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Marc Thiriet

Biology and Mechanics of Blood Flows

Part I: Biology

Springer

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Series Preface

The Centre de recherches mathématiques (CRM) was created in 1968 by the Université de Montréal to promote research in the mathematical sciences. It is now a national institute that hosts several groups and holds special theme years, summer schools, workshops, and a postdoctoral program. The focus of its scientific activities ranges from pure to applied mathematics and includes statistics, theoretical computer science, mathematical methods in biology and life sciences, and mathematical and theoretical physics. The CRM also promotes collaboration between mathematicians and industry. It is subsidized by the Natural Sciences and Engineering Research Council of Canada, the Fonds FCAR of the Province de Québec, and the Canadian Institute for Advanced Research and has private endowments. Current activities, fellowships, and annual reports can be found on the CRM Web page at www.CRM.UMontreal.CA.

The CRM Series in Mathematical Physics includes monographs, lecture notes, and proceedings based on research pursued and events held at the Centre de recherches mathématiques.

Montréal, Quebec, Canada *Yvan Saint-Aubin*

Contents

Introduction

> *"... there is a continuous and uninterrupted movement of blood from the heart through the arteries to the body as a whole, and likewise back from that body as a whole through the veins to the heart, with such flow and ebb that in such quantity and amount that it must somehow move in circle."*
> (A letter from W. Harvey to C. Hofmann [1])

As the title suggests, the present text, is aimed at providing basic knowledge and state of the art on the biology and the mechanics of blood flows. Due to its length, this book has been divided into two parts, taking into account the length scale at which the cardiovascular system is explored (length scale of cellular events [nanoscopic], cell and its interactions with adjoining cells and tissues [microscopic], and organs [macroscopic]).[1]

Part I, *Biology*, mainly deals with nanoscopic and microscopic scales. The nanoscale corresponds to the scale of biochemical reaction cascades involved in cell adaptation to mechanical stresses among other stimuli. The microscale is the scale of stress-induced tissue remodeling associated with acute or chronic loadings. Part I is composed of 10 chapters. Chapter 1 gives a survey of cell components, emphasizing cell fate, membranes of the cell and its organelles, mitochondrial production of energy, and cell cytoskeleton. The cytoskeleton is involved in cellular transport, in cell division, in cell adhesion and migration, and, last but not least, in cell adaption to mechanical stresses. Chapter 2 deals with the plasmalemma (cell membrane) and its relation to the cell environment. The extracellular matrix is a necessary medium for cell fate and tissue formation and remodeling due to applied mechanical stresses. Multiple cell processes are primed by activated ion carriers and receptors of the plasmalemma. Ion fluxes particularly generate action potential in the heart wall, which then travels through conduction (nodal) tissue and myocardium to trigger contraction of the cardiac pump. Heart contraction

[1] Part II, *Mechanics and Medical Aspects*, refers to the macroscopic scale. It contains chapters on anatomy, physiology, and continuum mechanics, as well as pathologies of the heart and walls of blood vessels, and their treatment.

requires calcium influx and energy (cardiomyocyte relaxation needs calcium efflux). Transduction of mechanical stresses applied both on the wetted surface of the vasculature and within the wall by the cells involves both ion carriers and receptors of the plasmalemma. Ion carriers and receptors to various ligands are thus introduced in Chapter 3. The signal propagates in the cell using specific pathways that are described in Chapter 4. The effector stage of the initiated signaling pathways is achieved by an activation cascade, with assembling of molecular complexes and reversible protein modifications, especially phosphorylation. Chapter 5 describes two cell processes in which the cytoskeleton is involved, molecular transport in the cell in interaction with its environment and cell migration for tissue adaptation and repair. The following chapters deal with biological tissues of the vasculature (histology) and their functioning. The first explored biological tissue is the blood, a concentrated suspension of circulating cells in a solvent, the plasma (Chapter 6). Blood is a peculiar type of connective tissue used for transport and body regulation. Blood is propelled throughout the circulatory system by the heart. Chapters 7 and 8 give the structure and composition of the heart and vessel walls, respectively. Chapter 7 highlights the nodal cells, which are responsible for action potential generation (heart automatism due to the natural pacemaker) and propagation, and the cardiomyocytes, which also propagate the action potential and contract to propel the blood. Chapter 8 underlines the activity of the vascular smooth muscle cells, which regulate the caliber of blood vessels. The endothelial cells at the interface between the flowing blood and vessel wall are assigned to Chapter 9, owing to their role in clotting, extravasation of flowing cells, and transduction of mechanical stresses. The mechanotransduction leads to the synthesis of several substances that regulate vasomotor tone. Chapter 10 discusses growth factors implicated in growth, remodeling, and repair of the vasculature.

Because the behaviors of vascular cells and tissues are tightly coupled to the mechanics of the flowing blood, the major features of blood flows and equations of mass and momentum conservation, the so-called Navier-Stokes equations, are introduced as the conclusion of Part I. The three basic natural sciences - biology, chemistry, and physics - interact with mathematics to explain the functioning of blood flow. The basic data of continuum mechanics, two analytical solutions of the Navier-Stokes equations, the steady Poiseuille flow and periodic Womersley flow, as well as certain information on fluid flow, are given in Appendix A. Because the architecture of the vasculature is complicated and blood circulation is complex, with a huge number of interacting elements that are difficult to measure, the Navier-Stokes equations in the macrocirculation cannot be solved analytically. Numerical simulations are then carried out (App. D).

The origin of this book is an invited contribution text on “Biochemical and Biomechanical Aspects of Blood Flows” included in the survey article

collection "Modeling of Biological Materials".[2] The present review also answers a pedagogical objective of the project "Mathematical Modeling for Haemodynamics" (HaeMOdel).[3] This book represents a much longer version of a draft given to the participants of the CRM–INRIA Spring School[4] on "Mini-invasive Procedures in Medicine and Surgery: Mathematical and Numerical Challenges," at the Centre de Recherches Mathématiques of the Université de Montréal in Québec, Canada.

The cardiovascular system, like any physiological system, has a complicated three-dimensional structure and composition. Its time-dependent behavior is regulated. This complex system is constituted by many components. The features of a complex system are not necessarily predictible from the behavior of its elementary constituents, which can non-linearly interact with feedback loops, contributing to system bulk behavior. The organization and bulk behavior of a complex system not only results from the simultaneous activities of its constituents, but also emerges from the sum of the interactions among its constituents. A complex system evolves between order and chaos, with sufficient order to auto-organize and freedom to adapt. A complex system adapts by changing its organization and possibly its structure to environmental stimuli.

A complicated system can be analyzed by decomposition into simple parts with identified functions. The combination of these functions allows us to deduce system functioning due to linear interactions. Deconstruction into parts of physiological systems may be necessary to understand part behavior as well as to determine between–part interactions. Besides, a part of a physiological system can represent a complex system, whatever its scale. The cell itself is a complex system. In any case, the simple still needs to be solved before investigating the complex. However, modeling of complex systems is now aimed at pursuing a bottom-up approach, starting from the acquired basic knowledge of system parts.

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[2] The book "Modeling of Biological Materials" belongs to the Series "Modeling and Simulation in Sciences, Engineering, and Technology", Bellomo N. (ed.), Birkhauser.

[3] HaeMOdel project corresponds to a Research Training Network of the fifth call of the framework program of the European community. The main objectives of HaeMOdel project are the development of numerical models including three-dimensional reconstruction from medical imaging, Navier-Stokes solutions in deformable domains, and the biochemical transport.

[4] The object of this combined school (short courses, May 16–20) and workshop (May 23–27, 2005) was to bring together several facets of mini-invasive procedures in medicine and surgery and identify issues, problems, and trends, as well as mathematical and computing challenges. The school was structured around the following themes: medical image processing and geometrical modeling, fluid–structure interaction in biomedical problems, static/dynamical design and control of (implantable) medical devices, finite element based computer-aided design/manufacturing.

Input data for integrative investigation of the complex dynamic cardiovascular system include knowledge accumulated at various length scales, from molecular biology to physiology on the one hand, and histology to anatomy on the other hand. Tier architecture of living system is characterized by its communication means and regulation procedures, allowing us to integrate environmental changes to adapt. Multiple molecules interacts to create the adaptable activity of the cells, tissues, organs, and body. The huge quantity of these molecules forms a complex reaction set with feedback loops and a hierarchical organization. Studies from molecular cascades primed by mechanical stresses to cell, then to tissues and organs need to be combined to study living systems with complex dynamics; but future investigations are still needed to mimic more accurately system functioning and interaction with the environment, using multi-scale modeling. An integrative model also incorporates behavior at various time scales, including response characteristic times, cardiac cycle (s), and diurnal periodicities (h), to efficiently describe the structure-function relationships of the explored physiological system.

> "*Tout organisme, quel qu'il soit, se trouve alors indissolublement lié, non seulement à l'espace qui l'entoure, mais encore au temps qui l'a conduit là et lui donne comme une quatrième dimension. [Any organism is necessarily linked not only to the surrounding space, but also to time which leads it to its present state and gives to it as a forth dimension.]* " (F. Jacob) [2]

Models that integrate nanoscale processes allow us to study reacting system rather than those that are inert. Furthermore, they can investigate responses of living tissues to administered substances, especially drugs delivered by nanotechnology-based methods or implanted medical devices. Such modeling takes into account the dynamics of cell processes, i.e., spatial and temporal organization of biochemical reactions and major molecular interactions with positive and negative feedbacks, which amplify or stabilize the response, respectively. The dynamics of a biochemical process can be represented by a set of equations that link the time variations of concentrations of implicated susbtances to production and consumption rates, which depends on concentrations of interacting molecules and spatial coordinates within the cell and possibly the extracellular compartment. The cell is an heterogeneous medium, even inside the cytosol and organelles. The loci of molecular interactions most often remain unknown, although most pathways have their first reaction steps at the cell membrane and cortex.

Multiple biochemical processes are involved in blood flow and blood function, including hematopoiesis, extravasation, coagulation, mechanotransduction, and wall remodeling on the one hand (Chap. 8), formation and development of the cardiovascular system, angiogenesis associated with tumors and tissue engineering, and defense mechanisms on the other hand (Chap. 10); and last but not least, pathogenesis of cardiovascular illnesses (Part II).

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1

The Cell

> " *Phenomena are manifestations of invisible things* "
> *(Anaxagoras).*

Anatomy deals with the macroscopic scale, histology with microscopic levels (tissue and cell structures). Regulated assemblies of cells produce biological tissues. The tissue morphology depends on the cell shapes, hence on cellular cytoskeletons and on cell adhesions, which are controlled by the mechanics of the cells subjected to forces. Biological tissues are, thereby, able to resist applied stresses and remodel.

There are four main tissue types involved in blood circulation [3–5]. Epithelial tissues (`type I`) are packed cell sheets that cover surfaces of the body and organs. The vascular endothelium is an epithelium that lines the wetted surface of the entire circulatory system. This living tissue reacts to flow forces and regulates cell and substance transport at the blood-wall interface. Connective tissues (`type II`), in which cells are separated in a matrix, bind the different structure components together, requiring adhesion molecules.[1] The mesenchyme, an embryonal and fetal connective tissue, is a precursor for the early formation of blood and blood vessels.[2] Muscle tissues (`type III`) are made of specialized elongated cells that contract to produce motion, using energy and biochemical reactions. Nervous tissues (`type IV`) send impulses to regulate the blood circulation. Blood is a peculiar type II tissue composed of cells suspended in a plasma, which replaces the matrix of connective tissues.

The cell is the major constituent of biological tissues. Any cell is aimed at surviving, at proliferating, and at migrating, as well as for certain cell types at differentiating, until disappearance. The cell is the smallest unit with structures and functions aimed at synthesizing assigned substances. Body cells,

[1] Connective tissues are either rich in fibers, which includes loose (poor in fibers, rich in ground matrix) and dense (rich in fibers, poor in ground substance) connective tissues, or rich in cells.

[2] The mesenchyme generates also connective and muscle tissues. Mesenchyme cells are pluripotent.

although specialized, have similarities. Among the shared features, the bulk structure with a cell membrane, a genetic code-containing nucleus, and a set of organelles. Water, proteins, and lipids are the main constituents of the cell (Table 1.1). Although the water content is quite high, the deformable cell can be considered as a slightly compressible material.

Tissue development, organization, and functioning depend on cellular interactions, both between the cell and its surrounding cells and between the cell and its matrix (Table 1.2). These interactions, indeed, occur either by transmission of chemical cues and stresses through the extracellular matrix (Chap. 2) of the microenvironment that separates neighboring cells (signaling pathways then convert stimuli into cell action; Chap. 4), or, for cells in contact, by stimulating between-cell adhesion complexes (inducing cell reactions via the cytoskeleton and via biochemical effectors) and by using intercellular communications (triggering chemical cascades). Among sent signals, exosomes secreted into the extracellular environment contain mRNAs and microRNAs. Force transmission to/by the extracellular matrix and cell contacts affects the cell maturation and assembly. The time and space variations in cell interactions regulate the cell fate.

Table 1.1. Approximative composition of a cell (Source: [6]).

Water	70
Proteins	18
Lipids	5
RNA, DNA	1.5
Polysaccharids	2
Small metabolites	3
Inorganic ions	1

Table 1.2. Tissue organization and development are governed by cell interactions with the environment. Between-cell communications are either direct via cell contacts or indirect via signaling molecules.

Inputs	Cell number and functions for tissue formation (cell molecular pathways) Matrix features
Cell fate	Growth factors Cell loadings Cell adhesiveness Migration potential, chemotactic factors
Growth control	Anchorage to cells and to the extracellular matrix State of the surrounding extracellular matrix (local degradation) Nutrient input

During organ motions, especially in heart and lungs, which bear large deformations during the cardiac and respiratory cycles, cells undergo stretch, shear, and possible torsion. Stretch stimulates growth, differentiation, migration, and remodeling. The cytoskeleton (Sect. 1.5) transforms from a solid-like to a fluid-like phase in response to transient stretches of relatively small amplitude [7]. Loaded soft cells quickly fluidize and then slowly re-solidify (slow relaxation). The closer the initial state from the solid-like state, the greater the fluidization, and the faster the subsequent recovery, except in the case of ATP depletion. The cell ability to fluidize suddenly in response to mechanical stresses and to subsequently resolidify appears to depend much more on stresses themselves than on molecular reactions triggered by stresses.

Ciliated epithelia generate flow (for mucus clearance in the respiratory airways, for cerebrospinal fluid transport in the cerebral ventricles). Oriented and coordinated motions of cilia of cell set is favored by flow. Motion orientation is aligned with the streamwise direction of imposed unidirectional flow during tissue development after a first programmed step of rough orientation, allowing cilia to produce directional flow [8].

1.1 Cell Components

The cell has a nucleus and several organelles (compartmentation) within its *cytoplasma* (Fig. 1.1). The cell elements, which undergo stresses resulting from the loading imposed by the cell environment, are wrapped in the plasma membrane, or *plasmalemma* (sarcolemma in muscle cells; Sect. 2.1). The cytoplasma, or cytoplasm, corresponds to the cell content outside the nucleus. The *cytosol* is the main compartment of the cytoplasm, excluding membrane-bound organelles. The characteristic sizes of the cell elements are given in Table 1.3.

1.1.1 Cellular Membranes

Cellular membranes separate the cell from the extracellular medium and compartmentalize the cell into a nucleus and functional organelles. The cell

Table 1.3. Characteristic sizes of the cell structural elements (nm; Source: [6]).

Mitochondrium thickness	500–2000
Lysosome (hydrolases)	200–500
Peroxysome (oxydative enzymes)	200–500
Plasmalemma thickness	4–5
Microtubule caliber	25
Actin filament bore	5–9
Intermediary filament bore	10

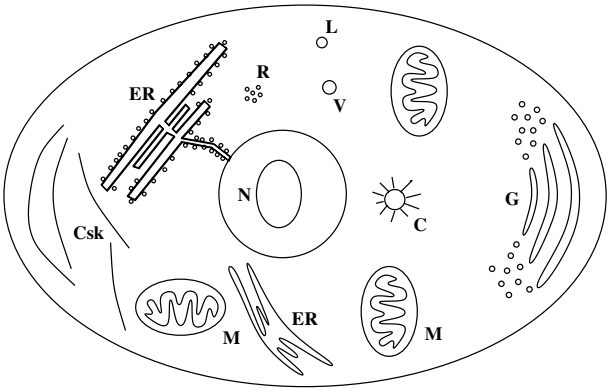

Figure 1.1. Scheme of the cell and its components. N: nucleus; ER: endoplasmic reticulum with its smooth and rough compartments; G: Golgi apparatus; M: mitochondrion; Csk: cytoskeleton; C: centrosome; R: ribosome; V: vacuole, vesicle; L: lysosome.

organelles have their own metabolism.[3] They regulate the active transport across them and transduce external cues. Biological membranes communicate between them. Vesicles indeed bud off from one membrane and fuse with another. Organelle identity, maintenance and function require membrane regionalization. Cooperation between compartments is done via many pathways.

The cell membrane is a phospholipid bilayer[4] (6–10 nm), characterized by fluidity and relative stability. The cell membrane acts as filters. It contains adhesion molecules and other compounds (proteins and polysaccharides) acting in cell junctions, in signaling and in transport (Table 2.5).

Proteins in the plasma membrane are not randomly distributed but are mainly partitioned into clusters. Proteins within the cluster can exchange with freely diffusing molecules. Nanodomains in which membrane proteins are sorted are stabilized by cholesterol; they indeed favor molecular interactions. Signaling molecules can regulate cell functioning by modulating protein translocation, recycling proteins to and from the plasmalemma. The plasmalemmal proteins are important for communication between the cytosol and the cell surface on the one hand, the cell and the adjoining cells or the extracellular matrix on the other hand. The membrane is bound to the cell skeleton proteins (actin, myosin, fodrin, supervillin[5], etc.).

The cell organelles are membrane-bounded compartments with given structures and functions. The composition of a given organelle can vary between different cells. Lipid bilayers form a barrier between organelle lumens

[3] Membranes ensure compartmentation of the cell and its organelles and prevent free crossing by ions and molecules.

[4] The hydrophobic chains are directed toward the cell and hydrophilic heads outward.

[5] Supervillin is associated with membrane rafts.

and the cytoplasm. These bilayers contain many transmembrane proteins, which interact with the cytoskeleton and signaling pathways. Organelles are composed of resident and transient molecules for given functions. The composition of organelles thus changes with time. Chemical elements are delivered to and transported from the organelles. The trafficking is mediated by components of the cytoskeleton and its associated compounds.

The cell organelles synthesize and degrade molecules, export manufactured substances and import nutrients, using transport vesicles. Accurate identification of target organelles requires activated GTPases[6] (Sect. 4.5.3) and label substances, such as phosphoinositides[7] (Sect. 2.1). Proteins involved in transport include: (1) coat proteins, which generate transport vesicles; (2) motor proteins, which move vesicles and organelles along the cytoskeleton within the cytosol; and (3) tethering proteins, which attach the vesicles to destination organelles before fusion.

Post-translational modifications[8] of proteins, reversible or not, regulate their function and structure. Protein activity, stability, location, and molecular interactions can then be modulated. The glycosylation of plasmalemmal proteins provides a means for recognition and communication between cells.

1.1.2 Cell Nucleus

The nucleus is bound by a membrane, which is connected to the cytoplasm by pores. The outer nuclear membrane is contiguous with the endoplasmic reticulum. The nucleus has a nucleolus, which produces ribosomal RNA (rRNA), and a nucleoplasm, with active euchromatine and dense inactive heterochromatine, composed of DNA, histones, and acidic nuclear proteins. Heterochromatin, generally, marks regions of silenced genes (centromeres, inactive X chromosome, telomeres, silenced genes among active genes), whereas euchromatin defines regions of active genes. Heterochromatin corresponding to chro-

[6] Rab and Arf GTPases yield organelle identity. Both Rabs and Arfs are located in specific organelles. The GTP-bound active form is associated with membranes, the GDP-bound inactive form with the cytosol. Proteins (GDP-dissociation inhibitor displacement factors, guanine nucleotide exchange factors), which regulate GDP/GTP cycle of Rab GTPases, are selection factors rather than linkers between the membrane lipid anchor and the Rab GTPase. Arf restricted distribution is also controlled by their guanine nucleotide exchange factors.

[7] PI(3)P belongs to early endosomes. PI(3,5)P2 is found in late endsomes [9]. PI(4)P is present in Golgi complex and plasmalemma. PI(4,5)P2 is a landmark for proteins targeting the plasmalemma. Diacylglycerol recruits protein kinases C at the plasma membrane. Phosphatidylserine (PSer) is implicated in protein recruitment to the plasma membrane.

[8] Post-translational site-specific modifications include conformational changes, phosphorylation either at a specific amino acid or at a small number of amino acids in a given protein, glycosylation, acylation, ubiquitination, redox processes, etc.

mosomal regions such as centromeres and telomeres, remain condensed whatever the cell life phase. Heterochromatin formation has a biochemical background at specific chromosomal regions, such as centromeres, involving the RNA interference pathway.

Histones participate in DNA packaging into chromatin. The nucleosome is composed of histone proteins (H2A, H2B, H3, and H4), the so-called core histones, which wrap DNA around them. The linker histone H1 bind to DNA in the nucleosome and linker DNA (DNA between each nucleosome), thereby favoring the compaction of nucleosomes into chromatin structures. Histones regulate not only the gene expression, but also DNA replication and repair.[9]

Long (few-meter) DNA is compacted into the nucleus. Numerous DNA damages occur per day. Genome damage and transcriptional noise increase during aging.[10] Chromosome breakage is associated with meiosis and DNA replication, as well as viral infection, cellular transformation, and recombination for adaptive immunity. Many factors cause mutations. Genetic damage is very dangerous to cells. Immediate repair is thus needed. DNA and chromatine remodeling allows structure repair and function restoration.

The maintenance of the genome requires three phenomena: damage sensing, cell-cycle regulation, and DNA repair. DNA damage sensors recruit specific kinases to the sites of damage. The major involved kinases include ataxia telangiectasia mutated kinase (ATMK) and ATM and Rad3-related kinase (ATRK). ATM and ATR phosphorylate components of the DNA damage response network, leading to the formation of protein complexes.[11]

The genes associated with the vasculature include two main sets: (1) the genes involved in cell signaling, and (2) the genes regulating the vascular structure via the connected cell and extracellular components (cell cytoskeleton, cell membrane, and extracellular matrix) [12].

