance that helps prevent oxidation reactions by donating its own electrons to a free radical

anus the opening through which undigested material exits the body from the rectum

aphotic zone in a body of water, the region where little light penetrates

apical dominance a phenomenon in which the growth at a terminal bud keeps nearby axillary buds dormant

Apicomplexa a phylum containing nonmotile, parasitic protozoa, including members of the former class called Sporozoa

apoplast in plants, the space external to the plasma membrane through which solutes flow, including the cell walls of xylem vessels

arachnid a member of the arthropod class consisting of mostly terrestrial invertebrates such as spiders, scorpions, mites, and ticks

Archaea the domain of life including prokaryotic, single-celled organisms of primitive origin that live in harsh or extreme habitats

archegonium the vase-shaped, female-gamete-producing organ of a plant

archenteron the cavity lined with endoderm and that develops into the digestive tract of animals

arteries blood vessels that carry blood away from the heart to body tissues

arthropod a member of the invertebrate phylum that includes animals with segmented bodies, jointed appendages, a chitinous exoskeleton, and a dorsal anterior brain connected to a ventral chain of ganglia

artificial selection the purposeful breeding of plants and animals to increase the prevalence of desirable traits

Ascomycota a phylum of fungi whose members produce sexual spores called ascospores in tubular sacs called asci

ascus (pl. asci) a tubular spore sac found in members of the fungal phylum Ascomycota

aseptic technique a manner of handling sterile equipment, supplies, and media in a manner that prevents contamination with any undesired microorganisms

asexual reproduction any form of reproduction involving only one parent and resulting in offspring that are genetically identical; includes binary fission, spore-formation, and budding

assisted reproductive technology (ART) procedures involving the retrieval of eggs and the manipulation of the eggs and sperm outside of the body in order to help a woman conceive; includes in vitro fertilization, gamete intrafallopian transfer, zygote intrafallopian transfer, and intracytoplasmic sperm injection

associative learning the type of learning acquired by the association or connection of two stimuli or a stimulus and a consequence

atmosphere the mass of air surrounding Earth

atom the smallest unit of an element that retains the properties of that element, consisting of a central nucleus that contains protons and neutrons surrounded by orbiting electrons

atrium one of the two upper chambers of the heart that receives blood from veins and passes the blood into a ventricle

australopiths extinct hominids of the genus *Australopithecus* that lived between 4 and 2 million years ago and had many near-human features, but had small brains

autocrine a type of cell-signaling in which a gland secretes a chemical substance that acts by binding receptors located on the surface of its own cells

autoimmunity a condition in which the body elicits an immune response against self-antigens, molecules that are a normal part of the body

autosome a chromosome that is not a sex chromosome

autotroph an organism that utilizes carbon dioxide or carbonates as its sole source of carbon and has the ability to synthesize organic molecules using energy from the sunlight or from the oxidation of inorganic substances

auxin a type of plant hormone that promotes cell elongation in plant shoots and also regulates other growth processes, such as root initiation

Aves the vertebrate class consisting of birds

axon an extension of a neuron that carries nervous signals from the cell body toward the target cells

bacteria prokaryotic, unicellular organisms with peptidoglycan in their cell walls and circular chromosomes. When capitalized (Bacteria), refers to one of the three domains of life

bacteriophage a virus that infects bacteria

barrier reef a coral reef that grows parallel to the shore but is separated from it by a strip of water

basal body a eukaryotic organelle formed from microtubules that plays a role in assembling cilia or flagella; or, the part of a prokaryotic flagellum that anchors the filament of the flagellum to the cell wall and plasma membrane

base a substance that releases hydroxide ions into a solution or that draws hydrogen ions out of solution

base pair two nucleotides held together by hydrogen bonds. In DNA, adenine forms a base pair with guanine, and cytosine forms a specific base pair with thymine

Basidiomycota a phylum of fungi whose members produce sexual spores called basidiospores in structures called basidia

basidium (pl. basidia) the sexual reproductive structure of the fungi belonging to the phylum Basidiomycota

basophil a type of white blood cell that contains granules of histamine and other chemical mediators involved in the inflammatory response

bathysphere a sphere-shaped diving vessel used for deep-sea observations

benthic relating to the seabed

bilateral symmetry an animal body plan exhibiting distinct right and left halves

binary fission a means of asexual reproduction in which one parent cell divides into two equally sized daughter cells

binge-eating disorder a condition characterized by the frequent ingestion of large amounts of food while feeling a loss of control over eating

binomial nomenclature the system of giving two names to a living organism, consisting of a generic name (genus) and a species name

biochemistry the study of chemical compounds and chemical processes that occur in living organisms or by living organisms

biodiversity also, biological diversity; the number and variety of different species in an environment

bioenergetics the study of biological processes involved in energy transformations and energy exchanges and transformations

biofuel a fuel composed of or made from biological materials, either living organisms or their metabolic products or waste

biogeochemical cycle a circular pathway involving biotic and abiotic components through which nutrients flow

bioinformatics a collection of methods for analyzing biological data (mostly molecular) using computers

biological oxygen demand (BOD) the relative amount of oxygen used by microorganisms in order to oxidize completely all of the organic and inorganic matter in water, thus serving as an indicator of water purity

biology the study of living organisms and life processes

bioluminescence light produced by a living organism

biomass collective dry weight of living organisms

biome any of the Earth's major ecosystems characterized by different types of vegetation and organisms that have adaptations suited to that particular environment, can be as large as an entire continent

biophysics the study of the physical principles involved in vital processes

bioremediation the use of microorganisms or natural biological processes to remedy an environmental problem, such as to clean up an oil spill

biosphere the entire portion of the Earth in which life is found; or, the collection of all the living organisms on the Earth

biotechnology the manipulation of living organisms or components of living organisms to carry out defined chemical processes for commercial applications; also, the techniques and applications used in such manipulations

biotic of or related to living organisms

bipedalism the characteristic of walking erect using only two feet

bivalve any of a class of mollusks that have a two-valve hinged shell, such as clams, oysters, mussels, and scallops

bladder a sac that stores urine prior to its exiting the body through the urethra

blade in plants, a leaf or the flat part of a leaf excluding the petiole, especially of an herb or grass; also the leaflike structure of a seaweed

blastocoel the cavity filled with fluid during the blastula stage of embryogenesis

blastocyst the modified blastula of a placental mammal having an outer layer consisting of the trophoblast

blastomere a cell in the developing embryo

blastopore the opening of the archenteron

blastula an early embryonic form characterized by a hollowed-out, fluid-filled cavity surrounded by a layer of cells

blood the substance in the arteries, veins, and capillaries that transports substances throughout the body

botany the scientific study of plants

Bowman's capsule the beginning portion of a nephron in the vertebrate kidney where the filtrate enters from the blood

brain part of the central nervous system in vertebrates or a concentration of nerves in invertebrates that integrates sensory information from inside and outside the body and controls involuntary actions (such as heartbeat and digestion), coordinates and directs motor control, and functions in thoughts and learning

brain stem part of the brain, found at the top of the spinal column, comprising the medulla, the pons, and the midbrain, and that controls many involuntary actions, such as breathing, heart rate, blood pressure, swallowing

bronchiole one of many smaller branches that extends from a bronchus and functions to transport air into an alveolus in the lungs

bronchus (pl. bronchi) one of the two tubes that branches from the trachea and transports air into the lungs

bryology the branch of botany concerned with the nonflowering plants comprising the mosses, liverworts, and hornworts

bryophyte a nonvascular plant that includes the phylum Bryophyta as well as the liverworts and the hornworts

bud on a plant, a small protuberance that may develop into a flower, leaf, or shoot; or, an outgrowth of an organism that can develop into a complete new organism or differentiate into a new structure on a developing organism

budding a form of asexual propagation characterized by the formation of outgrowths that pinch off of the parent and develop into new individuals or remain attached to form extensive colonies

buffer a substance that helps maintain a given pH by either accepting excess hydrogen ions or donating hydrogen ions to a solution

bulbourethral glands paired sex accessory glands located near the base of the penis in the human male that secrete a fluid into the semen during sexual arousal that lubricates the urethra and also neutralizes acids

bulimia an eating disorder characterized by compulsive eating followed by self-induced vomiting, laxative or diuretic abuse, or intense exercise

bundle-sheath cell a photosynthetic cell that is tightly packed around the veins of a plant leaf

C_3 pathway also known as the Calvin cycle, a carbon-fixation pathway in which the first product

of carbon fixation is the three-carbon molecule phosphoglyceraldehyde

C_4 pathway a carbon-fixation pathway in which the first product of carbon fixation is the four-carbon molecule oxaloacetate

caecilian a limbless, burrowing wormlike amphibian

calorie the amount of heat required at a pressure of one atmosphere to raise the temperature of one gram of water one degree Celsius

camouflage a type of disguise that causes one to blend in with the environment

CAM plants plants that use the C_4 pathway at night to fix carbon dioxide and the C_3 pathway in the daytime to synthesize sugars

cancer a tumor composed of cells that grow uncontrollably, can invade body tissues, and can potentially metastasize, or spread to other parts of the body

capillaries blood vessels that connect arteries and veins and have very thin walls through which diffusion of gases, nutrients, hormones, and other molecules occurs

capsid the protein coat of a viral particle made up of identical repeating subunits called capsomeres

capsomere an individual, repeating protein subunit that composes a viral protein capsid

capsule in microbiology, a substance surrounding some prokaryotic cells that is made of polysaccharides and that functions in attachment to surfaces and in avoiding phagocytosis by the immune system

carapace the dorsal part of the exoskeleton of some crustaceans, covering parts of the head and thorax

carbohydrate a type of biomolecule made of carbon, hydrogen, and oxygen; also called a sugar

carcinogen a substance that has been shown to cause cancer

carcinoma a cancer that originates from epithelial tissue

carnivore an animal that eats the flesh of other animals

carpel in a flowering plant, an ovule-bearing structure that makes up the innermost whorl of a flower

catabolism the metabolic processes involved in the breakdown of molecules into smaller parts, accompanied by the release of energy

catalyst something that enables a chemical reaction to occur at a faster rate or under different conditions. Within cells, chemical reactions are catalyzed by enzymes

cation a positively charged ion or molecule

cDNA library a collection of bacteria that contain plasmids with DNA inserts made from mRNA of a particular cell type, used in cloning experiments

cell the smallest structural unit of living matter capable of functioning independently, enclosed by a membrane and containing cytoplasm

cell cycle the sequence of events in a eukaryotic cell occurring between the formation of a new cell by division of its parent cell and the division of the new cell into two of its own daughter cells, composed of mitosis, G_1, S, and G_2 phases

cell membrane also called the plasma membrane, the phospholipid bilayer that surrounds a cell and acts as a selectively permeable barrier between the cell's interior and the external environment

cellular respiration catabolic reactions by which organisms generate ATP from energy stored in chemical compounds

cell wall protective layer external to the plasma or cell membrane, found in bacteria, plant cells, fungi, and some protozoans

centipede a member of the arthropod class Chilopoda that contains one pair of legs per segment and a pair of poisonous fangs

central nervous system the division of the vertebrate nervous system consisting of the brain and spinal cord

centriole also known as a basal body, the cellular structure that organizes the spindle fibers during mitosis and meiosis

centromere on a chromosome, the constricted region at a particular location where the kinetochore is found

centrosome a structure found in the cytoplasm that contains the centriole

Cephalaspidomorphi the class of the phylum Chordata that includes the jawless fishes called lampreys

cephalization in animal evolution, the development of an anterior structure (a head) containing a high concentration of sensory structures and nerves

Cephalochordata a small group of organisms of the phylum Chordata that includes the primitive chordates known as lancelets or amphioxus

cephalopod a member of the mollusk class Cephalopoda that includes squids, octopuses, and scuttlefishes

cercaria (pl. cercariae) a tadpole-shaped, larval trematode

cerebellum the part of the brain that controls muscle coordination and equilibrium, located between the brain stem and the back of the cerebrum

cerebrum the largest portion of the brain, involved in thoughts, memory, learning, and other mental activities

chelicerae the anterior, fanglike appendages of arachnids

Chelicerata the subphylum of the phylum Arthropoda that includes arachnids, horseshoe crabs, sea scorpions, and sea spiders

chelicerates arachnids that have fanglike feeding parts on their heads, includes spiders and scorpions

chemiosmosis a mechanism that uses energy stored in the form of a proton gradient to drive the synthesis of ATP by oxidative phosphorylation

chemolithotroph an organism that oxidizes reduced inorganic compounds as a source of energy and electrons and obtains carbon from carbon dioxide

chemoorganotroph an organism that oxidizes organic compounds as its source of energy

chemoreceptor a sensory organ that detects the presence of certain chemicals

chemosynthesis a process in which reduced inorganic compounds are oxidized to release energy to fix carbon and fuel the synthesis of organic compounds

chemotaxis movement of a cell or organism that occurs in response to a chemical

chemotherapy the use of chemicals in the treatment or control of disease

chemotroph an organism that obtains energy from the oxidation of chemical compounds

chiasmata a cross-shaped configuration of chromosomes that occurs during meiosis and is the physical equivalent of the genetic phenomenon of crossing over

chi-square test a type of statistical analysis performed to determine the probability of obtaining a set of observed results by chance alone given a specific hypothesis

chitin the polysaccharide from which arthropod exoskeletons and some fungal cell walls are formed

chlamydias a group of obligate, intracellular, parasitic bacteria

chlamydospore a thick-walled asexual fungal spore that develops within a hypha

chlorophyll a pigment that absorbs blue and red light, reflects green and yellow light, and is found in green algae and plants

chloroplast an organelle in plants and algae in which photosynthesis occurs

Chondrichthyes the chordate class containing cartilaginous fishes

Chordata a phylum of the kingdom Animalia consisting of animals that have at some time during their development, a notochord, a hollow dorsal nerve cord, pharyngeal slits, an endostyle, and a post-anal tail

chordate a member of the animal phylum Chordata

chorion the outer layer of the double membrane that surrounds amniote embryos (birds, reptiles, and mammals); in mammals it contributes to the formation of the placenta

chromatin the complex of DNA and protein that makes up eukaryotic chromosomes

chromatography a laboratory technique used to separate mixtures of molecules based on physical or chemical characteristics by passing a mobile phase containing the mixture through a stationary phase

chromomere a heavily stained mass of condensed chromatin occurring in a linear arrangement on a chromosome

chromosome a threadlike strand composed of DNA and protein that carries genetic information. Each species has a characteristic number of chromosomes

chylomicron a lipoprotein assembled from triglycerides and cholesterol during fat digestion in the epithelial cells of the small intestine and found in the bloodstream shortly thereafter

cilia short hairs on an organism that beat to create a water current or to swim

Ciliophora a phylum consisting of the ciliates, protozoa that have cilia

circulatory system a fluid-based system that transports oxygenated blood and nutrients throughout an animal's body

clade a taxonomic group that includes a common ancestor and all of its descendents

cladistics a phylogenetic classification system that uses shared characteristics and inferred evolutionary relationships to group organisms into taxa

classical conditioning a form of associative learning in which an animal develops a response to a previously neutral stimulus through repeated combined exposures of the neutral stimulus with a stimulus that naturally induces a response

cleavage a series of synchronized mitotic divisions that increases the number of cells and occurs immediately after fertilization during early embryogenesis

clone a molecule, cell, or organism that is genetically identical to a parent molecule, cell, or organism

Cnidaria an invertebrate animal phylum characterized by the presence of radial symmetry, specialization of tissues with no organization into organs, and the presence of cnidocytes; includes jellyfish, hydra, and sea anemones

cnidarian a member of the invertebrate phylum Cnidaria

cnidocytes stinging cells found on tentacles in cnidarians

coacervate an aggregate of colloidal droplets held together by electrostatic forces

coal a type of fossil fuel formed by intense heat and pressure acting on the organic remains of plant and algae over millions of years

codominance the phenomenon in which two alleles are both fully expressed in the heterozygous condition

codon a triplet of nucleotides that specifies a particular amino acid to be added during protein synthesis

coelom a fluid-filled body cavity

coelomate an animal with a true body cavity, one located entirely within the mesoderm

coenzyme a low-molecular weight organic molecule that is required by an enzyme to catalyze a biochemical reaction

coevolution reciprocal evolutionary change in interacting species

cofactor a nonprotein component of an enzyme that aids in catalysis, such as a metal ion or coenzyme

cognitive mapping the creation of an internal representation of the spatial relationships between objects within an environment

cohesion the state of sticking together

cohesiveness the property of having cohesion

coliform bacteria facultatively aerobic, gram negative, nonspore-forming, rod-shaped bacteria that ferment lactose and produce gas with 48 hours of incubation at 35°C. Because coliforms typically inhabit the digestive tract of animals, their presence in a sample serves as an indicator of fecal contamination

colony a group of individuals of the same species living together; in microbiology, a group of cells growing on a solid medium that are all derived from one original cell or cluster of cells

commensalism a symbiotic relationship in which one organism benefits but the other is neither harmed nor helped

communicable disease a disease that can be spread from one host to another

community the populations of organisms that live in and interact in a defined area

community ecology the study of the interactions between species in a biological community and how those interactions affect the community's structure

competition the demand by two or more members of a species (intraspecific) or two different species (interspecific) for a limiting environmental resource, such as nutrients or shelter

competitive exclusion principle the principle that states that two species cannot indefinitely occupy the same niche in a community

complement a group of proteins that circulate in the blood and nonspecifically destroy microbes with the aid of phagocytes

complementary in genetics, having the capacity to form specific base pairs with another strand of nucleic acid through hydrogen bonding

complete metamorphosis a dramatic change in body shape or form that occurs between the larval stage and adulthood

compound in chemistry, a combination of two or more elements

compound eyes sight organs made of many individual visual units

conidiophore an aerial hypha that produces condiospores

conidiospore an asexual fungal spore produced in a chain at the end of an aerial hypha

conjugation in bacteria, a one-way exchange of genetic material through direct contact via a pilus, the delivery system is encoded by sex plasmids in the donor cell; in ciliated protozoans, a means of sexual reproduction in which the cytoplasm of two organisms becomes joined and gametic nuclei are exchanged, resulting in the formation of zygotic nuclei in both participants

conservation biology the field of life science that is concerned with preserving biodiversity

continental shelf the submerged edge of a continental landmass

control group the part of an experiment that serves as a standard for comparison with another group that differs by a single factor (the independent variable)

convergent evolution the process by which organisms that are not closely related independently develop similar characteristics

coral atoll a ring-shaped coral reef that surrounds a lagoon

coral reef an underwater, rigid structure created from the skeletal deposits of coral polyps that serves as the basis for diverse marine communities

Coriolis effect an artifact of the Earth's rotation that affects moving bodies, such as the wind and oceans, causing deflection of midlatitude bodies to the right of the direction of motion in the Northern Hemisphere and to the left of the direction of motion in the Southern Hemisphere

corolla the outer envelope of a flower, composed of fused or separate petals

coronary of or relating to the heart

cotyledon the first leaf or one of the first pair or whorl of leaves developed by the embryo of a flowering plant

countercurrent exchange a mechanism whereby water entering gills flows in the opposite direction

as the blood in the capillaries, allowing for a more efficient exchange of gases

covalent bond a chemical bond in which two atoms share their valence electrons in order to achieve the maximum possible number of electrons in that electron shell

crop in vertebrate anatomy, the part of the digestive tract on some animals that is used for storing and grinding food

crossing over an exchange of segments of chromosomes that occurs between homologous chromosomes during meiosis

crude oil petroleum directly out of the ground in its natural state; unprocessed oil

Crustacea a subphylum of mostly aquatic arthropods, including lobsters, crabs, shrimp, crayfish, and barnacles, that have an exoskeleton, two pairs of antennae, and a pair of otherwise modified appendages on each segment

crustacean a member of the arthropod subphylum Crustacea

ctenoid scales thin, overlapping dermal scales with toothlike spines on the posterior edge, found in advanced bony fishes

ctenophore a member of the phylum Ctenophora resembling jellyfishes but having biradial symmetry and swimming by means of eight bands of transverse ciliated plates, also called comb jellies

culture in biology, the propagation or maintenance of organisms (such as bacteria or fungi), or cells (such as fibroblast cells), or tissues (such as epithelial tissue taken from a biopsy) in a prepared dish in the laboratory

cuticle a waxy coating that covers plant leaves and stems and protects against dehydration

cyanobacteria a group of photosynthetic bacteria, formerly called blue-green algae

cycloid scales thin, overlapping, dermal scales with smooth posterior edges, found in more primitive bony fishes

cytokine a chemical that is secreted by some cells, especially of the immune system, that communicates a signal to other cells, invoking a specific response

cytokinin a chemical substance in plants that stimulates growth and is often a derivative of adenine

cytology the study of cells, including their structure, function, reproduction, and diseases that affect them

cytoplasm all of the substance located inside the cell membrane but outside of the nucleus of a cell

cytosine a nitrogenous base that is a component of RNA and DNA

cytoskeleton an internal framework that provides cellular shape and support and plays a role in the motility of structures within the cells and in locomotion of cells via cilia and flagella

data factual information often collected during the course of a scientific investigation and that is used as a basis for reasoning or drawing conclusions about a phenomenon

deciduous having parts that fall off seasonally, such as leaves from a tree, or at a certain stage of development in a life cycle

decomposer an animal that breaks down dead and decaying organisms for food, and in the process recycles nutrients to the environment

degree of freedom in statistics, a measure of the amount of information from a sample set of data that has been used up. Degrees of freedom increase with the number of variables collected in a set of data and decrease with the number of variables that are estimated

deoxyribonucleic acid (DNA) the macromolecule that carries the genetic information. DNA is composed of a linear sequence of four different nucleotides and consists of two strands that are held together by hydrogen bonds to form a double helix

deoxyribonucleotide the monomer unit of deoxyribonucleic acid, each containing one deoxyribose sugar, one phosphate group, and one of four nitrogenous bases (adenine, thymine, cytosine, or guanine)

dependent variable a change in the test population not found in the control population that can be linked logically to the independent variable

depolarization a condition of an excitable cell in which the electrical charge inside of the cell is made less negative relative to the outside in comparison with the resting potential

dermis the inner layer of the skin

desert an arid biome that receives very little rain and has sparse vegetation

detritivore an organism, such as a fungus, that feeds on dead or decomposing organic matter

detritus nonliving, loose particulate organic matter, such as dead organisms, fecal material, or leaf litter

Deuteromycota a group of fungi that either have no sexual stage or for which the sexual stage is not yet known, also called imperfect fungi

deuterostome an animal whose blastopore develops into the anus instead of the mouth as in protostomes

development in biology, the natural process of growth, differentiation, or evolution by successive changes

diabetes mellitus an endocrine disorder due to inadequate production or utilization of the hormone insulin

diapsid an amniote of the lineage that includes most living reptiles and birds and is characterized by two pairs of temporal openings in the skull, one low in the cheeks and the other above those

differentiation the process whereby cells or tissues become structurally and functionally specialized or obtain their adult form or function

diffusion the natural, passive movement of a substance from an area of higher concentration to an area of lower concentration

digestion the process of breaking down food into small molecules that the body can absorb for nutritional needs

dioecious having male or female reproductive organs on different individuals

diploid containing two haploid sets of chromosomes, one inherited from each parent

dissection the systematic separation or opening up of the body of an organism to expose its internal parts for scientific study

disturbance in ecology, an event that causes change in the structure of a biological community

DNA fingerprinting a technique used to identify individuals based on highly variable minisatellites within the genome

dominant species in an ecosystem, the species that are most abundant or that have the highest biomass

dominant trait in genetics, a characteristic that is phenotypically expressed even when only a single copy of the corresponding allele for that gene is present

dorsal related to the backside

drift in genetics, the random fluctuation of gene frequencies that occurs when variation is neutral

ecdysis the process of molting or shedding of an exoskeleton or outer covering

ecdysone a hormone that triggers molting and metamorphosis in arthropods

ecology the study of the relationships and interactions between living organisms and their environment

ecosystem an ecological community and the physical environment in which organisms live

ectoderm the outermost of the three embryonic germ layers that develops into the outer layer of skin, the nervous system, and the sense organs

ectotherm a type of animal that regulates its body temperature by behavioral adaptations and by using environmental energy, sometimes called cold-blooded

egg a gamete produced by a female

electromagnetic receptors a sensory receptor that detects electromagnetic radiation, such as visible light

electromagnetic spectrum the entire spectrum of electromagnetic radiation consisting of wavelengths ranging from less than one nanometer to more than one kilometer

electronegativity the tendency of an atom or molecule to attract electrons

electron transport system in cellular respiration, a series of molecules embedded in the inner mitochondrial membrane of eukaryotic cells and the cell membrane of prokaryotic cells that shuttle electrons during cellular respiration from an oxidizable substrate to molecular oxygen (or another inorganic final electron acceptor in anaerobic respiration) by a series of redox reactions. The light reactions of photosynthesis also utilize an electron transport system to convert light energy into chemical energy

electrophoresis a technique used to separate biomolecules by placing them in a matrix (such as an agarose or polyacrylamide gel) saturated with a solution and subjecting them to an electric field, which causes the molecules to move through the matrix at different rates based on their physical and chemical characteristics

element a substance that cannot be transformed into any other chemical substance by chemical means, of which the smallest particle is an atom

elimination the physiological process of ridding the body of waste products

embryo the early developmental stage of a plant or animal following fertilization of an ovum

embryology the study of early animal development

embryophyte a type of plant that forms from multicellular embryos that develop within the maternal tissues, includes the bryophytes and tracheophytes

emulsifier a substance that promotes the conversion of two or more immiscible liquids into an emulsion

endergonic a type of chemical reaction characterized by a positive value for the change in free energy, indicating that the reaction cannot occur spontaneously

endocrine gland any gland that secretes hormones into the blood to regulate body processes

endocytosis a process for bringing substances into a cell during which a region of the plasma membrane of a cell reaches out to surround a substance and then pinches off to form an intracellular vesicle

endoderm the innermost of the three primary germ layers of an embryo, develops into the lining of the digestive tract, respiratory system, urinary bladder, digestive organs, liver, and other glands

endoplasmic reticulum a network of membranes in eukaryotic cells consisting of rough portions that contain bound ribosomes and process and

transport proteins and smooth portions that carry out lipid synthesis

endoskeleton a skeleton located underneath the skin

endosymbiotic theory a theory that explains the development of eukaryotic cells as arising from associations of small prokaryotic cells living within larger cells

endotherm a type of animal that regulates its body temperature by generating heat from metabolism

endothermic a type of chemical reaction that requires the input of energy

energy the capacity to do work

enterocoely a developmental process in which the coelom arises from an outpocketing of the archenteron or embryonic gut

enthalpy heat content of a system

entomology the scientific study of insects

entropy a measure of disorder in a system

envelope in some viruses, the outermost covering that surrounds the protein capsid and often contains receptors that resemble spikes

environmental science a multidisciplinary branch of science that seeks to understand natural environmental processes and how human activities affect them with the goal of addressing these issues

enzyme a protein that acts as a biological catalyst by lowering the activation energy of a biochemical reaction, thus increasing the reaction rate

eosinophil a type of white blood cell that plays a role in fighting infections by parasitic worms

epiblast the upper layer of cells in a blastoderm

epidermis the outer layer of cells covering the body of an animal

epilimnion the surface layer of a thermally stratified lake

epiphyte a plant that grows on a tree or other plant and obtains its moisture and nutrients from the air

epistasis a phenomenon in which one gene suppresses the effect of another different gene

epithelial tissue the type of tissue that covers the body and lines the internal body cavities and passageways or ducts

equational division cell division that maintains the number of chromosomes; the second stage of meiosis

equilibrium the state of having the forward and reverse rates of a chemical reaction equal one another, so the concentrations of reactants and products do not change

equilibrium constant at equilibrium, the ratio of the concentration of products over the concentration of reactants raised to the power equal to the stoichiometric coefficient

estrogen a steroid hormone produced by the ovaries that functions in female sexual development, the menstrual and ovarian cycles, and pregnancy

estuary the place where the lower end of a river flows into the sea, creating a unique, nutrient-rich habitat

ethology the scientific study of animal behavior under natural conditions

euchromatin loosely packaged chromatin that is accessible by transcription machinery

eudicot a clade of flowering plants including plants formerly called dicots that have two embryonic seed leaves

eukaryote an organism that has a membrane-enclosed nucleus and other membrane-bound organelles

eukaryotic a cell or organism characterized by a distinct membrane-bound nucleus and other membrane-bound organelles

eusociality a social behavior in which animals live in a cooperative group, often consisting of one female and several males that work together to care for the young and provide for the group

eutrophic lake a lake with high nutrient content, low oxygen content, and high biological productivity

evolution (biological) change over time that results from a species adapting to its environment

excretion the process of ridding the body of metabolic waste products such as nitrogenous wastes and carbon dioxide

exergonic a type of chemical reaction characterized by a negative value for the change in free energy, indicating the reaction can proceed spontaneously

exocytosis the process by which substances are released from a cell through a vesicle that fuses with the cell membrane

exogenesis the hypothesis that life on Earth originated elsewhere in the universe and was transferred to the Earth

exon part of a eukaryotic gene that encodes for a protein

exonuclease a nuclease that removes nucleotides from the ends of a DNA molecule

exoskeleton a hard, protective, external skeleton that covers an animal's soft body parts

exothermic releasing heat energy

experiment a procedure carried out under controlled conditions in order to test the validity of a hypothesis

extant still in existence

extinct no longer in existence

extremophile an organism that lives in extreme conditions, such as thermophiles, halophiles, or methanogens

facilitated diffusion the movement of a substance down a concentration gradient by a carrier protein

family in biological classification, a group of similar genera

fatty acid a long hydrocarbon chain with a carboxylic acid group at one end

feedback inhibition a method of control in which the product of a metabolic pathway binds to and allosterically inhibits an enzyme that catalyzes a reaction in the biochemical pathway leading to its own synthesis

fermentation the breakdown of carbohydrates resulting in the limited production of ATP without an electron transport chain and using an organic intermediate as the final electron acceptor

fertilization the joining of a sperm and an egg resulting in a zygote and initiating the development of an entire new organism

fetus a developmental stage of a vertebrate occurring after embryo until the individual hatches or is born

fiber with respect to nutrition and digestion, the indigestible material of food consisting mainly of cellulose from plant cell walls

filament stalklike part of the male reproductive structure of a flower, topped by the anthers

filter-feeding a method of obtaining food by filtration of organic material or tiny organisms from the water

fission in biology, the division of one parent cell into two equal daughter cells as a means of reproduction; in chemistry or physics, the splitting of an atomic nucleus accompanied by the release of a large amount of energy

fitness with respect to evolution, the relative reproductive success of an organism, including its probability of survival in order to reproduce

flagellum a long appendage that plays a role in locomotion, found in both prokaryotic and eukaryotic cells

follicle stimulating hormone (FSH) a hormone made in the anterior pituitary that stimulates the growth of follicles, the structures that contain ova, in females, and that stimulates the sperm-producing cells in males

food chain the hierarchical sequence of steps through which energy is transferred between organisms

food web the interconnections between food chains within a community of organisms

foraging a behavior associated with searching for food

fossil the trace, remnant, or impression of an organism from a past geologic age that has been preserved in sedimentary rock

fossil fuel a fuel, such as coal, oil, and natural gas, derived from former living organisms buried deep in the Earth. Over millions of years, the intense heat and pressure transforms the organic material into hydrocarbons

founder effect significant changes to the gene frequency in a population founded with a small sample of a larger population

frameshift mutation a genetic mutation that results in a disrupted reading frame, such as by the insertion or deletion of a number of nucleotides that is not evenly divisible by three

free radical atoms or molecules that contain an unpaired electron and are thus very reactive, also called reactive oxygen species

fringing reef a coral reef that borders the shoreline along the coast of a tropical island or continent

fruit the ripened ovary bearing seeds that develops following pollination and fertilization

fungus (pl. fungi) a member of the kingdom Fungi, consisting mostly of saprophytic, spore-producing eukaryotic organisms such as molds