1.1.2.1 Histone Acetyltransferases and Deacetylases

The histone code regulates gene expression via two families of enzymes. Histone acetyltransferases (HAT) relax chromatin, thereby facilitating DNA-binding transcription factor accessibility to DNA, whereas histone deacetylases (HDAC), generally antagonists of HATs, act in gene repression. Histone

[9] Histones are subject to post-translational modification (methylation, citrullination, acetylation, phosphorylation, sumoylation, ubiquitination, and ADP-ribosylation), which affects their interaction with DNA and nuclear proteins, and thus, their activity in gene regulation.

[10] Although the gene expression levels vary among cardiomyocytes of young heart, variation in transcript levels are much more pronounced in cardiomyocytes of old heart [10].

[11] Among these complexes, complexes that contain the tumor suppressor breast cancer BRCA1 and ubiquitin binding protein RAP80 form a complex at sites of DNA damage. These complexes bind either Abraxas, or DNA repair protein BACH1, or tumor suppressor protein CtIP [11].

deacetylases are classified as type 1 (widely expressed) or type 2 (tissue specific). In fact, the transcription repressors histone deacetylases include several sets: HDAC-I set (HDAC1, HDAC2, HDAC3, HDAC8), HDAC-II set (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9), and HDAC-III set (sirtuins; Sect. 1.2.1).

HDAC activity is mediated via transcription factor myocyte enhancer factor 2 (MEF2). Histone deacetylase 7 inactivation causes defaults in blood vessel patterning with loss of tight junctions between endothelial cells. Histone deacetylase HDAC7 represses MEF2 transcriptional activity and decreases matrix metalloprotease MMP10 activity, thus inhibiting degradation of proteins involved in vessel integrity [13]. Moreover, HDAC7 increases expression of tissue inhibitor of metalloproteinase TIMP1.

Inactivation of histone deacetylases HDAC5 and HDAC9 predisposes to cardiac hypertrophy. HDAC-II set members repress cardiac hypertrophy via gene expression, which depends on myocyte enhancer factor-2. HDAC-I set members repress antihypertrophic pathways. Histone deacetylase-2 allows reactivation of a fetal gene program, which leads to cardiac hypertrophy. In contrast, upregulation of the gene encoding inositol polyphosphate(5)phosphatase-f (which degrades PIP3) yields activation of glycogen synthase kinase GSK3β and reduces cardiac hypertrophy [14].

Nuclear serine-threonine homeodomain-interacting protein kinase-2 forms a complex with transcription factor AML1 and histone acetyltransferase p300. It phosphorylates (activates) AML1, which subsequently phosphorylates p300[12] to stimulate transcription and activities of histone acetyltransferases [15]. Deficits in p300 and AML1 induce defects in hematopoiesis, vasculogenesis, and angiogenesis.

1.1.2.2 Nuclear Filaments

Nuclear actin participates in the control of gene expression, especially in the regulation of coactivator MAL of the transcription factor serum response factor (SRF). Cytoplasmic MAL moves in the nucleus and binds monomeric G-actin [16]. Actin binding to MAL in the nucleus leads to MAL nuclear export. MAL dissociation from actin, G-actin depletion favor SRF activation by nuclear MAL.

The inner and outer nucleus membranes are related to a network of intermediate filaments including *lamins*[13] and lamin-associated proteins (emerin, lamina-associated polypeptides LAP1 and LAP2, and lamin receptor) and

[12] Modifications of histones (acetylation, phosphorylation, methylation, ubiquitination, and sumoylation) are required for the regulation of gene expression. Histone acetylation is regulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). Histone acetyltransferase p300 and related CREB-binding protein are co-activators of transcription factors.

[13] Lamin-A mutations can cause cardiomyopathy. Lamins-B are expressed during all embryonic stages; lamins-A only during differentiation.

with the endoplasmic reticulum, respectively. *Nesprins* are nuclear membrane-associated proteins that bind emerin and lamin [17]. Nesprins can be involved in cellular compartmentation of organelles, particularly in smooth muscle cells and in cardiomyocytes.

1.1.2.3 Nucleolus

The nucleolus is a dynamic nuclear compartment that is implicated not only in the genesis of ribosome subunits, but also in the cell cycle, in stress responses and in the production of many ribonucleoproteins (RNP). The nucleolus is composed of different regions: fibrillar center, and dense fibrillar and granular components [18].

The nucleolus disassembles during the cell division and reassembles after cell division. Moreover, various proteins associate with the nucleolus at different stages of the cell cycle, hence, regulating the cell-cycle evolution. Post-translational modifications (sumoylation, desumoylation, phosphorylation, and dephosphorylation) occurring throughout the cell cycle are regulated by the nucleolus. The nucleolus also sequesters proteins, such as telomerase reverse transcriptase by nucleolin, to regulate the cell cycle [18]. In cells subjected to stress (DNA damage, heat shock, or hypoxia), tumor suppressor p53 (Sect. 4.8.4) accumulates owing to the action with nucleolar p14ARF, and inhibits cyclin-D1–CDK4 and cyclin-E1–CDK2 via p21, causing cell-cycle arrest.[14]

The nucleolus responds to changes in metabolic activity by modifying the rate of ribosome production. The nucleolus contains ribosomal gene clusters. The 47S ribosomal RNA precursor is transcribed by RNA polymerase-1. This single precursor of mature 28S, 18S, and 5.8S ribosomal RNAs is cleaved and post-transcriptionally modified via interaction with small nucleolar ribonucleoproteins (snoRNP) and with other processing factors. Finally, it is assembled with many ribosomal proteins into ribosome subunits. The 5.8S and 28S rRNAs assemble with 5S rRNA to form the 60S subunit, whereas 18S rRNA assembles into the 40S ribosome subunit. The ribosome subunits are exported into the cytoplasma.

1.1.3 Endoplasmic Reticulum

The endoplasmic reticulum and the Golgi complex correspond to cell compartments of the first two stages in protein secretion. The endoplasmic reticulum (ER), is made of interconnected convoluted tubules and flattened sacs, which extends throughout the cytoplasm. The lumen (cisternal space) of central elements is connected to the nucleus. It produces several substances. It serves as

[14] HDM2 E3 ubiquitin ligase specific to p53 reduces p53 concentration due to degradation of ubiquitin-conjugated p53 in the cytoplasm. Nucleolar p14ARF links HDM2 and inhibits p53 ubiquitination and subsequent degradation.

a transport channel and a reservoir for certain chemical species. The endoplasmic reticulum then manufactures, stores, carries and releases materials. The two categories of endoplasmic reticula include the smooth and rough types, whether ribosomes cover its surface or not. The *rough endoplasmic reticulum* is the site of protein synthesis,[15] either resident or to be released by the smooth endoplasmic reticulum using vesicles for intracellular use or export from the cell. The *smooth endoplasmic reticulum* synthesizes lipids. The endoplasmic reticulum produces most of the lipids required for the generation of new segments of the cell membrane. These lipids include cholesterol, phosphatidylcholine, and phosphatidic acid. The latter is produced by the cleavage of phosphatidylcholine by the phospholipase D1 (PLD1). The endoplasmic reticulum sequesters calcium from the cytosol, especially in muscle cells, which have an abundant smooth endoplasmic reticulum, the *sarcoplasmic reticulum* (SR; Sect. 7.7). The mRNAs of proteins are translated at the endoplasmic reticulum. The exocytosis from the endoplasmic reticulum to the Golgi apparatus is mediated by the tubular–vesicular *endoplasmic reticululm–Golgi intermediate compartments* (ERGIC), smooth areas of the rough endoplasmic reticulum.

Among proteins of rough and smooth endoplasmic reticulum with a known function, most are implicated in protein synthesis, folding, and modification, whereas others interact with the actin cytoskeleton or act in metabolism [19].

Once malfolded polypeptides have been discovered in the lumen of the endoplasmic reticulum, a proteolysis occurs in the cytosol, which ensures the secretion of correctly folded proteins. This degradation requires the ubiquitin–proteasome system.

1.1.4 Golgi Apparatus

The Golgi apparatus, discovered by C. Golgi in 1898, processes newly synthesized proteins destined for secretion from the cell. These proteins are delivered to the cis face of the Golgi apparatus and exit from the trans face.

The Golgi apparatus is made of elongated layered cisternae (set of flattened sacs leading to pancakelike space stack) with vesicles. The Golgi body temporarily stores proteins. It also processes various newly synthesized molecules (proteins, and lipids) from the rough endoplasmic reticulum. It then packages them in vesicles, carried toward suitable organelles or the plasmalemma, where the content can be exported outside the cell. Hence, it selects the destination of the modified molecules (sorting according to the functioning site of the substance). In particular, the Golgi apparatus is the site of synthesis of glycosaminoglycans for the extracellular matrix. The Golgi apparatus can operate as a calcium store [20].

The entry area of the Golgi apparatus, the *cis Golgi reticulum*, follows ERGIC. The Golgi stack, the main processing zone, is composed of several

[15] Cytoplasmic proteins are synthesized on free ribosomes. The ribosomes bind to the endoplasmic reticulum to produce glycoproteins.

cisternae. Post-translational modifications, such as phosphorylation (Fig. 1.2), are aimed at regulating cell processes. The *trans-Golgi network* (TGN) is located in the vicinity of the nucleus. The trans-Golgi network is connected to the cisternae. Final reactions and sorting take place in the trans-Golgi network.

1.1.5 Mitochondria

The mitochondrion has two membranes, an outer and inner, with infoldings to increase the reaction surface area. Mitochondrial activity depends on mitochondrium number and working efficiency. This activity varies according to cell type.

Mitochondria (20–1000 per cell) are responsible for cell respiration, using delivered oxygen (mitochondria function in oxidative phosphorylation; Sect. 1.4). Oxidative phosphorylation is the process of ATP synthesis due to an electron transfer from nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) to oxygen by a set of electron carriers. The proteic complexes are located at the inner mitochondrial membrane. Oxidation and phosphorylation are coupled by a proton level difference across the inner mitochondrial membrane.

Mitochondria not only produce adenosine triphosphate,[16] but also regulate cell apoptosis and synthesize metabolites and reactive oxygen species. Mitochondria also act as buffers of cytosolic Ca^{++} concentration.

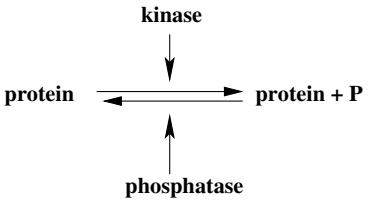

Figure 1.2. Reversible protein phosphorylation is aimed at controlling protein activities. Phosphates are added by kinases either on serine (Ser), threonine (Thr), or tyrosine (Tyr) of the protein from ATP to switch protein activity on or off. Phosphates are removed by phosphatases.

[16] Mitochondria store the energy in the form of ATP, which is used to power cell functioning, from protein synthesis to muscle contraction. ATP is generated when charged protons are transferred. In mitochondria, flavin mononucleotide is a primary electron acceptor within respiratory chain of the membrane. A set of iron–sulfur clusters transport electrons from dihydronicotinamide adenine dinucleotide (NADH) into the hydrophobic proton-pumping domain of the complex, avoiding generation of deleterious reactive oxygen species.

Mitochondria contain their own genome (mtDNA). However, mitochondrial proteins are mostly encoded in the nucleus.[17] Mitochondrial genes can be damaged by free radicals generated during energy production. Ribonucleotide reductase catalyzes the synthesis of deoxyribonucleotides from corresponding ribonucleotides.[18]

Most mitochondrial proteins are synthesized as preproteins in the cytosol and must be imported across outer and inner mitochondrial membranes. Proteins are selectively transported across the mitochondrial inner membrane through the translocase of the inner membrane Tim23, such which the electrochemical proton gradient, which drives adenosine triphosphate synthesis, is conserved.[19] Protein Tim50 maintains the membrane potential. Tim50 induces a rapid closing of Tim23 translocation channel [21]. Mitochondrial preproteins release Tim50-mediated inhibition to be translocated.

The heating system associated with brown adipose tissues is due to mitochondrial proton gradients that are uncoupled from ATP production, the chemical energy being converted directly into heat under certain environmental circumstances. Uncoupling protein-1 is required for thermogenesis.

1.1.6 Cytoskeleton

The cytoskeleton is composed of networks of various types of filaments, of associated motor proteins, and regulatory molecules (Sect. 1.5). It is particularly involved in substance transport within the cytosol, cell adaptation to its environment, cell motility and adhesion (Sect. 1.5). The *centrosomes* function as microtubule-organizing centers and spindle poles during cell division.

1.1.7 Vesicles

Vesicles and vacuoles transport molecules. Certain vesicles carry proteins and lipids between the endoplasmic reticulum and the Golgi complex. *Exosomes* are vesicles secreted into the extracellular medium after fusion of *endosomes* with the plasma membrane. They act in intercellular signaling. *Phagosomes* internalize large macromolecular complexes, especially pathogens, which are

[17] Heme, part of the oxygen-carrying hemoglobin, is made by mitochondria in bone marrow cells.

[18] Ribonucleotide reductase is made of two subunits: R1 and R2. The homolog of R2 subunit of ribonucleotide reductase p53R2 is generated by the tumor suppressor p53. In the S-phase of the cell cycle, most deoxyribonucleotides are synthesized by R1/R2 ribonucleotide reductase. However, in a nonproliferating cell, deoxyribonucleotides are produced by R1/p53R2 ribonucleotide reductase. p53R2 is involved in mitochondrial DNA synthesis as well as DNA repair.

[19] Tim23 complex contains the pore-forming protein Tim23 and three membrane proteins Tim17, Tim21, and Tim50. Tim23 has a transmembrane domain and a domain in the intermembrane space.

bound to plasmalemmal receptors for degradation. *Lysosomes* are degradative elements of the endocytosis. They digest targeted substances. They indeed contain many different hydrolytic enzymes and signaling proteins. Proteins of the endoplasmic reticulum and membrane rafts are observed in the lysosomal membrane. *Peroxisomes* are specialized for oxidative reactions, producing hydrogen peroxide. They are implicated in lipid metabolism (plasmalogen synthesis). They are linked to the actin cytoskeleton. *Macropinosomes* lead to massive membrane internalization from the cell surface. Clathrin-coated vesicles and calveolae are used in the endocytosis of various substances, such as signaling receptors, membrane pumps, and nutrients. Their formation at the Golgi network or the plasma membrane involves adaptor proteins 1 and 2.

Lipid droplets form cell dynamic stores of fatty acids and sterols. They consist of a core of neutral lipids, predominantly triacylglycerols or cholesteryl esters, surrounded by a monolayer of phospholipids and associated proteins (Rab and ADP-ribosylation factor GTPases, caveolins, phospholipase-D, and perilipin) [22]. The neutral lipids stored in lipid droplets are used for metabolism, for membrane and steroid synthesis, especially in adipocytes and steroidogenic cells.

1.1.8 Ribosomes

The ribosomes (15–20 nm in caliber) must be considered as *ribozymes* rather than organelles, being multimolecular complexes ($\sim$65% ribosomal RNA, $\sim$35% ribonucleoproteins) without membrane. The ribosomes disassemble into subunits when they do not synthesize protein. They are assembled in the nucleolus. They are the sites of messenger RNA translation, to manufacture proteins using amino acids delivered by transfer RNA (Sect. 1.2.3). The ribosomes are either free in the cytoplasm, producing intracellular proteins, or attached to the membrane of the rough endoplasmic reticulum, synthesizing export proteins.

1.2 Cell Fate

In the early embryo, cells decide between somatic fate or germ line. During maturation, germ cells undergo a transition from cell division to meiosis to differentiate as sperm or egg. Piwi-interacting RNAs (piRNAs), small noncoding RNAs bound by the Piwi proteins of Argonaute protein set, MIWI and MILI, repress transposon activity [23]. piRNAs participates in germ cell development.

In general, cell fate includes cell survival, division, proliferation, possible differentiation, self-renewal for stem cells, autophagy, apoptosis and death, as well as cell adhesion and motility (Chaps. 2 and 5). Cell fate depends on availability of nutrients and energetics, as well as environmental factors. Cells must respond specifically to different environmental stimuli in order to survive. The

pathways involved in sensing the stimuli and leading to signal transduction often share the same or homologous proteins (Chap. 4). Both simultaneous and temporally ordered cues achieve specificity using coordinated pathways that are connected and strictly controlled, avoiding useless cross-talk by inhibition at the appropriate pathway level.

Homeostasis without tissue growth implies that cell duplication is compensated by elimination of another cell. Pathologies are often associated with excessive or deficient cell death. Cell loss occurs acutely in infarction, and progressively in degenerative diseases. There are several cell death modes, either programmed or accidental, although biochemical cascades are triggered by cell stresses whatever the mode [24]. Morphological, biochemical, or immunological data alone cannot accurately define the cell death type. (1) Apoptosis is characterized by caspase activation and degradation by surrounding cells. (2) Massive autophagic vacuolization can induce cell death. (3) Cell necrosis, currently defined by ruptures of the plasmalemma that trigger inflammation, particularly involves calpains and cathepsins. (4) Mitotic catastrophe occurring during or shortly after a failed cell division results from a combination of deficient cell-cycle checkpoints and cellular damages, which activates self-destruction. Permeabilization of both mitochondrial membranes usually corresponds to the point of no-return in the cascade of events leading to cell death.

Cell adhesion and migration are regulated by coordinated activities of the cell cytoskeleton and junctions with adjoining cells and the extracellular matrix. Cell junctions are made of adhesion molecules (Chap. 2) and associated proteins, such as scaffolds, small GTPases, and enzymes (kinases, phosphatases, and proteases; Chap. 3 and 4). Structural proteins (vinculin, talin, and actinin) link the cell environment to the cytoskeleton. Signaling molecules (focal adhesion kinase, or paxillin) transduce the stimuli from the neighborhood to cellular effectors for organization of the cell adhesions and cytoskeleton. Many regulators of cell adhesions also control cell survival, growth, proliferation, and differentiation. Transport along microtubules is required for cell life and during cell division. Besides, vesicle transport is needed for the intracellular displacements of proteins, lipids, and other molecules between cell compartments. Myosin and its binding partners link the cytoskeleton to coated and uncoated vesicles.

Adipocytes regulate energy and nutrient homeostasis. Adipocytes secrete various adipokines (App. A.2). Adipocytes also store lipids for energy consumption. Adipocytes release fatty acids into the blood circulation, which are used when glucose level is low. Fatty acids generated from triacylglycerols contain more energy per unit mass than glycogen. Adipose depots, the so-called visceral fat, surround the heart and large vessels, among other locations. These depots are made of brown adipocytes in infancy. Brown adipocytes disappear

in adults, scattered brown adipocytes remaining within white fat pads.[20] Fat pads are innervated by sympathetic nervous fibers. The sympathetic activation elicits lipolysis.

Glucose homeostasis is due to concerted actions of several organs, the coordination being determined by the central nervous system. Pancreatic βcells (App. A) secrete insulin in response to elevated glucose level in the plasma. Insulin promotes glucose uptake by all cell types, particularly by adipocytes and by myocytes, and prevents glycogenolysis and gluconeogenesis in the liver. Conversely, low glucose concentrations lead to small insulin levels combined with elevated levels in antagonist hormones (glucagon, adrenaline, and corticosteroids) promote glucose production in the liver.

Cell internal state is kept stable within tolerable limits owing to integration of different cues and multiple adjustments (homeostasis). Mitogen-activated protein kinase pathways leading to ERK1 and ERK2 formation (Sect. 4.3.2.4), which transduce signals from the extracellular medium, are implicated in cell fate. During cell rest, unphosphorylated ERK1/2 interact with cytosolic anchors, thus remaining in the cytoplasm. Once stimulated, ERK1/2 relocalize in different cellular compartments, including the nucleus. Anchoring proteins can sequester ERK1/2 in these new sites. Retention of either unphosphorylated or phosphorylated ERK1/2 can then occur. MicroRNAs also affect the cell fate, for example, by modulating Hedgehog signaling (Sect. 3.2.7).

Cells must maintain ATP concentration at suitable levels for its energy-requiring functioning. ATP is mostly synthesized in mitochondria, which adapt the oxidative phosphorylation to the demand and according to external stimuli. Mitochondria induce a retrograde signaling that acts on nuclear gene expression, as well as on mitochondrial number and function. Members of the nuclear receptor superfamily relay signals to the genes, in association with certain inducible transcriptional coactivators to fit the energy demands. Members of the peroxisome proliferator-activated receptor-coactivator PPARγ family, PGC1α and PGC1β, and PGC1-related coactivator (PRC) serve in the regulation of mitochondrial activity and cellular energy metabolism [25].

A decay in ATP synthesis triggers an increased gene expression of oxidative phosphorylation and mitochondrial expression of PPAR1α to recover appropriate levels. The compensatory mechanism in response to reduced mitochondrial oxidative phosphorylation occurs owing to a burst of intracellular calcium followed by an AMPK activation and an increase in cAMP-response element binding (CREB) and transducer of regulated CREB (TORC) proteins, which promotes the production of the transcriptional coactivator PGC1α [26].

PPARγ coactivator-1α participates in mitochondrial genesis when new mitochondria are needed by the cell, and increases the energy substrate uptake.[21] PGC1α and PGC1β protect the cell against oxidative damages [27].

[20] White adipose tissues also contain endothelial cells, fibroblasts, leukocytes, and macrophages.

[21] PPARγ coactivator-1 interacts with several nuclear receptors, such as peroxisome proliferator-activated receptors PPARγ, PPARα, PPARβ, estrogen receptor-

1.2.1 Sirtuins

Sirtuins and related substances, which can coordinate metabolism with diet, especially during dietary restriction and excess, might also be involved in stress management and aging. Sirtuins Sirt1, Sirt6, and Sirt7 are nuclear sirtuins. Sirt7 is the single sirtuin located in nucleoli in association with RNA polymerase-1.

Sirtuin Sirt1 deacetylates certain transcription factors, including p53, forkhead subgroup O proteins (FoxO; Sect. 4.8.3) and DNA repair factor KU, thereby increasing the stress resistance of cells [28]. In white adipose tissue, Sirt1 inhibits adipogenesis in precursor cells and reduces fat storage in differentiated cells. It inhibits nuclear receptor peroxisome proliferator-activated receptor-γ. Sirt1 stimulates insulin secretion. In pancreatic βcells, Sirt1 represses the uncoupling protein gene UcP2, thus increases ATP synthesis and insulin secretion in response to glucose. Sirt1 also protects β-cells against oxidative stress by deacetylation and increased activity of the forkhead protein FoxO1. In the liver, Sirt1 deacetylases (activates) the PPARγ coactivator PGC1α, hence increasing the gene expression for gluconeogenesis.