G_0 a resting stage of the cell cycle that can occur during G_1 of interphase

G_1 the stage of the cell cycle that begins when cell division ends and ends with the initiation of DNA replication in S phase

G_2 the stage of the cell cycle that begins when DNA replication is completed and ends when cell division proceeds

gallbladder a saclike structure that stores bile produced in the liver

gametangia the multicellular, gamete-producing organs of the gametophyte stage in plants

gamete a haploid sex cell, usually an egg or a sperm

gamete intrafallopian transfer a form of assisted reproductive technology that involves the injection of eggs with treated sperm into the oviducts with the goal of achieving a pregnancy

gametogenesis the process of making gametes

gametophyte the haploid multicellular generation of plants that under an alternation of generations; or, the individual organism formed from a haploid spore and that produces haploid gametes by mitotic division

ganoid scales thick, nonoverlapping, rhombus-shaped scales found in primitive bony fishes

gap junction a connection between adjacent cells in which channels connect across the intercellular space to allow for the free exchange of ions or molecules between the cells

gastropod any of a class of mollusks with a distinct head and having sensory organs, including snails and slugs

gastrula the embryonic stage in which the three germ layers are established, occurring after the morula stage and before the blastula stage

gastrulation the process during embryonic development in which the three germ layers (endoderm, mesoderm, and ectoderm) are formed

gemmule an asexual reproductive structure consisting of an aggregate of cells, formed in sponges, that can develop into a new individual

gene the basic physical unit of inheritance, located at a specific position on a chromosome, that determines a particular characteristic of an organism

gene flow the movement of genes that occurs when organisms born in one location reproduce in another location

genetic code the key for assigning amino acids or stop signals to each of the 64 possible triplet (consisting of three bases) codons during protein synthesis

genetic drift a change in allele frequency due to random chance

genetic engineering the manipulation of the genetic makeup of an organism

genetics the science of heredity and variation in organisms

genome the entire complement of genes or the genetic material of an organism

genomics the study of genomes, including the sequencing of the DNA that composes an organism's genome and the applications of this information to biological research or medicine

genotype the genetic makeup of an organism

genus a category of biological classification just above the species level and below the family level containing organisms that share a particular characteristic

geochemical cycle the circuit of movement of an element or substance through an ecosystem by the action of geological and chemical processes

germ layer one of the three basic layers of cells (endoderm, mesoderm, and ectoderm) that develop into the tissues and organs of multicellular organisms

gerontology the study of aging and age-related processes and problems

gestation the time period during which a fetus grows inside a uterus

gibberellin a growth-stimulating hormone in plants

gill a structure that removes oxygen from the water

gizzard an organ found in some animals used to grind food mechanically before chemical digestion begins

glial cell a cell that supports the neurons of the brain and spinal cord by providing nutrients and other chemicals, synthesizing myelin, and repairing injured tissue

global warming a worldwide increase in the average surface temperature of Earth

glomerulus a structure consisting of numerous capillaries that project into a renal corpuscle

glucagon a hormone made in the pancreas that increases blood glucose levels

glucose a six-carbon, simple sugar that serves as an important metabolic intermediate and source of energy for the cell

glycocalyx a network of carbohydrate-rich molecules that coats the outermost portion of a cell

glycolysis a catabolic pathway that oxidizes sugar, resulting in two pyruvic acid molecules and two ATP

glycosidic linkage (glycosidic bond) a covalent linkage that joins two monosaccharides

Golgi apparatus the eukaryotic organelle made of flattened sacs of membrane that modifies and packages substances that are destined for secretion from the cell

G protein a cell membrane protein that binds guanosine nucleotides and plays a role in signal transduction

gradualism a pattern of evolution characterized by slow but continuous cumulative changes

granum (pl. grana) a stack of membranous thylakoids inside a chloroplast

grassland a biome dominated by grasses and other herbs as vegetation

gravitropism movement in response to gravity

greenhouse effect the warming effect caused by certain gases in the atmosphere, such as carbon dioxide and water vapor, that trap infrared radiation emitted from Earth's surface

groundwater water that flows underground through permeable rock or gravel

growth the process of getting larger or increasing in size, or in the case of populations, increasing in number

growth factor a substance that promotes cellular growth

guanine one of two nitrogenous purine bases that is a component of RNA and DNA

habitat the environment in which an organism or a community of organisms lives

habituation a type of learning in which an animal's response to a repeated stimulus decreases or is lost completely

half-life the amount of time it takes for half of the atoms of a radioactive substance to disintegrate

halophile a prokaryotic organism that lives in extremely salty environments

haploid having the same number of chromosomes as in a gamete, usually including one of each pair of homologous chromosomes

Haversian canals narrow, hollow channels in bone tissue that contain blood vessels and nerves

heart the organ that is made of cardiac muscle and is responsible for pumping blood throughout the circulatory system

heat capacity the amount of heat required to change the temperature of a given quantity of a substance by one degree Celsius

helminth a parasitic worm such as a tapeworm or fluke

herbivore an animal that eats mainly autotrophs such as plants or algae

heredity the transmission of characteristics genetically derived from one's ancestor to a descendant through the genes, or the sum of the characteristics derived from one's ancestors

hermaphrodite an animal that contains both male and female reproductive organs

hermaphroditism the condition of having both male and female gonads and being able to contribute both sperm and eggs during sexual reproduction

heterocercal a type of tail fin in which the upper lobe is larger than the lower lobe and the vertebral column ends in the upper lobe

heterochromatin chromatin that remains highly condensed during interphase and is visible using a light microscope

heterogamety the condition of having two different sex chromosomes, such as the X and Y chromosomes in human males

heterosporous producing separate male and female sporophylls

heterotroph an organism that requires organic compounds as their main carbon source

heterozygous possessing two different alleles for a given trait

histamine a chemical mediator released by mast cells and basophils that causes dilation of capillaries, contraction of smooth muscle, and stimulation of gastric acid secretion, and that is released during an allergic reaction

histology the study of living tissues and cells, usually at the microscopic level

histone a positively charged protein that associates with DNA in eukaryotic cells and that plays a key role in packaging the DNA into chromatin

holdfast the part of a seaweed that functions to secure the seaweed to a structure such as a rock and that resembles a root of a plant

hominid a primate that walks upright on two legs, includes the lineage that evolved into humans

hominoid a primate informally known as an ape; includes the gibbons, orangutans, gorillas, chimpanzees, and humans

homocercal a tail fin in which the upper and lower lobes are symmetrical and the vertebral column ends at the base, as in most bony fish

homogamety the condition of having two homologous sex chromosomes, such as two X chromosomes in human females

homologous chromosomes chromosomes that are essentially identical, carrying the same genes but not necessarily the same alleles

homologous structures anatomical traits that are derived from the same structure in a common evolutionary ancestor

homosporous producing only a single type of sporophyll

homozygous having two identical alleles for a given trait

hormone a chemical produced by cells that circulates and acts on target cells, often at a distant location, and causes some change in cellular activity

Human Genome Project an international research effort that began in the 1980s with the goal of mapping and sequencing all the genes in human DNA

human immunodeficiency virus (HIV) a retrovirus that causes acquired immunodeficiency syndrome (AIDS)

hybrid an offspring of two different varieties, breeds, species, or strains

hybridization in molecular biology, the coming together and formation of hydrogen bonds between two strands of nucleic acid that have complementary sequences

hydrogen bond a weak chemical bond formed by the attraction of a slightly positive hydrogen atom with a partially negative atom of another molecule or region of the same molecule

hydrophilic having an affinity for water

hydrophobic lacking an affinity for water, nonpolar

hydrophobic interaction the appearance of attraction between two nonpolar molecules or substances that results from the exclusion of the nonpolar molecules from electrostatic interactions with water molecules in a solution

hydrostatic skeleton in soft-bodied animals, an anatomical strategy for achieving shape and producing movement by filling an enclosed cavity with fluid

hydrothermal vent a crack or fissure on the seafloor that discharges volcanically heated, mineral-rich water and serves as the basis for unique benthic communities

hypersensitivity an exaggerated or inappropriate response of the specific immune system

hypha (pl. hyphae) a filament that grows from a germinating fungal spore

hypolimnion the bottom layer in a thermally stratified lake

hypothalamus a part of the brain that lies below the thalamus and that performs many autonomic homeostatic functions, also controls the pituitary gland

hypothesis a tentative explanation for a set of observations

ichthyology the scientific study of fishes

immunodeficiency a condition characterized by an insufficient number of functional T cells or antibodies, resulting in the inability of an individual to mount an effective immune response

immunoglobulin an antibody, abbreviated Ig

implantation the process of an embryo burrowing into the uterine lining and establishing a circulatory connection with the mother

imprinting a type of learned behavior acquired by an animal during a critical period of time

inclusive fitness an expansion of the concept of Darwinian fitness to encompass the consequences of altruistic behaviors, for example, by providing aid that enables close relatives to produce offspring

incomplete metamorphosis a process of change that occurs during the development of some insects in which the larvae resemble the adult form but are smaller and go through a series of molts before reaching adulthood

independent assortment law of heredity stating that each allele pair segregates independently during gamete formation. This law applies only when the two traits being examined are located on different pairs of homologous chromosomes or are sufficiently distant from one another on the same chromosome

independent variable the factor in an experiment that varies between the control group and the experimental group

indirect fitness the component of fitness due to the survival and transmission of copies of an individual's genes through nondescendant kin (carrying the same genes) producing offspring

indirect selection the phenomenon by which a gene is selected through pleiotropy (when one gene affects multiple traits) or linkage

induced mutation a type of mutation resulting from the action of a chemical, radiation, or insertion of a transposon

induction in embryology, the process of setting the fate of a cell or group of cells by physical proximity with neighboring cells

infection the growth of viable microorganisms that cause disease inside host tissues

infectious disease a disease caused by the growth of pathogenic microorganisms inside a host

inflammatory response a host response to tissue damage, characterized by swelling, redness, heat, and pain

ingestion the process of bringing in food for digestion

inhibitor a substance that slows down or prevents an enzyme from catalyzing a chemical reaction

inner cell mass the group of cells inside a primordial embryo, consisting of the epiblast, which gives rise to the three germ layers of the definitive embryo, and the hypoblast, which gives rise to extraembryonic tissues

insect a member of the class Insecta, characterized by a well-defined head, thorax, and abdomen, three pairs of legs, and one or two pairs of wings

insulin a hormone secreted by the pancreas that stimulates the uptake of glucose by cells

interferon one of a group of proteins that acts to fight viral infection or to destroy cancer cells, in addition to performing other immune related functions

intermediate filaments one of the three types of cytoskeletal elements, termed intermediate because the diameter ranges from 8 to 10 nanometers, in between the other two types of cytoskeletal elements (micofilaments and microtubules). They are very strong and durable and located in the cytosol between the nucleus and the cell membrane

interphase the stage of the cell cycle in between mitotic divisions

intertidal zone the region of shore exposed during the lowest tides and covered or splashed during the highest tides

intracytoplasmic sperm injection (ICSI) a form of assisted reproductive technology in which a single sperm is directly injected into an egg

intron a section of DNA within a eukaryotic gene that does not encode part of a protein. Introns are removed by a process called splicing that occurs after transcription and before translation

invertebrate lacking a spinal column; or, an animal lacking a spinal column

in vitro in an artificial environment such as a test tube or culture dish rather than in a living organism

in vitro fertilization (IVF) a form of assisted reproductive technology that involves giving a woman fertility drugs to induce ovulation, retrieval of the eggs at the proper time, fertilization of the eggs by mixing them with sperm in a glass dish in the laboratory, and the subsequent return of the embryos into the woman's body

ion a charged atom or molecule

ion exchange chromatography a type of chromatography that separates molecules in a solution based on differences in electrical charge

ionic bond an electrostatic attraction between two oppositely charged ions

islets of Langerhans the groups of endocrine cells within the pancreas that secrete the hormones insulin and glucagon

isomer one of two or more organic compounds that have the same number of atoms of the same elements but different structures, and therefore, different properties

isotope one of several forms of an atom of an element, each having the same number of protons but a different number of neutrons in the nucleus, and therefore different atomic masses

joint the part of a limb where moveable parts contact one another

karyotype an arrangement of an individual's chromosomes by size and shape, in homologous pairs, used for diagnosing chromosomal disorders

keratin a fibrous protein found in hair and nails

keystone species a species that has a large impact on the structure of the communities it inhabits

kidney the main organ of the excretory system that functions in osmoregulation and urine formation

kinesis a movement that lacks directional orientation and is dependent on a stimulus

kinetochore a disklike structure, located at the centromere of a chromosome, to which spindle fibers attach during mitosis

kinetoplast a granule containing a mass of DNA found inside flagellated protozoans called kinetoplastids

kin selection a form of selection in which individuals of a species increase their inclusive fitness by assisting relatives that are not their own offspring in their survival and reproduction

krill a type of shrimplike, zooplanktonic crustacean

labor the physical activities involved in giving birth

lacteal small ducts that protrude into intestinal villi and that drain into lymphatic circulation

lampbrush chromosome an enlarged mitotic chromosome found in amphibian eggs that has filamentous, lateral loops that give it a brushlike appearance

large intestine the portion of the vertebrate digestive tract following the small intestine and leading to the anus, consisting of the cecum, the ascending colon, the descending colon, the sigmoid colon, and the rectum. The main functions of the large intestine are the reabsorption of water and the formation of feces

larva the immature, wingless, wormlike feeding form that hatches from an insect egg and must undergo metamorphosis before reaching adulthood

larynx the modified upper portion of the trachea of air-breathing vertebrates, containing the vocal cords; also called the voice box

latent inactive, as in a virus that is not actively replicating within a host cell

law of conservation of energy the first law of thermodynamics stating that the total amount of energy in a closed system remains constant

law of conservation of matter the natural law stating that the sum of the masses of the reactants must equal the sum of the products following a chemical reaction

law of independent assortment (see independent assortment)

law of segregation (see segregation)

leaf a flattened, lateral outgrowth of a plant that functions mainly in photosynthesis in vascular plants

leukemia cancer of the blood, characterized by a marked increase in the number of white blood cells

leukocyte white blood cell

Leydig cell a cell found in the interstitium of the testes that produces steroid hormones

lichen a symbiotic association between a fungus and a photosynthetic organism, either a cyanobacterium or an alga

ligand a molecule that binds specifically to a receptor site of another molecule

ligase an enzyme that joins together two segments of DNA

light-dependent reactions the first stage of photosynthesis during which energy captured from light is used to generate NADPH and ATP

light-independent reactions the second stage of photosynthesis during which the NADPH and ATP are used to synthesized carbohydrates from carbon dioxide

lignin a phenolic polymer that serves to strengthen the walls of cells that make up the water-transporting tissues in vascular plants

limnetic of or relating to the well-lit, open surface waters of a body of freshwater

lipid one of a family of compounds that are not soluble in water

lithosphere the outer portion of Earth composed of rock and consisting of the crust and the outer layer of mantle

littoral related to the peripheral shoreline of a lake

locus, gene locus the position of a particular gene on a chromosome

loop of Henle the U-shaped part of the nephron of birds and mammals that lies in between the proximal and distal convoluted tubules and that

functions in water reabsorption during urine formation

lungs the respiratory organs of terrestrial vertebrates, snails, and spiders

luteinizing hormone (LH) a hormone made by the anterior pituitary that stimulates ovulation and the development of corpora lutea in females and the development of interstitial tissue in the testis in males

lycophyte a member of the plant clade Lycophyta, which includes club mosses, spike mosses, and quill worts

lymph the colorless fluid derived from the intercellular fluids that flows through the lymphatic vessels of vertebrate animals and contains white blood cells

lymphatic system a system of vessels and lymph nodes that returns fluids from the intercellular fluids to the blood circulatory system. The lymphatic system functions in conjunction with the immune system by filtering debris and foreign material from the bodily fluids

lymph node a mass of tissue distributed throughout the lymphatic system that contains phagocytes and other white blood cells and functions to filter debris and foreign material from lymph fluid

lymphocyte a type of white blood cell that matures in the bone marrow (B lymphocytes) or the thymus (T lymphocytes) and plays a role in specific immunity

lymphoma a malignant tumor of lymphoid tissue

lysogeny the persistence of a bacteriophage in the bacterial genome, where it remains inactive

lysosome a membrane-bound sac of digestive enzymes in eukaryotic cells

lysozyme an enzyme found in secretions like mucous and tears that breaks down peptidoglycan of bacterial cell walls

macroevolution evolution that occurs at the species level and higher, involving large and complex changes

macrophage a phagocytic cell of the immune system that is derived from a monocyte

magnification the degree of enlargement of an object when viewed under a microscope or magnifying lens

Malphigian tubules a group of long vessels that open into the digestive tract of insects and other arthropods. They function as excretory organs, removing nitrogenous waste from hemolymph and regulating osmolarity

mammal a member of the class Mammalia

Mammalia the amniote class of vertebrate animals that have mammary glands that produce milk

mantle in mollusks, the fold of skin that covers the dorsal surface of the body and encloses the internal organs. In some mollusks it secretes a shell

Mastigophora a phylum containing protozoa that have one or more flagella at some point during their life cycle; flagellates

matrix the region contained within the double membrane of mitochondria but outside of the cristae

matter anything that has mass and occupies space

mean in statistical analysis, a value calculated by dividing the sum of a set of values by the total number of values

mechanoreceptor a sensory receptor that detects physical changes to the body's environment, such as a change in pressure, touch, motion, or sound

medium (pl. media) a nutritive substance in or on which bacteria, fungi, and other microorganisms are grown for study

medusa the free-swimming stage of cnidarians and ctenophores

megaphyll a leaf with a branched vascular system

megasporangia the structure on megasporophylls that produces megaspores

megaspore a type of spore from a heterosporous plant that develops into female gametophyte

megasporophyll a type of leaf in heterosporous plants that produces megaspores

meiosis a specialized form of cell division that involves two stages and results in the production of haploid gametes for sexual reproduction

melanin a pigment colored yellow, various shades of brown, or black

melanocyte a type of cell, found in the inner layer of epidermis, that produces melanin

menstrual cycle a type of reproductive cycle in higher female primates, in which the endometrium is shed as a bloody discharge through the cervix and vagina in the absence of pregnancy

meristem plant tissue made of cells that remain capable of dividing indefinitely

merozoite a sporozoan trophozoite produced by asexual reproduction

mesoderm the middle germ layer of an embryo that develops into the notochord, bone, muscle, connective tissue, dermis, kidneys, gonads, and circulatory system

mesoglea the gelatinous substance between the endoderm and ectoderm of a sponge

mesohyl the gelatinous region between the two cell layers of a sponge

mesophyll the tissue within a leaf that is specialized for photosynthesis

metabolism the sum of the chemical reactions that occur within cells and serves to break down mole-

cules for energy and nutrients and subsequently to build up other molecules to sustain life processes

metalimnion the middle layer of a water column in a thermally stratified lake, characterized by a steep temperature gradient

metamorphosis a significant change in physical form or structure that occurs during development

metaphase a stage of nuclear division in which the chromosomes align along the equatorial plane of the cell

metazoan a multicellular organism with differentiated cells organized into tissues and a digestive cavity lined with specialized cells

methanogen an archaean that produces methane gas as a by-product of its metabolism

metric system a decimal system of weights and measures based on the meter and the gram

microbiology the branch of biology that deals with microorganisms, such as bacteria, archaeans, viruses, fungi, and protozoa

microevolution relatively minor evolutionary changes, both adaptive and neutral variations that occur in a population over a relatively short period of time

microfilaments solid rods made of the protein actin that are part of the cytoskeleton and that play a role in muscle contraction

microorganism a living organism that is too small to be seen with the naked eye

microphyll a type of leaf in lycophytes that contains a single vein

microsatellite DNA a type of repetitive DNA consisting of very short repeats (less than 15 base pairs)

microscopy the investigation of the structure of objects, including living organisms or parts of living organisms, using a microscope

microsphere a small spherical, proteinoid structure that may have been an important stage in the development of the first cells

microsporangia a structure in heterosporous plants that produces microspores

microspore a type of spore from a heterosporous plant that develops into a male gametophyte

microsporophyll a specialized leaf from a heterosporous plant that bears microsporangia, which produce microspores

microtubules long, hollow tubes that are made of the protein tubulin, provide cellular shape, and play a role in cell motility

micturition the act of urination

migration the mass movement of animals from one region to another, often seasonal and associated with breeding or feeding

millipede a member of the class Diplopoda that has two pairs of legs on most segments

mineral an inorganic substance; in nutrition, a chemical element other than carbon, hydrogen, nitrogen, or oxygen, that the body requires for proper development and functioning

minisatellite DNA a type of repetitive DNA consisting of repeats of 15–100 bp, found in clusters throughout eukaryotic genomes. The variation in size and number of repeated minisatellite sequences forms the basis of DNA fingerprinting

miracidium (pl. miracidia) the small, free-swimming, ciliated larval form of trematodes that seeks and infects host snails

miscarriage the spontaneous expulsion of the products of conception from the uterus during the first half of a pregnancy

missense mutation a change in DNA sequence in which a single nucleotide of a triplet codon is altered such that the codon specifies a different amino acid

mitochondrion (pl. mitochondria) the eukaryotic organelle responsible for cellular respiration and ATP synthesis

mitosis the process of nuclear division in which one cell divides into two nuclei that contain the same number of chromosomes as the parent and that are genetically identical to each other and to that of the parent cell

molarity the number of moles of a solute in one liter of solution

mold a type of fungus that grows in filaments called hyphae that form branched mycelial networks

molecule the smallest unit of a compound or substance that still retains the properties of that compound or substance and is composed of one or more atoms joined by covalent linkages

Mollusca the animal phylum containing mostly marine invertebrates characterized by soft, unsegmented bodies and most of which have a shell made of calcium carbonate

mollusk a member of the invertebrate phylum Mollusca, including snails, clams, octopuses, and squids

molt the process of shedding and replacing skin or a shell

monocot a member of a clade of flowering plants that has a single embryonic seed leaf and usually has parallel-veined leaves and floral organs arranged in cycles of three

monocyte a type of white blood cell that migrates into the tissues and matures into a macrophage

monoecious having both male and female reproductive organs on the same individual

monomer a subunit or building block of a polymer

monophyletic relating to a single group, consisting of an ancestral species and all of its descendents (a clade)

monosaccharide a simple sugar that serves as a monomer for polysaccharides or more complex carbohydrates

morphogenesis the development of structures in an organism, such as the formation and differentiation of tissues and organs

morula a solid ball of cells formed in early embryonic development after fertilization but before blastula formation

motor neuron a neuron that relays signals from the central nervous system to the muscles and other organs

M phase the stage of the cell cycle during which mitosis occurs

multiple alleles a type of inheritance in which several known alleles exist for one gene

mutagen something that causes a mutation to occur

mutation in genetics, a change in the nucleotide sequence of DNA

mutualism a symbiotic relationship in which both species benefit

mycelium the intertwining mass or colony formed by fungal hyphae

mycology the study of fungi

mycorrhiza (pl. mycorrhizae) a symbiotic relationship between a fungus and the roots of seed plants in which the fungi receives nutrition from the plant and the fungus increases the absorptive surface area of the plant roots, facilitating the uptake of water and nutrients from the soil

myelin the fatty insulation that surrounds some nerve fibers and is made by Schwann cells

Myxini a class of fish commonly called hagfish that are long and slender and have no larval stage

Myxomycota originally a group of fungi, but now considered to be more closely related to amoeba because of their feeding mechanisms; also called slime molds

NADH an electron carrier that plays an important role in cellular respiration

NADPH an electron carrier that plays an important role in photosynthesis

natural gas a gaseous fossil fuel consisting mostly of methane and other combustible hydrocarbons

natural selection the process by which organisms that are best adapted for survival and reproduction in their environment tend to survive and pass on their genetic information to their offspring while those that are more poorly adapted do not and die out as a result

nematocyst a stinging cell located on the tentacles of cnidarians

nematode a member of the phylum Nematoda, commonly known as roundworms

nephridia a tubular excretory organ found in some invertebrates

nephron the functional excretory unit of the vertebrate kidney involved in osmoregulation and urine formation

neritic relating to the narrow band of shallow water that adjoins the seacoast, generally corresponding to the continental shelf

nerve growth factor (NGF) a protein that stimulates the growth of certain types of nerve cells

nerve net a system of nerves that branch through the body in some invertebrates

nerves bands of nervous tissue that connect the brain with other body parts and that conduct electrical impulses

neural tube the hollow longitudinal, dorsal, vertebrate embryonic structure that is formed by the infolding of the ectoderm and that develops into the central nervous system

neuroembryologist one who studies the nervous system in developing unborn animals

neuron an excitable nerve cell capable of transmitting a nervous impulse

neurotransmitter a chemical messenger released from the tip of an axon at a synapse that diffuses across the synapse and stimulates or inhibits the adjacent neuron or effector cell (e.g., a muscle cell)

neutrophil the main type of phagocytic white blood cell

niche in an ecosystem, the role of a species with respect to its physical characteristics and its function in the biological community

node on the stem of a plant, a location from where a leaf is attached

nodes of Ranvier the interruptions in myelination between glial cells of a neuron that have a high concentration of voltage-gated ion channels and act to increase the speed of transmission of a neural impulse

nondisjunction the failure of homologous chromosomes to separate during meiosis I, resulting in gametes that contain either one too many or one too few chromosomes

nonpolar not having a dipole or a separation of charge, including compounds such as lipids that are insoluble in an aqueous solvent

nonsense mutation a change in the DNA sequence that converts a triplet codon that specified an amino acid into one of the three stop codons

notochord a supportive, longitudinal, flexible rod that runs along the dorsal axis in chordates

nucleic acid a polymer of nucleotide subunits that encodes genetic information (DNA) or plays a role in protein synthesis (RNA)

nucleolar organizer the region of a chromosome that contains the rRNA genes and that associates with the nucleolus

nucleolus a spherical body within the nucleus of a eukaryotic cell that contains multiple copies of the rRNA genes and that becomes enlarged during protein synthesis due to active transcription of the rRNA genes

nucleosome the smallest unit of DNA packaging in eukaryotes, consisting of a segment of DNA wrapped around an octamer of histone proteins

nucleotide a building block of nucleic acid consisting of a sugar, a phosphate group, and a nitrogenous base

nucleus the membrane-bound compartment within a eukaryotic cell that contains the DNA

null hypothesis in a statistical analysis, the hypothesis that an observed difference is due to chance alone rather than a specific cause

nutrient a nutritive substance, something with nutritional value that an organism uses for energy or to grow or replace damaged tissue

nymph the immature form of insects that do not have a pupal stage, resembling the adult but not fully mature

observation an act of recognizing and noting a fact or occurrence

ocean the continuous expanse of salt waters that covers more than 70 percent of Earth's surface

oceanic relating to the ocean, beyond the continental shelf

ocelli an eyespot on an invertebrate

oligochaete a member of the class Oligochaeta, hermaphroditic annelids with no distinct head

oligotrophic lake a lake with low nutrient content, abundant oxygen, and low primary productivity

omnivore an animal that eats other animals and plants or algae

oncogene a gene found in viruses or in a normal genome that can cause cancerlike characteristics

ontogeny the development or the course of development of an organism

oogenesis the process of making female gametes (i.e., eggs or ova)

operant conditioning a type of associative learning in which an animal learns that a specific behavior brings about either a positive consequence that reinforces the behavior or a negative consequence that discourages the behavior

operculum a structure that covers the gills in bony fishes

operon in prokaryotes, a sequence of adjacent genes that are under the same regulatory control and are transcribed as a single RNA

organ a specialized structure of the body that is made of several types of tissues and carries out a specific bodily function

organic chemistry the branch of chemistry concerned with carbon-containing compounds

organogenesis the formation of an organ

ornithology the study of birds

osmoregulation the regulation of osmotic pressure within the body of an organism

osmosis the movement of water through a semipermeable membrane from an area of low solute concentration to an area of higher solute concentration

osmotic pressure the pressure associated with the tendency for water to move across a semipermeable membrane from an area of low solute concentration to an area of higher solute concentration

ossicles small plates made of calcium carbonate that make up an echinoderm endoskeleton

ovary in flowers, the part of the pistil in which the egg-containing ovules develop; in animals, the structure that produces female gametes and reproductive hormones; in animals, the female gonad that produces steroid sex hormones and eggs

oviparous having the characteristic of laying eggs that develop outside of the female body

ovoviviparous having the characteristic of retaining fertilized eggs inside the body while they develop and laying them at or near the time of hatching

ovule the plant structure with the ovary containing the female gametophytes; site of fertilization and seed development

oxidation the loss of electrons

oxytocin a hormone that is made in the pituitary gland and that stimulates uterine contractions and milk secretion in mammals

pain receptors a specialized type of sensory receptor that detects pain, also called a nocireceptor

paleoanthropology the study of human origins

pancreas a vertebrate gland in the abdomen that secretes digestive enzymes and hormones such as insulin and glucagon

panspermia the hypothesis that life on Earth originated elsewhere in the universe

paracrine being secreted by one cell and acting on other nearby cells

paraphyletic relating to a group of species that consists of an ancestral species and some but not all of its descendents

parapodia fleshy appendages of annelids used in locomotion

parasite an organism that lives off the body fluids of a living host, often causing harm

parasitism a type of symbiotic relationship in which one organism lives off a host and usually harms the host in the process

parasitology the study of parasites, usually in relation to the harm they cause humans, animals, and plants

parathyroid glands four small glands located adjacent to the thyroid that produce parathyroid hormone

parathyroid hormone a hormone produced by the parathyroid glands that plays a role in calcium and phosphorus metabolism

parthenogenesis reproduction by the development of an unfertilized egg

partial dominance a type of inheritance in which no trait is fully dominant over another, sometimes called incomplete dominance