Amino acids can serve as carbon and energy sources in periods of energy deprivation. Sirtuins Sirt3 and Sirt4 are imported into the mitochondrial matrix. Sirt3 deacetylates acetyl-coenzyme-A synthetase-2. Sirt4 transfer ADP-ribose to glutamate dehydrogenase, which converts glutamate to α - ketoglutarate. Consequently, Sirt3 promotes the use of acetate, whereas Sirt4 represses the use of glutamate and glutamine into metabolisms.

Fat metabolism and thermogenesis arre stimulated in brown fat cells by peroxisome proliferator-activated receptor-γ coactivators PGC1α and PGC1β [28]. In slow twitch myocytes, which exclusively use oxidative metabolism for energy production, PGC1α stimulates the gene transcription for mitochondrial proteins by binding to transcription factors, such as nuclear respiratory factors-1 and 2 and estrogen-related receptors. PGC1α also activates fatty acid oxidation by binding to PPARα and PPARδ. In the liver, PGC1α activates

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related receptor ERRα, nuclear respiratory factor NRF1 and NRF2, as well as with the thyroid hormone receptor, the glucocorticoid receptor, estrogen receptor, retinoid receptors, farnesyl X receptor, pregnane X receptor, hepatic nuclear factor-4, liver X receptor, and non-nuclear receptors, such as myocyte enhancer factor MEF2, forkhead transcription factor-1, and Sry-related HMG box-9 [25]. Nuclear respiratory factors regulate the expression of mitochondrial transcription factor A acting on mitochondrial DNA, as well as the activity of nuclear genes encoding respiratory chain subunits and proteins of the mitochondrial function. To activate the transcription, PGC1α forms complexes with transcription factors and transcription regulators, such as proteins with histone acetyltransferase activity (steroid receptor coactivator-1, CREB-binding protein/p300), the chromatin remodeling increasing access of the transcription regulators, or such as activating complexes directly interacting with the transcription initiators (thyroid hormone receptor-associated protein/vitamin D receptor-interacting protein coactivator complex).

both fatty acid oxidation and gluconeogenesis by binding to transcription factors FoxO1 and hepatocyte nuclear factor-4α. In this organ, PGC1β, coactivator for the forkhead protein FoxA2, activates cholesterol synthesis and export to the blood by binding to the lipogenic transcription factors sterol regulatory element binding protein (SREBP) and liver X receptor (LXR).

1.2.2 Cell Growth

Cell growth means either cell reproduction or increase in cell size. Cells such as cardiomyocytes can increase in size because they are subjected to adaptation stimuli. Growth factors (Sect. 10.1) act via several effectors, such as phosphatidylinositol 3-kinase (PI3K; Sect. 4.2.2), phosphoinositide-dependent kinase-1 (PDK1), and protein kinase-B (PKB). Insulin signaling in the cardiovascular system involves two pathways: (1) the phosphatidylinositol 3-kinase pathway; and (2) the growth factor-like pathway with mitogen-activated protein kinase (Sect. 4.3.2.4). The mitogen-activated protein kinase signaling network regulates cell fate by transducing multiple growth-factor signals.

Phosphatidylinositol 3-kinase (PI3K) mediates increased uptake of glucose and amino acids required for cell development. PI3K family includes four sets (Sect. 4.2.2). PI3K set 4 includes related enzymes, such as mammalian target of rapamycin (mTOR). Mammalian target of rapamycin (mTOR), regulates cell growth (as well as cell proliferation and cell motility) [29]. mTOR integrates multiple cues from hormones (insulin), growth factors (IGF1/2), and mitogens. The PI3K/PKB pathway activates mTOR. mTOR also senses nutrient and energy levels (Fig. 1.3). The nutrient/energy/redox sensor mTOR complex 1 (mTORc1), composed of mTOR, regulatory associated protein of mTOR (raptor), among other components, controls protein synthesis and targets S6 kinase S6K1, which regulates cell growth [30]. Eukaryotic translation initiation factor eIF4E binding protein-1 (4E-BP1) is another effector of mTORc1 [29].[22] Phosphorylated 4E-BP1 is released from eIF4E. eIF4E then can bind mRNAs for recruitment to the ribosomal initiation complex and initiation of translation with initiation factor eIF4B, leading to protein synthesis. mTOR also stimulates transcription of rRNA genes by RNA polymerase-1, ribosomal protein genes by RNA polymerase-2, and tRNA genes by RNA polymerase-3. Hamartin TSC1 and tuberin TSC2 of the tumor suppressor gene syndrome tuberous sclerosis complex (TSC) stimulate mTOR via inhibition of GTPase Ras homolog enriched in brain (Rheb; Sect. 4.5.3).[23]

[22] The eukaryotic translation initiation factor eIF4E forms a complex eIF4F with scaffold proteins eIF4G1 and eIF4G2 and RNA helicase eIF4A, and binds to mRNA.

[23] Tuberous sclerosis complex is a genetic disorder caused by mutations in one of two tumor suppressor genes, TSC1 and TSC2. TSC genes are, in particular, involved in cell-cycle regulation and cell size control. The activity of TSC1 and TSC2 depends on phosphoinositide 3-kinase and protein kinase-B.

Rheb binds TSC2, acting as a GAP for Rheb and preventing Rheb activation of mTOR. TSC2 is phosphorylated (inactivated) by PKB and (activated) by AMPK. AMPK impedes mTOR. mTOR complex 2 (mTORc2), made of mTOR, rapamycin-insenstivie companion of mTOR (rictor), among other components, stimulates F-actin stress fibers, paxillin, and Rho GTPases. Rictor-mTOR complex also phosphorylates PKB and facilitates PKB phosphorylation by PDK1 [31].

1.2.3 Protein Synthesis

Proteins participate in cell life. They are required for cell structure and cell adhesion, as well as for cell mechanical and chemical functions. They intervene, at least as enzymes, in all metabolism. Proteins act in cell signaling and immune defense. They are implicated in the cell cycle.

Deoxyribonucleic acid contains the genetic code for cell development and functions.[24] The genetic code is a set of codons (three-nucleotide sets), with

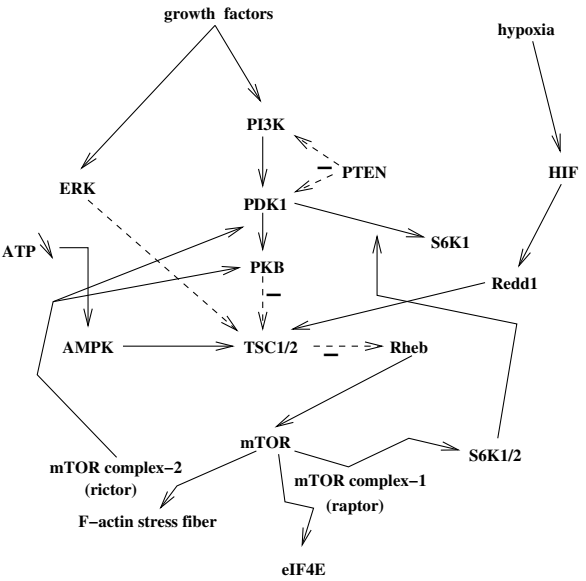

Figure 1.3. PI3K–PKB pathway activates mTOR, which can form two complexes, mTOR complex 1 with raptor, and mTOR complex 2 with rictor. mTOR complex 1 leads to activation of eukaryotic translation initiation factor (eIF4E) and S6 Kinase S6K1. mTOR complex 2 stimulates F-actin stress fibers. It phosphorylates PKB and facilitates PKB phosphorylation by PDK1. TSC: GTPase tumor suppressor gene syndrome tuberous sclerosis complex (Source: [31]).

[24] The genes are DNA segments with genetic instructions. The base sequence of a gene determines the messenger RNA composition, hence the corresponding protein composition. Other DNA sequences have structural or regulatory roles.

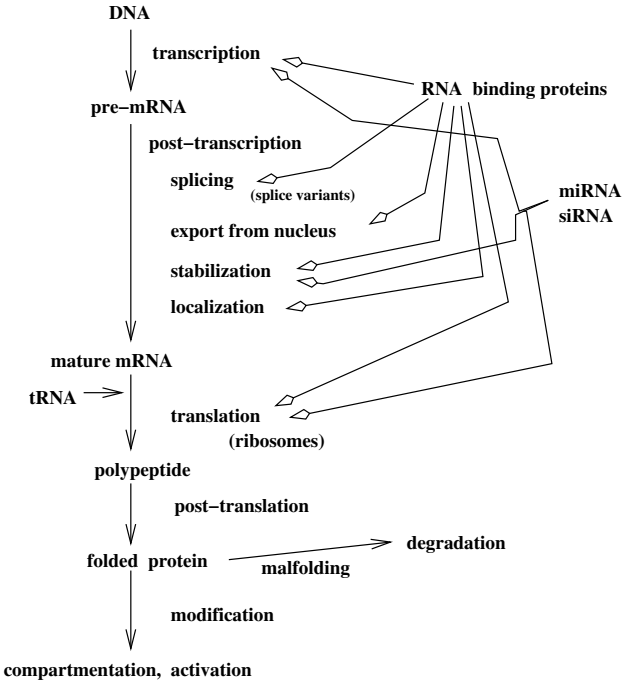

Figure 1.4. Main stages of the protein synthesis: transcription, post-transcription, translation, post-translation, and modifications. The genetic instructions are transcribed in the nucleus from DNA to pre-messenger single-stranded ribonucleic acid (mRNA) and conveyed into the cytoplasma by mRNA complementary coding sequences. Long pre-mRNA are cut into much shorter mature mRNAs. The genetic information is translated from RNA into protein using transfer RNA (tRNA) as an adaptor between mRNA and the amino acid. The exons, mRNA sequences, can be distributed discontinuously in the genome. Exons can be trimmed from primary transcripts and spliced (thus generating different mRNAs and leading to various proteins emanating from the primary transcript), while introns are degraded.

mapping between codons and amino acids, as some amino acids are mapped by more than one codon (redundancy). Proteins are assembled from amino acids using information encoded in genes. Main stages of the protein synthesis include transcription, post-transcription, translation, post-translation, and modifications (Fig. 1.4).

The genetic code in DNA is transcribed by RNA polymerase-2 (after binding to the promoter [initiation], running along the strand [elongation], and termination to avoid degradation) into premessenger RNA (transcription). Pre-messenger RNA is processed to form mature messenger RNA (post-transcription), translocated into the cytosol to become a template for protein synthesis in the ribosome (translation, also with three steps: initiation starting with fixation of tRNA and mRNA on specific ribosomal site, elongation

and termination when stopping codon is detected). The messenger RNA is linked to the ribosome and each codon is read and matched by anticodon of transfer RNA, which carries the corresponding amino acid. Post-translational modification and protein folding follow the synthesis.

Gene regulation occurs at multiple stages (Table 1.4). Pre-transcriptional regulation depends on cell signaling. Transcriptional regulation is associated with peculiar DNA sites. Post-transcriptional regulation is due to miRNAs, mRNA splicing, polyadenylation and localization, chromatin modifications, and mechanisms of protein localization, modification and degradation. Cytoplasmic aggresomes correspond to a cell response to misfolded proteins. Aggresome formation is associated with the redistribution of cytoskeleton proteins and the recruitment of proteasomes.

Promoters regulate DNA transcription. Augmenters and silencers enhance and impede gene transcription, respectively. Augmenter and silencer sorting is specific to each gene. Distinct combinations of activators and repressors control the transcription of different genes. Coactivator–activator complexes

Table 1.4. Gene regulation and protein modifications. Regulatory sites are often located upstream from protein-coding genes. Transcription factors control transcriptional regulation by binding to cis-regulatory sites. Post-transcriptional regulation is mediated by miRNAs, also by binding to cis-regulatory sites. Other stages of gene regulation include cell signaling, mRNA splicing, polyadenylation and localization, chromatin modifications, and mechanisms of protein localization, modification, and degradation. Protein modifications, such as myristoylation, palmitoylation, farnesylation, acetylation, and phosphorylation, assist in localizing the enzyme to various cell compartments. Except for phosphorylation, these changes do not significantly affect the protein activity. Ubiquitination is a reversible post-translational modification of proteins, in which ubiquitin is attached to lysine residues of target proteins. Monoubiquitination corresponds to the binding of a single ubiquitin to a protein, and multiubiquitination to the binding of a single ubiquitin to several Lys residues of a given protein. Monoubiquitination or multiubiquitination is required for endocytosis. Deubiquitination can follow monoubiquitination and allow protein activity. The protein–ubiquitin complex can attach further ubiquitins. Polyubiquitination directs the ubiquitinated protein to the proteasome. Subsequently, ubiquitin is recycled and ubiquitinated protein is degraded.

Transcriptional control	Post-transcriptional control
Transcription factors	mRNA
Chromatin state	miRNA
Cofactors	mRNA splicing
Promoters	Polyadenylation
	RNA-binding proteins
	Cell signaling
	Protein location
	Ubiquitination

bind to augmenters and repressors to silencers. Basis factors must aggregate on the minimal promoter in order to start the transcription. RNA polymerases-2 and -3 synthesize mRNA and tRNA associated with class 2 and 3 genes, respectively. Diseases occur by dysregulation of gene transcription and protein underproduction or overproduction.

Double-stranded RNAs (dsRNA) trigger suppression of gene activity in a homology-dependent manner (i.e., specifically targeting mature mRNA sequence homologous to dsRNA for degradation, other mRNAs being unaffected), the so-called RNA interference effect (RNAi). In contrast, antisense suppression of gene expression does not degrade mRNA. Single-stranded RNA fragments bind to mRNA, then block translation. RNA interference is a process can be used as a protective mechanism to keep transposons (mobile elements of the genome) silent and to prevent the production of defective proteins, as well as possibly in resistance to viral infection.[25] RNA interference also represents a technique in which exogenous, double-stranded RNAs complementary to targeted mRNAs are introduced into a cell to specifically destroy these mRNAs to reduce or abolish a given gene expression. Besides, dsRNA can spread between cells. dsRNA exerts its effect at the post-transcriptional level prior to translation. Therefore, RNA interference corresponds to post-transcriptional gene silencing. Furthermore, RNAi-like mechanisms keep chromatin condensed and suppress transcription in the vicinity of the condensed blocks of chromatin.

Both single-stranded antisense RNAs and sense RNAs either cannot cause or weakly induce gene silencing. Double stranded RNA is processed into double-stranded fragments, known as small inhibitory RNAs (20–25 nucleotides), which cleave mRNA. Small inhibitory RNAs are either called small interfering RNAs (siRNA) when they are exogenous or microRNAs (miRNA) when they are produced by RNA-coding genes within the cell. Mature endogenous miRNAs are nearly similar to siRNAs produced from exogenous dsRNA.[26] Pre-microRNAs are processed into single strand anti-sense RNA and then target mRNAs for degradation. Ribonuclease dicers produce microRNAs from their precursors and cuts long double-stranded RNAs into small double-stranded interfering RNAs.

Small RNAs contain both sense and antisense RNA sequences. Small non-coding RNA molecules can form with proteins ribonucleoprotein complexes, which affect the transcription, translation, replication, chromosome structure, and regulation of RNA processing. RNA-induced transcriptional silencing complex (RITS) is responsible for the maintenance of the genome structure and regulation of histone methylation. RNA-induced silencing complex

[25] RNA interference produces antiviral responses in plants. RNAi role in mammalian innate immunity is not well handled.

[26] Typical siRNAs have a perfect base pairing for mRNA, thus cleaving a single, specific target. miRNAs instead have incomplete base pairing to targeted RNAs, thereby inhibiting the translation of many different mRNAs with similar sequences.

(RISC) is the protein-containing complex responsible for mRNA destruction. RISCs also contain endonucleases argonautes, which cleave mRNA strands complementary to bound small inhibitory RNAs. Whereas passenger strand of small double-stranded RNA is degraded during RISC activation, a small antisense RNA (guide strand) links RISC complex to targeted mRNA strand by base-pairing. In the absence of perfect base pairing, antisense RNA blocks translation rather than triggers degradation of sense mRNA.

MicroRNAs are transcribed by RNA polymerase-2 within the nucleus and subsequently cleaved in the cytoplasm into functional RNA fragments [32]. Most of miRNA genes are found in intergenic regions or in antisense orientation to annotated genes, the majority of the remaining miRNA genes in introns. Other miRNAs are generated by intron processing of protein-coding genes. Because miRNAs form complexes that bind to specific mRNAs to inhibit synthesis of specific proteins, miRNAs modulate cell proliferation, differentiation, and apoptosis. They are required in developing timing control.

Protein activity is also regulated by cytoplasmic aggresomes, protein aggregates, which have the following features: (1) aggresomes occur either in response to unfolded or misfolded proteins or for regulation of protein functioning by quick sequestration and elimination, aggresomes containing proteasomes, ubiquitin and heat-shock proteins; (2) the aggresome formation is microtubule-dependent;[27] (3) aggresomes form in the neighborhood of the microtubule-organizing center, near the centrosome, using retrograde transport on microtubule tracks; (4) aggresome formation is associated with the collapse of intermediate filament around the aggresome; and (5) aggresome formation is accelerated by proteasomal inhibition.

The role of genotype in human diseases has been demonstrated. However, epigenetic modifications can associate alterations in gene expression leading to chronic diseases with the environment, particularly prenatal and early postnatal environmental (nutritional, chemical, and physical) factors [33]. During gestation, several coexisting generations (pregnant female, embryo, and its germ line) are exposed to environmental factors. Transmission of modified phenotypes is then possible.

Stable chromatin modifications during cell divisions can be inherited. Dynamic DNA methylation and histone post-translational modifications (Table 1.5) allow the recruitment of proteic complexes, which either activate or repress transcription. DNA methylation usually leads to transcription repression by reforming nucleosomes. Both DNA methylation and histone modifications of the promoters of tumor-suppressor genes and oncogenes are altered in cancers.

[27] The microtubule motor complex made of dynein–dynactin is thus involved in aggresome formation

Three possible targets in gene expression changes due to environmental factors include promoters, transposable elements adjacent to genes with metastable epialleles, and regulators of imprinted genes [35].[28]

1.2.4 Circadian Clock

Many physiological mechanisms (sleep-wake cycles, blood circulation, feeding, body temperature, hormonal secretions, and glucose, lipid, and mitochondrial oxidative metabolisms) follow cycles of repeated oscillations, being regulated

Table 1.5. Reversible histone post-translational modifications include acetylation, phosphorylation, methylation (mono-, di-, or trimethylation), ubiquitination, and sumoylation, as well as variant histones. Acetylation is activating, sumoylation repressing, and other modifications can have activating and repressing effects depending on targeted residues, combination of proteins, and the context (Source: [34]). Histone deacetylases, Ser/Thr phosphatases, demethylases, and ubiquitin proteases remove acetyl groups, phosphate groups, methyl groups, and ubiquitin, respectively. Deiminases alter arginine methylation, converting Arg to citrullin. Histone post-translational modifications can directly cause structural changes in chromatin, or indirectly acting via recruitment of effectors, either activators or repressors, both being able to target the same residue. Histone post-translational modifications occur during initiation, early and mature elongations, which are required for transcription activation. RNA polymerases cleave nucleosomes, histone–DNA contacts being reformed in the wake of the enzyme. Nucleosome clearance in the promoter and exchange of histone variants in the promoter and the open reading frames occur during transcription.

Agent	Recruited molecules
DNA-bound repressor	Histone deacetylases (negative modifiers)
DNA-bound activator	Histone acetylases (positive modifiers)
DNA-bound RNA polymerase	Histone methylases

[28] Metastable epialleles are loci that bear variable, reversible, epigenetical modifications leading to a phenotype distribution in genetically identical cells. The expression of imprinted genes results from only one of the two parental alleles. In mice, the paternal allele of the gene coding for insulin-like growth factor-2 is expressed, whereas the maternal allele remains silent. Insulin-like growth factor-2 receptor is expressed only by the maternal chromosome. However, both alleles yield IGF2R in humans. Imprinted genes are implicated in fetomaternal physiology. The alleles of imprinted genes differ mainly by DNA methylation, and also by chromatin conformation, histone modification, replication timing and recombination rate [35]. Aberrant imprinting disturbs development and causes various syndromes. Regulation alterations of imprinted genes, as well as their mutations, lead more easily to pathologies than when both alleles are expressed.

by the body clock, exhibiting mainly circadian rhythms that match the earth's rotation. The body clock is controlled by an interplay of numerous molecular pathways, with feedback loops leading to gene transcription and protein translation. The clock regulator consists of both transcriptional activators and repressors. Several genes are involved in the circadian rhythm, such as CLOCK, BMAL1, PER, CRY, and REV-erbα.

The hypothalamic suprachiasmatic nucleus is the master circadian clock driven by the daily light-dark cycle associated from retinal cues. It synchronizes the phase of circadian clocks in peripheral tissues. Heterodimers of circadian locomotor output cycles kaput/neuronal PAS domain protein-2 (Clock/Npas2) and brain and muscle ARNT-like-1 (Bmal1), bHLH-PAS transcription factors, regulate the circadian clock. The transcription factor Npas2 is a substitute for Clock in the suprachiasmatic circadian clock [36]. Clock and Npas2 can coordinately function and independently form heterodimers with Bmal1 in the suprachiasmatic nucleus to maintain rhythmicity.

The rhythm molecules include the major transcriptional repressors cryptochromes Cry1 and Cry2 and periods Per1 and Per2. The two transcription factors, Bmal1 and either Clock or Npas2, activate the production of Per and Cry. In turn, Per and Cry inhibit their own expression by repressing Clock/Bmal1 (negative feedback loop). Therefore, at low Per and Cry concentrations, Per and Cry are activated by a heterodimer that contains Bmal1 and Clock/Npas2.[29] Once their levels reach a threshold, Cry and Per bind to Bmal1-Clock/Npas2, subsequently canceling Bmal1-Clock/Npas2 activity. Consequently, Cry and Per transcription is reduced. Moreover, Cry forms a complex with F-box protein Fbxl3, a subunit of ubiquitin E3 ligases for degradation by proteasomes [37]. Per and Cry concentrations again reach low values due to the feedback loop and degradation; a new cycle of Cry and Per production then occurs. A mutation in Fbxl3 induces longer circadian rhythms of about 27 hours in homozygotes, delaying the Cry degradation rate and prolonging the duration of negative feedback [38].

The circadian molecular clock genes coordinate the regulation of behavior, energy balance, and metabolism. The circadian control of energy metabolism involves transcription coactivator PGC1α, which is required for glucose, lipid and energy homeostasis. PGC1α is stimulated by multiple environmental cues (temperature, nutritional status, and physical activity). PGC1α is rhythmically produced by the liver and skeletal muscles in mice [39]. PGC1α connects the activity of circadian clock genes to the regulation of energy metabolism via nuclear receptors RORα of the ROR family of orphan nuclear receptors.

1.2.5 Cell Division

When the cell does not undergo division, unraveled chromosomes are located in a more or less outer nucleus region (chromosome sorting by radial arrange-

[29] Per and Cry levels depend on phosphorylation by casein kinase CK1ε and CK1δ, and then on sumoylation.

ment). Each chromosome is, indeed, confined to a given region. The spatial organization in chromosome territory is implicated in genome stability and gene regulation [40]. The genome sequences in the various chromosome territories remain accessible to gene activators and inhibitors. The relative positioning of DNA sequences and their physical interaction can control the cell functions. The chromosome territory can move during cell differentiation. According to its activity, genome sequences can relocate from the nucleus periphery to the inner part.

DNA replication (copy of the genetic code) is required during cell division such that both daughter cells contain the parent genetic code. During cell division, the genetic material becomes visible and forms chromosomes. Each chromosome is constituted by two chromatids connected by a centromere with limbs of variable length terminated by a telomere. In chromosomes, chromatin proteins, such as histones, compact and organize DNA. DNA replication is done by multiproteic replisome, which simultaneously separates double DNA strands and produce a copy of each strand. The two DNA strands are separated. Helicases unwind the DNA double helix into single strands. Then DNA complementary to each strand is created by DNA polymerase.

The cell division cycle, or cell-cycle, includes four phases (Table 1.6): G1 (gap 1), S (synthesis), G2 (gap 2), and M (mitosis). The M phase is subdivided into two substages, actual *mitosis* (chromosome copy distribution between daughter cells) and *cytokinesis* (plasmalemma and cytoplasmic content division). In the transient absence of mitosis, cells are in a quiescent state (more or less long duration G0 phase). Senescent cells have permanently lost their ability to divide.

The *interphase* includes the cell-cycle phases (G1, S, and G2) before M phase. At the onset of the M phase (*prophase*; προ: first, before), chromatins condense to form replicated chromosomes with two twin chromatids bound at the centromere by the cohesion complex. The mitotic spindle is assembled to process the separation of the chromosomes. During the *prometaphase*, the nuclear membrane ruptures and allows the mitotic spindle to contact the chromosomes. During the *metaphase* (μετα: in the middle of), the chromosomes gather in the equatorial region of the mitotic spindle. During the *anaphase* (ανα: culminating), the chromosomes are split apart and pulled to the opposite cell sides. During the *telophase* (τελοσ: accomplishment), the nuclear membrane reassembles around each set of separated, decondensing chromosomes to form two nuclei. The mitosis refers to the process of chromosome duplication, whereas the following M-phase substage of *cytokinesis* divides the cytoplasm and plasmalemma. The plasmalemma wrinkles in its central part, perpendicularly to the mitotic spindle, due to the contractile ring, thereby ensuring cell division. The other components of the cell (plasmalemma, cytoskeleton, organelles, and cytosolic content) are distributed to the two daughter cells.

During mitosis, the spindle checkpoint allows the delay in transition between the metaphase and anaphase until all chromosomes are correctly

aligned and attached to microtubule spindles. Several kinases, which regulate microtubule-associated regulatory kinases, can activate the spindle checkpoint to avoid aberrant chromosomal segregation and aneuploidy.

Initiation factor 4B (eIF4B) binds to mRNA end (cap) to initiate mRNA translation. During mitosis, 14-3-3σ binds to several translation initiation factors, thereby regulating protein synthesis. 14-3-3σbound eIF4B cannot bind to the mRNA cap. During and immediately after the metaphase, cap-dependent translation is suppressed whereas cap-independent translation is activated, non-capped mRNAs for cell-cycle regulators containing internal ribosome entry sites for translation.

Cyclins are proteins involved in cell-cycle progression. Cyclins are either produced or degraded owing to ubiquitin ligases APC/C according to the stage of the cell cycle. Cyclin-A accumulates from late G1 phase and is destroyed before metaphase [41]. Cyclin-B is synthesized and degraded slightly later than cyclin-A. At low concentrations, cyclin detaches from cyclin-dependent kinases. Cyclin-dependent kinases (CdK) are serine/threonine protein kinases, which are activated by association with cyclins.[30] The M-phase promoting factor (MPF) is made of cyclin-B and CdK1. Cyclin–CdK complexes are

Table 1.6. Cell cycle. Both external and internal signals control the activity of cyclins and cyclin-dependent kinases (CdK), which govern DNA replication and chromosome segregation during the cell cycle. Before (but not after) the restriction step, regression to G0 is possible.

G0	Quiescence	Antimitogens
G0/G1	Transition from quiescence to division	Mitogens
G1	Gap 1 (cell growth)	Cyclin D–CdK4/6, Cyclin E–CdK2
G1/S	Transition	Cyclin D–CdK4, Cyclin E–CdK2, Cyclin A–CdK2
R	Restriction (no return point)	
S	DNA synthesis (chromosome duplication)	Cyclins A/E–CdK2
G2	Gap 2 (division preparation)	Cyclins A/B–CdK1
G2/M	Transition (control of DNA replication)	Cyclins A/B–CdK1
M	Mitosis (prophase, metaphase, anaphase, telophase)	Cyclins B–CdK1
M/G1 or G0	Transition Quiescence	Mitogens Antimitogens

[30] Orphan cyclins do not have identified CdK partners, but can be implicated in the cell cycle. There are two types of cyclins-A (A1, A2) and three types of cyclins-B (B1, B2, B3). CdK1 is activated by cyclin-A and cyclin-B, CdK2 by cyclin-A and

regulated by kinases and phosphatases. Cyclin-dependent kinases, except CdK9, regulate the cell cycle, as well as the transcription and mRNA hboxprocessing.

1.2.6 Cell Decision between Survival or Death

In case of stress, cell survival and death compete until one becomes dominant according to the context. Cell exposure to inflammatory cytokines, pathogens, and DNA damages: (1) either activates nuclear factor-κB (NFκB; Sect. 4.8.1), mitogen-activated protein kinases (Sect. 4.3.2.4), and interferon regulatory factors, which upregulate antiapoptotic proteins and cytokines, or cause either (2) autophagy or (3) apoptosis. Receptor interacting protein RIP1 is an adaptor kinase implicated in cell fate [42]. RIP1 forms integrator complexes of signaling pathways triggered by T-cell receptor (TCR), toll-like receptors TLR3 and TLR4,[31] and death receptors, such as tumor necrosis factor receptor TNFR1, TRAILR1 and TRAILR2. RIP1 also binds to death domain-containing adaptor proteins, such as tumor necrosis factor-receptor associated death domain (TRADD) and Fas receptor associated death domain (FADD), as well as with other types of adaptor proteins in order to recruit kinases, such as MAP3K1, MAP3K3, and RIP3.

When TNFα binds to its receptor TNFR1, TNFR1 recruits RIP1 and TNF receptor associated factor TRAF2 in plasmalemmal complex-I. Several pathways are then activated, such as NFκB, p38, and JNK (Sects. 4.3.2.4 and 4.8.1; Fig. 1.5). NFκB activation prevents apoptosis, its blockade causes apoptosis due to TNFα. Receptor endocytosis allows the recruitment by TRADD of FADD and caspase-8, which form complex-II, leading to apoptosis. Moreover, caspase-8 favors RIP1 cleavage. RIP1 ubiquitination in membrane rafts favors the recruitment of TAK1-binding protein TAB2 and the formation of the TAK1–TAB1–TAB2 complex. The latter activates IKK and NFκB.[32] Besides, TGFβ-activated kinase TAK1 also binds and phosphorylates MAP3K3, which activates NFκB. FasL can also activate NFκB via caspase-8 (Fig. 1.5), but its main activity is to elicit apoptosis.

1.2.7 Caspases

Caspases[33] have several functions, including inflammation, cell proliferation, differentiation and survival/apoptosis [43] (Table 1.7). All caspases are produced as inactive zymogens. Initiation of caspase activation needs adaptor

cyclin-E, CdK4 and CdK6 by cyclin-D1, -D2, and -D3, CdK7 by cyclin-H, CdK8 by cyclin-C, CdK9 by cyclin-T1, -T2a, -T2b, and cyclin-K, CdK11 by cyclin-L.

[31] Toll-like receptors TLR3 and TLR4 can activate the NFκB and the MAPK pathways. Stimulated TLR3 and TLR4 also activate interferon regulatory factor IRF3 and induce production of interferon-I.

[32] NFκB inhibitory protein A20 recruited to the TNFR1 complex impedes RIP1 ubiquitination.

[33] Caspases are cysteinyl aspartate proteinases, i.e., cysteine proteases, which cleave substrates targeting aspartate residues.

multiprotein complexes, such as apoptosomes, inflammasomes, etc. Then activated caspases target various effectors. The caspase substrates include cytokines, kinases, transcription factors, and polymerases. Many caspase inhibitors exist to control their activities. In addition to their catalytic activities, caspases have non-proteolytic functions for the recruitment of adaptors and effectors that modulate NFκB activation.

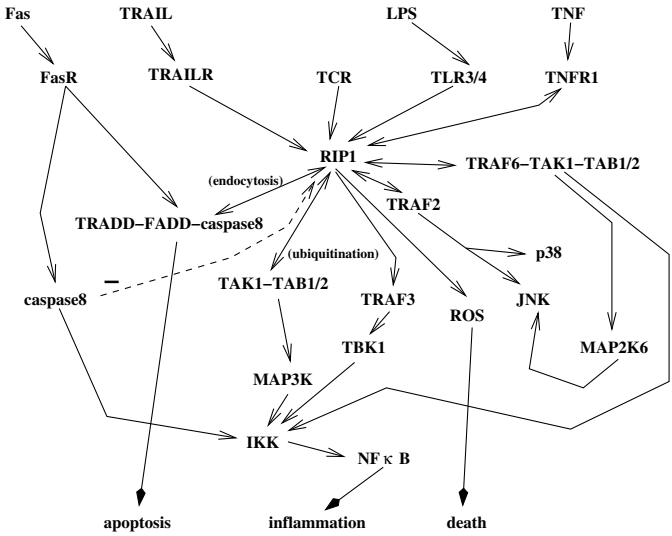

Figure 1.5. RIP1 integrates numerous signals to initiate different cellular responses, according to the type of RIP1-containing complexes, the complex location within the cell and the targeted substrates. TNFα binds to its receptor TNFR1 and activates either proapoptotic or antiapoptotic pathways. Activated TNFR1 recruits RIP1 and TRAF2, which forms plasmalemmal complex-I. RIP1 ubiquitination in membrane rafts favors the formation of the TAK1–TAB1/2 complex, which activates IKK and NFκB. The complex between TRAIL and its receptors TRAILR1 and TRAILR2 undergoes endocytosis and triggers either (most often) apoptosis or NFκB activation. TRAIL can stimulate JNK and p38, with cooperation of RIP1 and TRAF2, on the one hand and IKK, using RIP1, on the other hand, but at a lower magnitude and speed than TNFα. Fas recruits FADD and caspase-8 to the plasmalemma and forms the death-inducing signaling complex (DISC). When recruited to DISC, caspase-8 is activated and leads to apoptosis. Activated TLR3 recruits RIP1, TRAF6, TAK1, TAB1, and TAB2. This complex activates IKKβ and MAP2K6, and subsequently JNK. Under TNF stimulation, RIP1 translocates to the mitochondrion and reduces the interaction between adenine nucleotide translocase and cyclophilin-D, thus enhancing ROS production and reducing ATP synthesis. Reactive oxygen species (ROS) produced by mitochondria are involved in apoptosis (Source: [42]).

Caspase-1 mainly activates inflammatory cytokines (Sects. 6.2 and 10.1).[34] Inflammatory caspases (group 1 caspases) also include caspase-4 and -5 in humans [44]. They are activated by members of the NOD-like receptor (NLR) family, such as NAIP (neuronal apoptosis inhibitory protein), NALP (NACHT [NAIP domain], LRR [leucine-rich repeat], and PYD [pyrin domain] containing proteins), and IPAF (IL1β-converting enzyme protease-activating factor), which form multiproteic adaptor complexes, the inflammasomes. Inflammatory caspases are controlled at the inflammasome level by inhibitors to avoid excessive cytokine production.

Executioner caspases (caspase-3, -6, and -7) drives apoptosis. Apoptotic caspases are activated by initiator caspases (caspase-2, -8, -9, and -10), which are recruited by the formation of adaptor complexes [43]. Caspase-9 is the

Table 1.7. Caspase functions (Sources: [43–45]).

Caspase	Functions
Caspase-1	Cytokine maturation, inflammation, NFκB activation
Caspase-2	NFκB activation, Stress-induced apoptosis initiation, Erythrocyte formation
Caspase-3	Apoptosis, Erythrocyte and platelet formation
Caspase-4	Inflammation
Caspase-5	Inflammation
Caspase-6	Apoptosis
Caspase-7	Apoptosis
Caspase-8	Apoptosis initiation, Proliferation of T, B and NK cells, Maturation of monocytes into macrophages, NFκB activation
Caspase-9	Apoptosis initiation
Caspase-10	Apoptosis initiation
Caspase-11	Inflammatory cell migration, Caspase-1 activation

[34] Caspase-1, also called interleukin-1β-converting enzyme which targets interleukin-1β precursor (pro-IL1β, the production of which is induced by lipopolysaccharides) to generate active interleukin-1β, is the first known caspase. Caspase-1 is activated by P2X7 ion channel receptors stimulated by extracellular ATP. IL1β initiates and amplifies various events during microbial invasion and tissue injury. Caspase-1 also activates IL18. IL18 stimulates the production of interferon-γ by splenocytes and of proinflammatory cytokines, upregulates adhesion molecules, and stimulates natural killer lymphocytes. Both IL1β and IL18, activated by inflammasomes, activate their specific receptors (IL1R and IL18R) for signaling. Caspase-1 can activate NFκB.

main component of the mitochondrial intrinsic apoptotic pathway regulated by BCL2 protein family, because of its higher catalytic activity. Cytochrome-C release from the mitochondria favors the formation of septameric apoptosomes, composed of apoptotic protease-activating factor-1 (APAF1), which activates caspase-9. Apoptosome-bound caspase-9 cleaves (activates) caspase-3. Caspase-2 activation requires the assembly of a proteic complex that comprises adaptor RAIDD and p53-induced protein with a death domain (PIDD), the PIDDosome. Caspase-8, recruited to the death-inducing signaling complex (DISC), is implicated in the extrinsic apoptotic pathway triggered by tumor necrosis factor and death receptor Fas. Caspase-8 and -10 mediate NFκB-dependent inflammation in response to virus invasion. Caspase inhibitors for apoptosis regulation include flice-inhibitory proteins (FLIP), upregulated by the TNFα–NFκB pathway, and inhibitors of apoptosis proteins (IAP). Caspase-2 can be also involved in apoptosis. It also elicits MAPK and NFκB signaling.

Catalytic and non-proteolytic functions of caspases are implicated in cell differentiation, proliferation, and NFκB activation [45]. Although caspase-8 can initiate apoptosis, it also acts in the proliferation of immune cells (T, B, and natural killer lymphocytes), according to the caspase-8 adaptor recruitment level, hence to caspase-8 activation magnitude. T-cell receptor stimulates the recruitment of caspase-8 and its adaptor Fas-associated death domain–containing protein (FADD) to a suitable proteic complex required for TNF-induced NFκB activation. Caspase-3 targets substrates associated with the cell cycle control, such as cyclin-dependent kinase inhibitor and transcription factor nuclear factor for activated T cells. Caspase-2, -3, and -9 are transiently activated during erythroblast differentiation. Caspase-3 and -9 activation is involved in platelet formation from megakaryocytes. Both differentiation types lead to enucleated cells (erythrocytes, platelets); however, caspase-8 is implicated in the maturation of monocytes into macrophages.

1.2.8 Cell Autophagy

Cell autophagy occurs during cell differentiation and growth. It is associated with degradation and recycling of cellular macromolecules and organelles by lysosomes. Cell autophagy also allows the cell to survive against metabolic stresses. Catabolism of cell organelles generates energy that allows cell survival. However, prolonged autophagy induces programmed cell death, which differs from apoptosis. An increased cellular AMP/ATP ratio occurs during deprivation of nutrients and energy, such as hypoxia, ischemia, and glucose deprivation. Metabolic stresses thus lead to AMP accumulation, which activates AMP-activated protein kinase (Sect. 4.3.2.3).

Sensing of nutrient and energy availability, especially of cellular levels of adenosine monophosphate, is associated with the LKB1–AMPK pathway. The cyclin-dependent kinase inhibitor p27 is an effector of this energy-sensing

pathway.[35] p27 phosphorylation by AMP-activated protein kinase stabilizes p27kip1 and promotes cell autophagy in response to metabolic stress rather than apoptosis [46].

Autophagy is initiated by the formation of autophagosomes, which fuse with lysosomes to become autolysosomes. The autolysosomal content is then degraded by lysosomal hydrolases. The process is governed by autophagy-related genes (ATG) and gene products (Atg). TOR kinase senses nutrient status, regulates cell growth, and inhibits autophagy, acting on autophagy-execution proteins. Under nutrient-rich conditions, an activated target of rapamycin inhibits ATG1. Under conditions of starvation, ATG1 inhibits S6-kinase by blocking its phosphorylation [47]. Otherwise, activated S6K promotes cell growth. Reactive oxygen species, especially H_2O_2, intervene in autophagy induced by amino acid deprivation [48].

Autophagy and ubiquitin-proteasome pathways particularly protect cardiomyocytes. Altered autophagy has been observed in various heart diseases. The autophagy-related gene ATG5 produces Atg5 protein required for the formation of autophagosomes, which carry proteins and organelles destined for autophagy to lysosomes [49]. In the absence of Atg5, heart hypertrophy and failure occur. Conversely, autophagy plays a beneficial role in the heart in response to pressure overload or β-adrenergic stresses.

1.2.9 Cell Senescence

Cell senescence occurs as soon as the cell loses its ability to divide and to maintain its genetic material. The permanent suspension of division can be induced either by strong expression of oncogenes, such as HRAS, which involves the Arf–p53 pathway, or by shortened telomeres, which also implicate tumor suppressor p53.

DNA damages, which cannot be repaired but that do not lead to apoptosis, such as shortened telomeres, are responsible for cell senescence. Telomeres are repetitive DNA sequences at the ends of chromosomes, which shorten with the rising number of cell divisions. Cumulated oxidative stresses could also induce telomere shortening. Short telomeres are assumed to trigger the onset of senescence. Aging is also characterized by a decaying ability to respond to stress and to retain cell homeostasis.

Telomerase can synthesize new telomeric repeats and restore telomere length. Telomerase activity is regulated by protein kinase-B, protein kinase-C, and extracellular signal-regulated kinase-1/2 (Sects. 4.3 and 4.3.2.4). Nitric oxide (Sect. 9.5) can prevent senescence of endothelial cells by activating telomerase [50].

[35] p27 inhibits cyclin-dependent kinases, leading to cell-cycle arrest. Both CdK2 and CdK4 can act in case of p27 depletion. Depletion of CdK2 and CdK4 promote autophagy. Small interfering RNAs (siRNA) downregulate p27kip1 and cause apoptosis.

Cell senescence can be accelerated by unusual stress levels with defective DNA repair enzymes. Oxygen metabolized by mitochondria is converted to superoxide ions, and subsequently to hydrogen peroxide, hydroxyl radicals and other reactive species. These molecules can generate free radicals that damage structural proteins and DNA. Metal ions can participate. Glucids can react with amino acids and DNA bases and produce carbohydrate adducts (glycation). These compounds can form reactive species that target proteins or DNA, impairing their functions.

Restricted dietary intake without malnutrition favors longevity. Certain genes and corresponding pathways increase survival under conditions of dietary restriction. FoxO can be implicated (Sect. 4.8.3). It influences survival of the whole body, particularly controlling hormone production.

1.2.10 Cell Apoptosis

Apoptosis is characterized by morphological changes, and cell shrinkage leading small round cells with nuclear pyknosis (chromatin condensation) and karyorhexis (nuclear fragmentation). Apoptosis pathways drive fast cell death. Apoptosis includes two pathways: the extrinsic and intrinsic pathway. The receptor-mediated extrinsic pathway, implicated in the elimination of unwanted cells during development, is initiated by ligand-induced activation of the death receptors at the plasmalemma. The mitochondrion-mediated intrinsic pathway is triggered by stresses (DNA damage).

Both proteolytic pathways involve the activation of cysteinyl aspartate-specific proteases, the so-called caspases. Both initiator caspases and executioner caspases are required. Initiator caspases are inactive monomers activated by dimerization [51]. In the intrinsic pathway, the apoptotic stimulus triggers the assembly of the apoptosome. Cytochrome-C has two main functions: electron transport and caspase activation. Once released in the cytosol from mitochondria, cytochrome-C is sensed by apoptosis-protease activating factor-1 (Apaf1) and recruited to the apoptosome. Cytochrome-C then activates Apaf1 in the apoptosome, which is required for the recruitment of procaspase-9 and its stimulation to form caspase-9[36] and caspase-3, leading to the cell apoptosis. However, mitochondrial release of cytochrome-C can lead to caspase activation for cell differentiation, such as monocyte–macrophage differentiation. In the extrinsic pathway, apoptosis is initiated by the ligand-dependent plasmalemmal death-inducing signaling complex (DISC). An extracellular ligand, such as FasL and TRAIL, binds to a transmembrane receptor, thus inducing DISC formation and activating caspase-8 and -10. Caspase-8 activation is prevented by FLIP. The catalytic domain of caspases include large and small units. Cleavage of linkers these units of the dimer leads to caspase activation. Caspase-9 and -8 cleaves pro-caspase-3 and -7. Cleaved, active caspases achieve apoptosis.

[36] Caspase-9 activation requires the ATPase activity of Apaf1. Caspase-9 and its downstream effectors are inhibited by XIAP.

Mitochondrial dysfunction disrupts energy production and can trigger apoptosis. Mitochondrial outer membrane permeabilization releases mitochondrial intermembrane proteins, such as cytochrome-C. BCL2-family proteins[37] interact to promote or prevent cell apoptosis. Cytochrome-C liberation from the mitochondrion requires the activation of cell-death mediator BAK of the BCL2 family, which is located in the mitochondrial outer membrane, where it remains inactive unless activated. Another member of the BCL2 family, proapoptotic protein BAX is also needed to activate caspases. BH3-only members initiate apoptosis by engaging the multiple pro-survival relatives in guarding BAK and BAX [53].