parturition the process of birth

pathogen a disease-causing microorganism

pecking order a social hierarchy system in birds and mammals characterized by a linear order of precedence for access to food and mates

pedigree in genetics, a pictorial representation of a family's genetic history for one or more specific traits covering multiple generations

pedipalps mouthparts of arachnids used for grasping food

pelagic related to the ocean's open sea or midwaters

penicillin a chemical substance produced by the mold *Penicillium* with antibacterial properties

penis the male copulatory organ

peptide bond a covalent linkage between two amino acids

peptidoglycan a complex network of polysaccharides and protein that serves as the main structural component of bacteria cell walls

pericarp the thick wall of a fruit or ovary of a flowering plant

periderm the outer protective layer of tissue that replaces the epidermis in plants during secondary growth

peripheral nervous system the division of the vertebrate nervous system consisting of the sensory and motor neurons that carry information to and from the central nervous system

peristalsis successive waves of smooth muscle contraction that propels substances through a hollow tube such as in the digestive tract

petal a modified leaf of a flowering plant, often colorful, that helps attract pollinators

petiole the stalk of a leaf that joins the leaf to the stem of a plant

phage short for bacteriophage

phagocyte a type of white blood cell that ingests and digests microorganisms and foreign matter

phagocytosis a type of endocytosis in which the cell engulfs large particulate matter

pharynx part of the upper digestive tract in some animals, used in sucking and swallowing; in mammals, the region located between the nasal cavity, the mouth, and the esophagus

phenotype the physical and physiological traits of an organism

pheromone a volatile chemical secreted externally and detected through olfaction that influences the physiology and behavior of other members of the same species

phloem the type of vascular tissue that distributes organic nutrients throughout a plant body

phospholipids molecules that have a polar head and hydrophobic tails and are a major component of biological membranes

photic relating to light, especially by the Sun

photon a quantum of radiant energy

photoreceptors an electromagnetic receptor that detects visible light

photorespiration a light-dependent process in some plants that involves the oxidation of glycolic acid and the release of CO_2

photosynthesis the conversion of sunlight into chemical energy

photosystem a group of molecules that cooperate to capture energy in the form of light and convert it into chemical energy in the form of NADPH and ATP

phototaxis movement in response to light

phototroph an organism that obtains its energy from light

phototropism an orientation of an organism by movement or differential growth in response to the presence of light

phragmoplast in land plants, a complex of microtubules created at the midline of a cell during cell division

phylogenetic tree a branched picture that depicts the evolutionary relationships of organisms

phylogeny the evolutionary history of a species

phylum in biological classification, a major subdivision of a kingdom, encompassing many classes

physical dependence a physiological state related to drug or substance use reached when an individual suffers withdrawal symptoms upon discontinuing use of the drug

physiology the study of the functions and activities of cells, tissues, and organs of living organisms

phytoalexin a chemical secreted by plants that destroys or inhibits the growth of microorganisms

phytoplankton photosynthetic plankton

phytoremediation the use of plants to clean up contaminated soil or water

pigment a substance that absorbs specific wavelengths of light and reflects others, giving an organism color

pilus a long, tubular structure through which genetic material passes from one bacterial cell to another

pinocytosis a type of endocytosis in which the cell brings droplets of extracellular fluid into the cytoplasm of the cell

pistil female reproductive structure of a flower, including the stigma, style, and ovary

pith the spongy ground tissue internal to the vascular tissue in the stem of a vascular plant

pituitary gland a small structure hanging in the base of the brain that works with the hypothalamus to produce and release many hormones in the body

placenta the organ consisting of both embryonic and maternal tissue that connects a developing embryo to the uterus of the mother in placental mammals and allows for the exchange of materials in the blood supplies

placoid scales scales with a basal plate of dentin embedded in the skin and a backward-pointing spine tipped with enamel, found in cartilaginous fishes

plankton the tiny plants and animals that float near the surface of bodies of water and often comprise the bottom of a food chain

plant a member of the kingdom Plantae

Plantae a kingdom consisting of multicellular eukaryotic organisms that carry out photosynthesis

plasma the liquid part of blood

plasma membrane also called the cell membrane; the phospholipid bilayer that surrounds a cell and acts as a selectively permeable barrier between the cell's interior and external environment

plasmid a small, closed, circular piece of DNA that exists separately from the main bacterial chromosome and that replicates independently. Plasmids are mostly found in bacteria, but also in some fungi and plants

plasmodesma (pl. plasmodesmata) a channel in the cell wall of a plant that connects the cytosol between two adjacent cells

platyhelminth a flatworm

point mutation a change in the sequence of DNA at a single nucleotide pair

polar covalent bond a type of covalent bond that generally forms between two atoms with a difference in electronegativities of 1.7 or greater

polarity the condition of having opposite properties (such as different charges or partial charges) in opposite parts or ends, or having a directionality

pollen an immature male gametophyte that develops in the anthers of flower stamens

pollination the transfer of pollen from the stamen to the pistil of a flower

polychaete a member of the annelid class Polychaeta, the marine segmented worms

polygenic inheritance a type of inheritance characterized by an additive effect of two or more gene loci on a single phenotypic character

polymer a large chain consisting of numerous identical or similar linked subunits

polymerase chain reaction (PCR) a technique for making multiple copies of a specific DNA fragment by using a thermostable DNA polymerase and numerous repeated cycles of heating and cooling

polymorphism the coexistence of two or more forms of a trait, gene, or DNA sequence in a population

polyp the nonswimming stage of cnidarians, usually a hollow cylindrical body closed and attached at one end and opening at the other by a central mouth surrounded by tentacles

polypeptide a long chain of amino acids linked together by peptide bonds

polyphyletic belonging to a group that consists of species derived from two or more ancestral forms

polysome also called a polyribosome; the structure consisting of several ribosomes simultaneously translating the same mRNA molecule

polytene chromosome giant chromosome found in certain fly larval tissues that results from numerous rounds of replication without separation

population a group of individual members of the same species that occupy a defined area

population ecology the study of the interactions between populations of biological species and their environment

Porifera an invertebrate phylum comprising simple aquatic animals like sponges that have no organized tissues or organs

poriferan a member of the invertebrate phylum Porifera

preformation the idea that an entire preformed tiny organism exists inside an egg

prey any animal hunted by another animal for food

primary consumer an herbivore

primary producer an autotroph, usually a photosynthetic organism; an organism at the lowest trophic level of an ecosystem

primary production the amount of light energy that autotrophs convert to chemical energy in an ecosystem over a certain time period

primary succession the process by which a community becomes established in a lifeless area in the absence of soil, such as after a dramatic ecological disturbance such as glacial formation and retreat or when a new island is formed by volcanic activity

prion an infectious protein particle that has no nucleic acid and causes spongiform

encephalopathies such as Creutzfeldt-Jakob disease and mad cow disease

progesterone a female steroid hormone, produced by the corpus luteum in early pregnancy and then by the placenta, that maintains the uterine lining during pregnancy and plays a role in preparing the uterine lining before implantation

prokaryote an organism that does not have a nucleus or other membrane-bound organelles, includes members of the domains Archaea and Bacteria

prokaryotic a type of cell or organism that has no membrane-enclosed organelles

prolactin a hormone produced by the pituitary gland that influences the activity of FSH on the ovaries and stimulates milk production

prophage a bacteriophage that has undergone lysogeny

prophase the first stage of nuclear division, during which the chromosomes condense and the mitotic spindle forms

prostate a gland in the male reproductive tract that secretes a milky substance that nurtures and promotes the survival of sperm

protandry a type of sequential hermaphroditism in which the animal is a male first, then switches to become a female

protein a macromolecule composed of a chain of amino acids and that plays important structural, regulatory, hormonal, enzymatic, and other roles

proteobacteria members of the phylum Proteobacteria, including gram-negative bacteria of diverse metabolisms and nutritional modes

proteome the entire complement of proteins expressed in a cell, tissue, or organism

proteomics the study of the complete sets of proteins produced by genomes

protist a single-celled, eukaryotic organism. If the protist is photosynthetic, it is considered algae, and if a protist is not photosynthetic, it is called a protozoan

protobiont an aggregate of abiotically produced organic molecules that may have given rise to living cells

protocell a structure that gave rise to primitive cells

protogyny a type of sequential hermaphroditism in which the animal begins as a female then switches sex to become a male later in life

protooncogene a normal cellular gene that has the potential to become a cancer-causing gene if altered in a certain way

protostome an animal whose blastopore develops into a mouth, as opposed to an anus as in deuterostomes

protozoa a motile, unicellular eukaryotic organism that does not possess a cell wall or chlorophyll and obtains its nutrition by the ingestion of food

pseudocoelomate an animal with a body cavity between the mesoderm and the endoderm

pseudogene a gene that once encoded a functional protein but that has been inactivated by mutation

pseudopodium (pl. pseudopodia) a cytoplasmic extension in amoebas used for gathering food and locomotion; "false foot"

psychrophile a type of prokaryotic organism that lives in extremely cold temperatures

pteridology the study of ferns

punctuated equilibrium a pattern of evolution characterized by long periods of stasis interrupted by short periods of rapid change

Punnett square a square used in genetics to calculate the frequencies of the different genotypes and phenotypes among the offspring of a cross

pupa the nonfeeding, immobile stage of an insect's life cycle, occurring after the larval stage and before the adult stage

purine a class of double-ringed nitrogenous bases found in nucleotides

pyrimidine a class of single-ringed nitrogenous bases found in nucleotides

radial symmetry an animal body plan in which the body parts are arranged around a central point, similar to spokes on a wheel

radical (*See* free radical)

radioactivity a phenomenon in which some elements spontaneously emit energetic particles from their atomic nuclei; also, the rays emitted during the spontaneous decay

radula in mollusks, a tonguelike structure used to scrape food off surfaces or to tear food

receptor a protein that specifically recognizes and binds another molecule

recessive trait a genetically inherited trait that does not appear in a individual unless its gene is present in two copies. Recessive traits can be masked in the presence of a dominant gene

reciprocal cross a cross with the phenotype of each sex reversed in comparison to the original cross

recombinant DNA DNA made by joining together DNA from different sources

recombination a type of genetic transfer that occurs between chromosomes during meiosis or DNA of different organisms; also called crossing over

rectum the end of the large intestine, right before the anus, where solid waste material is stored prior to elimination

reduction the gain of electrons

reductional division a nuclear division that results in a reduction in the number of centromeres in each daughter nucleus, each receiving half as

many as the parent. Reductional division occurs during the first stage of meiosis

redundancy with respect to the genetic code, the fact that different triplet codons encode for the same amino acid

reflex a quick, simple, nervous response that is processed through the brain stem or spinal cord

regeneration the process by which a body part is restored after injury, also a mechanism for asexual reproduction

replication in genetics, the synthesis of new DNA from an existing DNA template

repolarization the return of the membrane potential of an excitable cell to its resting potential

reptiles members of the class Reptilia

Reptilia a class of amniote vertebrates that includes tuatara, lizards, snakes, turtles, crocodilians, and birds

reservoir with respect to environmental science, an artificial lake that collects and stores water; in epidemiology, a living or nonliving natural source that harbors a pathogenic organism

resolution the ability to distinguish between objects as separate entities when viewed under a microscope

respiration the exchange of oxygen and carbon dioxide gases across a moist surface; distinct from cellular respiration

restriction enzyme (restriction endonuclease) an enzyme that specifically recognizes and cuts DNA at a particular sequence

restriction fragment length polymorphism (RFLP) a variation within a population in the length of a fragment formed by digestion of DNA with a restriction enzyme

reticuloendothelial system a network of connective tissues that is heavily endowed with macrophages and that acts as a passageway within and between organs

retrovirus a virus that has RNA as its genetic material. After infecting a host cell, this class of virus makes and uses reverse transcriptase to synthesize DNA from the RNA, and then the DNA integrates into the host's genome

reverse transcriptase a retroviral enzyme that makes DNA using RNA as a template

rhizoid rootlike structure that anchors bryophytes to the ground but, unlike a root, does not play a role in water and mineral absorption

rhizome a horizontal plant stem that usually grows just below a surface and often has thickened areas that serve as deposits of food reserves

ribonucleic acid (RNA) a type of nucleic acid made from ribonucleotide subunits that functions in protein synthesis. Three types exist, messenger RNA, ribosomal RNA, and transfer RNA

ribonucleotide a monomer unit of ribonucleic acid that consists of a ribonucleotide sugar, a phosphate group, and one of four nitrogenous bases (adenine, uracil, cytosine, or guanine)

ribosome a cell organelle made from RNA and protein that is the site for protein synthesis

ribozyme RNA that can act as a biological catalyst, like a protein enzyme

RNA interference the silencing of a targeted gene by a double-stranded piece of homologous RNA

root a part of a vascular plant that anchors the plant to the ground and that functions in water and nutrient absorption from the soil

rubisco an enzyme that adds CO_2 to ribulose bisphosphate in the first step of the Calvin cycle of photosynthesis; abbreviation for ribulose bisphosphate carboxylase

salamander an amphibian that resembles a lizard, but has thin, moist skin and an aquatic larval stage

saltatory conduction the rapid transmission of a neural impulse along the length of a myelinated axon by jumping between the nodes of Ranvier, skipping over the myelinated regions

sap the fluid part of a plant that circulates through its vascular system

saprobe, saprophyte an organism that feeds off dead or decaying organic material

Sarcodina a phylum containing protozoa that have pseudopodia, or false feet; commonly known as amoebas

sarcoma a malignant tumor that originally forms from tissue derived from mesoderm, such as connective tissue, bone, cartilage, or muscle

Sarcopterygii lobe-finned fishes

satellite DNA highly repetitive DNA that makes up a significant portion of eukaryotic genomes, does not undergo transcription, and is not found in centromeric regions

savanna a grassland or plain with relatively few trees and that is generally drought resistant

scales small, flattened plates that cover the body, especially of a fish

schizocoely a development strategy in which solid masses of mesoderm initially split to form the body cavity

scientific method a set of commonly used procedures for the systematic pursuit of knowledge; includes the recognition of a problem, the development of a hypothesis, the collection of data through observation, and experimentation to test the validity of the hypothesis

scientific theory a hypothesis that explains some phenomenon, is based on observation, experimentation, and reasoning, and has withstood repeated experimental testing

secondary consumer a carnivore, or a member of the trophic level that feeds off of primary consumers

secondary succession the process by which a community becomes established following an ecological disturbance that leaves the soil intact, such as by a forest fire

second messenger a small molecule that relays a signal from the exterior of a cell to the cytoplasm on the interior. IP_3, Ca^{2+}, and cAMP are examples of second messengers

seed a plant structure containing an embryo packaged in a tough protective covering that also contains food stores

segmentation an evolutionary development in animals characterized by a series of repeated, similar units

segregation law of heredity stating that allele pairs separate during gamete (sex cell) formation and then randomly reform pairs during the fusion of gametes at fertilization

semen the combination of sperm, seminal fluid, and other male reproductive secretions

seminal vesicle one of a pair of glands in the male reproductive tract that secretes a milky substance that nurtures and promotes the survival of sperm

senescence the progressive deterioration of many bodily functions associated with aging

sensory neuron a neuron that receives information from the external or internal environment and sends information from a sense organ to the brain

sepal in angiosperms, a modified leaf that helps protect a flower bud before it blooms

septum (pl. septa) a wall that divides regions such as the chambers of the heart or two cells in the process of cytokinesis

Sertoli cell one type of cell forming the stratified epithelium of seminiferous tubules that nourishes developing sperm cells that are embedded in them

sessile permanently attached to a surface

sex-linkage the condition of genes or traits being located on one of the sex chromosomes, usually the X chromosome

sexual reproduction production of offspring by the joining of the genomes from two gametes made by two partners

sexual selection natural selection for characteristics that increase success at mating

shoot a new outgrowth, such as of a stem or branch of a plant

signal transduction the process by which a cell responds to an extracellular signal, often involving numerous second messengers and biochemical pathways

silent mutation a change in the nucleotide sequence of a triplet codon that does not result in a change in the amino acid for which it encodes

sister chromatids duplicated forms of a chromosome attached at the centromere. Sister chromatids separate into two individual chromosomes during mitosis or meiosis

size exclusion chromatography (gel filtration chromatography) a type of chromatography that separates molecules in a solution based on differences in size by using porous beads as the column resin

slime mold also called myxomycetes, a group of eukaryotic organisms formerly classified as fungi because they reproduce by spores, but now recognized to be distinct. Two main types of slime molds include the plasmodial slime molds and the cellular slime molds

small intestine the longest region of the digestive tract, where the majority of enzymatic digestion of food and the absorption of nutrients occurs

sociobiology the study of the evolutionary basis for animal behaviors

solvent a fluid capable of dissolving other substances

solute a substance that is dissolved in a solvent

somatic cell nuclear transfer the placement of the nucleus from a somatic cell into the cytoplasm of an oocyte; the basis of the most successful method for cloning organisms to date

somatic embryogenesis the initiation of embryos from previously differentiated somatic cells

somite a mass of mesoderm arranged segmentally in a series alongside the neural tube of an embryo

spatial learning the ability of an animal to recognize differences in the arrangement of objects with respect to one another within an environment

speciation the development of new biological species

species a group of organisms with common characteristics that can interbreed

sperm a gamete produced by a male, short for spermatozoa; also, semen

spermatogenesis the process of making male gametes, or sperm

spermatozoa the male gamete, also referred to simply as sperm

S phase the stage of the cell cycle during which DNA synthesis occurs

spinal cord the major nerve pathway between the brain and the rest of the body running lengthwise from the base of the brain downward, also coordinates some reflexes

spindle apparatus a eukaryotic cytoskeletal complex that plays a role in mitotic and meiotic division, consisting of centrosomes and spindle fibers (microtubules)

spiracles in arthropods, small openings through which air enters the body
spirochetes long, helical-shaped bacteria that have axial filaments
spontaneous generation a discredited theory that stated organisms arise from nonliving matter
spontaneous mutation a change in the DNA sequence of an organism that occurs by natural processes as opposed to by the presence of mutagenic chemical substances
sporangiophore a type of hypha that produces sporangiospores
sporangiospore an asexual fungal spore that forms in an enclosed sac at the end of an aerial hypha
sporangium a sac on the tip of a sporangiophore that encases asexual fungal spores called sporangiospores; also, the organ of the sporophyte stage of plants that contains the spore-producing sporocytes
spore a general term for the dispersal structure for fungi, can be produced asexually or sexually; also a single-celled structure that can develop into a gametophyte body-type in plants
sporocyte a type of cell that undergoes meiosis to produce haploid spores
sporophyll a specialized leaf that produces sporangia, which produce spores
sporophyte in organisms that undergo an alternation of generations, the multicellular diploid form that results from the union of two haploid gametes and that undergoes meiosis to generate haploid spores
Sporozoa formerly, a class of nonmotile, parasitic protozoa, now part of the phylum Apicomplexa
sporozoite a sporelike cell produced by members of the protozoan phylum Apicomplexa following sexual reproduction
stamen the male reproductive structure of a flower, including an anther and a filament
standard deviation in statistical analysis, the square root of the variance
stele the central, cylindrical, vascular tissue of a stem or root of a plant
stem the shoot of vascular plants that supports the leaves and reproductive structures
steroid a molecule belonging to a class of lipids characterized by a backbone consisting of four fused carbon rings
stigma part of the female reproductive structure of a flower to which pollen grains stick, located at the tip of the style
stipe the stemlike portion of a seaweed
stolon a horizontal stem that extends along the ground surface from the base of a plant and that can produce new plants from buds at the tips or nodes; also called a runner
stomata pores in the epidermis of plant cells through which gas exchange occurs
stratosphere the layer of atmosphere that starts just above the troposphere (which ends at 5–9 miles or 8–14.5 km) and extends to about 31 miles (50 km) from Earth's surface
stroma the region in between the double membrane of a chloroplast and the grana inside
stromatolite a rocklike structure formed from fossilization of layers of cyanobacteria, calcium carbonate, and sediment
style part of the female reproductive structure of a flower, separates the stigma from the ovary
substrate a substance upon which an enzyme acts
succession in ecology, the sequential process by which groups of species recolonize a habitat after a disturbance
sulfonamides a class of chemical agents that kill bacteria
sustainability in environmental science, the use and management of resources without depleting them or causing permanent damage to them or the environment
symbiosis the situation of two different species of organisms living together in direct contact
sympatric speciation the origin of new species without geographic separation
symplast cytoplasm connected by plasmodesmata between adjacent plant cells
synapse a junction between a neuron and another cell through which signals are transmitted
synapsid an amniote belonging to the lineage of early reptiles that evolved into mammals and characterized by a single hole on each side of the skull
syngamy during sexual reproduction, the fertilization of one gamete by another to form a zygote
systematics the science of analyzing and classifying living organisms based on evolutionary relationships
taproot the main vertical root of a plant from which many smaller lateral roots branch
taxonomy the science of classifying and naming living organisms
telomere the repetitive sequence that caps the end of a linear chromosome and serves to protect it and provide stability to the chromosome
teleost a modern bony fish
telophase the final stage of mitosis during which the spindle disassembles and the nuclear envelope reforms around each chromosome set, often occurs simultaneous with cytokinesis, or cell division; also the final stage of meiotic division
temperate forest one of Earth's major terrestrial biomes located in midlatitude regions with enough moisture to support the growth of deciduous trees

temperate grassland a terrestrial biome characterized by grasses and forbs

tentacle an armlike organ used for gathering food and for touch

tertiary consumer an organism that feeds on secondary consumers

testcross a cross between a homozygous recessive individual and an individual of an unknown genotype, performed for the purpose of identifying the unknown individual's genotype

testis (pl. testes) the primary reproductive organ in males that functions in sperm and testosterone production

testosterone the steroid hormone produced by Leydig cells in testes of males that stimulates the development of secondary male sexual characteristics and maintains sexual function and fertility in adult males

tetrapod a vertebrate animal that has four limbs; includes amphibians, reptiles, birds, and mammals

thalamus a relay station between the spinal cord and the cerebral cortex

thallus the plantlike body of a seaweed; also a plant body that is flattened and not organized into roots, stems, or leaves

thermocline a region of the water column that separates warmer surface water from cooler deeper water and across which the temperature rapidly changes

thermodynamics the study of energy transformations

thermophile an organism that grows optimally at temperatures between 122 and 140°F (50–60°C)

thermoreceptor a sensory structure that detects heat or cold and relays the information through neurons to the central nervous system

thermoregulation the mechanism by which an organism controls its body temperature

thigmomorphogenesis a plant response to a mechanical environmental stimulation

threshold potential the potential that an excitable cell must reach in order to generate an action potential

thylakoid a flattened sac of membrane that contains the pigments and photosystems that carry out the light-dependent reactions of photosynthesis within a chloroplast

thymine one of two pyrimidine nitrogenous bases found in deoxyribonucleotides of DNA

thyroid an endocrine gland found in vertebrates in front of the trachea on the neck that secretes hormones that regulate metabolism, growth, and development and that regulate calcium levels in the blood

thyroid hormone an iodine-containing hormone secreted by the thyroid gland, either thyroxine or triiodothyronine, that functions in the regulation of metabolism, growth, and development in vertebrates

tissue a collection of cells that perform a specific function

tolerance a state of drug use when an individual's body makes biochemical or physiological adjustments such that higher doses of the drug are required to achieve the same desired effect

totipotent having the capability of developing into any specialized tissue type or into a complete organism

trachea the cartilaginous, hollow tube that carries air from the nasal passages to the bronchi in vertebrates. In arthropods, one of a network of tubules forming the respiratory system

tracheids long, tube-shaped cells that make up xylem in vascular plants

transduction the process in which bacteriophage carry bacterial DNA from one host to the next

transformation in cell biology, the conversion of a normal animal cell into a cancerous cell; in genetics, the uptake and assimilation of foreign DNA into a cell

transgenic containing genes from other organisms

transpiration the passage of water vapor from plant tissue through stomata

transposable element a piece of DNA that can move from one position in a genome to another; also called a transposon

tricarboxylic acid cycle a circular pathway of biochemical reactions central to metabolism in which acetic acid is oxidized; also called Kreb's cycle or citric acid cycle

triglyceride a lipid molecule made by attaching three fatty acids to a glycerol by ester linkages

trophoblast the outer layer of a mammalian blastocyst that forms the fetal portion of the placenta

trophozoite the actively feeding or vegetative stage of a protozoan life cycle

tropism a curvature by differential growth toward or away from a stimulus, such as the orientation of a plant toward a light source

troposphere the portion of atmosphere where all life exists and where most weather phenomena occur, extending from the surface of the Earth about five to nine miles (8–14.5 km) upward

true-breeding occurs when self-fertilization gives rise to the same traits in all offspring generation after generation; homozygous

tubal ligation the surgical sterilization procedure performed in females that involves sealing,

tying, or cutting the oviducts in order to prevent pregnancy

tumor-suppressor gene a gene that encodes a protein that inhibits cell division, and therefore prevents a cell from becoming cancerous

tundra a biome characterized by sparse, shrubby, or matlike vegetation

Turner's circling a behavior exhibited by ants in which they move in circles as they approach a ground nest

ultraviolet radiation high energy radiation with wavelengths smaller than visible light but longer than X-rays, between 100 and 400 nanometers, and that is harmful to living organisms

undershoot the stage in the conduction of an action potential characterized by a membrane potential more negative than the resting potential due to the potassium channels remaining open longer than the sodium channels following depolarization

Uniramia a group of mostly terrestrial arthropods that have legs with only one branch, including insects, millipedes, centipedes, and their relatives

uracil a nitrogenous base that is a component of RNA

ureter one of the paired tubes that carries urine from the kidneys to the bladder

urethra the tube that carries urine from the bladder to the body's exterior during micturition

Urochordata a group of organisms belonging to the phylum Chordata; commonly known as tunicates or sea squirts

vaccine a weakened or killed bacterium or virus (or part of one) that is injected into a human or animal, stimulating active immunity to that particular organism

vacuole a membrane-bound sac or vesicle in the cytoplasm of a cell. Vacuoles serve diverse functions in different cells: food vacuoles formed during phagocytosis contain food particles destined for hydrolysis, contractile vacuoles in plants contain excess water, and gas vacuoles in aquatic bacteria help maintain buoyancy

valence electrons the electrons contained within the outermost shell of an neutral atom

valence shell the outermost electron shell of a neutral atom

valve a movable part that controls the passage of a fluid, such as a valve that controls blood flow between two chambers in the heart

variation a difference between members in the same species

vas deferens the duct that carries sperm from the epididymis and joins the duct from the seminal vesicle to form the ejaculatory duct, which carries sperm to the urethra

vasectomy the surgical sterilization procedure that involves the sealing, tying, and cutting of a man's vas deferens in order to prevent sperm transport from the testes to the penis

vector a plasmid or bacteriophage that carries DNA into a cell during a cloning procedure

veins blood vessels that carry blood from body tissues to the heart

ventral related to the front side or belly, opposite the dorsal side

ventricle a chamber of the heart that fills with blood and contracts to pump blood into arteries

Vertebrata subphylum of the phylum Chordata, consisting of animals with backbones, including mammals, reptiles, birds, amphibians, and fishes

vertebrate an animal with a bony skeleton and a spinal column

viroid a plant pathogenic agent composed only of a closed, circular RNA molecule

virology the study of viruses and viral diseases

virus a microscopic, acellular agent composed of nucleic acid surrounded by a protein coat

vitamin an organic compound that is essential in minute quantities for animals and plants. Many vitamins act as coenzymes and regulate metabolic processes

viviparous having the characteristic of eggs developing inside the mother and offspring born as juveniles

vivisection a dissection performed on a living animal for scientific study or experimentation

watershed the region that drains into a particular stream or river

wetland land area covered with sufficient water to saturate the soil and support the growth of aquatic plants

wobble the loose (not strict) pairing of the third nucleotide (5′ end) of a tRNA anticodon when it forms hydrogen bonds with a codon on the mRNA

X-ray crystallography a technique in which an X-ray beam is passed through a crystal of a macromolecule in order to figure out its structure. The atoms that comprise the crystal deflect the X-rays, creating a unique pattern that can be analyzed to determine the position of the atoms within the molecule

xylem a vascular tissue in complex plants that functions mainly in transport of water and minerals from the roots up through the plant

yolk sac one of four extraembryonic membranes that surrounds the yolk of vertebrate embryos and is the site where blood cells first form

zoology the scientific study of animals

zooplankton animallike plankton

Zygomycota a phylum of fungi whose members produce sexual spores called zygospores

zygospore a type of sexual spore formed by members of the fungal phylum Zygomycota

zygote a fertilized egg, the diploid product that results from the joining of two haploid gametes. After cleavage begins the product is referred to as an embryo

zygote intrafallopian transfer a form of assisted reproductive technology in which a fertilized egg that has not yet begun cleavage is placed into the oviduct in hopes of achieving a pregnancy

Appendix III
Further Resources

BOOKS

Campbell, Neil A., Jane B. Reece, Lisa A. Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, and Robert B. Jackson. *Biology*. 8th ed. San Francisco: Pearson Benjamin Cummings, 2008. Widely used, college-level, introductory biology textbook.

Cobb, Alan, ed. *Animal Sciences: Macmillan Science Library*. 4 vols. New York: Macmillan Reference USA, 2001. Covers biological concepts, history of zoology, biographies of scientists in the field, and ethical issues.

Considine, Glenn D., ed. *Van Nostrand's Scientific Encyclopedia*. 10th ed. 3 vols. New York: John Wiley & Sons, 2008. Comprehensive general reference containing more than 10,000 entries on topics ranging from all scientific disciplines, including the life sciences, Earth and atmospheric sciences, physical sciences, medicine, and mathematics, as well as many areas of engineering and technology.

The Diagram Group. *The Facts On File Biology Handbook*. Rev. ed. New York: Facts On File, 2006. Includes more than 1,000 glossary entries covering all aspects of biology, including organisms, organs, processes, and basic terminology; more than 400 biographies; a chronology; and useful charts, tables, and diagrams.

Eldredge, Niles, ed. *Life on Earth: An Encyclopedia of Biodiversity, Ecology, and Evolution*. Santa Barbara, Calif.: ABC-CLIO, 2002. An examination of nature's biological diversity and the human activities that threaten it.

Encyclopedia of Life Sciences. 26 vols. London: Nature Publishing Group, 2002–2007. Features more than 4,100 articles spanning the entire spectrum of life sciences.

Encyclopedia of the Biosphere. 11 vols. Farmington Hills, Mich.: Gale Group, 1999–2001. Comprehensive coverage of the Earth's ecosystems, their characteristics, their operation, and how human activities have transformed them.

Gillispie, Charles C., ed. *Dictionary of Scientific Biography*. 18 vols. New York: Charles Scribner's Sons, 1970–1981. *New Dictionary of Scientific Biography*. 8 additional vols., 2007. More than 5,000 biographies of scientists and mathematicians from around the world.