The activated program within the cell leads to DNA fragmentation, cytoplasm shrinkage, membrane changes and cell death without damage to neighboring cells. Viable cell plasmalemma displays markers that inhibit phagocytosis. Apoptotic cells lose these plasmalemmal molecules and exhibit other markers, such as phosphatidyl serine, for phagocytosis. Phagocytes release anti-inflammatory molecules, such as IL10 and TGFβ. Certain kinds of apoptotic cells display plasmalemmal calreticulin. They are targeted by dendritic cells for T-cell–mediated immune response.

1.2.11 Cell Necrosis

Apoptosis is a regulated cell death program, that eliminates the cell without generating any inflammatory response. In contrast, after necrosis, cell fragments are taken up by phagocytes, which produce pro-inflammatory cytokines. The subsequent inflammatory response can damage neighboring cells.

When tissues are not sufficiently oxygenated, concentrations in transcription factor HIFα and HIF prolyl hydrolase PHD2 rise. Both molecules avoid large tissue necrosis by favoring angiogenesis (Sect. 10.4.2) and contribute to repair damaged tissues after a slight, initial hypoxia to prevent later, more important lesions.

1.3 Cellular Membranes

The cell, its nucleus, and organelles have their membranes. Biological membranes mainly are mixtures of many types of lipids and proteins.[38] Biological

[37] BCL2 family proteins, identified in B-cell lymphoma/leukemia tumor cells, are classified into three sets, the antiapoptotic proteins (BCL2, BCLXL, MCL1, BCLW, and A1/BFL1), proapoptotic proteins (BAX, and BAK), and BH3-only proteins. BH3-only inactivators responding to cell-death stimuli bind to anti-apoptotic BCL2 family proteins, hence releasing BH3-only activators of pro-apoptotic BCL2 family proteins [52].

[38] Almost all membrane proteins are oligomeric, and many are hetero-oligomers.

membranes are organized fluid structures. The lipid composition of a membrane defines the organelle identity, flexibility and permeability of the bilayer, and its interaction partners.

Lipid bilayers are characterized by lateral static and dynamic heterogeneity due to nanodomains. The composition differs between functionally distinct domains of patchy biological membranes. Distinct regions have given structure and function, generating functional complexes with a given thickness associated with lipid–protein matching.

Cell membranes present a mixture of disordered and ordered lipid phases depending on lipid composition and organization. Cholesterol is one of the most important regulators of lipid organization. Membrane proteins preferentially associate with a peculiar type of phase according to the type of anchors[39] and interactions with membrane components. Protein translocation channel, or *translocon*, mediates the insertion of membrane proteins into the phospholipid bilayer.

Both fluidity and lateral organization are needed for membrane functioning. Various techniques have been applied to investigate this lateral heterogeneity. Scanning probe methods, such as x-ray crystallography or atomic force microscopy, give limited information on composition and organization on length scales of tens to hundreds of nanometers (i.e., on length scale greater than the characteristic size of protein assembly, about 10 nm). Optical methods (light microscopy, and infrared and coherent Raman imaging techniques) have limited spatial resolution. Fluorescence microscopy is specific to the labeled component and can alter between-compound interactions. Secondary-ion mass spectrometry with isotopic labeling of molecular species allows detection of variations in the gel phase composition of trapped fluid phase nanodomains with a lateral resolution of about 100 nanometers within a phase-separated lipid membrane [54].

Membrane remodeling occurs during molecular transport and organelle genesis. It requires specialized proteins that sense and create membrane curvature. Protein interactions and proteic cluster formation during such processes limit the bending energy [55]. Proteins with BAR domains[40] generate local membrane curvatures over a tiny length scale ($\sim 3\,\mathrm{nm}$), especially in vesicle formation and T-tubule organization in myocytes, which match the curvature of the BAR domain [56].

The inner layer of the plasmalemma has a high concentration of cholesterol, whereas the outer leaflet has high levels of sphingomyelin and glycosphingolipids. The membrane of the endoplasmic reticulum has low cholesterol and large unsaturated lipid contents. Membrane lipids are needed in membrane trafficking and signal transduction. Membrane lipids can also act as

[39] Src kinases associate with ordered domains, whereas Ras GTPases prefer disordered domains.

[40] BAR stands for Bin/amphiphysin/Rvs.

cofactors for certain membrane proteins. For example, cytochrome-C oxidase requires cardiolipin.

Biological membranes, which determine the configuration[41] of the cell and its organelles,[42] are curved.[43] The curvature of deformable membranes results from interactions (particularly electrostatic ones) of membrane constituents, lipids and proteins,[44] and applied forces.

Tiny membrane domains are also characterized by their curvature. The curvature of membrane patches is modulated by (1) lipid composition,[45] (2) inserted proteins and scaffold proteins at the membrane periphery, and (3) cytoskeletal activity. Membrane patch curvature is required in vesicle formation.

Membrane proteins either are contained within the bilayer or have structural ectodomains (outside the lipidic layer), which can be larger than the protein endodomain. Certain proteins associate and dissociate with lipids during their activities. Many membrane proteins are inserted as soon as they emerge from the ribosome via a protein-conducting channel, avoiding membrane leakage. Protein insertion across the membrane bilayer is catalyzed by a translocon complex. The translocon also acts on the topology of the inserted protein parts, the cytoplasmic side of membrane proteins being positively charged. Afterward, flexible tertiary and quaternary protein structures are built.

Membrane lipids interact with adjoining lipids and proteins. Mutually compatible lipids and proteins of the cell membrane form intimate contacts[46] to create a functioning barrier. The fluidity of the lipid bilayer membrane can

[41] The biconcave disc shape of erythrocytes, which do not have internal membranes, provides the optimal surface-to-volume ratio necessary for fast oxygen exchange between hemoglobin and tissues. Furthermore, erythrocytes change their shape to navigate in blood vessels of various size. Cells that generally have internal membranes can also change their shape, especially during migration. Moreover, cell configuration varies when the cell undergoes stresses, particularly sheared endothelial cells.

[42] Organelle shapes maximize the surface area for a low internal volume for efficient transport of molecules. The Golgi stack is much rather saccular and fenestrated, the endoplasmic reticulum rather tubular with small cisternae, both configurations having similar transfer efficiency.

[43] Membrane shapes can be described by the principal curvatures, κ_1 and κ_2 or the radii of curvature, $1/\kappa_1$ and $1/\kappa_2$. The curvature is small if the radius of curvature is much larger than the membrane thickness. Two indices can be used, the total curvature ($\kappa_1 + \kappa_2$) and the Gaussian curvature ($\kappa_1 \cdot \kappa_2$).

[44] The small GTPase Sar1 curve lipid bilayers. Adaptor proteins, coat proteins, amphiphysin, epsin, dynamin, and endophilin are involved in membrane curvature. Moreover, certain proteins might sense membrane curvatures [57].

[45] Lysophosphatidic acid and phosphatidic acid induce opposite curvatures.

[46] The lipid bilayer provides the basic barrier into which are plugged membrane proteins with particular functions (transport, and environment sensing). Proteins inserted in the plasmalemma must operate within the lipid bilayer without introducing any leak and without disturbing the membrane permeability. Lipids

affect the lateral diffusion of molecules and molecular interactions. Higher membrane fluidity can modify the conformation of protein receptors embedded in the lipid bilayer.

The lipid bilayer is a composite dynamic structure built by hydrophobic and between-constituent interactions. Spatial heterogeneity in the lipid bilayer is associated with a distribution of lateral pressure within the membrane thickness. Membrane tension leads to changes in membrane thickness associated with variations in hydration level of the lipid bilayer. Membrane stretching reduces repulsive interactions and elevates interfacial tension because of increased exposure of hydrophobic lipid tails to water, thus modifying the lateral pressure profile [59]. Membrane shearing not only affects the lateral pressure profile but also bilayer membrane density profiles [60]. Variations in the plasmalemmal lateral pressure profile are able to activate mechano-sensitive membrane proteins, shifting the protein conformation toward the appropriate state.

1.4 Mitochondrion

Mitochondria supply cells with energy. They produce ATP, the energy source of multiple biochemical reactions within the cell. Mitochondria convert substrate to energy using two different complexes. *Complex-I* reflects the global cellular NADH production from fatty acid oxidation, the tricarboxylic acid (TCA) cycle, and glycolysis (Fig. 1.6). The TCA cycle-dependent *complex-II* receives FADH2 directly from succinate dehydrogenase. The ATP production-to-oxygen consumption ratio varies in mitochondria of different cell populations. Heart mitochondria use more oxygen, but produce ATP at a faster rate than liver ones [61]. In the heart, the TCA energy-converting cycle maximizes the oxidative phosphorylation production of ATP in mitochondria (Fig. 1.7). F-ATPases in mitochondria are membrane-bound complexes that couple the transmembrane proton motor to ATP synthesis. The latter depends on a conformational change driven by the passage of protons from the inter-membrane space into the matrix via channels.

Electron transfer in cell respiration is coupled to proton translocation across mitochondrial membranes.[47] The resulting electrochemical proton gradient is used to power energy-requiring reactions, such as ATP synthesis. Cytochrome-C oxidase is a major component of the respiratory chain. It attaches oxygen as a sink for electrons and links O_2 reduction to proton pumping. Electrons from cytochrome-C are transferred sequentially from donors

can form a shell around inserted proteins. Phospholipids with their fatty acyl chains interact with the hydrophobic surface of the protein [58].

[47] Proton transfer to the O_2 reduction site is associated with electron transfer from heme a to the O_2 reduction site. This initial driving step of proton pump is followed by proton release on and uptake from the two aqueous sides of the membrane.

(CuA and heme a sites) to O_2 reduction site of cytochrome-C oxidase. This electron transfer initiates the proton pump [62]. Cytochrome-C in the mitochondrial intermembrane space thus serves as an electron shuttle between complex 3 and complex 4 of the respiratory chain and links to cardiolipin.

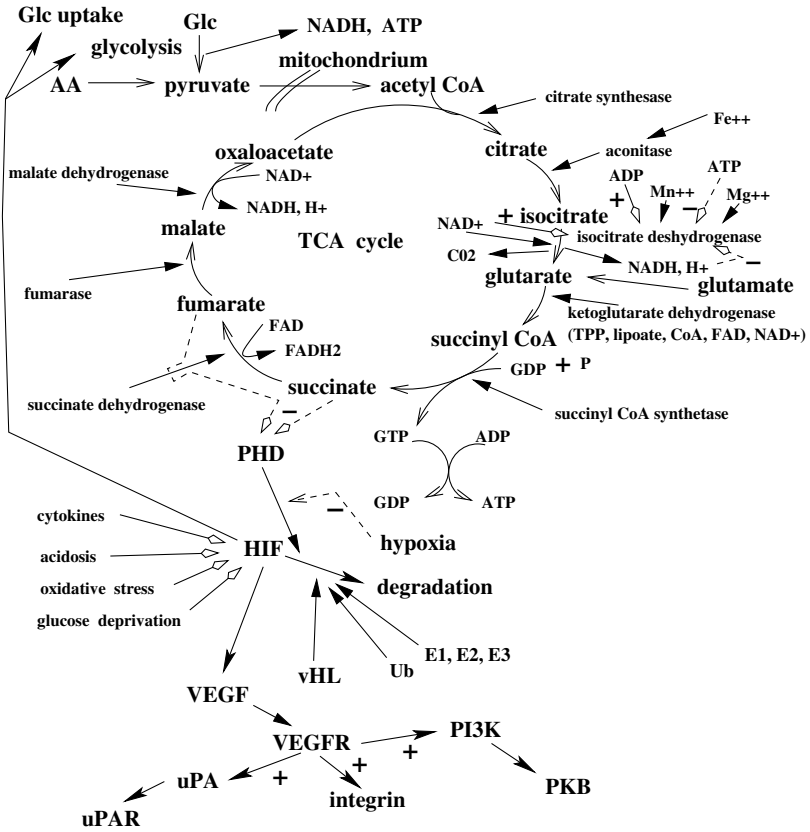

Figure 1.6. The tricarboxylic acid cycle (TCA or Krebs cycle) serves as a metabolic platform. During each TCA, three nicotine adenine dinucleotide (NAD+) are converted into NADH and one flavine adenine dinucleotide (FAD) in FADH2. TCA leads to electron transport and manufactures ATP. The hypoxia-inducible factor (HIF) is degraded by the von Hippel-Lindau protein (vHL), after hydroxylation by the prolyl hydroxylase (PHD). PHD is inhibited by increased concentrations in fumarate and/or succinate as well as local hypoxia. HIF is promoted not only by hypoxia but also by glucose deprivation, acidosis and cytokines. HIF upregulates vascular endothelial growth factor (VEGF) and promotes glucose (Glc) uptake and glycolysis, hence ATP and pyruvate formation. Once bound to its receptor, VEGF upregulates integrin expression and stimulates several pathways, via particularly urokinase plasminogen activator (uPA), phosphatidylinositol 3-kinase (PI3K), and nitric oxide. HIF degradation also involves the ubiquitin (Ub)/proteasome system with Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzymes.

Cytochrome-C–mediated electron transfer is due to the iron atom within the heme prosthetic group.

Mitochondrial respiration produces ATP from ADP phosphorylation. The energy production by mitochondria generates by-products, the *reactive oxygen species* (ROS), electrons reacting with oxygen to form superoxide. The ROSs[48] are highly reactive molecules that include free radicals, anions composed of oxygen, such as superoxide and hydroxyl, or compounds containing oxygen, such as hydrogen peroxide, which can produce free radicals or be activated by them.

The ROSs can have signaling activities. At physiological concentrations, ROSs can act as second messengers, particularly in cell growth and proliferation, but they can be toxic. These oxidants can indeed interact with other chemical species and produce derived products, which can cause oxidative damage. Therefore, the cell has antioxidant defenses with a set of enzymes, as well as other proteins, such as peroxisome proliferator-activated receptors PPARγ coactivator-1.

Under normal conditions, ROS are cleared from the cell by the action of *superoxide dismutase* (SOD), which targets superoxide, and catalase, or *glutathione peroxidase*, which degrades hydrogen peroxide. The increased production of reactive oxygen species rises oxidative protein damage and lipid peroxidation. Under pathological conditions, elevated ROS levels damage polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Such damages lead to cell dysfunction and death. The balance between beneficial and deleterious effects also depends on cellular localization and possibly on ROS sources.

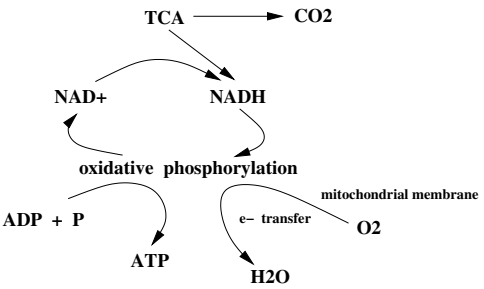

Figure 1.7. Last stage of catabolism of nutrients into wastes. This stage produces a great quantity of ATP from phosphate and ADP. NADH produced by the tricarboxylic acid cycle is used for ATP synthesis during oxidative phosphorylation in the mitochondrium.

[48] ROS sources are mainly aerobic respiration, peroxisomal oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and specific enzymes.

The complex NADH–ubiquinone oxidoreductase, which produces superoxide rather than hydrogen peroxide, is a major source of reactive oxygen species in the mitochondrion. The production rate of superoxide is determined by electron transfer between fully reduced flavin to oxygen, the reacting flavin level depending on concentrations of NADH and NAD^+ [63]. Superoxide production is enhanced when the NAD+ pool is reduced.

Cardiolipin (diphosphatidyl glycerol) is a component of the mitochondrial membrane, mainly on the inner membrane, especially in the cardiomyocyte. There are several types of cardiolipin, a major cardiac cardiolipin, tetralinoleoyl–cardiolipin, and minor cardiolipin species. This phospholipid stabilizes the activity of protein complexes implicated in the mitochondrial electron transport system or the respiratory chain. It generates an electrochemical potential for substrate transport (cardiolipin is required for translocation and not for binding) and ATP synthesis. It interacts with multiple mitochondrial proteins, such as NADH, ubiquinone oxidoreductase, cytochrome-C oxidase, and cytochrome-C. It thus acts on enzymes involved in oxidative phosphorylation. The early oxidation during cell apoptosis is catalyzed by a cardiolipin-specific peroxidase activity of cardiolipin-bound cytochrome-C. Furthermore, cardiolipin is involved in the osmotic stability of the mitochondrial membrane. The affazin is an enzyme involved in the synthesis of cardiolipin. A kind of cardiomyopathy results from the mutation in the gene coding for affazin (Barth syndrome).

Mitochondria have diverse tissue-dependent metabolic functions. In liver, mitochondria process a wide variety of molecules. Because the enzymes are predominantly in the matrix, the substrates are transported across the mitochondrial inner membrane. The mitochondrial metabolite transport system requires manifold specific carriers.[49] The mitochondrial membrane is impermeable to small ions and permeable to small uncharged molecules such as oxygen. Calcium uptake is catalyzed by a Ca^{++} channel in the inner membrane which exchanges Ca^{++} for H^+. The mitochondrial membrane is impermeable to nicotinamide adenine dinucleotides (NAD+, NADH, NADP+, and NADPH). Two main routes are then employed, the glutamate–aspartate and the dihydroxyacetone phosphate paths. The former uses 2-oxoglutarate–malate and glutamate–aspartate exchangers, as well as NADH and a transaminase enzyme. The latter relies on two different glycerol-3-phosphate dehydrogenase enzymes, the first one using NAD/NADH couple and the second FAD-linked membrane bound enzyme.

Mitochondrial oxidative phosphorylation depends on the intramitochondrial reducing power, i.e., on (1) the concentrations of NADH ([NADH]) and of NAD+ ([NAD+]), indexed by the [NADH]/[NAD+] ratio; (2) the cytoplasmic energy state (the relative concentrations in ATP [ATP] and ADP [ADP])

[49] These carriers include the phosphate/OH^- exchanger, the adenine nucleotide transporter, the tricarboxylate transporter, the transporters for citrate/isocitrate, malate/succinate, pyruvate, and 2-oxoglutarate carrier, etc.

given by [ATP]/([ADP][Pi]) ratio; and (3) intracellular oxygen pressure [64]. The mitochondrial respiratory rate is determined by the rate of cell ATP use. The cellular energy state at a given rate of ATP utilization is determined by the activity of mitochondrial dehydrogenases expressed as [NADH]/[NAD+] and the local oxygen pressure. NADH concentration in the mitochondrium hence is affected by the activity of mitochondrial dehydrogenases, by the cytosolic phosphorylations related to the energy state, and by cytochrome-C oxidation in the respiratory chain.[50]

The synthesis or hydrolysis of ATP by the mitochondrium occurs in its matrix space. Nevertheless, ADP and ATP can cross the mitochondrial membrane, via the adenine nucleotide transporter[51] (ANT) and the phosphate transporter[52] (Fig. 1.8).

The creatine kinase (CK), located at sites of energy demand and production, catalyzes the reversible transfer of phosphate from phosphocreatine (PCr) to ADP, which generates ATP and creatine (Cr). CK then controls the energy homeostasis of cells with high, fluctuating energy requirements, such as cardiomyocytes. Creatine kinases have subcellular compartmentations to be coupled to sites of either energy production (glycolysis and mitochondrial metabolism) or energy consumption (actomyosin ATPase, sarcoplasmic reticulum Ca^{++}-ATPase). They form a regulated energy distribution network, the so-called PCr circuit (or PCr shuttle). The main pools of CK include mitochondrial (miCK), cytosolic[53] (cyCK), and myofibrillar (mmCK) creatine kinases (Fig. 1.8). Mitochondrial creatine kinase binds to mitochondrial membranes and forms a complex with porin (or voltage-dependent anion channel VDAC) and adenine nucleotide translocase[54] (ANT) for export of phosphocreatine into the cytosol. The adenosine monophosphate-activated protein kinase (AMPK), an energy sensor, binds to CK, then phosphorylates CK and inhibits its activity. AMPK is regulated by both ATP/AMP and PCr/Cr ratios.

Under some circumstances, substantial amounts of acetate are produced that must be processed by acetyl-CoA synthetase. Moreover, acetate must be converted for energy production. Acetyl-CoA synthetases are abundant in the heart and skeletal muscles. The mitochondrial matrix acetyl-CoA synthetase-2 is activated by reversible deacetylation induced by sirtuin [66].

The mitochondrial nitric oxide (NO) synthase forms nitric oxide. Nitric oxide inhibits cytochrome-C oxidase, and may act as an oxygen sensor. Low

[50] Oxidation of NADH is related to cytochrome-C oxidation.

[51] The adenine nucleotide transporter catalyzes the exchange of ATP for ADP across the inner mitochondrial membrane. Entry of ADP and exit of ATP are favored.

[52] The transport involve either exchange of $H_2PO_4^-$ for OH^-, or cotransport of $H_2PO_4^-$ with H^+.

[53] There are several cytosolic creatine kinase isoforms: MM, MB, and BB.

[54] The adenin nucleotide translocator is the most abundant protein of the inner mitochondrial membrane. This transmembrane channel is responsible for the export of ATP in exchange with ADP (antiport) across the inner membrane.

oxygen supply can inhibit nitric oxide production, and hence increase oxygen affinity of cytochrome-C oxidase to maintain NADH oxidation and electron transfer. The mitochondrial nitric oxide synthase also leads to the production of superoxide anion O_2^-, especially after ischemia/reperfusion episodes. Peroxynitrites ($ONOO^-$, PN), ROSs, are released by the reaction between NO and O_2^-. Peroxynitrites inactivate mitochondrial creatine kinases.

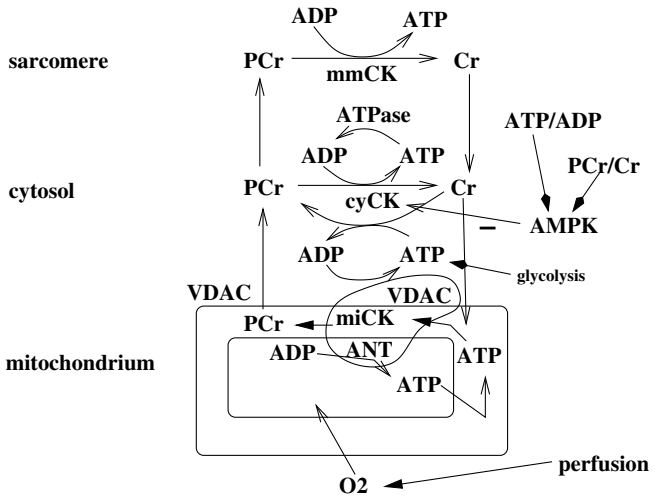

Figure 1.8. Phosphocreatine circuit model. Phosphocreatine (PCr) and creatine (Cr) represent an important network for the cell energy production, distribution, and consumption: mitochondrium, cytosol, and sarcomere (ATP-consuming site). Adenosine triphosphate (ATP) losing a phosphate group is transformed in adenosine diphosphate (ADP). Conversely phosphocreatine can give its phosphate group, thereby regenerating ATP from ADP at high rates. PCr/Cr circuit connects localized creatine kinase (CK), especially found in cells with high energy demands. Creatine kinase catalyzes the reversible transfer of phosphate group from phosphocreatine to ADP. CKs are specifically located at locations of energy demand (cytosol, sarcomere, where ATPases act, such as actomyosin ATPase and sarcoplasmic reticulum Ca^{++} ATPase in the cardiomyocyte) and energy production (mitochondrium, and glycolysis sites). Cytosolic creatine kinase (cyCK), in conjunction with Ca^{++} pumps, is required for the energetics of Ca^{++} homeostasis. Cytosolic CKs (cytosolic muscle-type MM, MB and BB) are co-expressed in a tissue-specific fashion with mitochondrial isoforms. Mitochondrial creatine kinase (miCK) in the mitochondrial intermembrane space cross-links mitochondrial inner and outer membranes. MiCK forms a complex with porin (VDAC) and adenine nucleotide translocase (ANT) for export of PCr into the cytosol. MiCK phosphorylates ATP produced by the mitochondrium into PCr, subsequently expelling to the cytosol via VDAC. The main myofibrillar CK (mmCK) converts PCr into ATP. The PCr/Cr reaction is regulated by AMP-activated protein kinase (AMPK) via ATP/AMP and PCr/Cr ratios. AMPK is activated by the low PCr/Cr ratio. In myocytes, the CK system corresponds to an energy buffer, allowing nearly constant ATP levels during muscle activity.

Reactive oxygen species that react with nitric oxide generate *reactive nitrogen species*, which include not only peroxynitrite, but also nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3). These substances induce a nitrative stress.

The number of mitochondria increases during both cardiac development and hypertrophy. Lack in mitochondrial chaperone Hsp40[55] in cardiomyocytes causes progressive respiratory chain deficiency and decreased level in mitochondrial DNA, which lead to cardiomyopathy [65].

Mitochondria play a major role in apoptosis (programmed death of damaged cells), and then protect against cancer. Involved proteins are located in the mitochondrial outer membrane. The set of various antiapoptotic molecules are counteracted by proapoptotic factors when cell apoptosis becomes imperative.

1.5 Cell Cytoskeleton

Cell deformation and motility is due to the cytoskeleton (Csk). The cytoskeleton includes three filament networks and their cross-linking and partner proteins. The cytoskeleton is built by a fibril network with articulation nodes from which the cytoskeleton can reorganize itself. In particular, it undergoes stresses and responds to minimize local stresses. Moreover, the cytoskeleton contracts and forms stiffer bundles to rigidify the cell. Its anchorage on adjacent tissue elements allows cellular ensemble deformation. The cytoskeleton is malleable in order to accommodate large strains and sufficiently stiff to transmit the stresses. Hence the cells, supported by their cytoskeleton, adapt to imposed loads. The cytoskeleton, which is in a constant state of rearrangement driven by the applied loads, can accommodate important changes of loading.

The cells, especially smooth muscle cells, can deform while maintaining their optimal functions. Smooth muscle cells, indeed, undergo large strains associated with vessel deformation during cardiac and respiratory cycles. Manifold molecules and fibers form this dynamic cell framework, which also determines cytosol organization and intracellular displacements [67]. The energy supply and protein–protein interactions that reorganize the cytoskeletal are regulated.

There are three classes of cytoskeleton filaments: microfilaments, microtubules, and intermediary filaments. The *spanning network* is a fourth element, which have a tiny weft and fills the whole cytosol. It can determine the sites of protein synthesis and the assembling locations of filaments and microtubules. It acts on cell organelle motility. Dynein, myosin, and kinesin constitute the three major families of cytoskeleton-based motor proteins. Dynein is much bigger than myosin-2 and kinesin.

[55] Hsp40 is known as Dnaja3 or Tid1.

1.5.1 Microfilaments

The microfilaments contain several proteins, especially actin. *Actin* filaments (length 0.1–1 μm, thickness 7–10 nm) are involved in cell configuration, adhesion, and motility.[56] Permanent exchanges exist between actin filaments and cytosolic nonpolymerized actin [68], associated with a sol–gel transition.[57] Actin filaments form bundles and are peripherally concentrated to build a felting in the cell cortex, which is connected to the plasma membrane. The actin filaments are indeed directly anchored on the cell membrane, using mooring proteins, *talin* and *vinculin* [69]. The microfilaments can be used as mooring and transmission lines in a stress field, and as towlines during motion. Moreover, myosin-5 transports various cellular cargoes along actin filaments.

Using atomic force microscopy, growth velocity of a branched actin network is found to be load-independent over a wide range of applied forces [70]. Furthermore, when force was decreased on a growing network, the velocity increased to a value greater than the previous velocity at the same load (hysteresis). Growth velocity depends on loading history and not only on the instantaneous load.

The actin cytoskeleton dynamics is maintained by the balance between *actin-binding proteins* (ABP) and *actin-severing proteins* (ASP), because actin polymerization–depolymerization cycle is required to fit needs of processing cells (Fig. 5.5). ABPs transform actin sol into gel, lengthening and branching actin filaments. Filament assembling necessitates ATP. Actin aggregation into a filamentous gel is induced by *filamin* in particular, whereas *profilin* (Pfn) inhibits actin polymerization. However, this actin-sequestering protein has a subtle activity, being able to favor polymerization of actin onto the barbed ends of actin filaments. Profilin indeed stimulates assembly of actin filaments; once complexed with G-actin, it attracts monomers to the actin end. Polyphosphoinositids inhibit ASP fixation on actin and allow filament lengthening. The translocated *cortactin* increases actin polymerization. Activation of Arf1 activates the recruitment of actin, cortactin, and dynamin-2 to Golgi membranes [71].

The depolymerizing activity of *cofilin*, an actin-severing protein, is inhibited by phosphorylation by *LIM kinases*[58] (LIMK). Cofilin not only breaks the actin filament, but also prevents lengthening. *Slingshot* (Ssh) phosphatases[59] and LIMK regulate actin dynamics via a reversible inactivation of *actin-depolymerizing factor* (ADF) and cofilin.[60] *Chronophin* is a phosphatase that

[56] The cell motility requires contractile proteins (actin, and myosin) with adequate ATP supply under the control of regulatory proteins.

[57] The sol state corresponds to a suspension of large particles. The gel is stiffer. Gelation is associated with the conversion of actin into actin filaments.

[58] LIMK is a serine/threonine kinase containing a LIM domain.

[59] Slingshot protein phosphatase dephosphorylates LIM kinase and cofilin.

[60] A protein complex consisting of Ssh1L, LIMK1, actin, and a scaffold protein regulates, with kinase PAK4, the ADF/cofilin activity [72].

dephosphorylates cofilin [73]. Both chronophin and LIMK are required to control cofilin activity. The *α-actinin* favors formation of actin stress fibers. The α-actinin is found in adhesion plaques, in filament–membrane binding sites, and within actin filaments.

The mechanical properties of the actin cytoskeleton are defined by the local activation of different actin cross-linking proteins [74]. These cross-linkers, which belong to a subset of actin-binding proteins, determine the structure of actin networks. Cross-linkers vary: (1) according to the actin-binding affinity of their binding domains; and (2) in the structure, number, and organization of their spacing rod domains, which separate the binding domain. There are short bundling cross-linkers, such as plastin and fascin, and long cross-linkers, such as α-actinin or filamin.[61] There are rigid cross-linkers, such as scruin, and compliant cross-linkers. The actin elastic modulus depends on the concentration of cross-linkers.

The *myosin filaments* (6 nm thickness) are localized along the actin filaments. They have about the same bore as actin filaments; they are thinner than those in muscle cells. There are different myosin types. Myosins-1 and -2 are the most abundant. Myosin-2 is found in muscle. Myosin-1 in association with actin displace cell organelles. Myosin-2 and actin bind weakly in the presence of ATP, and strongly with MgADP. Actin binding leads to conformational changes in myosin and to subsequent sequential release of hydrolysis products of ATP, phosphate, and then ADP. Myosins move toward the barbed plus-end of actin filaments, except myosin-6, implicated in cellular transport, which moves toward the minus-end.

A 3D fiber *geodesic dome* (geodome) surrounds the cell nucleus. From its vertices, filament bundles project toward the cell cortex. Geodome foci contain α-actinin and edges tropomyosin, whereas fibers attached to the vertices that extend peripherally contain actin, α-actinin, and tropomyosin.

Actin and myosin not only function as molecular motors for muscle contraction, cell motility, and cell division, forming actomyosin complexes, but are also involved in transcription[62] of DNA into RNA [75]. β-Actin and actin-related protein BAF53 associate with chromatin-remodeling complex BAF [76]. Myosin-1, a single-headed myosin, is observed in the nucleus [77]. Motions of transcriptional complexes and/or DNA are necessary to transcribe the genetic code. RNA polymerase-2 could move transcriptional complexes relative to DNA. Hence, nuclear actin and myosin-1 might be auxiliary motor to RNA polymerase-2. They also might have a structural role. Actin and actin-related proteins remodel chromatin. Actin interacts with the heterogeneous nuclear ribonucleoprotein complexes.[63] Conversely, the eukaryotic translation

[61] Short cross-linkers form filaments from isolated bundles.

[62] Transcription requires chromatin remodeling, formation of preinitiation complexes, binding of transcription factors to regulatory regions of DNA, recruitment of RNA polymerase, etc.

[63] Heterogeneous nuclear ribonucleoproteins are involved in newly transcribed RNA processing and transport.

elongation factor 1A (eEF1A or EF1), a G-protein that delivers aminoacyl-tRNA to the elongating ribosome, is implicated in the actin cytoskeleton [78].

1.5.2 Microtubules

The *microtubules* (internal and external diameters of $\sim$ 15 and 24–25 nm, length of 10–50 μm) are long polymers of α and β *tubulins* with polarity. They are characterized by a dynamic instability, evolving between growth and shrinking. The microtubule length adjusts to the cell size. The filament, indeed, collapses when it comes into contact with the cell edge. Microtubule dynamics can be regulated by applied stresses. The microtubules are thicker and less stable than microfilaments. They are the stiffer element of the cytoskeleton.

Tubulin subunits link into microtubules, which in turn aggregate to form more complex bodies. The tubulin polymerizes in the presence of guanosine triphosphate and calcium. α and β-Tubulin heterodimers associate laterally[64] and longitudinally to form polar, cylindrical microtubules. α and β-Tubulin polymerization is favored by the guanine nucleotide bound to β-tubulin. GTP-tubulin, indeed, leads to a conformation suitable for interactions between the dimers of the protofilaments. When GTP-tubulin is incorporated into the microtubule lattice, it is hydrolyzed because microtubules are mainly built from GDP-tubulin at inter-dimer contacts. GTP-tubulin is added to the microtubule end for microtubule growth.

The dynamic microtubule cytoskeleton regulates microtubule lengths. Microtubule dynamics, i.e., the balance between microtubule growth and microtubule depolymerization, is regulated by several *microtubule-associated proteins*. Certain can form a scaffold for the recruitment of other proteins exerting growth and/or stabilizing functions. Microtubule-associated proteins (MAP) hence facilitate microtubule assembling. Both MAP1 and MAP2 enhance the microtubule growth rate. Other microtubule-associated proteins only stabilize the microtubule skeleton. Among the microtubule-associated proteins, there are the plus-end tracking proteins, such as protein end binding-1, which accumulates at the growing microtubule plus end.

Guanosine triphosphate bound to β-tubulin is hydrolyzed to guanosine diphosphate during microtubule assembly.[65] Tubulins in microtubules are arranged in many protofilaments, parallel to the microtubule axis. Microtubules continuously grow and shrink during the polymerization and depolymerization of tubulin. GTP hydrolysis, associated with the microtubule depolymerization, promotes protofilament outward curvature [79]. In the presence of microtubule-associated proteins or certain divalent cations, GDP-tubulin bend back on themselves to self-assemble into ring-like structures. GDP-bound subunits of the main microtubule are straight (preferred conformation) because

[64] Long, wide tubulin sheets close to form cylindrical microtubules.

[65] In the presence of tubulin and constant GTP levels, microtubule ends grow and then switch to shortening (structural instability).

of intra- and inter-dimer interactions in the tubulin lattice, except at the ends, which can capture GTP and store strain energy. GTP binding induces a conformational change that straightens the dimer, and allows formation of lateral contacts.[66] Depolymerization releases the strain energy, even in the presence of high concentrations of GTP-tubulin.

The microtubules are organized as a scaffold within the cytoplasma. They control cell–organelle distribution. Mitochondria and the endoplasmic reticulum are located along the microtubule network. The microtubules are required for vesicle formation and traveling across the cytosol. Moving microtubules are used for simultaneous displacement in the two directions of vesicles and other organelles, such as mitochondria with their ATP stores, using ATP as energy source owing to associated ATPase [80]. The heavier the moving body, the slower the motion. Hence, the microtubules serve as information transmission lines.

Two families of motor proteins move along microtubules, kinesins and dyneins, like actin along myosin, having ATPase sites. Most kinesins move toward the microtubule plus-end, whereas some kinesins and dyneins move toward the minus-end. Intracellular transport of organelles involves both dynein and kinesin. The family of ATPases associated with various cellular activities, the Aaa proteins,[67] are involved particularly in the motion of microtubule motor dynein [81]. Minus-end–directed dynein is regulated by several proteins (dynactin, LIS1, NudE, and NudEL) [82].

Dynein and kinesin move along the microtubule at rates of about 0.5 to $3\,\mu m/s$ and generate forces of about 1 to 5 pN. Myosin and kinesin family members undergo conformational changes coupled with ATPase cycle, rotating their heads around the cargo-binding tail site. Dynein can change its transport mode (forward or backward motion) and motion rate (slow or fast). Dynein undergoes ATP-dependent rotation around the head–tail junction; the tail motion is responsible for quick sliding along the microtubule, the head drives slow motions and the ATP hydrolysis rate [83].

Dynein has its motor domain in the heavy chain, which also contains a ring of six mechanoenzymes, ATPases associated with diverse cellular activities. Dynein uses its mechanoenzymes for ATP hydrolysis to amplify conformational changes and generate motion along microtubules toward the minus end. Dynein heavy chain folds and forms three structural domains, the tail with binding sites, Aaa1–Aaa6 ring, and long ($\sim 15\,nm$) antiparallel coiled-coil stalk with its globular tip, which contains the microtubule-binding site. Dynein has two modes of microtubule sliding, one driven by the power stroke of the tail, the other for slower sliding associated with Brownian motion or active tilting of the stalk against the Aaa ring [83].

[66] GDP-tubulin curved dimers hamper the formation of lateral contacts. Furthermore, GDP-tubulin cannot be incorporated into microtubules.

[67] Aaa proteins form a set of enzymes, having an ATPase domain, which induce conformational changes (protein remodeling).

Kinesins participate in cell displacement, providing coordination between cell regions. The kinesin KIF16B, which contains a phosphatidylinositol-3-phosphate–binding Phox (PX)-homology domain, transports early endosomes to the plus end of microtubules, the process being regulated by the small GTPase Rab5 and its effector, the phosphatidylinositol-3 kinase VPS34 [84]. Using optical tweezers, the interaction force of a single macromolecule kinesin with the microtubule has been measured and found to be about 1 pN [85].

Several motor and non-motile microtubule-associated proteins compete for binding to microtubules. Total internal reflection fluorescence microscopy shows which kinesin-1 (conventional type) waits in a strongly bound state on the microtubule with high bond densities until obstacle molecules unbind and frees the binding site for further kinesin processing [86].

ATP-dependent depolymerization of microtubules by motor proteins can use the kinesin-13 family. Kinesin motors reach microtubule ends by ATP-dependent translocation along microtubules. Kinesins undergo directed displacements on microtubules. Kinesins-13 transiently interact with microtubules at rates exceeding those of standard enzyme–substrate kinetics. Kinesins-13 follow a random path on the microtubule lattice during their transient diffusive interaction with the microtubule. Kinesins-13 diffuse according to a three-dimensional mechanism to a one-dimensional one to find the microtubule ends [87].[68]

The *centrosome* is a cell body from which radiate the microtubules [88]. Because the microtubules control actin and intermediary filament distribution, the centrosome organizes the cytoskeleton. It is also involved in intracellular transport. The centrosome contains two *centrioles*, each composed of nine cylindrical elements like a paddle wheel and three microtubules. The microtubules are thus involved in cell division.

Microtubules form a scaffold for the positioning of myosin filaments during sarcomere formation, when myoblasts give birth to striated muscle cell [89]. Myosin indeed moves toward microtubule plus-ends. Furthermore, microtubules are required for the stabilization of myosin-containing elements prior to their incorporation into mature sarcomeres.

1.5.3 Intermediate Filaments

The intermediate filaments (mature bore of 10–12 nm) cross the cytoplasma either as bundles or isolated elements, often in parallel to the microtubules. Intermediate filaments provide resistance to mechanical forces. Intermediate filaments cope with mechanical stresses in conjunction with cell junctions to ensure both rigidity and flexibility. Intermediate filaments are, indeed, linked to cell–cell junctions (desmosomes) by desmoplakin and cadherins and to

[68] The average MCAK time diffusion is equal to 0.83 s with a diffusion coefficient of $0.38\,\mu\,m^2/s$.

cell–matrix junctions (hemidesmosomes, focal adhesions) by plectin, BPAG1, and integrins.[69]

Intermediate filaments are assembled from fibrous proteins. In opposition to both microfilaments and microtubules, intermediate filaments do not have polarity and do not form seeds to which subunits add for filament growth. Cytoplasmic intermediate filament proteins laterally associate to form filaments which can secondarily elongate and reduce their bore. Nuclear lamin assembly is done by simultaneous lateral and longitudinal association of dimers.

In opposition to microtubules and microfilaments, intermediate filaments are made of many different proteins. The intermediate filament proteins include *keratins*, *desmin*, *vimentin*, *lamins*, synemin, syncoilin, and nestin, among others. Desmin and vimentin intermediate filaments can form homopolymers. In the nucleus, intermediate filaments are assembled from lamins.

Intermediate filaments are characterized by a specific expression according to the cell type (mesenchymal vimentin, muscular desmin, epithelial keratin, etc.). Desmogleins and desmocollins anchor different intermediate filaments according to the cell type (keratins in epithelial cells, desmins in cardiomyocytes, and vimentin in endothelial cells). Desmins, abundant near Z-discs, connect sarcomeres to desmosomes in the cardiomyocyte. The coordination of microfilaments and intermediate filaments in the intercalated discs between cardiomyocytes involves plakoglobin and plakophilin. Muscle intermediate filament proteins, synemin and syncoilin, integrate into intermediate filaments via dimerization with vimentin, desmin, α-internexin, or neurofilament protein NF-L. Synemins can bind to α-actinin and vinculin, thus connecting with focal adhesions and microfilaments.

In the perinuclear region of endothelial cells, intermediate filaments are composed of vimentin (Vim). Vimentin associate with integrins, particularly at adhesion sites. The network of intermediate filaments can thus transmit mechanical forces sensed by the plasmalemmal integrins. Vimentin participates to vascular adaptation to flow features. It is implicated in the structural responses of blood vessels to changes in hemodynamic quantities. When vimentin lacks, the endothelial production of either nitric oxide (Sect. 9.5.3) or endothelin (Sect. 9.5.4) is defectuous. Vimentin can also play a role in molecule localization in the cell. Intermediate filaments can interact with molecular complexes involved in regulation and signaling cascades associated with mechanotransduction.

Intermediate filament remodeling and functioning as stress absorbers depend on the combined action of kinases, phosphatases and chaperones. Intermediate filament extensibility is much higher than the one of microfilaments and microtubules. They can bear 250% tensile strain and up to 400% shear strain [90].

[69] The collagen fibers of the extracellular matrix interact with cell integrins. Integrins are connected to membrane-associated collagens, microfilaments, and intermediate filaments.

Five types of intermediate filament proteins have been defined according to their amino-acid composition: types 1 and 2 correspond to acidic and basic keratins (keratins are heterodimers made of basic and acidic partners), type 3 to desmin and GFAP, type 4 to neurofilament proteins (NF-L, NF-M, NF-H, α-internexin, and peripherin), and type 5 to nuclear lamins. Another functional classification leads to three sets according to the assembly mode: keratins, vimentin-like proteins, and lamins. These three kinds can coexist in the same cell, contributing to the cell rheology.

Intermediate filaments build two networks, in the nucleus (attached to the inner nuclear membrane) and in the cytoplasm (connecting the cell junctions at the plasmalemma via integrins to the outer nuclear membrane via plectin and nesprin-3) [90]. The properties of cytoplasmic and nuclear intermediate filaments are different. These two networks withstand tensile and bending stress. Intermediate filaments thus adapt and stabilize the cell shape to environmental conditions. In the nucleus, intermediate filaments provide a skeleton for the assembly of nuclear proteic complexes (including emerin, lamina-associated proteins, lamin-B receptor, heterochromatin protein-1, SMAD proteins, and nesprins). Intermediate filaments can then contribute to the regulation of gene expression.

1.5.4 Modeling of the Cytoskeleton Mechanics

At rest, short actin filaments are surrounded by a pool of actin monomers bound to profilin. Myosin-2 exists in the bent state. Integrins (Sect. 2.2.3) are dispersed over the cell surface. The formation of stress fibers is triggered by an activation signal. Several parallel intracellular pathways exist. Adhesion to the extracellular matrix (Sect. 2.4) triggers a signaling pathway defined by the activity of profilin, cofilin, and gelsolin, which activates phospholipase-C, leading to calcium release from stores. Calcium influx activates gelsolin, which cleaves the capped actin filaments into tiny fragments for formation of long filaments. Calcium-triggered phosphorylation favors myosin-2 extended state for assembly of myosin filaments and formation of stress fibers. These fibers generate tension by cross-bridge cycling between the actin and myosin filaments. When the tension disappears, the stress fibers disassemble.