Hamblin, Jacob Darwin. *Science in the Early Twentieth Century: An Encyclopedia*. Santa Barbara, Calif.: ABC-CLIO, 2005. Alphabetical entries examining science between 1900 and 1950.

Haugen, Peter. *Biology: Decade by Decade*. New York: Facts On File, 2007. Chronicles the history of 20th-century biology.

Hine, Robert. *The Facts On File Dictionary of Biology*. 4th ed. New York: Facts On File, 2005. Contains more than 3,700 entries, 60 black-and-white drawings, tables, charts, bibliography, and pronunciation symbols.

Hutchins, Michael, ed. *Grzimek's Animal Life Encyclopedia*. 2nd ed. 17 vols. Farmington Hills, Mich.: Gale Group, 2003–2004. Taxonomically arranged resource for information about animals, including their life cycles, predators, food systems, overall ecology, and more.

Kress, John, and Gary W. Barrett, eds. *A New Century of Biology*. Washington, D.C.: Smithsonian Institution Press, 2001. A collection of essays written by notable biologists concerning problems their discipline must address in the 21st century.

Kusky, Timothy. *Encyclopedia of Earth and Space Science*. 2 vols. New York: Facts On File, 2009. Contains more than 200 entries on topics related to the NSES content standards for grades 9–12, a chronology, glossary, and further resources.

Lennarz, William J., and M. Daniel Lane. *Encyclopedia of Biological Chemistry*. 4 vols. Oxford: Elsevier, 2004. Compilation of more than 500 entries encompassing all aspects of biochemistry, as well as the extensions of this subject into the related fields of molecular biology, cell biology, genetics, and biophysics.

Lerner, K. Lee, and Brenda Wilmoth Lerner, eds. *Gale Encyclopedia of Science*. 4th ed. 6 vols. Farmington Hills, Mich.: Gale Group, 2007. Provides an overview of current knowledge in all

major areas of science, engineering, technology, mathematics, and the medical and health sciences, consisting of alphabetical entries of scientific concepts and terms.

Lewin, Benjamin. *Genes IX*. Sudbury, Mass.: Jones & Bartlett, 2008. Comprehensive, college-level text covering molecular biology and molecular genetics.

Nemeh, Katherine H., ed. *American Men and Women of Science: A Biographical Dictionary of Today's Leaders in Physical, Biological, and Related Sciences*. 25th ed. 8 vols. Farmington Hills, Mich.: Thomson Gale, 2008. Brief profiles of nearly 135,000 living scientists.

Oakes, Elizabeth H. *Encyclopedia of World Scientists*. Rev. ed. 2 vols. New York: Facts On File, 2007. Profiles nearly 1,000 scientists from around the world.

———. *International Encyclopedia of Women Scientists*. New York: Facts On File, 2002. Profiles approximately 400 notable women scientists from around the world, past and present.

O'Daly, Anne, ed. *Encyclopedia of Life Sciences*. 2nd ed. 13 vols. Tarrytown, N.Y.: Marshall Cavendish, 2004. Contains more than 470 articles covering all facets of life sciences.

Panno, Joseph. *The New Biology*. 6 vols. New York: Facts On File, 2004. Consists of six volumes on current topics in biology and medical research.

Pechenik, Jan A. *Biology of the Invertebrates*. 5th ed. New York: McGraw Hill, 2004. Concise, college-level textbook for invertebrate zoology courses.

Rice, Stanley A. *Encyclopedia of Evolution*. New York: Facts On File, 2006. Contains more than 200 entries that span from modern evolutionary science to the history of its development.

Richards, Julie E., and R. Scott Hawley. *The Human Genome: A User's Guide*. 2nd ed. Burlington, Mass.: Elsevier Academic Press, 2004. Discusses basic genetics information and analytical techniques and applies them to topics such as genetic testing and gene therapy.

Robinson, Richard, ed. *Biology: Macmillan Science Library*. 4 vols. New York: Macmillan Reference USA, 2001. Explains biological concepts, reviews history of biology, and explores related fields.

———. *Genetics: Macmillan Science Library*. 4 vols. New York: Macmillan Reference USA, 2002. Major themes include inheritance; genes and chromosomes; genetic diseases; biotechnology; ethical, legal, and social issues; history of the field; and careers.

———. *Plant Sciences: Macmillan Science Library*. 4 vols. New York: Macmillan Reference USA, 2000. Introduces fundamentals such as cells, transportation, and photosynthesis as well as related fields such as agribusiness, conservation, and ethnobotany.

Rosen, Joe, and Lisa Q. Gothard. *Encyclopedia of Physical Science*. 2 vols. New York: Facts On File, 2009. Contains more than 200 entries on topics related to the NSES content standards for grades 9–12, a chronology, glossary, and further resources.

Stone, Carol Leth. *The Basics of Biology*. Westport, Conn.: Greenwood Press, 2004. Introductory overview of the field, including its history, key concepts, and principles.

Tudge, Colin. *The Variety of Life*. New York: Oxford University Press, 2000.

Watson, James D., Tania A. Baker, Stephen P. Bell, Alexander Gann, Michael Levine, and Richard Losick. *Molecular Biology of the Gene*. 6th ed. San Francisco: Benjamin Cummings, 2007. Widely used, comprehensive, college-level textbook for molecular biology.

Yount, Lisa. *A to Z of Biologists*. New York: Facts On File, 2003. Profiles more than 150 biologists, discussing their research and contributions.

INTERNET RESOURCES

American Museum of Natural History home page. Available online. URL: http://www.amnh.org/. Accessed February 6, 2008. Contains links for the Center for Biodiversity and Conservation, research conducted by the museum, and updates on scientific topics.

ASU Ask a Biologist. Available online. URL: http://askabiologist.asu.edu/. Last modified on February 6, 2008. Intended as a resource for K–12 students and teachers.

Biology-Online.org. Available online. URL: http://www.biology-online.org/. Accessed February 6, 2008. Contains numerous links for information and articles related to biological subjects, tutorials, discussion groups, and other resources.

Cold Spring Harbor Laboratory. Dolan DNA Learning Center. DNA Interactive. Available online. URL: http://www.dnai.org/. Accessed February 8, 2008. Contains news about genetics, tutorials, movies, and a glossary. Organized into several sections: a timeline; how scientists cracked the genetic code; how DNA can be manipulated; an overview of genomes; how DNA science is applied to healthcare, forensic investigations, and human origins; and an exploration of the failings of eugenics.

Does, Amy, Norman A. Johnson, and Teresa Thiel. *Rediscovering Biology*. Online Textbook. Annenberg Media, Washington, D.C. Available online. URL: http://www.learner.org/channel/courses/

biology/textbook/. Accessed February 8, 2008. Focuses on topics that have undergone significant change in the preceding decade.

Encyclopedia of Life. Available online. URL: http://www.eol.org/. Accessed February 8, 2008. A work in progress that aims to serve as an online reference source for all identified species.

Farabee, Michael J. *Online Biology Book*. Available online. URL: http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookTOC.html. Accessed February 8, 2008. An e-book organized into chapters and containing diagrams and links to glossary terms.

Gilbert, Joanna. BiologyMad. Available online. URL: http://www.biologymad.com/. Accessed February 8, 2008. A Web site that contains topic notes, concept maps, and animations on subjects that include cells, genetics, human biology, biochemistry, ecosystems and the environment, and plant biology.

Howard Hughes Medical Institute. Biointeractive: Teach Ahead of the Textbook. Available online. URL: http://www.hhmi.org/biointeractive/. Accessed February 8, 2008. Includes virtual laboratory exercises, tutorials, lectures, videos, and animations on more than a dozen selected topics that are relevant to current medial research.

Kimball, John W. *Kimball's Biology Pages*. 2006. Available online. URL: http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/. Accessed February 8, 2008. A regularly updated online biology textbook.

Museum of Vertebrate Zoology at the University of California at Berkeley Web site. Available online. URL: http://mvz.berkeley.edu/. Accessed February 9, 2008. Includes descriptions of current projects and research at the MVZ as well as links to reputable sources of information about biodiversity, genomics, and evolutionary biology.

National Health Museum. Access Excellence. Available online. URL: http://www.accessexcellence.org/. Last updated February 5, 2008. Contains resources, graphics, animations, activities, and updates on issues related to biotechnology, health, and science.

———. National Human Genome Research Institute home page. Available online. URL: http://www.genome.gov/. Accessed February 10, 2008. Home page for the NHGRI, contains descriptions of their research, educational resources, and more.

National Institutes of Health. Office of Science Education. Available online. URL: http://science.education.nih.gov/. Accessed February 10, 2008. Educational resources for students or for the public.

National Library of Medicine and the National Institutes of Health. National Center for Biotechnology Information home page. Available online. URL: http://www.ncbi.nlm.nih.gov/. Last revised January 25, 2008. Home page for the NCBI, contains links for GenBank, literature databases, molecular databases, genomic biology, data mining tools, research at NCBI, and educational resources and tutorials.

National Oceanic and Atmospheric Administration home page. Available online. URL: http://www.noaa.gov/. Accessed February 10, 2008. Home page for the agency whose mission is to understand and predict changes in Earth's environment and conserve and manage coastal and marine resources to meet national economic, social, and environmental needs.

National Science Foundation. Directorate for Biological Sciences (BIO). Available online. URL: http://www.nsf.gov/bio/. Accessed February 10, 2008. Home page for the branch of the NSF whose mission is to enable discoveries for understanding life.

Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.). Available online. URL: http://www.ncbi.nlm.nih.gov/omim/. Accessed February 10, 2008. Contains links to enormous amounts of technical information regarding genetic disorders, locations of specific genes, statistics, and genome databases.

SciTechDaily Review. Available online. URL: http://www.scitechdaily.com/. Accessed February 10, 2008. Regularly updated science and technology coverage.

Sullivan, James A. "Cells Alive! Quill Graphics." Available online. URL: http://www.cellsalive.com/. Accessed February 9, 2008. Contains links to illustrations, micrographs, animations of cells and their processes, biological structures, and quizzes.

Tree of Life Web Project. Available online. URL: http://www.tolweb.org/tree/. Accessed February 9, 2008. A collaborative effort to organize and present information about every known species and group of organisms through Web pages linked via a virtual phylogenetic tree.

University of Arizona. Department of Biochemistry and Molecular Biophysics, College of Science. The Biology Project. Available online. URL: http://www.biology.arizona.edu/. Accessed February 6, 2008. An interactive online biology resource that contains numerous activities, tutorials, and additional Internet resources.

University of California Museum of Paleontology Web site. Available online. URL: http://www.ucmp.berkeley.edu/. Accessed February 9, 2008. A Web site that provides a vast amount of information about the history of life and the diversity of the Earth's biota.

University of Michigan Museum of Zoology. Animal Diversity Web. Available online. URL: http://animaldiversity.ummz.umich.edu/. Accessed February 9, 2008. An online database of animal natural history, distribution, classification, and conservation biology.

WGBH Educational Foundation and Clear Blue Sky Productions, Inc. *Evolution*. Available online. URL: http://www.pbs.org/wgbh/evolution/. Accessed February 9, 2008. A comprehensive learning resource about evolutionary science for teachers, students, and the general public based on a seven-part, eight-hour television broadcast that premiered on PBS in 2001.

PERIODICALS

The American Biology Teacher
Published by the National Association of Biology Teachers
12030 Sunrise Valley Drive #110
Reston, VA 20191–3409
www.nabt.org

The American Naturalist
Published for the American Society of Naturalists by the University of Chicago Press
1427 E. 60th Street
Chicago, IL 60637
www.journals.uchicago.edu/AN/home.html

American Scientist
Published by Sigma Xi, The Scientific Research Society
P.O. Box 13975
Research Triangle Park, NC 27709
Telephone: (919) 549-0097
www.americanscientist.org

Audubon
Published by the National Audubon Society
700 Broadway
New York, NY 10003
Telephone: (212) 979-3000
www.magazine.audubon.org

The Biological Bulletin
Published by the Marine Biological Laboratory
7 MBL Street
Woods Hole, MA 02543
www.biolbull.org

BioScience
Published by the American Institute of Biological Sciences
1444 I Street, NW, Suite 200
Washington, DC 20005
Telephone: (202) 628-1500
www.biosciencemag.org

Current Biology
Published by Cell Press, Elsevier, Inc.
600 Technology Square, 5th floor
Cambridge, MA 02139
Telephone: (617) 661-7057
www.current-biology.com

Discover
Published by Buena Vista Magazines
114 Fifth Avenue
New York, NY 10011
Telephone: (212) 633-4400
www.discovermagazine.com

Frontiers in Bioscience
Published by Frontiers in Bioscience
P.O. Box 160
Albertson, NY 11507-0160
Telephone: (516) 484-2831
www.bioscience.org

Journal of Biology
Published by BioMed Central Ltd.
Middlesex House
34-42 Cleveland Street
London W1T 4LB, UK
Telephone: +44 (0)20 7323 0323
jbiol.com/home

Natural History
Published by Natural History Magazine, Inc. in affiliation with the American Museum of Natural History
P.O. Box 5000
Harlan, IA 51593-0257
www.naturalhistorymag.com

Nature
The Macmillan Building
4 Crinan Street
London N1 9XW
Telephone: +44 (0)20 7833 4000
www.nature.com/nature

New Scientist
6277 Sea Harbor Drive
Orlando, FL 32887
Telephone: (888) 822-3242
www.newscientist.com

Oceanus
Published by Woods Hole Oceanographic Institution
WHOI
Mail Stop 40
Woods Hole, MA 02543
www.oceanusmag.com

PLoS Biology
Published by the Public Library of Science
185 Berry Street, Suite 3100
San Francisco, CA 94107
Telephone: (415) 624-1200
www.plosbiology.org

Science
Published by the American Association for the Advancement of Science
1200 New York Avenue, NW
Washington, DC 20005
Telephone: (202) 326-6417
www.sciencemag.org

Scientific American
415 Madison Avenue
New York, NY 10017
Telephone: (212) 754-0550
www.sciam.com

The Scientist: Magazine of the Life Sciences
400 Market Street, Suite 1250
Philadelphia, PA 19106-2501
Telephone: (215) 351-1660
www.the-scientist.com

SOCIETIES AND ORGANIZATIONS

American Association for the Advancement of Science (www.aaas.org) 1200 New York Avenue NW, Washington, DC 20005. Telephone: (202) 326-6400

American Institute of Biological Sciences (www.aibs.org) 1444 I Street NW, Suite 200, Washington, DC 20005. Telephone: (202) 628-1500

Association of Biology Laboratory Education (www.ableweb.org) c/o Michael O'Donnell, President, Trinity College, 300 Summit Street, Hartford, CT 06106.

Ecological Society of America (www.esa.org) 1707 H Street NW Suite 400, Washington, DC 20006-3915. Telephone: (202) 833-8775

Federation of American Societies for Experimental Biology (www.faseb.org) 9650 Rockville Pike, Bethesda, MD 20814. Telephone: (301) 634-7000

The Human Biology Association (www.humbio.org) c/o Darna Dufour, President (2005–09), Department of Anthropology, University of Colorado at Boulder, Campus Box 233, Boulder, CO 89309-0233. Telephone: (303) 492-6061

The Linnean Society of London (www.linnean.org) Burlington House, Picadilly, London, W1J 0BF, England. Telephone: +44 020 7434 4479

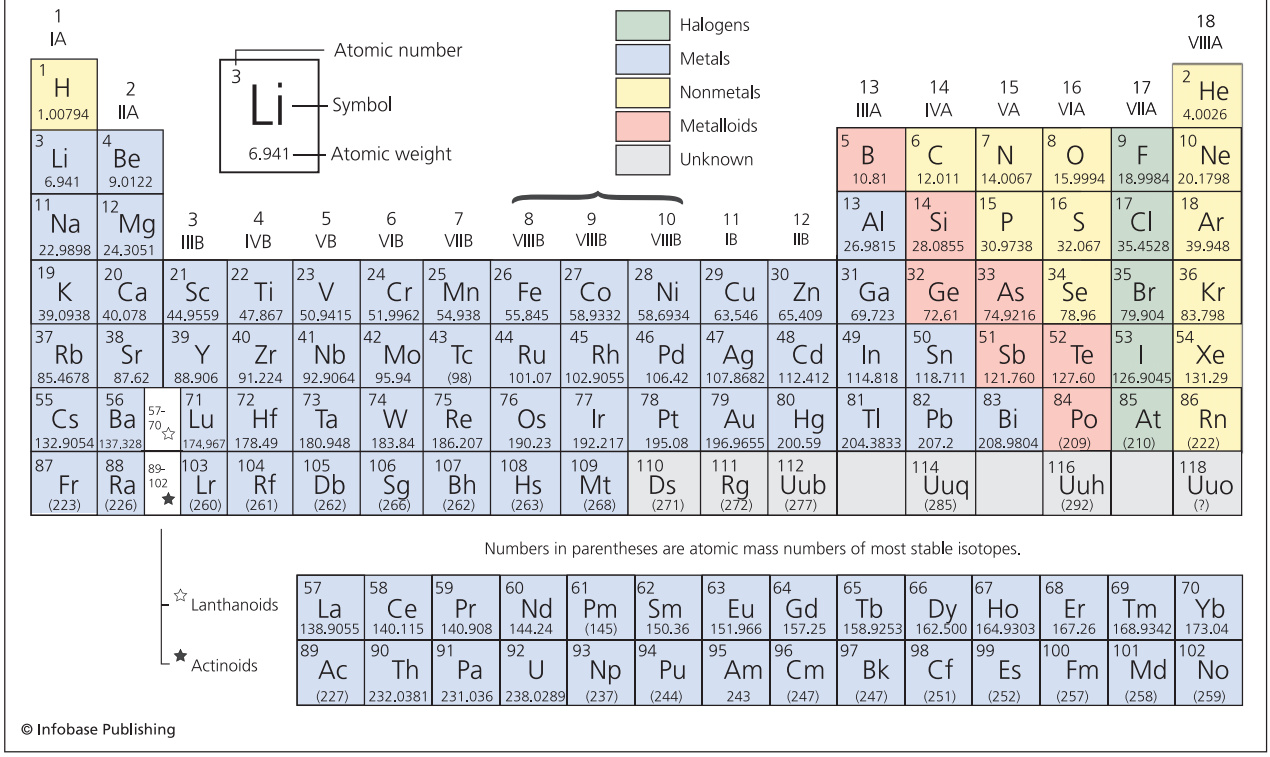
National Association of Biology Teachers (www.nabt.org) 12030 Sunrise Valley Drive, Suite 110, Reston, VA 20191. Telephone: (703) 264-9696 or (800) 406-0775

National Audubon Society (www.audubon.org) P.O. Box 52529, Boulder, CO 80322. Telephone: (800) 274-4201

The National Science Foundation (www.nsf.gov) 4201 Wilson Boulevard, Arlington, VA 22230. Telephone: (703) 292-5111, FIRS: (800) 877-8339, TDD: (800) 281-8749

Appendix IV
Periodic Table of the Elements

Periodic Table of the Elements



The Chemical Elements

(g) none
(c) nonmetallics

element	symbol	a.n.
carbon	C	6
hydrogen	H	1

(g) chalcogen
(c) nonmetallics

element	symbol	a.n.
oxygen	O	8
polonium	Po	84
selenium	Se	34
sulfur	S	16
tellurium	Te	52
ununhexium	Uuh	116

(g) alkali metal
(c) metallics

element	symbol	a.n.
cesium	Cs	55
francium	Fr	87
lithium	Li	3
potassium	K	19
rubidium	Rb	37
sodium	Na	11

(g) alkaline earth metal
(c) metallics

element	symbol	a.n.
barium	Ba	56
beryllium	Be	4
calcium	Ca	20
magnesium	Mg	12
radium	Ra	88
strontium	Sr	38

(g) none (c) metallics

element	symbol	a.n.
aluminum	Al	13
bohrium	Bh	107
cadmium	Cd	48
chromium	Cr	24
cobalt	Co	27
copper	Cu***	29
darmstadtium	Ds	110
dubnium	Db	105
gallium	Ga	31
gold	Au***	79
hafnium	Hf	72
hassium	Hs	108
indium	In	49
iridium	Ir ****	77
iron	Fe	26
lawrencium	Lr	103
lead	Pb	82
lutetium	Lu	71
manganese	Mn	25
meitnerium	Mt	109
mercury	Hg	80
molybdenum	Mo	42
nickel	Ni	28
niobium	Nb	41
osmium	Os****	76
palladium	Pd****	46
platinum	Pt ****	78
rhenium	Re	75
rodium	Rh****	45
roentgenium	Rg	111
ruthenium	Ru****	44
rutherfordium	Rf	104
scandium	Sc	21
seaborgium	Sg	106
silver	Ag***	47
tantalum	Ta	73
technetium	Tc	43
thallium	Tl	81
titanium	Ti	22
tin	Sn	50
tungsten	W	74
ununbium	Uub	112
ununquadium	Uuq	114
vanadium	V	23
yttrium	Y	39
zinc	Zn	30
zirconium	Zr	40

(g) pnictogen (c) metallics

element	symbol	a.n.
arsenic	As*	33
antimony	Sb*	51
bismuth	Bi	83
nitrogen	N	7
phosophorus	P**	15

(g) none (c) semimetallics

element	symbol	a.n.
boron	B	5
germanium	Ge	32
silicon	Si	14

(g) actinoid (c) metallics

element	symbol	a.n.
actinium	Ac	89
americium	Am	95
berkelium	Bk	97
californium	Cf	98
curium	Cm	96
einsteinium	Es	99
fermium	Fm	100
mendelevium	Md	101
neptunium	Np	93
nobelium	No	102
plutonium	Pu	94
protactinium	Pa	91
thorium	Th	90
uranium	U	92

(g) halogens (c) nonmetallics

element	symbol	a.n.
astatine	At*	85
bromine	Br	35
chlorine	Cl	17
fluorine	F	9
iodine	I	53

(g) lanthanoid (c) metallics

element	symbol	a.n.
cerium	Ce	58
dysprosium	Dy	66
erbium	Er	68
europium	Eu	63
gadolinium	Gd	64
holmium	Ho	67
lanthanum	La	57
neodymium	Nd	60
praseodymium	Pr	59
promethium	Pm	61
samarium	Sm	62
terbium	Tb	65
thulium	Tm	69
ytterbium	Yb	70

(g) noble gases (c) nonmetallics

element	symbol	a.n.
argon	Ar	18
helium	He	2
krypton	Kr	36
neon	Ne	10
radon	Rn	86
xenon	Xe	54
ununoctium	Uuo	118

a.n. = atomic number
(g) = group
(c) = classification

* = semimetallics (c)
** = nonmetallics (c)
*** = coinage metal (g)
**** = precious metal (g)

APPENDIX V

COMMON CONVERSIONS FROM U.S. CUSTOMARY TO METRIC UNIT VALUES

Quantity	To Convert From	To	Multiply By (Rounded to Nearest 1,000th)
mass	pounds (lb)	kilograms (kg)	0.454
	ounces (oz)	gram (g)	28.350
length	miles (mi)	kilometers (km)	1.609
	yards (yd)	meters (m)	0.914
	feet (ft)	meter (m)	0.305
	inches (in)	centimeters (cm)	2.540
area	square feet (ft^2)	square meter (m^2)	0.093
	square miles (mi^2)	square kilometers (km^2)	2.590
volume	gallon (gal)	liters (L)	3.785
	quarts (qt)	liters (L)	0.946
	fluid ounces (fl oz)	milliliters (mL)	29.574

APPENDIX VI

CONVERSIONS WITHIN THE METRIC SYSTEM

Prefix	Symbol	Factor of Base Unit
giga-	G	10^9
mega-	M	10^6
kilo-	k	10^3
hecto-	h	10^2
deka-	da	10^1
deci-	d	10^{-1}
centi-	c	10^{-2}
milli-	m	10^{-3}
micro-	μ	10^{-6}
nano-	n	10^{-9}
pico-	p	10^{-12}

1 meter (m) = 100 centimeters (cm) = 1,000 millimeters (mm)
1 centimeter (cm) = 10 millimeters (mm) = 0.01 meters (m)
1 millimeter (mm) = 1000 micrometers (μm) = 1 micron (μ)

1 liter (L) = 1,000 milliliters (mL)
1 cubic centimeter (cc or cm^3) = 1 milliliter (mL)
1 milliliter (mL) = 1,000 microliters (μL)

1 kilogram (kg) = 1,000 grams (g)
1 gram (g) = 1,000 milligrams (mg)
1 milligram (mg) = 1,000 micrograms (μg)

Appendix VII
Temperature Conversions

In the Celsius scale, 0°C is the freezing point of water and 100°C is the boiling point. In the Fahrenheit scale, 32°F is the freezing point of water and 212°F is the boiling point. To convert degrees Celsius (T_C) to degrees Fahrenheit (T_F):

$$T_F = \frac{9}{5} T_C + 32$$

To convert Fahrenheit (T_F) to degrees Celsius (T_C):

$$TC = \frac{5}{9} (TF - 32)$$

INDEX

Note: Page numbers in **boldface** indicate main entries; *italic* page numbers indicate photographs and illustrations.

A

AAV (adeno-associated virus) 373
abiogenesis 698
abiotic components 75, *275*
absence seizures 316
absorption
  in digestion 251, *253–255*
  by root system 620–621
abstinence 686, 689
abyssal zone 106, *107*
Acarapis woodi (tracheal mite) 15, 17
acetylation 194, 473
acid 737
acidophiles 40–41, 43
acid rain 304, *305*
acoelomates 35, *36*
acquired immunodeficiency syndrome (AIDS) **1–3,** 466, 468
Acquiring Genomes (Margulis) 530
Acrasiomycota 323, *323*
actin cytoskeleton *644,* 645
Actinobacteria 52
Actinopterygii 716–717, *717*
action potential 579–580, *580, 581,* 677
activation energy 311, *311*
activators 370
active transport *96,* 96–97
adaptation
  of bacteria 50
  in biology 104
  of birds 722
  in coevolution 275
  of desert plants 114
  of digestive systems 255
  in evolution 333, 506, 732–734
  identifying 334–335
  of plants 610–612, 629
  for thermoregulation 423–424
  traits as 334–335
addict (term) 4
addiction, biology of **3–8,** *7*
addition reactions 66, *66*
adenine
  Chargaff's research on 181
  in DNA structure 245
  in nucleic acids 122
adeno-associated virus (AAV) 373
adenosine triphosphate (ATP)
  in cellular respiration 171–172
  from chemiosmosis 172
  energy from 73
  from fermentation 172–173
  free radicals from 11
  from glucose 119
  in muscle function 572–573
  in photosynthesis 603, 604
ADH (antidiuretic hormone) 250, 425
ADHD (attention-deficit/hyperactivity disorder) 576–577
ADP ribosylation 473
adrenal glands *294*
adsorption 728
adult stem cells 751
adventitious buds 659
advertising, and obesity 609
aerobic respiration
  historical emergence of *413,* 413–414
aerosols
  for biological weapons delivery 98
affinity chromatography 189
AFM (atomic force microscopy) 555
Africa
  HIV pandemic in 1
agar 22, 168–169
agarose gel electrophoresis 280, 281, *655, 656*
Agassiz, Louis 400
agency
  in evolution 396
aggression 30, 523
aging **8–13,** *9,* 753
agnathans 715
Agouti gene 474
agriculture **13–20,** *19*
  biodiversity and 71–72
  biology in 105
  biotechnology in 131
  cloning applications in 210–212
  DNA cloning in 207
  environmental science in 310
  evolutionary biology and 342–343
  fungi and 363
  genetic engineering in 378–379, 749
  genetics in 383
  history of 14
  honeybees in 18
  pesticides used in 155
Agrobacterium 51
agronomy 14
AIDS (acquired immunodeficiency syndrome) **1–3,** 466, 468
air pollution 303
Akihito (crown prince [later emperor] of Japan) 203
alcohol 4, 6, *7*
alcoholic fermentation 173
aldosterone 425
Alexander, Albert 350
Alexander the Great 43, 44, 45
algae *20,* **20–22,** *339*
algin 22
alkalinophiles 40–41, 43
alleles. *See also* chromosomes
  in addiction 4
  frequencies of 333
  in inheritance 190, 472–476, 542–545, *543*
  polymorphic 713–714
allergies 463–464
alligators 721–722
allopatric speciation 336
allosteric enzymes 313
allosteric regulation 314–315
almonds, bee-pollination of 15
alpha-helix 119, *120*
alpha particles 653
Alphaproteobacteria 51
alpine tundra 117

alternation of generations
in algal reproduction 22, 664
in plants 611–612, 664
Altman, Sidney **22–26,** *23,* 158
altruism 692–693, *693*
alveolates 323
alveoli 666, *667*
Alvin (submersible) 458
Alzheimer's disease 12, *12,* 375
amebiasis 647
American foulbrood 15
amino acids. *See also* protein(s)
facilitated diffusion of 96
in molecular biology 559
prebiotic synthesis of 556, *557,* 588–589
in protein function 88–89
in proteins 119
in protein sequence alignment 85
structure of 119, *120*
ammonia 78–79, 343
amniotes 286, 717–718, *720*
amoebas 646, *646,* 647
AmpFLP (amplified fragment length polymorphism) *258,* 259
amphetamines 4, 5, 6, *7*
Amphibia 718–719
amphibians 717–719
Amphisbaenia 721
ampullae of Lorenzini 29
amygdala 5
amylase 66, 68
amyloid plaques 12
anabolic pathways 67, 171
anabolism 171, 173–174, 584
anaerobic bacteria, in bioremediation 125
anaerobic sludge digesters 739–740
anaphase 176, 660
anapsids *720,* 720–721
anatomic anomalies 26
anatomy **26–27**
Aristotle's work on 44
of birds 722
in botany 133
in cell biology 161
dissection in study of 256
of excretory system 344, *345*
Geoffroy Saint-Hilaire's theory of 388
physiology and 607
in systemics 90
in taxonomy 89
ancestral protist 326, *327*
Anderson, William 375
aneuploidy 138, 377, *379*
angel dust 4
angiogenesis 149
angiosperms 325, *325, 339, 616,* 618, *618,* 625–626
animal(s). *See also* zoology
asexual reproduction in 658, *659*
in chaparral 115
characteristics of 714–715
classification of. *See* biological classification
cloning 131, 208–213, 749–753
in coastal wetlands 109
community structure among 101
in coniferous forest 116
in coral reefs 108–109
in desert 114
diploid life cycles of 664
dispersal of 80–81
dissection of 256
in estuaries 109
fossil remains of 228–229
genetically engineered 379
genetically modified 749
historical emergence of 418
in intertidal zones 106–107
in lakes and ponds 109
in marine benthic zones 109
in mountaintops 117
in oceanic pelagic zone 107–108
overexploitation of 288
population density among 634
population structure among 101
reproduction in 409
in rivers 112
in savanna 114
sex determination of 682–684
sexual reproduction in 659
in temperate broadleaf forest 116
in temperate grasslands 115–116
tissue culture of 170
transgenic 210
in tropical rain forest 114
in tundra 116–117, *117*
variations in generations 236
in wetlands 111
animal behavior **27–31**. *See also* ethology
Aristotle's work on 44
evolution in 694
Lorenz's work in 520–523
social behavior 692–693
in systematics 89
Animal Breeding Organization (ABRO) 749
animal cognition and learning 28, **31–33,** 521, 709–710
animalcules 509
animal development. *See* embryology and early animal development
animal form **33–37**
animal husbandry 14
Animalia kingdom 93, 322, 761
Annelida (annelids) 37, 326, 489–490
anorexia nervosa 274
ANP (atrial natriuretic peptide) 425
ant(s) *708,* 708–709
antelopes 101
anthrax
as biological weapon 98, 130
Koch's work on 500–501
vaccine for 595
anthropogenic pollutants 303
anthropoids *438,* 438–439
anthropology 727
antibacterial antibiotics 37–38. *See also* penicillin and penicillin derivatives
fungi and 363
overprescription of 39–40
resistance to 39–40
antibodies
in DNA cloning 207
in host defenses 435, *436,* 436–437
Pauling's study of 599
vaccines and 348
antibody-antigen interaction 435
antibody-dependent cytotoxic reactions 464
antidepressants 5
antidiuretic hormones (ADH) 250, 425
antifungal drugs 38–39
antigen presenting cells (APCs) 435–436
antigens
in blood 199
in host defenses 434–435
in vaccines 711
antihelminth drugs 38–39
antimicrobial drugs **37–40.** *See also* antibacterial antibiotics; antifungal drugs; antihelminth drugs; antiprotozoan drugs; antiviral drugs
antiprotozoan drugs 38–39
antisense RNA 668–670
antiseptics 348–349, 390
antiviral drugs 39
ant lions 709–710
Anura 718–719
ANWR (Arctic National Wildlife Refuge) 299
APCs (antigen presenting cells) 435–436
aphotic zone
in lakes 109
in marine biomes 106, *107*
apical meristems 610–611
Apicomplexa (apicomplexans) 323, *323,* 646–648, *648*

Apis mellifera. *See* honeybees
Apistan™ 16
Aplysia 674
apomixis 659
apoptosis 174
appendicular skeleton 570
aquatic biomes. *See* biomes, aquatic
Arachnida 492–493
Archaea **40–43,** *41,* 50
  chromosomes of 191
  classification of 93, 529
  as domain 756–758, *757*
  at hydrothermal vents 460
  kingdoms in 93
archenteron 34, 284
Arctic National Wildlife Refuge (ANWR) 299
Arcturus Adventure, The (Beebe) 58–59
Aristotle **43–45,** 89
Aristotle's lantern 44
aromatic hydrocarbons 125
ART (assisted reproductive technology) **45–47,** 130
arteries 195, *196,* 344
arthritis 13
Arthropoda (arthropods) 37, 326, *327,* 490–493, 666
Arthus reaction 464
articulations. *See* joints
artificial selection 342–343, 505
Ascomycota 324, 362
aseptic technique 595
asexual reproduction 658–659
  cloning and 208
  of fungi 361, *361, 362, 363*
  genetic variation in 332
  of plants 625
aspartate aminotransferase 66
Assembling the Tree of Life (ATOL) 92
assisted reproductive technology (ART) **45–47,** 130
Association of Zoos and Aquariums (AZA) 225
associative learning 32
astronomy 429
atherosclerosis 13, 200, 250
atmosphere 126, 530–531
ATOL (Assembling the Tree of Life) 92
atom(s) 100, 183–184
atomic bomb 601
atomic force microscopy (AFM) 555
atomic weight 183
atonic seizures 316
ATP. *See* adenosine triphosphate
atrial natriuretic peptide (ANP) 425
attention-deficit/hyperactivity disorder (ADHD) 576–577
aura 318–319
Australian social spider 31
australopiths 439–440, *441*
autism 706
autoclaves 168
autoimmune disorders *465,* 466
autonomic nervous system 578, 666
autopsy 256, 726
autosomal dominant disorders 376
autosomal recessive disorders 376
autosomes 442
autotrophs
  in carbon cycle 76
  in nutrient cycles 75
  in phosphorus cycle 79
Avery, Oswald **47–49,** 247, 524
Aves. *See* birds
axial skeleton 570
axopodia 324
AZA (Association of Zoos and Aquariums) 225
azidothymidine (AZT) 2
azoic theory 700–701
azoles 38

B

B_{12} vitamin *421,* 421–422
Bacillariophyta 20
backcross 543
bacteria. *See also* antibacterial antibiotics; Archaea; Bacteria domain; infectious diseases; prokaryotes
  adaptation of 50
  Avery's work on 48
  beneficial 548
  biological classification of 528, *529*
  as biological weapons 98–99
  in bioremediation 124
  cell envelope of 642–643
  cell membrane of 38, 642, *644*
  cell walls of 37–38, 642–643, *644*
  cholera from 502
  chromosomes of 191
  culturing 168–169, *169,* 349–350
  discovery of 510
  diversity of 50–51
  in DNA cloning 205–206
  drug resistance in 39–40
  EGSs used against 25
  external structures of 643, *644*
  in food production 72
  in genetic engineering 379
  genetic information in 247
  genome of 384–385, *385*
  growth of 50, 696
  honeybees infected by 15–16
  immunity to 711–712
  internal structures of 643–645, *644*
  MacLeod's work on 524–525
  in nitrogen cycle 78–79
  nucleic acid synthesis in 38
  protein synthesis in 38, 39
  shape of 641, *642*
  in sulfur cycle 79
  transformation of 48–49, 50, 524, 534
  treatment of infection by 37–38
  variation from 713
  virulence of 48
Bacteria (Eubacteria) domain 40, *41,* **50–52,** 93
bacterial colony 50
bacteriology
  Brock's work in 138–141
  Fleming's work in 348–349
  McCarty's work in 534–535
bacteriophages
  DNA of 182, 247–248
  Hershey's work on 410
  life cycle of 729–731
  transduction by 50
balance 678–680, *679*
Ballard, Robert 457–459, 534
Banting, Frederick G. **53–56**
barbiturates 4
bar graph 238, *238*
barnacles 492
barrier contraception 688
barrier reefs 109
Barron, Moses 54
Barton, Otis 59
basal angiosperms 618–619
bases 737
Basic Local Alignment Search Tool (BLAST) program 84–85, 88
Basidiomycota 324, 362
basidiospores 324
basophils 199, *199,* 433
Batesian mimicry 733–734
Bathysphaera intacta 61
bathysphere 59–61, *60,* 533
bats 90
Bauhin, Gaspard 134
Beagle, HMS 233–235
Beebe, William **56–62,** 533
beer, pasteurization of 595
bees. *See also* honeybees
  chemical senses in 356–357
  language of 357–359, *358, 359*
  Mendel's work with 545
  Turner's work with 709
beeswax 15
behavior. *See* animal behavior; ethology
behavioral ecology 276. *See also* ethology

Beijernick, Martinus Wilhelm 494–495
benthic zones 106, *107,* 109, *110*
benzodiazepines 4
Berg, Paul 205, 260, 654
Bermuda 59
Bernal, John Desmond 419–420
Berzelius, Jöns Jakob 68
Best, Charles H. 54–56
beta decay 653
β-lactam ring 39, *40*
beta particles 653
beta-pleated sheets 119–120, *120*
Betaproteobacteria 51
Bigelow, Henry *62,* **62–65,** 533–534
bilateral symmetry 35, *36*
Bilateria 326, *327*
binary fission 658
binge-eating disorder 274
binomial nomenclature 89, 92
bioaugmentation 124, 221
biochemical reactions **65–67,** 278, 338
biochemistry **67–70,** *69*
  applications of 69
  in biological classification 90–91
  history of 68
  research in 68–69
biodiversity **70–72.** *See also* conservation biology
  in biogeography 81
  decreased plant growth and 297
  in temperate broadleaf forest 116
  in tropical rain forest 113, 296
bioenergetics **73–75.** *See also* energy
  in biochemistry 68–69
  in ecosystems 277–278
bioenergy 130, 297
bioengineering 130
biofuel 302
biogenetic laws 404–405
biogeochemical cycles **75–80.** *See also* carbon cycle; nitrogen cycle; nutrient cycles; phosphorus cycle; water cycle
biogeography **80–83,** *81*
  Buffon in 141
  evolutionary evidence from 340, 734
  Haeckel in 406
  Wallace in 733, 734
bioinformatics **83–89**
Biological and Toxin Weapons Convention 98
biological classification **89–94**
  Aristotle's 43–44
  of bacteria 51–52
  in biology 104
  Cuvier's 230, *230*
  data used for 89–91
  DNA in 322
  of Eukarya 322
  of fungi 361–362
  hierarchical organization in 92–94
  Humboldt's 453–454
  Lamarck's 504–506
  Linnaeus's 517, 518, *518,* 519
  Margulis's 528, *529*
  modern 93–94
  phylogenetic trees in 91–92
  of viruses 727–728, *729*
  Whittaker's 745–746
  Woese's 756–758, *757*
biological membranes **94–98,** *95*
  of bacteria 38, 642, *644*
  of eukaryotic cells 328
  potential energy in 97
  of primitive cells 590
  structure of 94
  transport across 94–98, *96, 97*
biological oceanography 63
biological oxygen demand (BOD) 741
biological sex 444, 562
biological symmetry 35
biological weapons **98–99,** 130
biologic therapy, for cancer 151
biology **99–106.** *See also* cell biology; conservation biology; evolutionary biology; forensic biology; marine biology; microbiology; molecular biology; sociobiology
  applications of 105
  characteristics of life in 102–104
  as process 104
  subdisciplines of 102, *103*
  themes of 104–105
Biology of the Cell Surface, The (Just) 499
bioluminescence 73, 107
biomass
  energy from 132
  in production efficiency 279–280
  as renewable energy source 301
biomes
  aquatic **106–112**
    freshwater 109–112
    marine 106–109
  in biology 105
  structure-function relationships in 102
  terrestrial **112–118,** *113*
biomining 129
biomolecules **118–123.** *See also* carbohydrate(s); lipid(s); nucleic acids; protein(s)
  from anabolism 173–174
  in biochemistry 67
  in biology 104–105
  characterization of 68
  electrophoresis of 280–283
  elements in 183–184
  hydrogen bonds in 599
  purification of 68
  structure and function of 599
biophysics 756
bioreactors 124, 739–740
bioremediation **123–125,** 207, 221, 550
biosphere **125–129**
  abiotic factors affecting 127
  definition of 125–126
  as physiological system 530–531
  structure of 126–127
biostimulation 124
biosynthesis
  in nutrition 583–585
  of tRNA 23–24, *24*
biotechnology 19, **129–131.** *See also* genetic engineering
bioterrorism 98
biotic components 75
bipedalism 440
birds (Aves)
  DDT's effects on 153–155
  protection of 223–224
  as vertebrates 718, *720, 722,* 722–723
birth, in population density 633
birth control 687–689, *689*
bisphosphoglycerate mutase 67
bivalves 488
black bears 222–223
black reaction 391–392
black smokers *458,* 458–459
bladder 346
BLAST (Basic Local Alignment Search Tool) 84–85, 88
blastocoel 34
blastoderm 284
blastopore 34, 35–36
blastula 34, 284, *285*
blastulation 283–284
blood
  circuit of 198, 407–408, *408, 525*
  in circulatory system 195
  clotting of 199
  composition of 198–199, *199*
  oxygen transport in 667–668
blood glucose 248–249, 424
blood pressure, high 200
blood type 199
bluegills 31
blue orchard bees *(Osmia lignaria)* 18
Blueprint for a Cell (Duve) 269
BMI (body mass index) 583

BOD (biological oxygen demand) 741
body equilibrium 678–680, *679*
body mass index (BMI) 583
body plans 35–37
bone marrow 750, 751
bone tissue 570–571
Bonpland, Aimé 453–455
bony fishes 665–666, 716–717
botany **131–135**
  Buffon's work in 141
  forensic 352
  Gray's work in 398–399, 400
  history of 134
  Hooker's work in 431–432
  Humboldt's work in 452–453
  Ivanovsky's work in 494–495
  Lamarck's work in 504–505
  Linnaeus's work in 516–519
  Schleiden in 671–672
  subdisciplines of 133–134
  uses of 132–133
bottom-up control model of communities 218
botulism 99
Boveri, Theodor **135–138**
bovine spongiform encephalopathy. *See* Creutzfeldt-Jakob disease
bowerbirds 30
Boyer, Herbert 205
Boyle, Robert 426–427
Boyle's law 426–427
bracket fungi 324
Bragg, Lawrence 226
brain. *See also* epilepsy; seizures
  addiction and 5–6, *7*
  in ADHD 577
  in nervous system 575–578
  resection of 318
  in sensation 678
brain stem 5
branch points, in phylogenetic trees 91, *91*
breast cancer 150, 377, 685
breastfeeding 452
breathing 666–667
Briggs, Robert 208
brightfield microscopy 551, *552*
broadleaf forest, temperate 116
broad spectrum antibiotics 38
Brock, Thomas 42, **138–141**
Brongniart, Alexandre 229–230
Bronx Zoo 57
broth, in bacteria culture 168–169
Brown, Robert 134, 160
brown algae (Phaeophyta) 20–21, 324
Bryophyta (bryophytes) 325, *325*, *611*, *612*, 612–614
bubble hypothesis 590
bubonic plague 98
Buchner, Eduard 68
bud (plant) 622
budding 658, *659*
Buffon, Georges-Louis Leclerc, comte de **141–142,** 504
Buffon's law 142
bulbs 659
bulimia nervosa 274
bulk feeders 250
bundle-sheath cells 606
buoyant density gradient centrifugation 179–180

C

caecilians 718
Caenorhabditis elegans 674–675
caffeine 4
calcium regulation 425–426
California Institute of Technology (Caltech) 263–264, *565*, *597*, *598*
caloric restriction 11–12
calories 583
Calvin, Melvin **143–148,** *556*
Calvin cycle 148, 604–605, *605*
Cambrian 416, *416*
Campbell, Keith 209, 752–753
cAMP-mediated pathway *165*, 166–167
cancer
  age-related risk of 12–13
  biochemical research on 69
  biology of **148–151**
  causes of 149–150, 178, 377
  cell cycle regulation and 176–178
  cell differentiation and 148–149
  chromosomes in 138
  epigenetics and 474–475
  gene therapy for 371–374
  immortalized cells in 170
  metastasization of 177–178
  reproductive 685
  treatment of 150–151
  types of 150–151
  vitamin C and 601
cannabis 4, 6, *7*
Cape Haze Marine Laboratory 202
capillaries 525
capillary action 735
carbohydrate
  as biomolecule 118–119
  biosynthesis of 174
  monomers of 67
  in nutrition 583–584
carbon
  atomic structure of 184, *184*
  in biomolecules 118
  in organic chemistry 587
  during photosynthesis 144–148, *145*, *146*, *147*
  structure-function relationship in 100
carbon cycle *76*, 76–77, 132
carbon dating 653
carbon dioxide
  for bioenergy 130
  in carbon cycle 76
  collection of 637, *638*
  increase in, and plant growth 296–297
  photosynthetic reduction of 144, 605–606, 640
  in respiration 664
carbon fixation 605–606
Carboniferous 418
carbon monoxide 641
carcinoma 150
cardiac muscle 572
cardiovascular disease 13, 199, 608
carnivores
  diet of 250
  digestive systems of 255
  in ecosystems 277
  vertebrate 724
Carpenter, William Benjamin 700–701
Carrageen 22
carrier proteins 96
carrying capacity 635, *635*
Carson, Rachel **151–156,** *152*, 308
Casiquiare 453, 454
castes 693
CAT. *See* computed tomography
catabolic pathways 67, 171
catabolism 171–173, 582, 584
catalysis 22, 24–25, 68
catalysts 67, 74, 310–311
catastrophism 230
catecholamines 5
categorizing (cognitive ability) 32–33
cat pheromones 29
cattle 44
Caudata 718, *719*
Cavendish, Henry 640
CCD (colony collapse disorder) 16–17
CD (circular dichroism) 747
cDNA (complementary DNA) library 205
Cech, Thomas 25, **156–159,** *157*
cell(s)
  in biology 104–105
  development of 162
  in Eukarya 321–322
  fractionation of 162, 179, 268
  life span of 9–10
  as model organisms 675
  origin of term 428

oxidation of *65*
in plants 671
primitive *590–591*
surface area of *195*
surface receptors of 164
cell biology **159–163,** *674*
cell communication **163–167,** *289–290*
cell culture **167–170,** *168, 169, 170*
by aseptic technique 168
of bacteria 168–169, *169*
of cancer cells 149
in cell biology 162–163
in DNA cloning 207
of fungi 169
Levi-Montalcini's use of *514*
cell cycle. *See* cellular reproduction
cell differentiation
cancer and 148–149
cloning after 208, 209–210
epigenetics in 473–474
in plants 623–624
research on 162
of stem cells 750
cell division cycle *(cdc)* genes 176
cell envelope, of bacteria 642–643
cell-mediated specific immunity 435–436
cell membrane. *See* biological membranes
cell senescence 170
cell theory
in biology 104
in botany 671
foundation of 160
modern 160
of Schwann and Schleiden 672–673, 726–727
cellular metabolism **170–174,** *173*
cellular phones, CCD and 16–17
cellular reproduction **174–178,** *175, 177*
in cancer 149
chromosomes during 135–136
cellular respiration
bioenergetics of 74
in metabolism 171, *173*
photosynthesis and 602
cellular slime molds 324, 690, *691*
cellulose 100–101, 119, *119*
cell walls
of archaea 41
of bacteria 37–38, 642–643, *644*
of eukaryotic cells 328
Celsius, Olaf *516–517*
centipedes 492
central nervous system (CNS) *575–578*
centrifugation *178,* **178–180,** *179*
Cephalaspidomorphi 715, *715*
cephalization 35, 327
cephalopods 489
cerebellum *577–578*
cerebral cortex *5, 7*
cerebrum *5, 575–576*
cervical cancer 685
Cesalpino, Andrea 134
Cestoda 486
Cetus Corporation *566–568*
CFCs (chlorofluorocarbons) 303–304
CFTR gene 372
Chain, Ernst 37, 350–351
Challenger, HMS 701–702
chaparral 114–115
Chargaff, Erwin **180–182,** 226, 743
Chargaff's rules 181
Charles I (king of England) 408
charophyceans 610
Chase, Martha **182–183,** 247–248, 410
Checkmite+ 16
Chelicerata 492
chemical(s)
in agriculture 19
atomic structure of 183–184
as basis of life **183–187**
bonds in 184–187, *185, 186, 597–599*
in estuaries 112
in rivers 112
chemical degradation DNA sequencing 260
chemical synapse 581–582
chemical weapons 99
chemiosmosis 172
chemistry
in biology 104
Hodgkin's work in 419
Lamarck's work in *507*
Pauling in *597–602*
Priestley's work in 637–641
chemoautotrophs 51, 413
chemoheterotrophs 51
chemolithogrophs 73
chemoorganotrophs 73
chemoreceptors 678
chemosynthesis 460
chemotaxis 28
chemotherapy
for cancer 150–151
definition of 37
for microbial infections 37–40
chemotrophs 73
chicks, dissection of 44
Child, Charles Manning 461–462
childhood obesity 608–610
Chilopoda 492
chimaeras 716, *716*
chimpanzees 439, *440*
chi-square test *244,* 244–245
chlamydia 686
Chlamydiae 51, *52*
chlorinated hydrocarbons 125
chlorofluorocarbons (CFCs) 303–304
chlorophyll 603
Chlorophyta 20, 21
chloroplasts
of eukaryotic cells 329–330, 385–386
origin of 414, *415*
Choanoflagellates 326, *327*
cholera 502, *595*
cholesterol 290, 420, *585*
cholesterol levels 200
Chondrichthyes 716, *716*
Chordata (chordates) 37, 328, 493–494, *494*
chorology. *See* biogeography
chromatin 191, 194
chromatography **187–189,** *189*
chromosomal disorders 377–378, *379*
chromosomal theory of inheritance 160, 189–190, 382, 761
chromosomes **189–195**
of archaea 42
of bacteria 643, *644*
Boveri's work on 135–136
of eukaryotes 191–192, *192*
genes on 472
genetic difference in 136–137
in genome 442
in human reproduction 444
identification of 193, *193*
in inheritance 137, *562*
inversion of 263
number of, by species *190*
of prokaryotes 191
in reproduction *658*
in sex determination 562, 683–684, *699*
Stevens's work on 698–699
structure of 190–194
in tumor development 138
Chrysophyta (golden algae) 20–21, *323,* 324
chyme 253
Chytridiomycota 324, 362
cilia 330, *331,* 372, 646, *647*
ciliates 323, *323*
Ciliophora 646, *646, 647*
circular dichroism (CD) 747
circulatory system **195–200.** *See also* blood; heart
Aristotle's work on 44
of birds 723
excretory system and 344, *345*
Harvey's work on 407–408, *408, 525*

health of 199–200
human 195–197, *196*
Malpighi's work on 525
CITES (Convention on International Trade in Endangered Species of Fauna and Flora) 287, 288
citric acid cycle 160, 171–172
cladograms 91
clams, at hydrothermal vents 458, 460
Clark, Eugenie **200–204,** *201,* 534
classical conditioning 32
classical genetics 382
classification. *See* biological classification
cleavage 283–284, 450, 497
Clements, Frederic 745
climate
biosphere and 127, *128*
water in 736
climate change
biodiversity and 72
in habitat destruction 287
humans in 304–306, *306*
in species distribution 82
clocks 427
clone
definition of 207–208
cloning of DNA **204–207,** 567, 632–633, 656
cloning of organisms **207–213.** *See also* asexual reproduction
animals 131
applications of 210–212
from genetically modified organism 753
history of 208–209, *209*
humans 212–213
plants 129, 625
policies on 213
process of 209–210
Wilmut's work in 749–753, *754*
cloning of skin 130
closed system 73
club mosses 325
Clustal algorithm 84, 88
cnidarians 326, *327*
digestive systems of 251
hierarchical level of organization of 34
as invertebrates 483–484, *484, 485*
CNS (central nervous system) 575–578
CO_2. *See* carbon dioxide
Coacervates 590
coal 301
coastal wetlands 109
cocaine 4, 5, 6, *7*
cockroaches 710
codeine 4
CODIS (Combined DNA Index System) 259
codominance 479
codons 367–369, *368, 369,* 559
coelom 35, *36,* 327
coelomates 35, *36*
coevolution 275
cofactors 311
cognition. *See* animal cognition and learning
cognitive mapping 32
Cohen, Stanley 205, 514, *515*
cohesiveness 735–736, *736*
cold (rhinovirus) 601
Cold Spring Harbor Laboratory (CSHL) 744
coliform bacteria 741
collagenous fibers 35
Collip, J. B. 55–56
colony collapse disorder (CCD) 16–17
color vision. *See* vision
Columbia University 181, 265–266
column chromatography 188, *188*
comb jellies 326, 485
cometabolism 125
commensalistic symbiosis *215,* 215–216, 527
communicable disease 466
communication
in animal behavior 28–29
among bees 357–359, *358, 359*
among cells **163–167,** 289–290
in feeding behavior 30
communities
in biology 105
disturbance of 218–220, *219*
at hydrothermal vents 459–461
species interaction in 214–216
structure-function relationships in 101
structure of 216
community ecology **213–220,** 744–745
compact bone tissue 570
comparative anatomy 26–27
Cuvier's work in 230–232
evolutionary evidence from 338–340, *339*
in taxonomy 89
comparative ethology 321
comparative pathology 726
competition 214, 530
competitive exclusion principle 214
competitive inhibitor antibiotics 38
competitive inhibitors *314,* 315
complementary DNA (cDNA) library 205
complement system 434
compound(s) 183
compound eyes 681
compound microscopes 159, 551, *552*
compulsive eating 274
computational biochemistry 69
computed axial tomography. *See* computed tomography
computed tomography (CT) 27, 318
concentration 73
condensation 79
condensation reactions 66
condoms 688
confidence interval 242–243
congenital immunodeficiencies 465
coniferous forest 116
connective tissue 35, 149, 251–253
consciousness, in animals 33
conservation biology **220–225.** *See also* biodiversity
Carson and 156
efforts of 220–221
goals of 220–221
hot spot identification in 221
conservation of energy 73–74
conservation management plans 220–221, 222–224
Conservation Study Institute 225
constipation 255
continental shelf 106, *107*
continuum community hypothesis 745
contraception 687–689, *689*
Convention on Biological Diversity 71
Convention on International Trade in Endangered Species of Fauna and Flora (CITES) 287, 288
convict fish 204
Copeland, Herbert F. 93
coral atoll 109
coral reefs *108,* 108–109, 484
corals 326
corpus callosotomy 318
cosmology, Aristotle's work on 45
Cosmos (Humboldt) 456
Costa Rica 297
cotransport 97, *97*
Coulson, Alan 260
courtship behavior 30, 704
Cousteau, Jacques-Yves 534
covalent bonds 184–185, *186,* 587
crassulacean acid metabolism (CAM) plants 606
creationism 396
CRE-binding protein (CREB) 167
Crenarchaeotes 42–43
Cretaceous 418
Creutzfeldt-Jakob disease (CJD) 649–651, 731

Crick, Francis **225–227**
Chargaff and 181
DNA structure discovered by 248
in molecular biology 558
Rosalind and 354–355
Watson and 742–744
cri du chat 377–378
crime scenes 352
crocodiles 721–722
Crocodilia 721–722
cross-linked proteins 12
crossover interference 564, 713
crude oil 301
Crustacea 492
cryopreservation 748–749
Cryptophyta 20
crystals 592–593. *See also* X-ray crystallography
CSHL (Cold Spring Harbor Laboratory) 744
CT. *See* computed tomography
Ctenophora 326, *327,* 485
cumulus cells 209
customary system 547–548
cutting 659
Cuvier, Georges, Baron **227–232,** 388–389
C vitamin 601–602
cyanobacteria 51, *52*
in history of life 417
of Lake Mendota 140
photosynthesis in 603
cyclic electron flow 603
cyclins 176
cystic fibrosis 372–373, 480
cysts 645
cytogenetics 382, 536–537
cytokinesis 175, 176
cytology 26, 671, 698–699
cytoplasm
in bacteria 643, *644*
and diminution 138
in inheritance 137–138
cytoplasmic genetics 527
cytoplasmic membrane. *See* biological membranes
cytosine
Chargaff's research on 181
in DNA structure 245
in nucleic acids 122
cytoskeleton
in Eukarya 321
of eukaryotic cells 330, 414
of prokaryotic cells *644,* 645

D

dancing, in bees 357–358, *358*
Dancing Naked in the Mind Field (Mullis) 569
darkfield microscopy 553
dark reactions. *See* light-independent reactions
Darwin, Charles **233–238,** *234*
on adaptation 334
on competition 214
Gould on 394–395
Gray and 399–400
Hooker and 431
human ethology and 321
on natural selection 334
Wallace and 732
Darwin, Erasmus 235
Darwin Bay 58
Darwin's frog 31
data presentation and analysis **238–245**
graphs *238,* 238–241, *239, 240, 241*
in scientific investigation 673, 676
statistical analysis 241–245
DDT (dichlorodiphenyltrichloroethane)
bioremediation of 125
effects of 153–155, *154*
organic chemistry and 588
death
from infectious disease 467–469
in population density 633
from substance abuse 3
decomposition
in biogeochemical cycles 75
in bioremediation 124
in carbon cycle 77
in ecosystems 277, 278
microorganisms in 549
Deep Ocean Engineering 272
Deep Ocean Exploration and Research (DOER) 273
Deep Rover (submersible) 272
deforestation 287
degrees of freedom 242
dehydration syntheses 66
deimination 473
delayed hypersensitive responses 464–465
Deltaproteobacteria 51–52
demographics 634–635
denatured proteins 120
density gradient centrifugation 179, *180*
deoxyribonucleic acid. *See* DNA
dependent variable 239–240, *240*
dermal tissue 620
dermis 481
desalination 300
Descent of Man, The (Darwin) 237
Description of Helioscopes, A (Hooke) 427
desert biomes 102, 114, *115*
desertification 295–298
detritivores 277, 278
Deuteromycota 324, 362
deuterostomes 36–37, 326, *327*
developmental anatomy 26, *675*
developmental biology 338
Devonian 417–418
diabetes **248–250**. *See also* insulin
age-related risk of 13
obesity and 608
therapeutic cloning and 212
diabetes insipidus 250
diamonds 456
diaphragm (contraceptive) 688
diaphragm (respiratory) 666
diapsids 721
diarrhea 255, 468
diatoms 21, *323,* 324
dichlorodiphenyltrichloroethane. *See* DDT
dicots 618
Dictionnaire de botanique (Lamarck) 505
dideoxy chain termination DNA sequencing *261,* 261–262
diet. *See* nutrition
differential centrifugation 179
differential interference microscopy 553
differentiation
cancer and 148–149
cloning after 208, 209–210
epigenetics in 473–474
in plants 623–624
research on 162
of stem cells 750
of tissue 672
digestion 250–251
digestive systems **250–255,** *252, 255*
digestive tract 251–253
digger wasp 32, 703–704
dihybrid cross 478, *478,* 543–545, *544*
dikdik 101
diminution, cytoplasm and 138
dimorphic species 30–31
dinoflagellates 21, 323, *323*
Dinophyta 20
diploid life cycle 664
diplomonads 322, *323*
Diplopoda 492
direct fitness 692–693
disaccharides 119
discrete community hypothesis 745
disease. *See* infectious diseases
dissecting microscope 553
dissection **256**
in anatomy 27
Aristotle's use of 44
Harvey's use of 408
dissimilatory sulfur reduction 52
diving bell 533

diving helmet 58, *59*
diving suits 533
DNA (deoxyribonucleic acid) **245–248**
  in aging 9–10
  in bacteria transformation 48–49, 534
  in biological classification 322
  as biomolecule 120–122
  in chromosome structure 190–191
  cloning of. *See* cloning of DNA
  electrophoresis of 280–281
  in Eukarya 321
  Franklin's work on 354–355
  function of 122
  in gene therapy 371–373
  genetic importance of 47, 48–49
  as genetic information carrier 182, 247–248, 410–411, *411,* 524, 534–535, 558
  in genome 442
  Hershey's work with 410
  HIV and 1–2
  hydrogen bonds in 187
  mitochondrial 11, *527*
  in molecular biology 558–560
  in molecular genetics 382–383
  Mulis's work with 566
  mutations in 629–632
    in aging 11
    repairing 247, *631,* 631–632
  nucleotide composition of 181
  packaging of *191,* 191–192, *192*
  radiation exposure and 302
  repetitive 386–387
  replication of. *See* DNA replication
  in reproduction 658
  spectrophotometry of 696–697
  strands of, in transcription 365
  structure of *121,* 245–246, *246,* 558
    Chargaff's work on 181
    discovery of 225–227, 742–744, 747–748
    Franklin's work on 354–355
    Pauling's work on 600
    Wilkins's work on 747–748
  sugars in 120–122
  synthesis of
    in cellular reproduction 175
    in viruses 728–729
  X-ray photographs of 226, 354–355, 742–743, 747
DNA fingerprinting 130, **256–260,** *257, 259,* 353
DNA polymerase
  in DNA replication 246–247
  in PCR 566–568
  in viruses 39
DNA polymorphisms. *See* DNA fingerprinting
DNA replication 246–247
  in bacteria 38
  in viruses 39
DNA sequencing **260–262**
  in biological classification 90
  evolutionary evidence from 338, 756
  in genetics 756
  of human genome 442
  of recombinant DNA 657
  sequence alignment algorithms and 84–87
DNA transcription
  in bacteria 38, *367*
  in gene expression 365–367, *366*
  histone code in 194
  in origin of life 589
  variations from 712–713
  in viruses 39, 728–729
DNA translation 367–369, *369*
DNA vaccines 712
Dobzhansky, Theodosius **262–266**
DOER (Deep Ocean Exploration and Research) 273
dogs, in insulin studies 54
Dolly (sheep) 209, *209, 748,* 753, *754*
domains 93, 321–322
domestication 522
dominant alleles 542–545, *543*
dominant species 216
donor eggs 47
donor sperm 47
dopamine 5, 6–7, *7*
double-helix. *See* DNA, structure of
Double Helix, The (Watson) 744
double-reciprocal plot 313, *313*
dredging 701
Drosophila
  in embryology research 675
  genetic research on 262–264, *563,* 563–564, 699
  mating song of 29
  sex determination in 683–684
  sex-linked inheritance in 562–563, *564*
drug cocktail 2–3
drug resistance, in bacteria 39–40
drugs. *See also* medications
  addiction to **3–8,** *7*
  in brain chemistry 5, *7*
  delivery methods for 4–5
  tolerance to 6
  types of 4
ducks 523
duodenum 253
Duve, Christian de **266–269**
dyes, in DNA sequencing 262