A model for the cell contractility hence takes into account three processes governing the dynamic remodeling of the cytoskeleton: (1) the activation of actin polymerization and myosin phosphorylation; (2) the tension-dependent assembly of the actin and myosin into stress fibers; and (3) the cross-bridge cycling between actin and myosin that generates tension [91]. The fiber formation rate dependent on the activation signal is coupled to a dissociation rate dependent on the tension. The stress fiber contraction rate depends on the tension via the cross-bridge dynamics. The model predicts the main features: (1) the dependence on substrate compliance, (2) the influence of cell shape and boundary conditions, and (3) the high concentration of the stress fibers at the focal adhesions (Sect. 2.2.5).

Rounded cells commonly have a microfilament mesh in the cytoplasm cortex, just below the membrane. A bi-dimensional model of the cytoskeleton dynamics has been developed to describe stress-induced interactions between actin filaments and anchoring proteins [92]. A small shear induces rearrangement of the four filament population[70] toward an orientation parallel to the streamwise direction.

Reactive flow model of contractile networks of dissociated cytoplasm under an effective stress c_{eff} in a square domain is associated with a system of nonlinear partial derivative equations with boundary conditions [93]. This system requires the following variables: space- and time-dependent network ϕ_{n} and solution ϕ_{s} volume fractions, an effective pressure and velocity field, with the given rheological properties, the network and solution shear $G_{\text{n/s}}$ and dilation $E_{\text{n/s}}$ viscosities, and the network-solution drag coefficient κ_{d}, which are involved in the mass and momentum conservation equations. Two main dynamical modes are observed during contraction of a dissociated wholly polymerized contractile network.[71] (1) Rending-type contraction is related to violent contractions that tear the network. (2) Squeezing-type contraction describes progressive global contractions with gradual expulsion of fluid. Three dimensionless parameters govern the dynamical behavior of the network. According to the value of the rending number, which depends on κ_{d}, G_{n}, and E_{n}, the network contracts into a single mass (small values) or breaks up into clumps (large values). Crucial dynamical factors of cytoskeleton mechanics are: (1) the viscosity of the contractile network associated with an automatic gelation as the network density enlarges, without undergoing large deformation; (2) a cycle of polymerization–depolymerization; and (3) a control of network contractibility and plasmalemmal adhesion.

Continuum hypothesis is supposed to be valid because the problem length scale, although small with respect to the cell size, remains greater than the cell organelle size. It is then assumed that the cell microstructural elements can be neglected. However, cytoskeleton elements are in general smaller than a required size of fifty times greater than the typical cell component. Actin filament behavior is then investigated in a domain that contains a solution of cytoskeleton components rather than the cell itself. The stripped cytoskeleton can also be considered as a discrete structure of stress-bearing components.

Filamentous proteins arranged in a homogeneous, isotropic, cross-linked mesh, uniformly loaded, stiffen from low to intermediate strains, without requiring a specific architecture or multiple elements with different stiffnesses, assuming affine deformations [94]. Stiffer filaments, like F-actin or collagen, stiffen at a few per cent strain, whereas more flexible filaments like vimentin

[70] The population of actin filaments include two subpopulations, whether filaments are moored or not on the cell membrane, each family having two subsets according to presence or absence of connections to other actin filaments.

[71] Experimentally, various components of the cytosolic network are put in a solution in a depolymerized form and polymerize secondarily.

stiffen only at larger strains, approaching 100%. Biological tissues then adapt to loading, using not only the nonlinear passive behavior, i.e., the strain-stiffening, but also stiffness changes associated with contraction of nanomotor proteins of the cytoskeleton.

Tensegrity models[72] of cytoskeleton mechanics are aimed at depicting the essential features of stress-subjected cytoskeleton distortion. Tensegrity models consider deformable cells as a set of beams and cables that sustain tension and compression [95]. For instance, the cytoskeleton model, subjected to 1D traction, can contain six rigid struts (compression-resistant elements that mimic the microtubules), to which pulling forces are applied. The struts are connected to 24 elastic prestressed cables (tension-resistant elements that represent actin filaments), either via frictionless looped junctions (free slip)[73] or pin joints (cross-links)[74] [97]. The stiffness depends on the prestress level, and for a given prestress state, to the applied stretch, in agreement with experimental findings [98]. Non-linear elastic elements can be used to take into account the cell stiffening (strain-hardening) response due to the nonlinear stress–strain relationship. Using a theoretical model of a 30-element tensegrity structure, normalized element length and elastic tension are found to govern the mechanical response of the structure for three types of loading (extension, compression, and shear) [99]. The tensegrity model can also be composed of viscoelastic prestretched cables (Voigt bodies), arranged in a network of 24 cables, associated with 6 rigid bars [100]. The normalized viscosity and elasticity moduli are found to be almost independent or dependent of changes in normalized initial internal tension, respectively. Both quantities depends on the normalized length of the structure.

[72] Tensegrity stands for tensile integrity. Tensegrity structures made of bars linked by cables have several advantages, resistance and high deformability, for a minimal material weight. Tensegrity structures can easily remodel. Tensegrity models are used in civil engineering. This building principle was first described in architecture and constructed by a Fuller's student, the sculptor K. Snelson [96].

[73] The tension in the cables depends on the total cable length, and only tension and compression are transmitted to the model components.

[74] The tension in each cable depends on its length and forces acting at element ends reduced to a single force.

2

Plasma Membrane and Cell Environment

The plasmalemma is the barrier between the extracellular (ECF) and the intracellular fluids (ICF). The plasmalemma thus connects the cell to its environment: (1) neighborhing cells, especially in epithelia, and (2) the extracellular matrix (Sect. 2.4), as well as in the case of endothelium, (3) the flowing blood (Chap. 9).

2.1 Plasmalemma

The phospholipid bilayer of the plasma membrane embeds proteins and glucids.[1] Proteins embedded in the phospholipid bilayer and carbohydrates attached to the membrane surface are required for cell communication and transport across the membrane. The membrane has indeed specialized sites for exchange of information, energy, and nutrients, essentially made from transmembrane proteins. The membrane then has multiple functions. (1) Its continuous sheet separates the cytosol from the extracellular space, but certain membrane elements connect these two interacting compartments. (2) It controls molecule transport, providing a selectively permeable barrier. It contains the carriers for the substance transport across it, from both compartments. (3) It responds to external stimuli, via signal transduction. (4) It has components for cell recognition and adhesion. (5) It organizes cellular biochemical activities, through vesicle transport associated with a submembrane and cytoplasmic scaffold. (6) It maintains cell polarity. (7) It is involved in energy transfer from glucids and lipids to adenosine triphosphate (ATP). The approximate composition of the cell membrane is given in Table 2.1 for the red blood cell.

The deformable plasmalemma has constituents that regulate the flux of materials, react to ligand binding, and interact with the cell environment.

[1] The two layers of different types of lipids, rich in proteins, are asymmetrical.

Three main kinds of lipid are present in cell membranes: phospholipids (Table 2.2), with hydrophilic and hydrophobic ends, cholesterol, and glycolipids. Cholesterol is a major structural and functional component of cellular membranes. Minor disturbances in membrane cholesterol content cause strong changes in membrane physical properties, affecting intracellular signaling and transport. The phospholipid bilayer is impermeable to water soluble molecules and ions. The cell membrane contains functional nanodomains. Rafts allow protein attachment. Certain junctions and signaling pathways use nanodomains.

Most or all plasmalemmal proteins are incorporated in cholesterol-enriched domains (size from 30 to 700 nm, raft regions or non-raft regions) connected to the cortical cytoskeleton and separated by protein-free membrane portions poor in cholesterol [102].

Random movements contribute to cell membrane heterogeneity, although diffusion, which corresponds to the macroscopic effect of the Brownian motion, favors homogeneity.[2] Molecule diffusion in cell membrane differs from pure Brownian diffusion. In a multiphase medium, molecules are trapped

Table 2.1. Approximative composition of an erythrocyte plasmalemma (Source: [6]).

Cholesterol	23
Phosphatidylethanolamine	18
Phosphatidylcholine	17
Phosphatidylserine	7
Sphingomyelin	18
Glycolipids	3
Others	14

Table 2.2. Phospholipid distribution in the two layers of the erythrocyte plasmalemma (Source: [101]).

	External layer	Internal layer
Sphingomyelin	∼20	∼5
Phosphatidylcholine	∼20	∼10
Phosphatidylethanolamine	∼10	∼25
Phosphatidylserine		∼10
Phosphatidylinositol	∼1–2	∼2–3

[2] Under thermal agitation, molecules permanently and randomly move and collide. Homogeneity results from the random continuous spreading of molecules from regions with high molecular concentrations to those with lower concentrations. The spreading rate is given by the diffusion coefficient. The smaller the molecular size, or the lower the molecule density, or the smaller the friction, or the higher the temperature, the greater the diffusion coefficient.

in domains in which positive interactions occur with the nearest neighbors, and negative interactions are minimized. Membrane protein transport is affected by interactions with molecular complexes. Sphingolipids and glycosyl-phosphatidylinositol (GPI)–anchored proteins undergo transient confinements in nanodomains [103]. Cholesterol is packed with glycosphingolipids to form membrane rafts. Lipids thus compartmentalize cell membranes. Cell membrane organization into nanodomains impedes the diffusion of transmembrane proteins. Besides, enzyme activities, membrane recycling, and signaling events permanently disturb the equilibrium of cell membranes and favors nanodomain formation [104]. Moreover, the cytoskeleton hinders lateral transport of transmembrane proteins, thus confining materials into nanodomains. However, lipid compartmentalization in nanodomains is independent of the cytoskeleton.

Membrane rafts and caveolae are membrane nanoregions used for cell transport. A membrane raft is defined as a specialized, tiny (size of a magnitude order O[10 nm]), dynamic (half-life O[100 ns]) membrane domain with signaling and transport functions, formed by GPI-anchored protein clusters associated with cholesterol, phospholipids, and glycosphingolipids. Any activation could modulate the size and stability of rafts. Membrane-raft proteins are involved in signaling. They are stabilized through interactions with the cytoskeleton. They can aggregate into larger platforms in response to various stimuli. Caveolae, flask-shaped invaginations of the plasmalemma differ from membrane rafts. Caveolae contain caveolin, whereas rafts are transient nanodomains stabilized or generated by protein cross-linking. Caveolin, thereby distinguishes caveolae from other membrane rafts. Membrane nanodomains represent connecting platforms of transport and signaling by clustering signaling and trafficking molecules [105].

Integral membrane proteins either cross the lipid bilayer (*transmembrane proteins*), some being able to span it, or are attached to bilayer lipids. *Peripheral membrane proteins* are attached to the integral membrane proteins. The membrane proteins function as carriers, receptors, adhesion sites, or markers (cell recognition). Membrane proteins select ions and molecules to cross the plasmalemma from or to the cytosol. Cell membranes then allow development of concentration gradients. The cell membrane also contains histocompatibility antigens (H-antigens, protein–carbohydrate complexes), that determine the interaction with the immunological system. H-antigen formation is controlled by the major histocompatibility complex (MHC) on chromosome 6.

Membrane proteins represent approximately 30% of the proteome. The activity of plasmalemma proteins is regulated by switching among inactive, active and possible intermediate states, particularly by phosphorylation. Numerous plasmalemmal proteins are attached to the cell membrane by a glycosyl-phosphatidylinositol anchor. GPI-anchored proteins can be removed by phospholipase-C.

Proteases irreversibly cleaves peptide bonds. The membrane proteases include presenilin/γ-secretase, signal peptide peptidases, and rhomboids. The

latter can cut the membrane-docked precursor of the epidermal growth factor (Sect. 10.1), then releasing the extracellular domain, which transmits signals to surrounding cells.

The plasmalemmal prohibitin-domain proteins bind cholesterol and ion channels, especially those involved in mechanotransduction, such as epithelial sodium channels and TRP channels (Sect. 3.1.1). The family includes raft-associated flotillin, podocin, prohibitin, stomatin, etc. The plasmalemmal prohibitin domain proteins regulate the activity of associated ion channels.

Changes in conformational dynamics between folded and unfolded protein states affect the protein stability and binding affinities. Proteins translocate across or into membranes, either in folded or unfolded manner, using or not pre-existing translocons, to function. The translocon allows protein migration across and integration into membranes. The translocon contains a non-specific protein-conducting channel embedded in the membrane and its associated molecules.

Proteins that are secreted (messengers) or inserted in the plasmalemma (sensors and receptors of the cell environment cues), once translated by ribosomes, have a short terminal sequence that assigns the final destination. They are then translocated across or into the cell membrane, using a *translocase*, which can form a complex with the ribosome [106]. The translocase is a protein-conducting channel in the membrane that conveys newly synthesized proteins bearing the appropriate address label.

2.1.1 Glycerophospholipids

Phosphatidylcholine is abundant in cell membrane, corresponding to a large fraction of phospholipids. *Plasmalogen* provides an important quantity of choline glycerophospholipids of the myocardium. Myocardial phospholipase A2 (iPLA2), which is mainly associated with the sarcolemma and does not require calcium, acts on plasmalogen. Activated iPLA2 produces lysoplasmenylcholine and arachidonic acid. Lysoplasmenylcholine alters calcium fluxes. Arachidonic acid decrease gap junction conductance.

Besides, trans-arachidonic acids are produced by NO-dependent NO2-mediated isomerization of arachidonic acid within the cell membrane. They induce time- and concentration-dependent apoptosis of endothelial cells. Trans-arachidonic acid effects are mediated by thrombospondin-1 [107]. *Nitrative stress* leads to microvascular degeneration and ischemia.

Glycerol-3-phosphate is acylated to form lysophosphatidic acid (LPA), which is thought to be further acylated by transmembrane lysophosphatidic acid acyl transferase[3] (LPAAT) to form phosphatidic acid. Phosphatidic acid is a precursor for glycerophospholipids (phosphatidylethanolamine, and phosphatidylinositols). Low LPAAT activities have been assigned to endophilin

[3] LPAATα is localized to the endoplasmic reticulum. It has ubiquitous tissue distribution, whereas LPAATβ has a more limited one.

and CtBP (or BARS), particularly implicated in stabilization of membrane curvature at budding vesicles; but LPAAT activity can be a purification artefact [108].

2.1.2 Phosphoinositides

Phosphoinositides (or phosphatidylinositol PtdIns or PI), although small in amount in the cell membrane among the phospholipids, are involved in numerous cell-life events (Table 2.3). Phosphoinositides are also found on the cytosolic face of intracellular membranes and the nucleoplasm. They particularly regulate nuclear functions, cytoskeletal dynamics, cell signaling, and endocytosis. Their metabolism involves PI kinases and phosphatases (Table 2.4).

Table 2.3. Functions of phosphoinositides (Source: [109]).

	Location	Functions
PtdIns(3)P	Endosomes	Endocytosis
PtdIns(4)P	Golgi	Transport
PtdIns(5)P	Nucleus	Apoptosis
PtdIns(3,4)P2	Plasmalemma	Signaling, Cytoskeletal dynamics
PtdIns(3,5)P2	Endosomes	Osmotic stress, Signaling
PtdIns(4,5)P2	Plasmalemma	Endocytosis
	Nucleus	Cytoskeletal dynamics
PtdIns(3,4,5)P3	Plasmalemma	Signaling, Cytoskeletal dynamics

Table 2.4. Enzymes and their isoforms implicated in the phosphoinositide metabolism for transport and signaling (PIK: phosphatidylinositol kinase; PIPK: phosphatidylinositol phosphate kinase; PTEN: phosphatase and tensin homologue; SHIP: SH2-containing inositol phosphatase; Source: [110]).

PI3K	p100α–p100δ
	PI3K-C2α–PI3K-C2γ
PI4K	PI4K-IIα, PI4K-IIβ
	PI4K-IIIα, PI4K-IIIβ
PI5K	
PIP4K	PIP4Kα–PIP4Kγ
PIP5K	PIP5Kα–PIP5Kγ
3-Phosphatase	PTEN1, PTEN2
4-Phosphatase	Synaptojanin-1, synaptojanin-2
5-Phosphatase	Synaptojanin-1, synaptojanin-2
	SHIP1–SHIP2
	5-Phosphatase-2, 5-phosphatase-4

Phosphoinositides serve as platforms for molecular assemblies recruiting and/or activating effectors at given locations and time. The turnover of membrane phosphatidylinositol and its phosphorylated products, the phosphoinositides,[4] occurs after tissue stimulation. Each of the seven phosphoinositides has a cellular distribution often characterized by a predominant location in membrane subsets.[5] Phosphoinositide segregation on different membranes ensure directional transport from one cell compartment to another. Moreover, phosphoinositides are converted by kinases and phosphatases to adapt their activities to the demand. They also facilitate enzyme recruitment to the appropriate membrane for catalysis when substrates have reached the membrane.

Phosphoinositides also are precursors of intracellular signaling molecules, thereby, involved in smooth muscle cell contraction (Sect. 8.2) and endothelial cell production of vasoactive molecules (Chap. 9). Phosphoinositides can specifically interact with proteins having lipid-binding domains. Phosphoinositides are implicated in variability of membrane lipidic composition between the various cell compartments as well as in heterogeneity within a given membrane, associated with cell trafficking and signaling pathways. Inositol polyphosphates and inositol phospholipids are metabolically and functionally interconnected. Inositol polyphosphates are involved in biosynthesis and protein phosphorylation. Inositol(1,4,5)trisphosphates are required in intracellular calcium signaling. Inositol phospholipids are effectors in acute signaling and regulate the transport across cell membranes and within the cytosol, as well as the cytoskeleton organization.[6] Moreover, they are constitutive cues that define organelle identity.

Phosphoinositides differ in phosphorylation at hydroxyl positions 3, 4 and 5 of the inositol ring. Hence, there are mono- (PIP), bi- (PIP2), and trisphosphate (PIP3 or PtdIns(3,4,5)P3) derivatives (Fig. 2.1). Membrane–bound PIs hence include: (1) singly phosphorylated phosphatidyl inositol (PI(4)P, much more abundant than PI(3)P and PI(5)P); (2) doubly phosphorylated phosphatidyl inositol (PI(4,5)P2, with a much higher level than PI(3,4)P2 and PI(3,5)P2); and (3) triply phosphorylated phosphatidyl inositol PI(3,4,5)P3. The activity of PIs depends on their cellular distribution. PI actions are regulated by PI kinases, PI phosphatases, and PI transfer proteins.

[4] Reversible phosphorylation of the inositol ring of the precursor phosphatidylinositol at positions 3, 4, and 5 generates seven phosphoinositides.

[5] PtdIns(4)P is observed in the plasmalemma, mainly in the Golgi apparatus, and in the endoplasmic reticulum, PtdIns(3)P in early endosomes. PtdIns(4,5)P2 and PtdIns(3,4,5)P3 are located at the plasmalemma. PtdIns(3,4)P2 is mostly found at the plasmalemma and in the early endocytic vesicles, PtdIns(3,5)P2 in early and late endosomes.

[6] Phosphoinositides bind to cytosolic proteins and to cytosolic domains of membrane proteins. They, thereby, regulate the function of membrane proteins and recruit to the membrane cytoskeletal and signaling components.

Phosphatidylinositol(4,5)bisphosphate[7] of the inner leaflet of the cell membrane is synthesized from phosphoinositide by phosphoinositide 4-kinase (PI4K) and phosphoinositide 5-kinase (PI5K). Phosphatidylinositol(4,5) bisphosphate can undergo dephosphorylation by 5-phosphatase to create phosphatidylinositol(4)monophosphate. Phosphoinositide 3-kinase-1 phosphorylates phosphatidylinositol(4,5)bisphosphate and produces phosphatidylinositol (3,4,5)trisphosphate, which regulates small guanosine triphosphatases Rho (Sect. 4.5). PI(3)P can be produced by the action of phosphatidylinositol 3-kinase (PI3K) on PIs or by dephosphorylation of PI(3,4)P2 and PI(3,4,5)P3.

The plasmalemmal distribution and sequestration of highly charged PIP2 is affected by electrostatic interactions and Ca^{++}–calmodulin. Phosphatidylinositol(4,5)bisphosphate and phosphoinositides phosphorylated at the 3 position have direct signaling roles. Phosphatidylinositol(4,5)bisphosphate transduces extracellular signals, either via concentration fluctuations or its metabolites, generating three second messengers (diacylglycerol, inositol triphosphate, and phosphatidylinositol trisphosphate). Cleavage by phospholipases, such as phospholipase-C and phospholipase-A2, produces signaling metabolites. Dephosphorylation mainly by 5-phosphatase switches off its signaling. Phosphorylation by phosphoinositide 3-kinase produces phosphatidylinositol-(3,4,5)trisphosphate, a second messenger for the activation of protein kinase-B and phosphoinositide-dependent kinase-1, in response to growth factor stimulation. Dephosphorylation of PtdIns(3,4,5)P3 at the 3 position by phosphatase

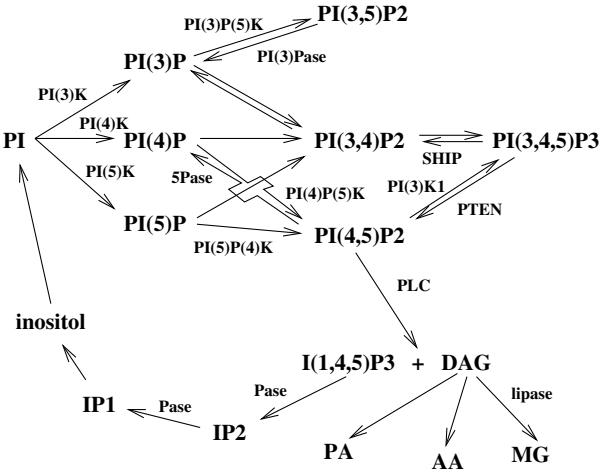

Figure 2.1. Main components of the phosphoinositides (PI) and their relationships (IP: inositol phosphate; AA: arachidonic acid; MG: monoglyceride; PA: phosphatidic acid).

[7] Phosphatidylinositol(4,5)bisphosphate is also known a bisphosphophosphatidylinositol.

and tensin homologue (PTEN) switches off its signaling (Fig. 2.1). Dephosphorylation of PtdIns(3,4,5)P3 by 5-phosphatase, such as SH2-containing inositol phosphatase SHIP1 and SHIP2, produces functional PtdIns(3,4)P2 (Sect. 4.4).