E

ear 678–680, *679*
Earle, Sylvia **270–273,** *271,* 534
Earth Summit (United Nations Conference on Environment and Development) 71
earthworms *252,* 327, 490
East African dikdik 101
eating disorders **274–275**
Ecdysozoa 326–327, *327*
Echinodermata 493
ecological footprint 636
ecological pyramids 278–280, *279*
ecology **275–277.** *See also* community ecology
  Beebe's work in 58
  of behaviors 694
  in botany 133
  careers in 276–277
  Carson's work in 156
  chemistry in 183
  definition of 214
  fungi in 362–363
  Haeckel in 406
  history of 275–276
  Humboldt in 456
  ice in 736
  microbial 139
  microorganisms in 549
  in physiology 607
  specializations in 276
  Whittaker in 744
economics, in botany 133–134
economy, algae in 22
ecosystems **277–280**
  algae in 22
  biodiversity in 70–71
  biogeochemical cycles in 75
  in biosphere 126
  changes in 277
  climate change and 305–306, *306*
  in coral reefs 108
  endangered species in 288–289
  fragmentation of 221
  geological events and 82
  at hydrothermal vents 109, 457–458
  microorganisms in 549
  natural change in 295
  nitrogen cycling in 51
  productivity of 70–71, 745
  as resource 308–309
  services of 275
  specialized study of 276
  stability of 71
ectoderm 34, 284, 285–286
ectoparasites 216
ectoparasite varroa mite *(Varroa destructor)* 15
ectothermy 423
Edge of the Sea, The (Carson) 153

education, about environmental concerns 306
efficacy 396
eggs
donor 47
in inheritance 137–138
polarity of 284
EGSs. *See* external guide sequences
Ehrlich, Paul 37
ejaculation 446
Elasmobranchii 716
elastic fibers 35
elasticity 427
Eldredge, Niles 394–395
electrical synapse 580–581
electrical work 73
electroencephalogram 317, *317*
electrolyte imbalance 274
electromagnetic receptors 678
electromagnetic spectrum 695, *695*
electron capture 653
electronegativities *185,* 185–187, *186, 598–599*
electron microscopy 160–161, 162, *553–555*
electrons. *See also* valence electrons
in atomic structure 183–184, *184*
in photosynthesis 603, 604
in radioactivity 652–653
electron shells 184, *184*
electron transport system 172, *173,* 413
electrophoresis **280–283,** *281,* 442
elements 45, 183
Elephas primigenius 229
elimination 251, 253–255, *254*
elimination reactions 66, *66*
ELISA (enzyme-linked immunosorbent assay) 2
Elledge, Stephen J. 3
elongation 368, *369*
Elton, Charles 279
embryo(s)
cryopreservation of 748–749
Langerhans cells in 55
embryology and early animal development **283–286**
Drosophila used in 675
epigenetics in 473–474
human 450–452, *451*
Just's work in 496–499
Levi-Montalcini's work in 512–514
Morgan's work in 561
of plants 612
of reptiles 719
sex determination in 684
in sexual reproduction 663
in taxonomy 89
Wilmut's work in 748–749
embryonic germ cells 751
embryonic stem cells. *See* stem cell(s)
Embryophyta 325, *325, 611, 619,* 620. *See also* plant(s)
emigration 633
emotion 321, 609
emperor penguin 31
empirical method 45
endangered species 212, **286–289**
Endangered Species Act (ESA) 224, 286–287
endergonic reactions 74
endocrine system 10, **289–295,** *292, 293–294. See also* hormones
endocytosis 97, *97,* 728
endoderm 34, 284, 286
endogenous opioids 5
endomembrane system 329
endonucleases 655
endoparasites 216
endoplasmic reticulum (ER) 322, 329, *331*
endoskeletons 570
endospores 50, 501, 645
endosymbiotic theory 322, 331–332, 414–416, *415,* 527–530
endothermic reaction 74
endothermy 423
energy. *See also* bioenergetics
in cellulose 101
conservation of 73–74
in ecosystems 277–278
first cells and 412–413
in living organisms 104
nonrenewable sources of 300–302
nutrition in 582–583
renewable sources of 301
Enterococcus, vancomycin-resistant 40
enterocoely 35, *36*
enthalpy 73–74
entomology, forensic 352
entropy 74
enucleation 209
environment
in aging 8, 10–12
in animal behavior 28
body plans and 34
epigenetics and 474
in evolution 507
gene expression and 28
in heredity 265, 405–406
in obesity 609–610
plant response to *628,* 628–629
in sensation 677
in sex determination 683
environmental concerns, human-induced **295–307**
in agriculture 19–20
in biodiversity 70, 71–72
bioremediation of 123–125
botany and 133
in ecology 276
extinction 287
land degradation 295–299
in marine biology 272–273
nonrenewable energy sources 300–302
pesticides 153–155
pollution 302–304
sustainability 306
water supply 299–300
Environmental Protection Agency (EPA) **307–308**
bioremediation under 124
conservation under 221–224
water safety under 741
environmental science **308–310**
enzyme(s) **310–315**
allosteric 313
in biochemical reactions 65–67, 74
in cellulose breakdown 101
in DNA cloning 205
in DNA replication 246
expression of 314
function of 310–311, *311, 312*
in human digestion 253
latency of 267–268
in molecular biology 558
in recombinant DNA 655, *656*
regulation of 314–315
enzyme inhibitors 39
enzyme kinetics 311–313, *313*
enzyme-linked immunosorbent assay (ELISA) 2
eosinophils 199, *199,* 433
EPA. *See* Environmental Protection Agency
epidemiology 131, 467
epidermis 481
epigenetic information 194
epigenetic inheritance 194
epigenetics 473–475
epilepsy **315–319,** *317*
epilimnion layers 110
epiphytes 113
epithelial tissues 34–35
commercial availability of 170
differentiation of 149
in digestive system 251
Epsilon Proteobacteria 52
equilibrium, in bioenergetics 74–75
equilibrium constant 74
equilibrium gradient centrifugation 179–180
equilibrium model of island biogeography 81, *81*
ER (endoplasmic reticulum) 329, *331*
error theories of aging 10–12

erythrocytes *198*, 198–199, *199*
ES. *See* stem cell(s)
ESA (Endangered Species Act) 224
Escherichia coli 51, 550, 674
essential nutrients 174, 278
estuaries 109, 300
ethics, of cloning humans 212–213
ethology **319–321**. *See also* animal behavior; behavioral ecology
  Tinbergen's work in 703–706
  Turner's work in *708*, 708–709
Eubacteria domain. *See* Bacteria domain
euchromatin 193
eudicots 619
eugenics 265
euglenoids 322–323
Euglenophyta 20, 21
euglenozoans 322–323, *323*
Eukarya 40, *41*, **321–328**
  chromosomes of 189, 191–192, *192*
  classification as 93
  as domain 321–322
  emergence of 414–416
  gene expression in 369–370
  genome of 385–386
  infections by 38–39
  kingdoms in 93–94
  life cycles of 664
  microbes in 548
  repetitive DNA in 386–387
eukaryotic cells **328–332**
  cell walls of 328
  cytoskeleton of 330
  endomembrane system of 329
  endosymbiotic theory of 331–332, 527–530
  external structures of 330–331
  historical emergence of 414
  membranes of 94, 328
  nucleus of 328, *331*
  organelles of 329–330, *331*
  photosynthesis in 602–603
  reproduction of 658
  structure of *331*, 414
  tricarboxylic acid cycle in 171
Eumetazoa 326, *327*
European nursery spiders 30
Euryarchaeotes 42
eusociality 693
Eustigmatophyta 20–21
eutrophication 79, 111, *111*, 302
evaporation 79
evidence, biological 352–353
evolution, theory of **332–341**. *See also* human evolution; natural selection
  in animal behavior 694
  Aristotle and 45
  in biogeography 82
  in biological classification 89–91
  in biology 104, 105
  Buffon and 141–142
  competition in 530
  DNA sequencing and 756
  ecology and 275
  environment in 507
  of eukaryotic cells 331–332
  evidence for 338–340, *339*
  genetic variation in populations 332–333
  Geoffroy Saint-Hilaire and 388
  Gould on 394–395, 396–397
  Gray on 399–400
  Haeckel's work with 404–405
  Hooker and 431
  of humans. *See* human evolution
  humans and 265–266
  improvement and 340–341
  inheritance in 264–265
  Lamarck and 231, 505–507
  macroevolution 335–338
  mechanisms of 333–335
  misconceptions about 340–341
  modern synthesis of 264, 342
  mutations in 263
  natural selection in 235–238, 732–734
  origin of life and 341
  of plants 399–400
  polymorphism in 714
  serial endosymbiotic theory in 527–530
  social behaviors and 692–693
  Wallace on 732–734
  in zoology 761
Evolution and Adaptation (Morgan) 562
evolutionary biogeography 734
evolutionary biology **341–343,** 607
evolutionary genetics 383
evolutionary psychology 321
excision repair *631*, 631–632
excretory system **343–346**
  of birds 723
  mammalian 344, *345*
  mechanisms of 343–344
  nephrons in 344–346
exercise 609
exergonic reactions 74
exhalation 666, *667*
exocytosis 97, *97*
exogenesis 591
exons 205
exoskeletons 569
exothermic reaction 74
ExPASy (Expert Protein Analysis System) 87–88
experiment 673–676
exponential population growth 635, *635*
Expression of the Emotions in Man and Animals, The (Darwin) 321
external fertilization 662–663
external guide sequences (EGSs) 25
extinction
  in biodiversity 81
  causes of 287–288
  Cuvier on 229
  Darwin on 236
  in evolution 337–338
  Lamarck on 505
extracellular digestion 251
extrapolation 239
extremophiles 40–41, 126, 457–458
eye 680–682, *681*

F

Fabrici, Girolamo 407
facilitated diffusion 96
facultative aerobes 51
fallopian tube *447*, 448
families, obesity and 609–610
FAPs (fixed action patterns) 28, 521–522
fat 583
fatty acids 122, 584
fauna. *See* animal(s)
feces 738
feedback
  in cell cycle regulation 176
  in global ecology change 297
feedback inhibition
  in enzyme regulation 314–315
  in metabolism regulation 174
feeding behavior 29–30, 705, *705*
female reproductive system *447*, 447–448
fermentation
  as biological process 593
  as biotechnology 129
  in cellular metabolism 172–173
  microbes in 549, 593
ferns *339*, *616*, 616–617
Ferris, James 589
fertilization
  Boveri's study of 136
  in embryonic development 283
  in human reproduction 450
  Just's work on 497–499
  in sexual reproduction 662–663
fetal development 450–452, *451*
fetal stem cells 751
fever 434
fiber (dietary) 584–585
fibrous proteins 120
fibrous root systems 620
Filipchencko, Yuri 263
fimbriae 643, *644*
finches 235, *235*

fingernails 481
fingerprints 353
Fire, Andrew 668–670
fire algae (Pyrrophyta) 20, 21
Firmicutes 52
Fish and Wildlife Service, U.S. (FWS)
  Carson with 152–153
  Clark at 201
  ESA under 224, 286–287
Fishes of the Western North Atlantic (Bigelow) 64
fission 653
Fitzroy, Robert 233–234
Five Kingdoms (Margulis and Schwartz) 528–529, *529*
fixed action patterns (FAPs) 28, 521–522
flagella 330, *331,* 643, *644*
flagellates 645–646, *646*
flatworms 251, 485
Flavr Savr tomato 379
Fleming, Alexander 37, **347–351,** *348*
Flemming, Walther 190
flora. *See* plant(s)
flora (microbiotic) 548
Flora of North America (Gray and Torrey) 398–399
Flore française (Lamarck) 504–505
Florey, Howard 37, 350–351
flowers 622, 625–626, *626*
fluid feeders 250
fluid mosaic model of membrane structure 94
flukes 486
fluorescence microscopy 553, *553*
flying 723
follicle (ovarian) *447,* 448
food
  fungi in 363
  for honeybees 17
  microbes in 549
  in nutrition 583–585
food chains 216, *217,* 362–363
food supply 71–72, 132
food webs 216, *217*
foraging behavior 29–30, 357–359, *358, 359*
foraminiferans *323,* 324
forensic biology **351–353**
forensic sciences 351–352
  biology used in 105
  biotechnology in 130
  genetics in 383
  human genome used in 443
forest
  coniferous 116
  degradation of 295–298
  temperate broadleaf 116
formalism 388–389
fossil(s). *See also* history of life
  animals identified from 228–229
  in biological classification 90
  as evolutionary evidence 336–337, 340
  gradualism and 394–395
fossil fuels
  biodiversity and 72
  in biogeochemical cycles 75
  in carbon cycle 77
  environmental concerns from 301
  plants in 132
fossilization 75, 430
foundation species 218
fractionation 162, 179, 268
fragmentation 658, 659
frameshift mutations 630
Franklin, Benjamin 470, 636–637
Franklin, Rosalind 226, **353–355,** 742–743, 747
free radicals, in aging 11
Freeze, Hudson 139
freeze-etching 554
freeze-fracture replication 162
frequency-dependent selection 713–714
frequency distributions *243,* 243–244
freshwater biome 109–112
freshwater supply, and biodiversity 72
fringe reefs 108–109
Frisch, Karl von 320, **355–360,** 522, 523, 706
frogs 31, 718–719
fruit
  bee-pollination of 15
  in plant morphology 622
  in plant reproduction 627–628
fruit fly. *See Drosophila*
function 100. *See also* physiology
functional groups 65–66, 118, *118*
functionalism 388–389
fungal infections 38–39, 363–364
Fungi (fungi) **360–364**
  in bioremediation 124
  culture of 169
  ecological importance of 362–363
  in history of life 417
  impact of 363–364
  as kingdom 93, 322, 324
  in succession 219
fusion inhibitor drugs 2
FWS. *See* Fish and Wildlife Service, U.S.

G

Gaia hypothesis 530–531
Galápagos Hydrothermal Expedition 457–459
Galápagos Islands
  Beebe's work in 58–59
  Darwin's work in 234–235, *235,* 334
Galápagos: World's End (Beebe) 58–59
Galen 406, 407
gamete(s) 659
gamete donation 47
gamete intrafallopian transfer (GIFT) 46–47
gametogenesis 662
gametophyte 611–612, 626
gamma decay 653
Gammaproteobacteria 51
gap junctions 163
Garcia canister 222
Garrod, Archibald 558
gases 637–639
gastrin 255
gastropods 488
gastrovascular cavity 251
gastrula 34, *285*
gastrulation 34, 284, *285*
gas vacuoles 644–645
Gaucho (imidacloprid) 16
geese 520–521, 523
gel electrophoresis 442
gel filtration chromatography 188, *189*
gel mobility shift assay (GMSA) 283
Gelsinger, Jesse 375
gene(s)
  in addiction 3–4
  in aging 8, 9, 12
  in Alzheimer's disease 12
  in animal behavior 27–28
  of archaea 42
  in cancer 12–13, 148, 149, 150
  cloning 204–205, 371
  DNA and 47, 48–49, 247–248, 410–411, *411*
  encoding, identifying 442
  free radical damage and 11
  in inheritance 472
  linked 563
  location of 562
  in maize 536–537
  mobile 537–539
  in molecular biology 558, 559
  mutation of. *See* mutation
  for NGF 515
  in prion diseases 650–651
  promoters on 365–366
  recombining 536
  relocated 537–538
  sequencing, in biological classification 90
  sex-linked 479–480, 562–563, *564*
  in vaccines 712

gene expression **365–371**
in clones 753
crossover interference in 564
environment and 28
eukaryotic 369–370
in gene therapy 374
in molecular biology 559
prokaryotic 369–370
regulation of 370
RNA interference in 668–670
steroid stimulation of 291
studies of 207
transcription in 365–367
translation in 367–369
gene flow 335
gene library 205
gene mapping 382, 563–564, 657
gene pools 333
generalized seizures 316
Generelle Morphologie (Haeckel) 404–405
gene therapy **371–376,** *374,* 670
genetically modified organisms (GMOs)
animals 749
in biotechnology 131
CCD and 17
cloning 753
genetic engineering of 379
genetic code, universality of 87
genetic counseling 383, 480
genetic disorders **376–378**
genetic drift 335
genetic engineering **378–381.** *See also* biotechnology; recombinant DNA technology
in agriculture 19, 131
for bioremediation 124, 125
v. biotechnology 129
microbes used in 550
of vaccines 712
genetics **381–384.** *See also* inheritance
in addiction 3–4
animal research in 675
applications of 383–384
bioinformatics used in 83
chemistry in 183
in development of organisms 104
history of 381–384
in learning 33
McClintock's work in 536–537
Mendel and 541, 545
in obesity 583, 609
in origin of life 589
subdisciplines of 382–383
Genetics and the Origin of Species (Dobzhansky) 264, 266
genetic variation. *See* variation
genital herpes 687
genital warts 687
genomes **384–387**
of archaea 42, 758
bioinformatics used for 83–87
in biological classification *91,* 91–92
databases of 87–89
human. *See* Human Genome Project
manufacturing 380–381
recombinant 654
in reproduction 658
sequencing 262
in speciation 530
of viruses 727
genomic imprinting 474
genomic plasmid library 205
genomics 387
bioinformatics and 87
in biological classification *91,* 91–92
uses of 443
genotype 39, 332, 472
genus 92
Geoffroy Saint-Hilaire, Étienne 231–232, **387–389**
Geographical Distribution of Animals, The (Wallace) 734
geological events, ecosystems and 82
geological features, in climate 127
geological oceanography 63
geological processes, in nutrient cycle 278
geology
Buffon's theory of 142
Cuvier's work in 229–230
Hooke's work in 430
Humboldt's work in 454
Lamarck's work in 507
Lyell's theory of 234
geomagnetism 455–456
Georges Bank 64
germination 628
germ layers 284–286, *285*
germ plasm theory 160
germ theory of disease **389–390,** 548, *595*
germ theory of fermentation 593
gestation 450–451
gestational diabetes 249
GFP (green fluorescent protein) 207
giant tortoise 234–235, *235*
giardiasis 510, 647
Gibbs energy 74–75
GIFT (gamete intrafallopian transfer) 46–47
Gilbert, Walter 260
Gilchrist, Joe *55*
gills 664–666, *665*
glaciers 82
Glaucophyta 20
Gleason, Henry 745
glial cells 392
global climate change. *See* climate change
global warming. *See* climate change
globular proteins 120
glucagon 161, 267, 424
glucose
as biomolecule 119
in blood 248–249, 424
in cellulose 119, *119*
in plants 100–101
glutamate-ammonia ligase 66
glycocalyx 330–331, 643, *644*
glycogen 583
glycolysis
in cellular respiration 171
discovery of 68, 160
glycoproteins 1
GMO. *See* genetically modified organisms
GMSA (gel mobility shift assay) 283
golden algae (Chrysophyta) 21, 324
Golgi, Camillo **391–393**
Golgi apparatus 329, *331,* 392–393
Golgi staining 391–392, *392*
Golgi's tendon organs 393
gonadal dysgenesis 662
gonorrhea 686
Gould, John 236
Gould, Stephen Jay 334–335, **393–397,** *394*
gout 13
government, agricultural policies of 20
GPP (gross primary production) 277–278
G protein (guanosine triphosphate binding protein) 164
G protein-linked receptors 164
gradualism 336, *337,* 394, *395*
grafting 659
gram-negative archaea 41
gram-negative bacteria 37, 38, 51, 642
gram-positive archaea 41
gram-positive bacteria 37, 38, 51, 52, 642–643
Gram staining 162, 553, 642
grand mal seizures 316
graphs *238,* 238–241, *239, 240, 241*
grasslands
as biome 105
degradation of 295–298
temperate 115–116
gravitation 677
gravitropism 629
Gray, Asa **397–401**
gray wolf 288–289, *289*
grazing 298
Great Fire of London 428
green algae 324–325

green fluorescent protein (GFP) 207
greenhouse gases 305
Greenland 704
Grew, Nehemiah 134
Griffith, Frederick 48, 247, **401**
grooming behavior 29, *30*
gross anatomy 26
gross primary production (GPP) 277–278
ground tissue 620, 621
groundwater, bioremediation of 124
growth factors 512–515
guanine
  Chargaff's research on 181
  in DNA structure 245
  in nucleic acids 122
guano 455
guanosine triphosphate (GTP) 157
Guided Tour of the Living Cell (Duve) 269
Gulf of Maine 63
gulls 706
Gurdon, John 208
Gymnodinium breve 22
Gymnophiona 718
gymnosperms 325, *325*, *339*, *616*, 618

H

HAART (highly active antiretroviral therapy) 2–3
habitat destruction 220, 287
habit formation 5–6
habituation 31–32
Haeckel, Ernst 93, **403–406**
hagfish 715
hair 723
hair follicles 481
Haiti 59
Hales, Stephen 134
hallucinogens 4, 6
halophiles 41, 42
Hamburger, Viktor 512
Hamilton, William 692–693
Hamilton's rule 692–693
haplodiploid sex determination 684, 693
haploid life cycle 664
Harvard University 63, 64, 399
Harvey, William **406–409,** *407*, *525*
hashish 4
Hawkes, Graham 272
hazardous waste disposal 303
HCFCs (hydrochlorofluorocarbons) 304
hearing 678–680, *679*
heart. *See also* circulatory system
  Aristotle's work on 44
  obesity and 608
  structure and function of *197*, 197–198
heartbeat 198
heat, in biological processes 73
heat capacity 736
heat exchange 423
heat loss 424
hematopoiesis 435
hemispherectomy 318
hemoglobin 600, 667–668
hemolytic disease of the newborn 464
herbivores
  cellulose energy and 101
  in communities 215
  diet of 250
  digestive systems of 255
  in ecosystems 277
  as vertebrates 724
heredity. *See* genetics; inheritance
hermaphroditism 662, 683
heroin 4
herpes simplex viruses 687
Herrick, Clarence Luther 707
Herring Gull's World, The (Tinbergen) 705–706
Hers disease 269
Hershey, Alfred 182, 247–248, **409–411**
Hertwig, Richard 135
hESCs (human embryonic stem cells) 750–751
heterochromatin 193
heterogamy 22
heterosporous plants 615
heterotrophs 51, 75, 79
heterozygote advantage 713
hexose phosphates *145*, 145–146
hierarchical levels of organization 34, 104
high blood pressure 200
highly active antiretroviral therapy (HAART) 2–3
hippocampus 5
Hirudinea 490
histogram 239, *239*
Histoire des animaux (Buffon) 142
Histoire naturelle (Natural History) (Buffon) 141
Histoire naturelle des poisons (Cuvier) 232
histology 26, 391–392
histone modifications 473
histone proteins 191, *192*, 194
Historia animalium (The History of Animals) (Aristotle) 43, 44
history of life **411–418,** *417*. *See also* origin of life
  aerobic respiration 413–414
  colonization of land 417–418
  eukaryotes 414–416
  first appearance 411–413
  multicellularity 416–417
  photosynthesis 413
HIV. *See* human immunodeficiency virus (HIV)
Hoagland Laboratory 47–48
Hodgkin, Dorothy **419–423**
Holocephali 716, *716*
Holst, Erich von 522–523
homeostasis **423–426**
homing *708*, 708–709
hominids 439–441
hominoids *438*, 439
Homo genus 440–441, *441*
homology 388
homosporous plants 615
homozygous traits 476–477
honeybees
  in agriculture 15–18
  communication among 29
  eusociality of 693
  genetic variations in 16
  smell in 356–357
  stresses in 17
  threats to 15–16
  Turner's work with 709
  vision in 355–356
Honolulu technique 209
Hooke, Robert **426–430**
  botany work of 134
  in cell biology 159–160
  Leeuwenhoek and 510
Hooker, Joseph Dalton 236, 237, **430–432**
Hooke's law 427
hormones 289–290
  action of 290–292, *291*
  in aging 10
  in animal behavior 28
  for birth control 688
  in cancer treatment 151
  in cell communication 163
  in digestion 255
  eating disorders and 274
  in humans 292–294
  in plants 623, 628
  synthesis of 290–292
  transport of 290–292
hormone X. *See* insulin
horn-faced bees *(Osmia cornifrons)* 18
hornworts 613–614
horseshoe crabs 493
horticulture
  genetics in 383
Hortus cliffortianus (Linnaeus) 518
Hospital for Sick Children (Toronto) 53
host defenses **432–437**
Howard Hughes Medical Institute (HHMI) 158–159
HPV (human papilloma virus) 687

Hubbs, Carl 201
human(s)
biological classification of 45, 92, 437
Buffon on 142
cloning 212–213
DDT toxicity in 155
dependence on biodiversity 70
digestive system of 251–255
domestication of 522
endocrine system of 292, 293–294
environment impact of 308–309. *See also* environmental concerns, human-induced
ethology applied to 706
microbes hosted by 510–511
population growth of 635–636
sex determination in 684
skeleton of 570, *571*
survivorship curve of 635
human embryonic stem cells (hESCs) 750–751
human ethology 321
human evolution 237, 265–266, **437–441**
Human Genome Project **441–443**
biotechnology and 130–131
DNA sequencing used in 85–86
Watson in 744
human immunodeficiency virus (HIV) 1–3, 466, 468
human-induced environmental concerns. *See* environmental concerns, human-induced
Human Microbiome Project 443
human papilloma virus. *See* HPV
human reproduction **444–452**
Humboldt, Alexander von 275, **452–457**
Humm, Harold 270
humoral specific immunity 435–436
humors 389
humpback whales 272
Huntington's disease 375
Huxley, Thomas Henry 237
Huygens, Christiaan 427
hybridization (DNA) 205–207, 657
hybridization (orbital) 598
hybrid sterility 263–264
hydra 483–484, *659*
hydrocarbons
bioremediation of 124, 125
environmental concerns from 301
hydrochloric acid (HCl), in digestion 253
hydrochlorofluorocarbons (HCFCs) 304
hydrogen, atomic structure of 184, *184*
hydrogenation 65
hydrogen bomb 99
hydrogen bonds *186*, 187
in biomolecules 599
in DNA 245, *246*
in water 735
hydrogen peroxide, in bioremediation 124
hydrolases 66
hydrologic cycle. *See* water cycle
hydrolysis reactions 66
hydrophobic interaction 187, 736–737
hydrophytes 111
hydrosphere 126, 127
hydrostatic skeletons 569
hydrothermal vents **457–461**
communities of *459*, 459–461
ecology of 109, 140
in origin of life 589
hydroxyl groups 587–588
Hyman, Libbie Henrietta **461–462**
hyperglycemia 249
hypersensitivities 463–465
hyperthermophiles 42, 139–140
hyphae 360
hypolimnion 110
hypothalamus 5, 293, *293*
hypothesis 244, 673, *675*
hypotonicity 95

I

IAPV (Israeli acute paralysis virus) 17
ice 736
ichthyology 200–204
ICP (Institute of Cellular and Molecular Pathology) 269
ICSI (intracytoplasmic sperm injection) 47
ICSS (intracranial self-stimulation) 6
imidacloprid (Gaucho) 16
immigration 81, 633
immune complex reactions 464
immune system
in aging 10
cellular therapies and 751–752
in host defenses 433
infectious disease and 467
in vaccination 711–712
immune system disorders **463–466**
immunodeficiencies 465–466
immunological theory of aging 10
immunology 48
immunotherapy. *See* biologic therapy
imprinting 28, 520, 521, 523
inactivation 474
inclusion bodies 643–644, *644*
inclusive fitness 692
incomplete dominance 478–479, *479*
independent assortment 190, 477–478, 713
independent variable 239–240, *240*
India 431–432
indicator species 71
indirect fitness 692–693
induced fit model 311, *312*
induced mutation 631
induced-pluripotent cells (IPCs) 752
induction, in organogenesis 284
Industrial Revolution 14
industry
biochemistry used in 69
botany used in 132–133
DNA cloning used in 207
environmental science used in 310
fungi used in 363
microbes used in 550
nonrenewable energy sources used in 300
species overexploitation by 288
infectious diseases **466–470,** *467*. *See also* inoculations; vaccines
in honeybees 15–16
microorganisms in 501–502, 548–549
Pasteur's study of 595–596
theories of 389–390, 595
in water 738, 741
infertility 46
inflammatory response 434
influenza 469–470
Ingenhousz, Jan **470–472**
ingestion
in digestion stages 250–251
of drugs 4
in human digestion 253
inhalants 4
inhalation 666, 667
inhalation anthrax 98
inheritance **472–481**. *See also* gene(s); genetics
of acquired characteristics 265
in addiction 3–4
in ADHD 577
Aristotle's work on 44
cancer and 150
chromosomal theory of 160, 189–190, 382, 761
chromosomes in 137
complex patterns of 478–479, *479*
cytoplasm in 137–138
epigenetic 194
in evolution 264–265
germ plasm theory of 160

Mendel's work on 541–545, *542, 543,* 545–546
Morgan's work on 562–563
nucleus in 137–138
in obesity 583, 609
pedigree analysis 480, *480*
sex-linked 479–480, 562–563, *564*
symbiogenesis in 530
inhibitors *314,* 314–315
injection of drugs 4
inoculations 470–471
insectivores 724
insects 251, *252, 491,* 491–492
in situ bioremediation (ISB) 124
insoluble fiber 584–585
Institute of Cellular and Molecular Pathology (ICP) 269
insulin
in diabetes 248–250
discovery of 53–56
Duve's work on 267
Hodgkin's work on 422
in homeostasis 424
role of 248–249
integumentary system **481–482**
intelligence, in sharks 203
intelligent design 396
intercostal muscles 666
interference
crossover 564, 713
RNA 25, 375, **668–670**
interferons 39, 434
intermediate filaments 330
internal fertilization 663
interphase 174–175
interpolation 239
interstitial fluid 196–197
intertidal zones 106–107, *107*
intracranial self-stimulation (ICSS) 6
intracytoplasmic sperm injection (ICSI) 47
intranasal drug delivery 4
intrauterine devices (IUDs) 688
introns 157, 205, 338
invagination 284
inversion of chromosomes 263
invertebrates **482–494**
circulation in 195
classification as 328
eyes of 680–681
Hyman's work on 461–462
Lamarck's work on 505–506
marine 533
in rivers 112
Invertebrates, The (Hyman) 461–462
in-vitro fertilization (IVF) 46
ion(s) 183, 598
ion exchange chromatography 188–189
ionic bonds 185–186, *186*
ionic interaction 187
IPCs (induced-pluripotent cells) 752
iron-sulfur world theory 556, 589–590
irrigation, in agricultural history 14
ISB (in situ bioremediation) 124
islands, biogeography of 81, *81*
isletin. *See* insulin
islets of Langerhans 53–54, 249
isogamy 22
isolating mechanisms 265
isomerizations 66–67, 473
isomers 66
isotopes 183, 652–653
Israeli acute paralysis virus (IAPV) 17
IUCN—World Conservation Union 224–225
IUDs (intrauterine devices) 688
Ivanovsky, Dmitri **494–495**
IVF (in-vitro fertilization) 46

J

jawless fish 715
Jefferson, Thomas 455
Jeffreys, Alec 256–257
jellyfish 326, 484
Jim suit 272
joints 570
Joint United Nations Programme on HIV/AIDS (UNAIDS) 1
Jurassic 418
Just, Ernest **496–499,** *497*

K

Kalacoon 58
Kandel, Eric 674
Kandler, Otto 757–758
kangaroo rats 102
Kartabo 58
Kashefi, Kazem 42
kelp 22
keratin 482
ketoacidosis 249
ketogenic diet 318
Kew Gardens 432
keystone species
in biodiversity 71
in community structure 216–218
endangered 288–289
in habitat destruction 220
in structure-function relationships 101–102
kidneys 344, *345*
kinesis 28
kinetoplastids 322–323
King, Thomas J. 208
kingdoms
in classification 92–93
in Eukarya 322
formerly in Protista 322–324, *323*
King Solomon's Ring (Lorenz) 522
kin selection 333, 692–693, *693*
Klug, Aaron 355
Koch, Robert 390, **500–503,** *501,* 548
Koch's postulates 501
Korarchaeotes 42, 43
Krebs cycle. *See* tricarboxylic acid cycle
kuru 650

L

labor 451–452
Labyrinthulids 323, *323*
lactation 452
ladybird beetles 262, 263
lagging strand 246
lakes 109–111, *110*
Lamarck, Jean-Baptiste 231, **504–508**
Lambert, Harry 350
laminar flow hoods 168, *168*
lampbrush chromosomes 193–194
lamprey 715, *715*
land degradation 295–299
Langerhans, Paul 53
language, of bees 357–359, *358, 359*
large intestine 253–255
Lavoisier, Antoine 639
leading strand 246
leafcutter bees *(Megachile rotundata)* 18
learning. *See* animal cognition and learning
leaves 615, 622, *623*
Leeuwenhoek, Antoni van **508–511**
germ theory of disease and 390
Hooke and 428–429
in microbiology 159–160
legislation
on childhood obesity 609
on cloning 213
on conservation 221–224
on endangered species 287. *See also* Endangered Species Act
on environment *307*
length, unit of 547
leukemia 150
leukocytes 199, *199,* 433
Levi, Giuseppe 512
Levi-Montalcini, Rita **511–516,** *512*
Lewontin, Richard C. 334–335
lichen *218,* 219, 362, 417
life. *See* history of life; origin of life

life expectancy 8, 212–213
ligand binding 163–164
ligand-gated ion channels 164
ligases 66
ligation reactions 66
light
  and biosphere 127–129, *128*
  through crystals *592–593*
  in primary productivity 278
  in spectrophotometry *695–696*
  in water, penetration of 106, 112
light-dependent reactions 602–603, *604*
light-independent (dark) reactions 144, 602–603, 604–605
light microscopy *551–553*, *552*
light reactions 144
limbic system *5*
limnetic zone 109, *110*
Lindeman, Raymond 278–279
line graphs 239–240, *240*
LINEs. *See* long interspersed elements
LINEs (long interspersed elements) 386
Lineweaver-Burke plot 313, *313*, *314*
linker DNA 191, *192*
Linnaeus, Carl 89, 92–93, 134, **516–520**
lipid(s)
  as biomolecules 122, *123*
  biosynthesis of 174
  monomers of 67
  in nutrition 583–584
lipid hormones 290–292
liposomes 371, 373
liquid chromatography 188
liquid column chromatography 188
Lister, Joseph 390
lithosphere 126, 127
littoral zone 109, *110*
liverworts *612*, 612–613
live vaccines 711
living organisms
  characteristics of 102–104
  chemical basis of **183–187**
lizards 721
lobe-finned fishes 717
lock-and-key model 311, *312*
Loeb, Jacques 498–499
logging 297
long interspersed elements (LINEs) 386
Lophotrochozoa 326–327, *327*
Lorenz, Konrad 28, 320, **520–523**, 704
Lovecock, James 530–531
Lovley, Derek 42
LSD. *See* lysergic acid diethylamide
Lucy 439–440
lungs 666
lyases 66
Lycophyta (lycophytes) 325, *325*, 615
Lyell, Charles 234, 236, 733
lymphatic system 196–197, 433
lymphocytes 1–2, *198*, 199, *199*, 433, 435
lymphoma 150
Lysenko, Trofim *265*
lysergic acid diethylamide (LSD) 4
lysosomes 268–269, 329
lysozyme 349, 433

M

M1 RNA 24
MacLeod, Colin Munro 48, **524–525**
Macleod, John James Richard *53–56*
macroevolution 332, 335–338, *337*
macromolecules. *See* biomolecules
mad bee disease 16
maggots 697
magnetic resonance imaging (MRI) 27, 318
magnification, in microscopy *551*
maize 536–537
major histocompatibility complex (MHC) 436
malaria 393, 469, 647–648, *648*
male reproductive system 444–446, *445*
malignancy 149, 177–178
Malpighi, Marcello 134, **525–526**
Malpighian layer *526*
Malpighian tubes 344
Malthus, Thomas 236, 733
mammals *720*, 723–725
  excretory systems of 344, *345*
  osmoregulation in 425
  respiratory systems of 666–667, *667*
mammary glands 723–724
mangroves 111
Marfan syndrome 26
Margulis, Lynn 331, 414, **526–532**
marijuana 4
marine benthic zone 106, *107*, 109
marine biology *532*, **532–534**
  Aristotle's work in 44
  Beebe's work in 59–61
  Carson's work in 151–153
  Earle's work in 270–273
  history of 533–534
  Just's work in 497–498
  research in 533
  Thomson's work in 700–702
marine biome 106–109, *107*
Mars 591
marshes 111
mass, unit of *547*
mass extinction 70, 288, 338, 418
Masson's trichrome 162
Mastigophora 645–646, *646*
mating behavior 30–31
mating songs 29
matter 183
maturation-promoting factor (MPF) 176
Maxam, Allan 260
Max-Planck-Institut für Verhaltensphysiologie *522–523*
McCarty, Maclyn 48, **534–535**
McClintock, Barbara **535–540**, *538*
mean 242, *242*
measles 469
measurement 546–548
mechanical work 73
mechanoreceptors 677–678
Medical Research Council (MRC) 23
medications
  for addiction 7–8
  in brain chemistry *5*, 7
  for epilepsy 318
  genetically engineered 379–380
  for HIV 1, 2–3
medicine (field)
  biochemistry used in 69
  biology used in 105
  biotechnology used in 130
  botany in 133–134
  cloning applications in 210, 212, 753
  DNA cloning used in 207
  endangered species and 289
  evolutionary biology and 343
  fungi and 363–364
  gene therapy used in 371
  genetic engineering used in 379–380
  genetics in 383
  human genome used in 443
  organic chemistry in 588
  physiology in 607
  radioactivity used in 653
  RNA interference and 670
  stem cells used in 750–752
medusa 483, *484*
Megachile rodundata (leafcutter bees) 18
Megan (lamb) 752
megasporia *616*, 617, *617*, 626
Mehring, Josef von 53
meiosis
  in algal reproduction 22
  discovery of 135
  in sexual reproduction 659–660, *661*

Mello, Craig 668–670
membrane filter test 741
membrane lipids 41–42
membrane potential 97, *579*
memory cells 437, 711
Mendel, Gregor 189–190, 342, 381–382, **540–546,** *541, 562*
Mendota, Lake 140
menopause 10
menstrual cycle 10, 448–450, *449*
Menten, Maud 311–313
Merostomata 493
mescaline 4
mesoderm 34, 284, 286
mesolimbic dopaminergic pathway 6, *7*
mesophiles 42
mesophyll cells 606
messenger RNA (mRNA) *369*
  in gene expressions 366–367
  in translation 367–369
metabolic pathway 67
metabolism 582–583, *589*. *See also* cellular metabolism
  in bioremediation 123–124
  in thermoregulation 424
metalimnion layers 110
metamerism. *See* segmentation
metanephridia 344
metaphase 175–176, *660*
metastasis 177–178
Metazoa 326, *327*
metazoans 416–417
meteorology *507*, *545*
methane, for bioenergy 130
methanogens 41, 42, *756*
methicillin-resistant *Staphylococcus aureus* (MRSA) 40
methylation 194, 473
methylphenidate *576–577*
metric system *546,* **546–548,** *547*
MHC (major histocompatibility complex) 436
mice *675*
Michaelis, Leonor 311–313
Michaelis constant 312–313
Michaelis-Menten model 311–313, *313, 314*
microbial ecology 139
microbial infection 37–40
microbiology 139, **548–550**
microbiota 548
microevolution 332
microfilaments 330
Micrographia (Hooke) 426, 428–429, 510
microorganisms. *See also* microbiology
  in bioremediation 123–124
  in cellulose breakdown 101
  in disease 389–390, 466–467
  ecology of 549
  habitats of 549
  in human body 510–511
  Leeuwenhoek's discovery of 509–510
  spontaneous generation and 697–698
  in water treatment 739–740
microphylls 615
microRNAs 473, 475
microsatellites 259, 386
microscopy **550–555**
  in cell biology 159, 160–161, 162
  electron *553–555*
  Hooke's work in 428–429, *429*
  Leeuwenhoek's work in 508–511, *509*
  light *551–553*, *552*
  Malpighi's work in *525–526*
  in pathology 726
microspheres 590
microsporangia 617–618
microsporidia 322, *323*
microtubules 330, *331*
middle repetitive DNA 386
migrant song birds 223
migration 28, *29,* 724
Miller, Frederick *53*, *54*
Miller, Stanley **555–558,** *588–589*
millipedes 492
minerals (dietary) 584
mining
  bacteria used in 129
  dangers of 301
  microbes used in 550
minisatellites 256–257
Minkowski, Oskar 53
Mirbel, Charles-François 134
MIS (mullerian-inhibiting substance) 684
miscarriage 47
mismatch repair 631
mitochondria
  development of 322
  of eukaryotic cells 329, *331,* 385–386
  origin of 414, *415*
mitochondrial disorders 378
mitochondrial DNA 11, *527*
mitosis *177*
  in algal reproduction 22
  in cell reproduction 174, *175,* 175–176
  chromosomes during 193
  in eukaryotic reproduction 658
MND (motor neuron disease) 753–755
model organisms 674–675
model systems 674–675
molecular biology **558–560**
  E. coli used in 674
  founding of 68
  history of *558–559*
  of HIV 1–2
  research in 560
  scope of *559–560*
molecular genetics 382–383
molecular orbital theory (MO) 598
molecular variation 714
molecules
  definition of 183
  polar 186–187
  structure-function relationship in 100
Mollusca *488,* 488–489
Monera 40, 93
monerans 404
monism 405
monocots 618, *619*
monocytes 199, *199,* 433
Monogenea 486
Monograph of the Pheasants, A (Beebe) 57
monomers 67, 118
monophyletic organisms 91–92
monosaccharides 119
monounsaturated fatty acids 122
Morag (lamb) 752
Morgan, Thomas Hunt 262–263, **560–565,** *561*
morphine 4
morphology
  Haeckel's work in 404–405
  of plants 620–622, *621*
  in systematics 89
morula 34
Moses sole 203
mosses 219, 325, *339, 613,* 614, *614*
most-probable number (MPN) test 741
Mote Marine Laboratory 203
moths 710
motion, Aristotle's work on 45
motor division 578
motor neuron disease (MND) 753–755
mountains, and climate 127
mountaintops 117
movements, in animal behavior 28
MPF (maturation-promoting factor) 176
MPN (most-probable number) test 741
MRI (magnetic resonance imaging) 27, 318
mRNA. *See* messenger RNA
MRSA (methicillin-resistant *Staphylococcus aureus*) 40
mucociliary clearance 372

mucous membranes 433
mucus 372
Müller, Paul Hermann 153
mullerian-inhibiting substance (MIS) 684
Mullis, Kary 140, 258–259, **565–569,** 632
multicellularity *416,* 416–417
multifactorial disorders 377
multiple birth, assisted reproduction and 47
multiple sequence alignments 84, 88
Murray, John 703
muscle
  contraction of 35, *572–574, 573*
  in respiration 666
  types of *572, 573*
muscular tissue 35, 149, 251
musculoskeletal system **569–574,** *571*
  bone tissue in *570–571*
  joints in *570*
  types of skeletal systems *569–570*
mushrooms 324
mutagens 150
mutation
  in evolution 333
  in medical disorders 371
  point *566,* 629–632, 712
  replacement 630
  silent 338
  spontaneous 630–631, 712
  in variation 712
mutualism *527*
mycorrhizae 362, 671
mycoses. *See* fungal infections
myoclonic seizures 316
Myxini 715
myxobacteria 51–52
myxomycotes 322, *323*

N

NAc (nucleus accumbens) 6, *7*
Naegleria kingdom 323, *323*
Nägeli, Karl Wilhelm von 134
Nanoarchaeotes 42, 43
nanoparticles 373
narrow spectrum antibiotics 37
National Oceanic and Atmospheric Administration (NOAA) 224, 272–273, 286–287
National Park Service 225
National Petroleum Reserve-Alaska (NPRA) 299
natural bioremediation 124
natural gas 301
Natural History (Histoire naturelle) (Buffon) 141
natural pest control 14
natural selection. *See also* evolution, theory of
  in adaptation 334
  in behavior 694
  in biology 104
  competition in 214
  ecology and 275
  in evolution 236–238, 333, 732–734
  Haeckel on 405
  inclusive fitness in 692
  in nonliving systems 590–591
  in polymorphism 713–714
  in structure-function relationships 100
  Wallace on 732–734
nature, unity in 455
Neanderthals 441
Needleman and Wunsch algorithm 85
negative staining 554
Neisseria 51
Nematoda 486–487, 674–675
neo-Darwinism 342
Neognathae 722
nephron 344–346
Nereis 497
neritic zone 106, *107*
nerve growth factor (NGF) 512–515, *513*
nervous system **575–582.** *See also* epilepsy
  in addiction 5–6, *7*
  cell theory and 392
  differentiation of 149
  embryonic development of 284
  Levi-Montalcini's work on 512–515
  organization of *575–578*
  in sensation 677
nervous tissue 35
net primary production (NPP) 277–278
neural impulses *579–580, 580*
neurokinin 5
neurons 5, 316, 513–514, *578–579, 579*
neuron theory 392
neuropathies 250
neurotransmitters 5, 163, *581,* 581–582
neurotrophins 515
neutrons 183–184, *652–653*
neutrophils 199, *199,* 433
Newton, Isaac 429–430
New World monkeys 438–439, *439*
New York University 201
New York Zoological Society 57
NGF (nerve growth factor) 512–515, *513*
niches, in communities 214
nicotine 4, 6, *7*
Nistrosomonas 51
nitrate 79
nitrite 79
nitrogen
  atomic structure of 184, *184*
  fixing 625
  in plants 625
nitrogen cycle 51, *77,* 77–79
nitrogenous waste 343
NOAA (National Oceanic and Atmospheric Administration) 224, 272–273, 286–287
Nobel Prize
  for Altman 22, 25, 158
  for Banting 56
  for Buchner 68
  for Calvin 143, 148
  for Cech 25, 158
  for Cohen 515
  for Crick 227, 744, 748
  for Duve 269
  for Fire 668
  for Fleming 350–351
  for Frisch 359, 522, 523, 706
  for Gilbert 260
  for Golgi 392
  for Hershey 410
  for Kandel 674
  for Klug 355
  for Koch 502
  for Konrad 706
  for Levi-Montalcini 515
  for Lorenz 522, 523
  for Macleod 56
  for McClintock 539
  for Mello 668
  for Morgan 565
  for Mullis 568
  for Pauling 600, 601
  for Prusiner 651
  for Sanger 260
  for Spemann 208
  for Svedberg 179
  for Tinbergen 522, 523, 706
  for Watson 227, 744, 748
  for Wilkins 227, 744, 748
  X-ray crystallography and 759
Noble, E. C. 55
noncompetitive inhibitors *314,* 315
noncyclic electron flow 603
nonnative species, introduction of 287–288
nonnucleoside reverse transcriptase inhibitor drugs 2
nonseptate fungi 324
nonspecific defenses 433–434
nori 22
normal distribution *242,* 242–243
North Pacific ecosystem 277
Nosema apis 15

Nosema ceranae 15–16, 17
NPP (net primary production) 277–278
NPRA (National Petroleum Reserve-Alaska) 299
nuclear energy 302, 653
nuclear transfer 208, 209–210, *210,* 749, 751–752
nuclear weapons 99, 601, 653–654
nucleic acids. *See also* DNA; RNA
  as biomolecules 120–122
  Franklin's work on 354–355
  monomers of 67
nucleic acid synthesis, in bacteria 38
nucleoid 643, *644*
nucleoside 121–122
nucleoside reverse transcriptase inhibitor drugs 2
nucleosomes 191, *192*
nucleotide(s)
  in chromosome structure 190–191
  in DNA *121,* 181
  in DNA sequence alignment 85
  formation of 122
  point mutations of 629–632
nucleotide chain 122
nucleus (atomic) 184
nucleus (cellular)
  in Eukarya 321
  of eukaryotic cells 328, *331*
  in inheritance 137–138
nucleus accumbens (NAc) 6, *7*
nutrient cycles 75–79, 278, *549,* 745
nutrients
  essential 174
  fungi absorption of 360
  genetic engineering for 379
  in primary productivity 278
  transport of, in plants 624
nutrition **582–586**
  diabetes and 249–250
  eating disorders and 275
  in epilepsy treatment 318
  first cells and 412–413
  of mammals 724
  obesity and 609
  of plants 624–625
  of sponges 482

O

O_2. *See* oxygen
OAC (Oregon Agricultural College) 597
obesity 583, 608–610
obligate aerobes 51
obligate anaerobes 51
observation 673
ocean contour map 702
oceanic pelagic zones 107–108
oceanic zone 106, *107*
oceanography. *See also* marine biology
  Beebe's work in 58–61
  Bigelow's work in 62–64
  Thomson's work in 700–703
Oceanography, Its Scope, Problems, and Economic Importance (Bigelow) 63
ocean water 300
octet rule 184
oil prospecting 299
oil spills
  bioremediation of 124, 221
  in Persian Gulf 273
  pollution from 302–303
Old World monkeys 438–439, *440*
olfaction 356–357, 680, 704
Oligochaeta 490
oligomer restriction assay 566
oligosaccharides 119
oligotrophic lakes 111
omnivores 250, 255, 724
On Aggression (Lorenz) 523
oncogenes 149
On the Origin of Species (Darwin) 236–237, 400, 733
ontogeny 404–405
oocytes *447,* 447–448
oogenesis 447, *447*
Oomycota *323,* 323–324
oomycotes 324
Oparin, Aleksandr 555–556, 588
open system 73
operant conditioning 32
operons 384–385, *385*
opiates 4, 5, 6, *7*
opium 4
opportunistic infection 2
optimal foraging theory 30
oral contraceptives 688
Ordovician 416–417
Oregon Agricultural College (OAC) 597
organelles 162, 329–330
organic chemistry, its relevance to life science **587–588**
organic molecules
  biomolecules as 67
  in origin of life 588–589
  prebiotic synthesis of 555–556, *557,* 588–589
organisms
  characteristics of life 102–104
  cloning of. *See* cloning of organisms
  model 674–675
organogenesis 284–286
organs, tissues of 34
organ-system level of organization 34
organ transplantation
  biotechnology in 130
  cloning and 212
  xenotransplantation 212, 380
origin of life **588–591**. *See also* history of life
  evolution and 341
  historical record of 411–413, *412*
  prebiotic synthesis in 555–556
ornithology 57–58
Osborn, Henry Fairfield 57
Osmia cornifrons (horn-faced bees) 18
Osmia lignaria (blue orchard bees) 18
osmoregulation 424–425
osmosis, across membranes 94–95
Osteichthyes 716–717
osteoarthritis 13
osteoporosis 13, *13*
ovarian cycle 448–450, *449*
ovaries *294, 447,* 448
oviparous animals 663
ovoviviparous animals 663
oxidases 269
oxidation-reduction reactions 65
oxygen
  atomic structure of 184, *184*
  discovery of 639
  in history of life 413–414
  from photosynthesis 144
  in respiration 664, 667–668
oxygen therapy 471
ozone 303
ozone layer 303–304, *304*

P

p53 gene 149–150, 176, 178
PABA (para-aminobenzoic acid) 38
Pacific Railroad Surveys 399
Paenibacillus larvae 15
PAGE (polyacrylamide gel electrophoresis) 281, *282*
pain receptors 678
Palade, George 268
Paleognathae 722
paleontology 228–229, 394–395
pancreas
  diabetes and 53–56, 249
  in endocrine system *294*
panspermia 591, *591*
paper chromatography 145, 188
pap smear 685
para-aminobenzoic acid (PABA) 38
parabasalids 322, *323*
paracrine signaling 163
paraphyletic organisms 92
parasites, in honeybees 15–16, 17
parasitism, in communities 216, *216*

parathyroids *294,* 426
parental care 30, 31
Parkinson's disease *375*
parthenogenesis 498, *662*
partial seizures 316
particulate matter (PM) 303
Partners in Protection 223–224
Pasteur, Louis **592–596**
  in biochemistry 68
  in cell biology 160
  in microbiology 390, *548*
  spontaneous generation and 698
Pasteur Institute *596*
pasteurization *594*
pathfinding *708,* 708–709
pathogens 216, 466–467. *See also* infectious diseases
pathology 726
Pauling, Linus 185, 226, **596–602,** *600,* 743
Payen, Anselme 68
Payne, Fernandus 562
PCBs (polychlorinated biphenyls), bioremediation of 125
PCP (phencyclidine) 4
PCR. *See* polymerase chain reaction
pea(s). *See Pisum*
pearls 519
peat moss 614
pedigree analysis 480, *480*
pelagic zone 106, *107*
pelvic inflammatory disease (PID) 686
penetration, in viral life cycle 728
penguin 31
penicillin and penicillin derivatives
  discovery of 37, 347, *349,* 349–350
  fungi and 363
  Hodgkin's work on 420–421
  resistance to 39, *40*
  synthesis of 350–351
penis 446
pepsin 420
peptidoglycan 37
peregrine falcons 223
periodic abstinence 689
periodic table of elements 183, *185*
peripheral nervous system (PNS) *578*
peritoneum 35
permafrost 116–117
Permian 418
peroxisomes 268–269, 330
Persoz, Jean-François 68
Perutz, Max 227
pest control, natural 14
pesticides
  effects of 153–155
  honeybees threatened by 16, 17–18
PET (positron emission tomography) 318
petroleum products. *See* hydrocarbons; oil spills
Petty, William Fitzmaurice (Lord Shelburne) 639
PFGE (pulsed-field gel electrophoresis) 280–281, *281*
PGD (preimplantation diagnosis) 47
pH 313, 737
Phaeophyta (brown algae) 20–21, *323,* 324
phage 656
phagocytosis *97,* 97–98, 434
pharmaceutical industry
  biochemistry in 69
  DNA cloning used in 207
  genetic engineering in 380
pharming 131
phase contrast microscopy *553*
phencyclidine (PCP) 4
phenotypes 333, 472–476
pheromones 29
Philosophie zoologique (Lamarck) 506
phloem 615, 620, 624
phlogiston 639
Pholidichthys leucotaenia 204
phosphatase 267–268
phosphate 79
phosphodiester linkage 122
phosphoglycerate 145–146
phospholipase C 167
phospholipids
  in biological membranes 94, *95*
  as biomolecule 122, *123*
phosphorus cycle *78,* 79
phosphorylation
  in cell communication 164
  chromosomes and 194
  in enzyme regulation 314
  epigenetic modification from 473
photic zone 106, *107,* 109–111
photoautotrophs 51, 602
photoheterotrophs 51
photon 653
photoreactivation repair 631
photoreceptors 678, 680–682
photorespiration 606
photosynthesis **602–607**
  Calvin's work on 144–148
  in carbon cycle 76, 132
  carbon during 144–148, *145, 146, 147*
  carbon fixation in 605–606
  compounds produced during *145,* 145–147, *146, 147*
  in coral reefs 108
  discovery of 471
  energy in 277
  historical emergence of 413
  light-dependent reactions of 602–603, *604*
  light-independent reactions of 144, 602, 604–605
  in marine biome 106
  nutrients from 624–625
  oxygen generated by 144
  in plants 131, 132
  Priestley's study of 639–640
phototaxis 28
phototrophs 51, 73
phototropism 628–629
phyletic gradualism 394, *395*
phylogenetic trees *91,* 91–92, *92*
phylogenies 89–90, 404–405
physical activity, and obesity 609
physical-chemical oceanography 63
physical laws, and body plans 34
physics 429–430
physiology **607–610**
  anatomy and 26
  Aristotle's work on 44
  of birds 722–723
  in botany 133
  in cell biology 161
  dissection in study of 256
phytoplankton 107
PID (pelvic inflammatory disease) 686
pie chart 238–239, *239*
pilus 643, *644*
pineal gland *294*
pinocytosis *97,* 98
Pisum 541–546, *542, 543*
pituitary glands 10, 293, *293*–294
PKA (protein kinase A) *165,* 166–167
plague, as biological weapon 98–99
planarians 485, *486*
planetary motion 429–430
plankton 20, 107
Plankton of the Offshore Waters of the Gulf of Maine (Bigelow) 63
plant(s). *See also* botany
  adaptations in 610–612
  asexual reproduction in 208, *659, 659*
  biological classification of 133
    by Gray 398
    by Hooker 431
    by Lamarck 504–505
    by Linnaeus 517, *518,* 519
  breeding 14–19
  bryophytes 612–614
  cells in 671
  cellulose in 100–101
  in chaparral 115
  cloning 129, 208
  in coastal wetlands 109

community ecology of 744–745
competition among 214
in coniferous forest 116
in desert 114, *115*
development of 622–624
dispersal of 80–81
diversity of **610–620**
in food production 72
form and function of **620–629**
genetically engineered 379
growth of, and increase in carbon dioxide 296–297
hermaphroditism in 662
historical emergence of 417–418
in intertidal zones 106–107
in lakes and ponds 109
marine 532–533
morphology of 620–622, *621*
in mountaintops 117
nutrition of 624–625
overexploitation of 288
photosynthesis in 131, 132
reproduction of 625–628, *627*
response to environment *628*, 628–629
in savanna 114
seedless vascular plants 614–617
seed plants 617–619
symbiotic 215
in temperate broadleaf forest 116
in temperate grasslands 115
temperature increase and 297
tissue culture of 129, 170
transgenic 19
transport in 624
in tropical rain forest 113–114
in tundra 116, *117*
viruses in 494–495
in wetlands 111
Plantae kingdom 93, 322, 324–325, *325*
plant cytology 671
plasma membrane. *See* biological membrane
plasmids 384
plasmodial slime molds 324, 690, *690*, *691*
plastids 322, 414
platelets 199, *199*
Plato 43
Platythelminthes 485–486
pleiotropy 263
plow 14
pluripotent cells 750
plus-and-minus DNA sequencing 260
PM (particulate matter) 303
pneumatic chemistry 637–639
pneumatic trough 637, *638*
pneumococcal transformation 48
Pneumococcus 48, 401
pneumonia 467–468
pneumonic plague, as biological weapon 98–99
PNS (peripheral nervous system) 578
point mutations 39, 566, **629–632**, 712
polar covalent bonds *186*, 186–187
polar molecules 186–187
Polge, E. J. Chris 748
pollination 15, 72, 626–627
pollinators 18
pollution 302–304
polyacrylamide gel electrophoresis (PAGE) 281, *282*
Polychaeta *489*, 489–490
polychlorinated biphenyls (PCBs), bioremediation of 125
polyenes 38
polygenic disorders 377
polygenic inheritance 479
polymerase chain reaction (PCR) **632–633**
DNA cloning and 207
in DNA fingerprinting *258*, 258–259
invention of 566–568
in thermophiles 140
polymers, in biomolecules 118
polymorphism 713–714
polyp 483, 484, *484*
polypeptide 368–369, 559–560
polypeptide chains 119–120, 600
polyphyletic organisms 92
polysaccharides 119
polytene chromosomes 193, *194*
polyunsaturated fatty acids 122
ponds 109–111
population(s)
in biology 105
dispersion of 634
genetic variations in 332–333
growth of *635*, 635–636
size and density of 633–634
stabilization of 309
structure-function relationships in 101
population ecology 276, **633–636**
population genetics 264–265, 342, 383
Porifera 326, *327*, 482–483, *483*
Portuguese man-of-war 484, *485*
positron 653
positron emission tomography (PET) 318
postzygotic barriers 336
potable water 738–739
prebiotic synthesis 555–556, *557*
precipitation 79
predation 214–215
preformation 44, 142, 409
pregnancy 1, 450–451
preimplantation diagnosis (PGD) 47
prezygotic barriers 336
Priestley, Joseph 470, **636–641**
primacy of the egg 409
primary cultures 170
primary immunodeficiencies 465
primary producers 277
primary succession *218*, 218–219
primates
ancestors of 437–438, *438*
grooming behavior in 29, *30*
primitive cells 590–591, 758
prions 649–651, *650*, 731
probability 477
problem-solving, cognitive ability for 32–33
producers 106, 132, 277
production efficiency 278, 279–280
productivity 70–71, 745
profundal zone 109–110, *110*
progenotes 758
programmed theories of aging 8–10
prokaryotes. *See also* Archaea; Bacteria domain
chromosomes of 189, 191
gene expression in 369–370
genome of 384–385, *385*
infections by 37–38
kingdoms of 93
marine 532
microbes 548
photosynthesis in 603
prokaryotic cells **641–645**
conjugation by 50
membranes of 94
reproduction of 658
shape of 641
tricarboxylic acid cycle in 171
as universal ancestor 411–412, *412*
prometaphase 175–176
promoter 365–366
prontosil 350
prophase 175, 660
prostate cancer 685
prostheses, brain-driven 130
protease inhibitors 39
protein(s)
in biological classification 90
in biological membranes 94, *95*
as biomolecules 119–120
in cell communication 164
characterizing 83, 86
databases of 87–89
denaturation of 120
electrophoresis of 281–283
in eukaryotic chromosomes 191, *192*
fibrous 120

functions of 120
as genetic information carrier 182
globular 120
in molecular biology 559
monomers of 67
mutations in, and function 88
in nutrition 583–584
prion 649–651
sequence alignment algorithms for 84–87, *86*
structure of
and function 88–89
Pauling's work on 599–600
predicting 87
primary 89, 119
secondary 89, 119
for transport across membranes 96
variation in 714
X-ray crystallography of 759–760, *760*
protein crystallography 420
protein folding 119–120, *120*
protein hormones 290, *291*
protein kinase A (PKA) *165,* 166–167
protein phosphatases 167
protein sequencing 600
protein synthesis
in bacteria 38, 39
chromosomes and 193, 747
in molecular biology 559–560
in origin of life 589, 590
regulation of 314–315
RNA in 668
in translation 367–369
proteobacteria 51
proteomics 387
Protista kingdom 93, 322–324, *323,* 404
protobionts 590–591
Protoctista kingdom 93, 528, *529*
protonephridia 343–344
protons 183–184, 652
protooncogenes 149
protoplasmic streaming 671
protostomes 36–37, 326, *327*
protozoa **645–649,** *646*
infections by 38–39
pathogenic 647–648
types of 645–647
proximate questions 320
Prusiner, Stanley **649–651**
Prymnesiophyta 20
pseudocoelomates 35, *36*
pseudogenes 338
psilocybin 4
psychrophiles 42–43
Pteridophyta 325, *325, 615,* 615–616
puerperal fever 390
pulsed-field gel electrophoresis (PFGE) 280–281, *281*
punctuated equilibrium 336–337, 389, 394–395, *395*
Punnett squares 476, *476*
purines 121–122
Purkinje cells 391–392
Pycnogonida 493
pyrimidines 121–122
Pyrrophyta (fire algae) 20, 21

Q

Q fever, as biological weapon 99
quantitative genetics 383
quantum mechanics 598
quartz 745
Quaternary 418
quiescence 752
quinine 39

R

RAAS (renin-angiotensin-aldosterone system) 425
rabies 595–596
radial symmetry 35, *36*
radiation, cancer from 150
radiation therapy, for cancer 151
radioactive decay 652–653
radioactivity 302, **652–654**
radiolarians *323,* 324, 403
radiological weapons 99
Ras protein 167
rat(s) 675
rate of living theory of aging 11–12
rate-zonal centrifugation. *See* density gradient centrifugation
ray(s) 716
ray-finned fishes 717, *717*
rebreathers 271
receptors
in cell communication 163–164
for protein hormones 290
sensory 677–678
for steroid hormones 292
stimulation of 628
recessive alleles 477, 542–545, *543*
recombinant DNA, synthesis of 205
recombinant DNA technology 19, 129, **654–657**. *See also* genetic engineering
recombinatorial repair 632
red algae (Rhodophyta) 20, 21, 22, 323
red blood cells. *See* erythrocytes
Rede, Francesco 697
Red Sea 202, 204
red tide 22
reduction 604
reductive pentose phosphate cycle. *See* Calvin cycle
redundant species 71
regeneration 562
regional anatomy 26
relativity 677
release mechanisms 521
renal arteries 344, *345*
renewable energy sources 301
renin-angiotensin-aldosterone system (RAAS) 425
replacement mutations 630
repressors 370
reproduction **657–664**. *See also* asexual reproduction; assisted reproductive technology; sexual reproduction
adaptation in 335
of amphibians 718
Aristotle on 44
in biology 104
of birds 723
Buffon's theory of 142
of fungi *361,* 361–362, *362, 363*
Harvey's work on 409
human. *See* human reproduction
of mammals 724–725
of plants 622, 625–628
of sponges 482–483
by spontaneous generation 697–698
stem cells in 750
reproductive cancers 685
reproductive isolation 336, *337*
reptiles 683, 718, 719–722, *720*
RES (reticuloendothelial system) 433
reservoir (nutrient) 75
resolving power 551
resonance 598
respiration and gas exchange **664–668**
bioenergetics of 74
in birds 723
in carbon cycle 76–77
historical emergence of *413,* 413–414
Hooke's work on 427
in mammals 666–667, *667*
systems of 664–666
respiratory infections 467–468
restriction enzymes 205, 655, *656*
restriction fragment length polymorphism (RFLP) 257, *258*
restriction point 176
reticular fibers 35
reticular theory 392
reticuloendothelial system (RES) 433
retinal 682
retinoblastoma 150

retrovirus. *See also* human immunodeficiency virus
DNA synthesis in 39
in gene therapy 371, *375*
reverse transcriptase inhibitor drugs 2, 39
reverse transcription 369
reward, in habit formation 5–6, *7*
RFLP (restriction fragment length polymorphism) 257, *258*
Rhaphidophyta 21
rheumatoid arthritis 13
Rh factor 464
rhinovirus 601
Rhizobium 51
rhizomes 659
Rhodophyta (red algae) 20, 21, 22, 323, *323*
ribonuclease P (RNase P) 23–24, *24*
ribonucleic acid. *See* RNA
ribonucleic acid viruses. *See* human immunodeficiency virus; retrovirus
ribosomal RNA (rRNA)
Cech's work with 156–157
in gene expression 366
as multiple copy gene 386
in translation 367–369
Woese's work on 756
ribosomes
of archaea 42
in bacterial protein synthesis 38
of eukaryotic cells 330, *331*
of prokaryotic cells 643, *644*
in translation 367–369
Woese's work on 756
riboswitches 25
ribozymes, discovery of 157–158, *158*
Rice, Mary Blair 57
ricin, as biological weapon 99
rinderpest 502
Ritalin 576–577
rivers 112
RNA (ribonucleic acid)
catalysis in 22, 24–25, 157–158, *158*
function of 122
in origin of life 589, 590
sugars in 120–122
in transcription 365, *366*
RNA editing 369
RNA interference 25, 375, **668–670**, *669*
RNase P. *See* ribonuclease P
RNA sequencing 90
RNA viruses. *See* human immunodeficiency virus; retrovirus
RNA world hypothesis 589
Robertson, Hugh 23–24
Rockefeller Institute 48
roots 615, 620–621
Rosén, Nils 517
Roslin Institute 209, 749
rotenone 201–202
Rotifera 487, *487*
rotors, of centrifuges 178, *178*
roundworms 486–487
Royal Botanical Gardens 432
Royal Society 427, 509
rRNA. *See* ribosomal RNA
rubeola 469
ruminants, and cellulose energy 101

S

Saccharomyces cerevisiae 674
sac fungi 324
Sagan, Carl 527
salamanders 718, *719*
salinity
of aquatic biomes 106
of estuaries 109
salt marshes 111
salvarsan 37
sand dollar 493, 498, 499
sandworms 497
Sanger, Frederick 260
sap 624
Sarcodina 646
sarcomas 150
Sarcopterygii 716–717
satellite DNA 386
satellite subviruses 731
Sauria 721
savanna 114
scanning electron microscopy (SEM) 554–555
scanning tunneling microscope (STM) 555
scatter plots 240–241, *241*
scavengers 352
schizocoely 35, *36*
schizophrenia in twins 474
Schleiden, Matthias **671–672**
botany work of 134
cell theory of 160, 392
Schwann and 672–673
Schwann, Theodor **672–673**
cell theory of 160, 392
Schleiden and 671
SCID 375
scientific investigation **673–676**
scientific laws 675–676
scientific method 673–676
in agricultural history 14
Aristotle and 45
in medical research 726
scientific theory 104, **676–677**
SCNT. *See* somatic cell nuclear transfer
SCNT (somatic cell nuclear transfer) 210, *211*, 752
scope, in evolution 396
scorpion 31
scrapie 650
SCUBA 534
SDS (sodium dodecyl sulfate) 282
SDS-PAGE electrophoresis 282, *282*
sea anemones 326
Sea Around Us, The (Carson) 153
sea cucumber 493
sea gooseberries 485
sea levels 306
sea slug 674
sea spiders 493
sea star 493
sea stations 59
sea urchin 44, 493, 499
SEB (staphylococcal enterotoxin B), as biological weapon 99
sebaceous glands 482
secondary consumers 277
secondary immunodeficiencies 465–466
secondary production 278
second messengers
in cell communication 164, *166*, 166–167
protein hormones and 290
sectioning 554
seed 622, 627
seedless vascular plants *611*, 614–617
seed plants *611*, *616*, *617*, 617–619
segmentation 37, 327–328
segmented worms 489–490
segregation 190, 472–477, *476*
seizures 315, 316
selective media 169
selective serotonin reuptake inhibitors (SSRIs) 5
selectivity 589
self-awareness, in animals 33
self-fertilization 537
Selzentry 2
SEM (scanning electron microscopy) 554–555
semen 446
semisynthetic antibiotics 37
senescence 8, 170
sensation **677–682**
sense RNA 668–670
senses. *See* olfaction; vision
sensory transduction 678
sentiment intérieur 506–507
sequence alignment algorithms 84–87
sequential hermaphroditism 662
Sequoia and Kings Canyon National Parks 222–224
Serengeti Research Institute 706
serial endosymbiotic theory (SET) 527–530

serotonin 5
Serpentes 721, *721*
serpentine 745
serum sickness 464
sewage treatment 549–550, **737–741,** *739, 740*
sex determination 444, 562, **682–684,** 693, 699
sex-linked inheritance 562–563, *564*
sexual and reproductive health **684–690**
sexually transmitted diseases (STDs) 685–687
sexual reproduction 659–662, *663*
  of animals 682
  of fungi 361–362, *363*
  life cycle and 663–664
  of plants 625–628, *627*
  variations from 713
sexual selection, in animals 30–31
shark repellent 203
sharks *202,* 202–204, 716
sheep, cloning 209, *209,* 749–753, *754*
Shelburne, Lord. *See* Petty, William Fitzmaurice
shelf fungi 324
shoot system 621–622
short interspersed elements (SINEs) 386
short tandem repeat (STR) analysis *258,* 259, *259*
sickle cell disease 600
Sierra Interagency Black Bear Group 222–223
signal transduction pathways 165–167
significance level 244
sign language, used by animals 33
silent mutation 338
Silent Spring (Carson) 153–155, 308
silicosis 56
silkworm diseases 594–595
Silurian 417–418
simultaneous hermaphroditism 662
SINEs (short interspersed elements) 386
single-gene disorders 376–378
single-lens eye 681
single sequence alignment 84
sinoatrial node 198
siphonophores 61
site-directed mutagenesis 657
size exclusion chromatography 188, *189*
skates 716
skeletal muscle 572, *573*
skeletal systems 569–570
skin 130, 433, 481–482, 723–724
slime molds 324, *690,* **690–692,** *691*
slime nets 323, 324
small intestine 253–255, *254*
smallpox
  as biological weapon 98
  inoculation against 470–471
smell 356–357, 680, 704
Smith, James 519
Smith, John 24
smoke alarms 653
smooth muscle 572
snakes 721, *721*
snowmelts 300
social behavior of animals **692–694**
sociobiology **694**
sodium dodecyl sulfate (SDS) 282
sodium-potassium pump *96,* 97
soil 129
solar radiation, and biosphere 127–129, *128*. *See also* light
soluble fiber 584
solvent, water as 736–737
somatic cell(s), stem cells from 210, *211*
somatic cell nuclear transfer (SCNT) 210, *211,* 752
somatic nervous system 578
songbirds
  DDT's effects on 153–155
  protection of 223
souls, Aristotle on 45
sounding 702
South America 234, 453–454
Southern blotting 281
Soviet Union, biological science under 265
sow bugs 28
spatial learning, in animals 32
speciation. *See also* evolution, theory of
  in evolution 335–337, *337*
  Gould on 394–395
  Margulis on 530
species
  classification of 92
  definition of 336
  distinctiveness of 142
  dominant 216
  foundation 218
  identification of 102
  interactions of, in communities 214–216
  keystone 71, 101–102, 216–218, 220
  native, threats to 287–288
Species plantarum (Linnaeus) 519
species richness 81–82
specific immunity 434–437, 711
spectrophotometry **695–697,** *696*
Spemann, Hans 208
sperm 444–446, *445,* 510–511
sperm, donor 47
spermatogenesis 444–446, *445*
spermicide 688
Sphenodonta 721, *721*
spider crabs 460, *460*
spiders 707
spinal cord 578
Spirochaetes 51, 52
Spirogyra 21
sponges 326, 482–483, *483*
spongy bone tissue 570–571
spontaneous generation 390, 511, 593–594, *594,* **697–698**
spontaneous mutation 630–631, 712
sporangia 612
spores
  in fungal reproduction 361, *361, 362, 363*
  in plant reproduction 625
sporophylls 615
sporophyte stage 614–615
springs 427
SSRIs (selective serotonin reuptake inhibitors) 5
staining 391–392, *392*
  in cell biology 162
  in microscopy 551–553, 554
standard deviation *242,* 242–243
staphylococcal enterotoxin B (SEB), as biological weapon 99
Staphylococcus aureus, methicillin-resistant 40
Stark, Benjamin 24
statistical analysis 241–245
STDs (sexually transmitted diseases) 685–687
stem cell(s) 750–752
  in biotechnology 130
  from cell reprogramming 210, *211*
  in gene therapy 374
  in genetic modification 749–752
  research on 162
  from somatic cells 210
  in therapeutic cloning 753–755
stems (plant) 621–622
stepwise process 673
stereochemistry 592–593
sterilization 689
steroids 122, *123,* 290–292
Stevens, Nettie **698–699**
Steward, F. C. 208
stimulants 4, 576–577
stimuli, response to 104
STM (scanning tunneling microscope) 555
stolons 659, *659*
stomach 253, *254*
stomata 606, *606,* 624

stramenopiles 323, *323*
STR (short tandem repeat) analysis *258*, 259, *259*
Strasburger, Eduard 134
streams 112
stresses, in CCD 17
stroke 200
stromatolites 412, *412*, 413
structure. *See also* anatomy
  of atoms 183–184
  definition of 100
  formalist view of 388
  living organisms' need for 102–104
  of viruses 727, *728*
structure-function relationships 100–102
  adaptations and 334
  at atomic level 183
  Cuvier on 229, 231–232
  in DNA 248
  at molecular level 183
Structure of Evolutionary Theory, The (Gould) 396–397
Study of Instinct, The (Tinbergen) 705
Sturtevant, Alfred H. 563
subcutaneous layer 481
submersibles 272, 533
sub-Saharan Africa, HIV pandemic in 1
substance abuse 3
substrate feeders 250
succession *218*, 218–220
sugars. *See also* glucose
  facilitated diffusion of 96
  in nucleic acids 120–122
  in photosynthesis 145–146
sulfate-reducing bacteria 52
sulfonamides 350
sulfur bacteria 51, 603
sulfur cycle 79
sulfur hot springs 139
sulfur-reducing bacteria 52
SUMOylation 473
Sun, and bees 358–359
sunlight. *See* light
supernatural theory of disease 389–390
surface tension 735–736
surgical sterilization 689
surveying 517
survival, adaptation in 335
survival of the fittest 341. *See also* evolution, theory of; natural selection
survivorship curves *634*, 634–635
suspension feeders 250
sustainability 306, 308, 309
Sutton, Daniel 471
Svedberg, Theodor 179
swamp gas 740
swamps 111
sweat glands 481
symbiogenesis 528
symbiosis, in communities *215*, 215–216
sympatric speciation 336
synapse 5, 580–582, *581*
synchronous hermaphroditism 662
synthesis phase 175
synthetase 66
synthetic work 73
syphilis 37, 348, 686–687, *687*
Systema naturae (Linnaeus) 92–93, 517–518
systematics 89, 133
Systematische Phylogenie (Haeckel) 406
systemic anatomy 26
system state 73

T

taiga. *See* coniferous forest
tapeworm infections 39
tapeworms 486, *486*
taproot systems 620
Taq polymerase 140, 568
taste 680
taxis, in animal behavior 28
taxonomy. *See* biological classification
TB. *See* tuberculosis
technology, in modern agriculture 14
Tee-Van, John 58
Tektite II 271–272
teleology 45
telomeres
  cancer and 13
  in cell life span 9–10
  discovery of 537
  HHMI research on 158
telophase 176, 660
TEM (transmission electron microscopy) 162, 554, *554*
temperate broadleaf forest 116
temperate grasslands 115–116
temperature
  in deep ocean 700–701
  in enzyme reaction velocity 313
  plant response to 297, 629
  regulation of. *See* thermoregulation
temporal lobectomy 318
terminator 366, *367*
Ternate essay 733
terrestrial biomes. *See* biomes, terrestrial
territoriality 704, 725
terrorism. *See* bioterrorism
Tertiary 418
testcross 477
testes *294*, 444, 750
testicular cancer 685
tetrafoil cleavage 136, *136*
Tetrahymena thermophila 156–157
tetranucleotide hypothesis 181
tetrapods 717–723
thallus 324
Thane, Elswyth 59
Theophrastus 134
Theorell, Hugo 267
theory. *See* scientific theory
therapeutic cloning 212, 752, 753
thermodynamics, in bioenergetics 73–74
thermodynamic spontaneity 74
thermophiles 40, 42, 139–140
thermoreceptors 678
thermoregulation 423–424, 736
Thermus aquaticus 139–140, 568
thigmomorphogenesis 629
Thiobacillus ferrooxidans 129
Thomson, C. Wyville 533, **700–703**, *701*
thought, in animals 33
threatened species 286
thrombus 200
thymine
  Chargaff's research on 181
  in DNA structure 245
  in nucleic acids 122
thyroid *294*
time, units of 547
"time since perturbation" hypothesis 82
Tinbergen, Nikolaas **703–707**
  in ethology 320, 523
  fixed-action patterns work of 521
  spatial learning experiment of 32
tissue
  bone 570–571
  commercial availability of 170
  culture of 169–170, *170*
  differentiation of 672
  hierarchical levels of organization of 34
  microscopy of 526
  of plants 620, 621–622
  in skin 481
  types of 34–35, 149
tissue transplantation 130
T lymphocytes 1–2
TMOF (trypsin modulating oostatic factor) 379
toads 718
tobacco mosaic virus (TMV) 355, 494–495, 743
toenails 481
testcross 477
testes *294*, 444, 750
testicular cancer 685
tetrafoil cleavage 136, *136*
Tetrahymena thermophila 156–157
tetranucleotide hypothesis 181
tetrapods 717–723
thallus 324
Thane, Elswyth 59
Theophrastus 134
Theorell, Hugo 267
theory. *See* scientific theory
therapeutic cloning 212, 752, 753
thermodynamics, in bioenergetics 73–74
thermodynamic spontaneity 74
thermophiles 40, 42, 139–140
thermoreceptors 678
thermoregulation 423–424, 736
Thermus aquaticus 139–140, 568
thigmomorphogenesis 629
Thiobacillus ferrooxidans 129
Thomson, C. Wyville 533, **700–703**, *701*
thought, in animals 33
threatened species 286
thrombus 200
thymine
  Chargaff's research on 181
  in DNA structure 245
  in nucleic acids 122
thyroid *294*
time, units of 547
"time since perturbation" hypothesis 82
Tinbergen, Nikolaas **703–707**
  in ethology 320, 523
  fixed-action patterns work of 521
  spatial learning experiment of 32
tissue
  bone 570–571
  commercial availability of 170
  culture of 169–170, *170*
  differentiation of 672
  hierarchical levels of organization of 34
  microscopy of 526
  of plants 620, 621–622
  in skin 481
  types of 34–35, 149
tissue transplantation 130
T lymphocytes 1–2
TMOF (trypsin modulating oostatic factor) 379
toads 718
tobacco mosaic virus (TMV) 355, 494–495, 743
toenails 481

tolerance, development of 6
tonic clonic seizures 316
tool use 440
top-down model of communities 218
topoisomers 66
tornaries 326
Torrey, John 398
totipotent cells 750
tourism, and endangered species 289
toxins
  as biological weapons 98–99
  bioremediation of 124
  vaccines for 711–712
toxoid 712
trace fossils 90
tracheal mite *(Acarapis woodi)* 15, 17
Tracheophyta 325
traits, inheritance of 472–478
transcription factors 370
transcriptomics 387
transduction 50
transfer RNA (tRNA)
  biosynthesis of 23–24, *24*
  in gene expression 366
  structure of 23
  in translation 367–369, *369*
transformation
  of bacteria 48–49, 50, 524, 534
  of recombinant DNA 656
  in variation 713
transformism 505–506
transgenic animals 210
transgenic plants 19
translation 367–369, *369*
transmission electron microscopy (TEM) 162, 554, *554*
transpiration
  in plants 606, 624
  in water cycle 79–80
transport 624
transport proteins 96
transposons 386, 474, 537–539
trees
  in chaparral 115
  in coniferous forest 116
  species migration of 82
  in temperate broadleaf forest 116
  in tropical rain forest 113–114
trefoil cleavages 137
Trematoda 486
Triassic 418
tricarboxylic acid cycle 160, 171–172
trichomoniasis 647
triglycerides 122, *123,* 173–174
tRNA. *See* transfer RNA
trochophores 326
Trojan horse vaccines 712
trophic levels 278–279, *279*
trophozoite 645
tropical habitats, species richness in 82
tropical rain forest 113–114
  degradation of 298–299, *299*
  global change and 296–298
  large-scale experiments in 296–297
tropical research 58
Tropical Wildlife in British Guiana (Beebe) 58
tropism 628–629, 709
troposphere 126
trypsin modulating oostatic factor (TMOF) 379
t-test 245
tuataras 721, *721*
tubal embryo transfer. *See* ZIFT
tubal ligation 689
tuberculin 502
tuberculosis (TB) 468–469, 501–502
tube worms, vestimentiferan *459,* 459–460
tularemia, as biological weapon 99
tumor 138, 514
tumor suppressor genes 12–13, 149–150
tundra 116–117, *117*
Turbellaria 485–486
Turner, Charles Henry **707–710**
twins 474
Type 1 diabetes 249
Type 2 diabetes 249
tyrosine 5
tyrosine-kinase receptors 164

U

ubiquitination 473
ultimate questions 320
ultracentrifuge 179
ultrasound imaging 27
ultraviolet spectroscopy 696
ultraviolet-visible spectrophotometry 696
umbilical cord stem cells 751
UN. *See* United Nations
"Undersea" (Carson) 152
Under the Sea-Wind (Carson) 152, 153
unicellular organisms 34, *36*
uniformitarianism 394
Unirama *491,* 491–492
United Nations Conference on Environment and Development (Earth Summit) 71
United Nations (UN) conservation programs 224
United Nations Convention to Combat Desertification (UNCCD) 298
United Nations Environmental Programme (UNEP) 224
United States Department of Agriculture (USDA) 20
United States Fish and Wildlife Service. *See* Fish and Wildlife Service, U.S.
universal ancestor 411–412, *412,* 758
University of Toronto 54–56
Upjohn Company 139
uracil 122
Ural Mountains 456
uranium
  bioremediation of 125
  in nuclear energy 302
urea 343
Urey, Harold 555–556, 588–589
uric acid 343
urine 343–346, *345*
U.S. Exploring Expedition 398, 399
USDA (United States Department of Agriculture) 20
uterus *447,* 448

V

vaccines **711–712**
  for HIV 3
  inoculation and 347–348
  Pasteur's development of 390, 595–596
  for smallpox 470–471
vacuoles 329
vagina *447,* 448
vaginitis 687
vagus nerve 318
valence bond theory (VB) 598
valence electrons 184
  in covalent bonds 184–185
  in ionic bonds 185–186
vancomycin-resistant *Enterococcus* (VRE) 40
Vanderbilt, Anne 202
van der Waals forces 187
variable number tandem repeats (VNTRs) 257, *258*
variation (genetic) **712–714**
  in asexual reproduction 658
  in clonal bacteria 50
  in evolutionary theory 332–333
  fixed traits from 342
  in honeybees 16
  in sexual reproduction 660
variation (statistical) 242–243
Varroa destructor (ectoparasite varroa mite) 15
varroa mites 15, 17
vascular tissue 620, 621

vasectomy 689
vectors 371, 655–656
vegetables, pollination of 15
vegetative propagation 659
veins
  in circulatory system 195–196, *196*
  color of 334–335
venom (honeybee) 15
Venter, J. Craig 380, 442
ventral tegmental area (VTA) 6, *7*
vertebrates **714–725**
  classification as 328
  endocrine system of 292–293
  eyes of *681,* 681–682
Vesalius, Andreas 256, 407
vestimentiferan tube worms *459,* 459–460
viral hemorrhagic fevers (VHFs), as biological weapons 99
viral load 2
Virchow, Rudolf 160, 671, 673, **725–727**
viroids 731
viruses and other infectious particles **727–731**. *See also* acquired immunodeficiency syndrome; bacteriophages; human immunodeficiency virus; infectious diseases
  antibiotics prescribed for 39–40
  as biological weapons 98
  cancer from 150
  chemotherapy for 39
  classification of 727–728, *729*
  EGSs used against 25
  in gene therapy 371, 372–373, *374*
  genetic variation from 713
  immunity to 711–712
  life cycles of 728–731, *730*
  polymorphic 714
  prions and 650
  RNA interference and 670
  structure of 727, *728*
  as vectors 656
  Watson's work on 743, 744
vision 355–356, 680–682, *681,* 709
vitalism 68, 672
vitamin B_{12} *421,* 421–422
vitamin C 601–602
vitamins 584, *585*
viviparous animals 663
vivisection 256, 408
VNTRs (variable number tandem repeats) 257, *258*
volcanic activity, in Galápagos islands 58
Volvox 34
Voyage of the Challenger, The (Thomson) 702
Voyages to the Equinoctial Regions (Humboldt) 456
VRE (vancomycin-resistant *Enterococcus*) 40
VTA (ventral tegmental area) 6, *7*

W

Wächtershäuser, Günter 589–590
Wallace, Alfred Russel **732–735**
  on competition 214
  Darwin and 236–237
Wallace Line 734
wastewater 737, *739,* 739–741, *740*
water
  biodiversity and 72
  biological importance of **735–737**
  bioremediation of 124
  biosphere and 127–129
  carbonated 637
  as electron donor 413
  human-induced threats to 299–300
  plant response to 629
  pollution of 302
  properties of *735,* 735–737, *736*
  transport of, in plants 624
  treatment of **737–741,** *738*
water balance 424–425
water bodies, and climate 127
water cycle 79–80, *80,* 127–129, 299–300
water molds 323–324
watersheds, in biodiversity 72
Watson, James D. 248, 558, **741–744**
  Chargaff and 181
  Crick and 225–227
  Rosalind and 354–355
waves 64
weather, and biosphere 127
weight, in diabetes risk 13
Weismann, August 190
Werner, Abraham Gottlob 455
western blot analysis 2, 282
wetlands 111, 287, 300
whales 272
whaling, ecological impact of 277
white blood cells. *See* lymphocytes
Whittaker, Robert 93, 322, **744–746**
WHOI (Woods Hole Oceanographic Institution) 63–64
Wilberforce, Samuel 237
wildlife recreation 289
Wilkins, Maurice H. F. 226–227, 354, 742–743, **746–748**
Willadsen, Steen 208–209, 749
William Beebe Tropical Research Station 61
Williams syndrome 377
Wilmut, Ian 209, *748,* **748–755**
Wilson, Edmund Beecher 699
Wilson, Edward O. 89, *694*
wind, in desert structure-function 102
Wind Waves at Sea, Breakers, and Surf (Bigelow) 64
wine pasteurization 594
withdrawal (contraception) 689
Woese, Carl **756–758,** *758*
  Archaea proposed by 40
  classification system of 93, 529
  domains proposed by 140, 322, 756–758, *757*
Wöhler, Friedrich 68
Wonderful Life (Gould) 396
Woodell, George M. 746
Woods Hole Marine Biological Laboratory 497, 561–562
Woods Hole Oceanographic Institution (WHOI) 63–64
woody magnoliids 619
work, in biological processes 73
World War I 57
World War II 56, 522
World Wildlife Fund 225
worm lizards 721
worms. *See* specific types
Wren, Christopher 428
Wright, Almroth 347–348

X

Xanthophyta (yellow-green algae) 20, 21, *323,* 324
Xenopus laevis 675
xenotransplantation 212, 380
X-inactivation 474
X-linked disorders 376–377
X-ray(s)
  for anatomic imaging 27
  plants exposed to 537
X-ray crystallography *759,* **759–760,** *760*
  of DNA 226, 742–743, 747
  Franklin's work with 353–355
  Hodgkin's work with 419–422
  Pauling's work with 597
xylem 615, 620, 624

Y

Yale University 25
yeasts 360, *361,* 549, 593
yellow-green algae (Xanthophyta) 20, 21
Yellowstone hot springs 139, 140
yolks 284

Z

zebra mussels 287–288
ZIFT (zygote intrafallopian transfer) 47
zinc fingers 87
zones, of marine biomes 106, *107*
zoology **761–762**
  Aristotle's work on 44–45
  Beebe's work in 57
  Haeckel's work in 403–404
  Hyman's work in 461–462
  Just's work in 498
  Lamarck's work in 505–506
  Turner's work in 707
  Watson's work in 742
zooplankton 107
zooxanthellae 305–306, *306*
Zygomycota 324, 361–362
zygote
  chromosomal composition of 136
  development of 283
  in plant reproduction 625
zygote intrafallopian transfer (ZIFT) 47