Phosphatidylinositol(4,5)bisphosphate regulates several ion carriers, such as the Na^+/Ca^{++} exchanger, the ATP-sensitive K^+ channel, and also the G-protein–dependent inward rectifier K^+ channel. Furthermore, phosphatidylinositol(4,5)bisphosphate activates enzymes and anchors proteins to the membrane. PtdIns(4,5)P2 is involved in cell transport because: (1) it binds clathrin adaptors and endocytic factors, such as dynamin; and (2) it acts on the actin cytoskeleton. Phosphoinositide-sequestering proteins, such as pipmodulins, buffer PtdIns(4,5)P2 and release it upon demand.

When stimulated by G-protein-coupled receptors (Sect. 4.5), phospholipase-C cleaves phosphatidylinositol(4,5)bisphosphate to manufacture the second messengers inositol(1,4,5)trisphosphate (IP3) and diacylglycerol (DAG). Diacylglycerol activates protein kinase-C. Diacylglycerol is either hydrolyzed by the diacylglycerol lipase into the messenger arachidonic acid or recycled into phosphoinositide synthesis, being phosphorylated by diacylglycerol kinase to phosphatidic acid. Phospholipase-D is associated with the reverse reaction (dephosphorylation), from phosphatidic acid to diacylglycerol by phosphatidic acid phosphohydrolase. Inositol(1,4,5)trisphosphate releases Ca^{++} from intracellular stores associated with inositol-trisphosphate receptors. Inositol(1,4,5)trisphosphate can be converted into inositol by specific phosphatases.

Small guanosine triphosphatases (Sect. 4.5) have a functional relationship with phosphoinositides [111]. Like phosphoinositides, small GTPases help in recruiting cytosolic proteins to specific membrane compartments.[8] Phosphoinositides regulate the recruitment of guanine nucleotide exchange factors and GTPase activating proteins to membranes and their activity. Several enzymes of the phosphoinositide metabolism are effectors of GTPases, inducing feedback loops. Membrane-bound GTPases form coreceptors with phosphoinositides to recruit cytosolic proteins.

2.1.3 Cholesterol

Membrane component lipids (cholesterol, sphingolipids, glycosphingolipids, and lysobisphosphatidic acid) are delivered to late endosomes and lysosomes to undergo hydrolysis. Extracellular lipids, such as low-density lipoproteins (LDL), are transported via endosomes; the LDL receptor recycles to the plasmalemma.

Cholesterol biosynthesis involves a great number of enzymes. The hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme. It is required in the conversion of HMG-CoA into mevalonate (Fig 2.2).

Excess cholesterol is esterified by acyl CoA-cholesterol acyltransferase (ACAT) in the endoplasmic reticulum. The esterified cholesterol is stored in

[8] Ras GTPases are implicated in signaling, Rho GTPases in actin regulation, Arf, Arl, and Rab GTPases in vesicular transport.

short-term cytoplasmic lipid buffers. Cholesterol esters are indeed processed by hydrolases in late endosomes and lysosomes (de-esterification) and released for cell use.[9] Two proteins in late endosomes, NPC1, a transmembrane protein, and NPC2, a luminal protein that binds to cholesterol, are required for efflux of cholesterol from late endosomes.[10] Cholesterol motion back to the cell membrane is done by vesicular and non-vesicular transports. Synthesized cholesterol in the endoplasmic reticulum is conveyed to the plasmalemma, mostly bypassing the Golgi complex.

The membrane sterol-cleavage activating protein transports the sterol-regulatory element-binding protein from the endoplasmic reticulum to the Golgi, thereby activating cholesterol synthesis when the cellular cholesterol level is low. Cholesterol accumulation in the ER membranes changes conformation of the sterol-cleavage activating protein so that this protein binds to Insig [112]. Insig-bound sterol-cleavage activating protein fails to carry sterol-regulatory element-binding protein to the Golgi and hinder cholesterol synthesis. Thereby, sterol-cleavage activating protein dictates the rate of cholesterol synthesis.

Three cellular pathways of cholesterol transport between cells and high-density lipoproteins (HDL; Sect. 6.1) include: (1) passive diffusion from the plasmalemma to plasma HDL; (2) efflux via scavenger receptors B1 (mainly in the liver and in the adrenal gland); and (3) active efflux via ATP binding cassette transporters to lipid-poor apolipoproteins, such as apolipoprotein A1. The two first processes involve bidirectional fluxes of cholesterol, whereas the latter is associated with unidirectional outward transfer of cholesterol.

The family of ATP binding cassette transporters (ABC) delivers cholesterol and phospholipids to apolipoproteins (Fig. 2.3) [113]. ABC-A1[11] pro-

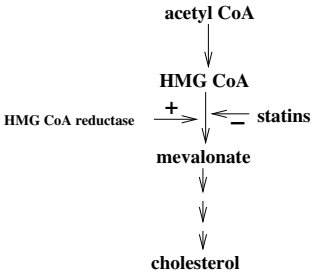

Figure 2.2. Main steps of the cholesterol synthesis.

[9] Esterification/de-esterification cycle represents a major part of cholesterol metabolism.

[10] Niemann-Pick disease C (NPC) is characterized by very slow efflux of cholesterol from late endosomes and subsequent cholesterol accumulation.

[11] ABC-A1 is particularly expressed in the liver, kidney, adrenal glands, intestine, and foam cells (transformed macrophages) of atherosclerotic lesions. ABC-A1 is localized on the plasmalemma, Golgi apparatus, endosomes, and lysosomes.

motes phospholipid transfer to lipid-poor apolipoprotein A1, which can then be transformed into HDL in the blood by lysolecithin-cholesterol acyltransferase (LCAT; Fig. 2.3). ABC-G1, expressed by macrophages and endothelial cells, favors cholesterol efflux from cells to HDLs, which binds to scavenger receptor-B1 in hepatocytes. ABC-G5 and ABC-G8, expressed in the liver, transport cholesterol, and other sterols into the bile.

The nuclear hormone receptors,[12] liver X (LXR) and retinoid X (RXR), are involved in cholesterol transport from peripheral tissues to the liver, as

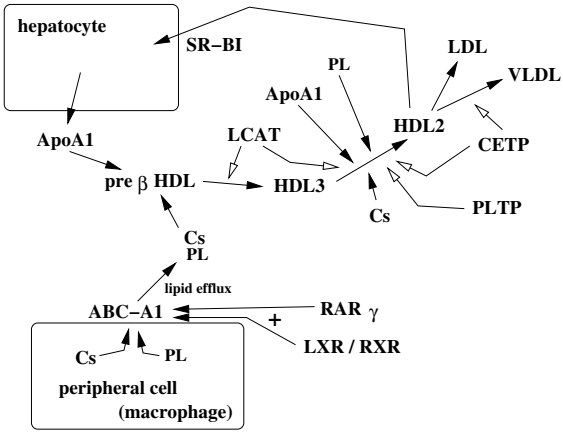

Figure 2.3. Cholesterol (Cs) and its transport via lipoproteins (Sources: [114, 115]). ATP binding cassette (ABC) transporters A1 of the cell membrane favor lipid efflux from cholesterol-loaded peripheral cells, such as macrophages. They are activated by nuclear liver X receptor/retinoid X receptors (LXR/RXR) and retinoid acid receptor-γ (RARγ). Cholesterol and phospholipids form preβ-HDL (a minor fraction of plasma HDLs) with apolipoprotein A1 (ApoA1), which are transformed into small high-density lipoproteins (HDL3) by lysolecithin-cholesterol acyltransferase (LCAT) and secondarily into large HDL2 by addition of Cs, phospholipids and ApoA1. HDL2 give birth to very low-density lipoproteins (VLDL) by cholesterol ester transfer protein (CEPT) and low density lipoproteins (LDL) HDL metabolism involves: (1) the various subspecies of HDLs (lipid-poor preβ-HDL; small, dense HDL3; large, light HDL2); (2) lipid components (apolipoprotein A1 (ApoA1), phospholipid (PL), cholesterol (Cs), cholesterol ester (CE)); (3) enzymes (lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), hepatic lipase (HL), endothelial lipase (EL)); and (4) receptors and transporters (ATP-binding cassette transporter A1 (ABC-A1), scavenger receptor BI (SR-BI)). HDL3 have antiatherogenic effects.

[12] Transcription of ATP-binding cassette carrier ABC-A1, which transports cholesterol and phospholipids across the plasmalemma, is regulated by oxysterol-activated nuclear receptor liver X receptor (LXR), a partner of retinoid X receptor (RXR), which is activated by specific oxysterols (transformed cholesterol). ABC-A1 is hindered by mevalonate products [116].

well as the cholesterol conversion into bile acids and excretion. Cholesterol overload in cells, particularly foam cells (Part II), activates liver X/retinoid X receptors, leading to cholesterol efflux, via activated receptors ABC-A1 and ABC-G1 and apolipoprotein E, and transport to the liver for secretion in bile,[13] using the plasma lipid transfer proteins CETP and PLTP. ABC-A1 might not only act as a phospholipid translocase, but also as a membrane raft.

2.1.4 Membrane Rafts

Membrane rafts are made of cholesterol and glycosphingolipids [117]. They compartmentalize the membrane of the cell and organelles into nanodomains. They are then able to fix or exclude specific lipids and proteins. They contain lipid-modified proteins, like glycosyl phosphatidylinositol-anchored proteins, as well as stomatin, flotillin-1[14] and flotillin-2 acting as scaffolds. Flotillin-1 resides in tiny patches of the plasmalemma, distinct from clathrin-coated pits and caveolae, as well as in certain clathrin-independent endocytic particles with glycosylphosphatidylinositol anchors.

Cytoskeletal proteins, such as spectrin, actin, bands 4.1 and 4.2 are partly associated with membrane rafts. Membrane rafts of the cell membrane form nanodomains with phosphoinositides,[15] which are involved in cell endocytosis. Moreover, they control cell signaling. Consequently, they participate to the regulation of various physiological processes [119]. Although the spatial organization of signaling proteins in the cell membrane is ascribed to membrane rafts, clustering and trapping can also be explained by protein–protein interactions between the relevant signaling molecules [120].

2.1.5 Proteins

The membrane proteins, embedded in the lipidic skeleton, are implicated in several cell activities (Table 2.5). Protein–lipid and protein–protein interactions control the binding of signaling molecules to membrane receptors as well as endocytosis. *Clathrin* and membrane rafts are both required for compound internalization from the cell surface. Glycosylphosphatidylinositol-anchored

[13] In the liver, cholesterol is excreted into the bile both as free cholesterol and after conversion to bile acids.

[14] Flotillin-1 is also called reggie-2.

[15] There are manifold phosphoinositides that interact with specific protein domains, with either a Ca^{++}-dependent or -independent mechanism. They have a restricted distribution. Phosphoinositides then control the localization of phosphoinositide-binding proteins to targeted organelles. Membrane rafts are used for phosphatidylinositol(4,5)biphosphate (PIP2) signaling. Distinct phospholipase-C-coupled receptors, either cholesterol dependent, for neurokinin A, or independent, for endothelin, can share the same PIP2 pool at the plasma membrane [118].

proteins, lipoproteins (nonreceptor tyrosine kinases, Ras, etc.) and transmembrane proteins interact with membrane rafts.

Ion pumps and channels, as well as gap junctions coordinate the electrical activity and molecular exchanges. The communication receptors are transduction molecules, generally proteins. Ligand fixation triggers synthesis of *second messengers*, such as cyclic nucleotides (cyclic adenosine monophosphate [cAMP], and cyclic guanosine monophosphate [cGMP]), phosphoinositides, etc., responsible for cell responses of the extracellular ligand (first messenger). Other molecules are involved in cell organization and adhesion. Protein insertions into target membranes depend on translocations, abundance of nonpolar residues in transmembrane helices, and position of polar residues within transmembrane segments [121].

2.1.6 Glucids

Membrane glucidic copulas of the external membrane layer contribute to the membrane asymmetry. Membrane glucids participate in the protein structure and stability of the membrane. Membrane glucids modulate the function of membrane proteins. Surface polysaccharids are directly used for cell recognition and adhesion. For example, laminin, a glycoprotein, allows adhesion of endothelial cells to collagen. The *macrophage migration-inhibitory factor* (MMIF) released by activated T lymphocytes, binds to membrane glucids of macrophages to inhibit their migration once they are on the working site.

Table 2.5. Membrane proteins

Function	Type	Effect
Mass transfer	Ion pump Ion exchanger Ion channel	Cell polarity, Cell functioning, Cell metabolism
	Vesicle (clathrin, caveolin)	Endo/exocytosis
	Gap junction (connexin)	Electrical activity coordination Molecule exchange
Transduction	Receptor	Signaling
Cell organization & adhesion	Adhesion molecules Cadherin, Selectin Integrin IgCAM	Tissue stability Signaling ECM-Csk connections Cell–cell interactions Extravasation

2.2 Adhesion Molecules

The coordinated expression by cells of a various types of adhesions, either with the extracellular matrix or adjoining cells, regulate the cell shape, tissue structure, and cell motility. In particuler, cellular junctions maintain the structural integrity of tissues.

Zipcode-binding proteins IMPs[16] (IMP1, IMP2, and IMP3), members of the RNA-binding protein family mainly located in the cytoplasm, are required for cell motility, cell adhesion, and cytoplasmic spreading.

Adhesion molecules are plasmalemmal proteins used for binding either other cells or the extracellular matrix. Adhesion molecules also serve as signaling components that control cell growth and division, cell differentiation, and cell apoptosis. Adhesion molecules are composed of three domains: (1) an intracellular domain interacting with the cytoskeleton, (2) a transmembrane domain, and (3) an extracellular domain associated with either the same kind (homophilic binding) or other types (heterophilic binding) of adhesion molecules. There are four main sets of adhesion molecules: (1) cadherins, (2) selectins, (3) integrins, and (4) adhesion molecules of the immunoglobulin superfamily. The first classes of substances are calcium-dependent adhesion molecules.

2.2.1 Cadherins

The cadherins, which contain calcium binding sites, connect cells together, one cadherin binding to another in the extracellular space (Table 2.6). They can mediate interactions between the cell cytoskeletons. In particular, cadherins mediate interactions between neighboring endothelial cells. The short cytoplasmic tail of cadherin interacts with catenins, which interacts with cytoplasmic proteins, such as actinin and vinculin, to link the cadherin–catenin complex to the actin cytoskeleton.

β-Catenins bind to the cytoplasmic tail of cadherins, whereas α-catenin can bind to both β-catenin and actin filaments, either directly or via actin-binding proteins. α-Catenins exist either as monomers or homodimers. Monomeric α-catenin preferentially binds to cadherin–β-catenin complexes, whereas its dimeric form is linked to actin filaments. However, cadherin–β-catenin–α-catenin complexes do not necessarily bind to actin filaments. α-Catenin homodimers hamper actin polymerization, which occurs in the presence of actin-related protein complexes Arp2/3 and Wiskott-Aldrich syndrome proteins [122]. α-Catenins transiently bound to cadherin–β-catenin complexes can dissociate from them and bind to actin.

[16] RNA locates in the cytosol according to attached RNA-binding proteins, which dictate the RNA destination. Insulin-like growth factor-2 mRNA-binding proteins (IMP) belong to the zipcode-binding protein family. The same notation is used to designate impedes mitogenic signal propagation compounds, Ras effectors modulating the sensitivity of the MAPK cascade.

Table 2.6. Cadherins (Sources: [123, 124].)

Molecule	Distribution
Cadherin VE (CD144)	Endothelium
Cadherin T, H	Heart
Cadherin, fibroblast 1	FB
Cadherin, fibroblast 2	FB
Cadherin, fibroblast 3	FB
Desmocollin-2	Myocardium, Lymph node
Desmoglein-2	All

Vascular endothelial cadherins (VE-cadherin) anchor the adherens junctions between endothelial cells to p120 catenin (p120Ctn) and β-catenin (βCtn). β-Catenin links VE-cadherin to the actin cytoskeleton via α-catenin. p120Ctn regulates the actin cytoskeleton via the Rho GTPases [125]. VE-cadherin can interact with p120Ctn to mediate cell locomotion and proliferation.

Displacements of endothelial and epithelial cells in cellular layers require coordinated cell junction movements. Although cadherins form adherens junction at the cell apical region, an additional cadherin population is distributed throughout the lateral between-cell adhesion loci. VE-cadherin motions in a basal-apical direction associated with reorganization of actin filaments occur at cell junctions between adjoining moving cells for sliding of contacting cell membranes [126].

2.2.2 Selectins

The extracellular domain of selectins, single transmembrane polypeptides, can reach sizes of about 50 nm [127]. The selectins are expressed in endothelial cells and blood cells for binding two cell surfaces in presence of Ca^{++} (Table 2.7). They slow intravascular leukocytes before transendothelial migration (Sect. 9.3). Three selectin kinds are defined according to the cell in which they were discovered. *L-selectin*[17] is expressed on leukocytes that target activated endothelial cells. *E-selectin*[18] is produced by endothelial cells after

[17] L-selectin is also refered to as CD62L (CD meaning cluster of differentiation with a number assigned by the international workshops on leukocyte typing) or LAM1 or Mel14.

[18] E-selectin is also refered to as CD62E or ELAM1.

Table 2.7. Vascular selectins with three main types: endothelium (E)-selectins, lymphocyte (L)-selectin, and platelet (P)-selectin (Sources: [123, 130–132]).

Molecule	Ligands	Distribution
L-Selectin (CD62L)	Glycosylated mucinlike molecules (Glycam-1, CD34, MadCAM1)	WBC
E-Selectin (CD62E)	Sialyated mucinlike molecules (CD15s or ESL1)	EC
P-Selectin (CD62P)	P-Selectin glycoprotein ligand-1 (PSGL1 or CD162)	EC, TC

cytokine activation. *P-selectin*[19] is preformed and stored for rapid release in platelet granules or Weibel-Palade bodies[20] of endothelial cells [129].

2.2.3 Integrins

The integrins are transmembrane glycoproteins (GP) that connect actin filaments of the cell cytoskeleton to the proteins of the extracellular matrix (ECM). The extracellular domain of integrins can reach sizes of about 20 nm [133]. Binding between integrin and its specific ligand is calcium and magnesium dependent. Integrins that affect organization and assembly of cytoskeletal signaling complexes mediate signaling to or from[21] the environment. These complexes contain cytoskeletal proteins, adaptor proteins, and protein tyrosine kinases, which, once assembled, initiate signaling cascades.[22] Integrin interactions with ECM ligands can trigger signaling events and provide paths for transduction of mechanical forces across the plasma membrane. Integrins

[19] P-selectin is also refered to as CD62P or GMP140 (granule membrane protein 140) or PADGEM. E-selectin and P-selectin have similar roles. P-selectin binds to P-selectin glycoprotein ligand 1 (PSGL1).

[20] Activated endothelial cells release Weibel-Palade bodies, which containing von Willebrand factor and P-selectin, which induce leukocyte rolling and platelet adhesion and aggregation. Weibel-Palade granules thus contain mediators that promote inflammation and coagulation. Prevention of Weibel-Palade body release might be a mechanism by which NO protects the vessel wall from inflammation [128].

[21] The extracellular matrix yields signals that control cell shape, migration, proliferation, differentiation, morphogenesis, and survival. These signals are derived from cryptic sites within matrix molecules (matricryptic sites) that are revealed after structural or conformational modifications of these molecules. Matricryptins refer to enzymatic fragments of ECM containing matricryptic sites [134].

[22] The various molecules that bind to the cytoplasmic part of integrins include tensin, paxilin, zyxin, actin, talin, vinculin, and α-actinin [135].

are also involved in the regulation of vascular tone and vascular permeability. Three main classes of proteic ligands interact with integrins: (1) ECM proteins, (2) plasma proteins, and (3) cell membrane proteins[23] [136].

Integrins are α/β heterodimeric metalloproteins. Their activity is modulated by divalent cations, Ca^{++}, Mg^{++}, and Mn^{++}. Integrins change from a curved, compact shape in low-affinity state to an extended conformation in high-affinity state [137]. Ligand binding induces the extension of the integrin extracellular domain. Ligand binding to the metal ion-dependent adhesion site of α_L I domain is modulated by the β_2 I domain. When integrin extends, its β_2 cytoplasmic and transmembrane domains separate, enabling signal transmission across the plasmalemma. Furthermore, integrin cytoplasmic domains can then cooperate with cytoskeletal components to trigger cell spreading.

Various integrins combine different kinds of two subunits α and β (Table 2.8). Integrins have a large extracellular domain, a membrane region and, most often, a short cytoplasmic part. They are subdivided into classes according to β subunits, which are associated with one or more α subunits [138]. Vascular cells express several members of the integrin family. β_1 Integrins (α_1–α_{11}, α_v),[24] in particular, are elements of platelets.[25] β_2 integrins (α_L, α_M, α_X, α_D) are leukocyte-specific receptors, involved in leukocyte adhesion and transmural migration, granulocyte aggregation, T-lymphocyte killing, and T-helper cell response [139]. Leukocyte integrins $\alpha_L\beta_2$,[26] $\alpha_M\beta_2$[27] and $\alpha_X\beta_2$[28] contain the same CD18 subunit. Leukocytes have $\alpha_1\beta_2$ and $\alpha_M\beta_2$ integrins, respectively, which bind to endothelial cells. β_3 Integrins (α_{IIb}, α_v) are observed in various cells (platelets, endothelial cells, monocytes, smooth muscle cells, etc.). Platelet β_3 integrin subunits bind to fibrinogen during clotting.[29] Other integrins include β_4 (α_6), β_5 (α_v), β_6 (α_v), β_7 (α_4, α_E), β_8 (α_v),.... Integrins

[23] Homophilic interactions means that similar molecules bind to one another, whereas heterophilic interactions refer to the attachment among different molecules.

[24] $\alpha_4\beta_1$ is also called very late antigen-4 (VLA4) or CD49d/CD29.

[25] Glycoproteins-Ia-IIa and Ic-IIa are also termed $\alpha_2\beta_1$ and $\alpha_5\beta_1$ integrins.

[26] $\alpha_L\beta_2$ Integrin is also refered to as leukocyte function associated antigen-1 (LFA1) or CD11a/CD18. It binds to intercellular adhesion molecule-1 (ICAM1) on endothelial cells to promote leukocyte adhesion and transmural migration. LFA1, once bound and activated by ICAM1, triggers abrupt, firm adhesion of rolling lymphocytes [140].

[27] $\alpha_M\beta_2$ Integrin is also termed CD11b/CD18 or Mac1 or CR3.

[28] $\alpha_X\beta_2$ Integrin is also termed CD11c/CD18 or CR4.

[29] Platelet $\alpha_{II}\beta_3$ integrin recognizes fibrinogen, in presence of agonists (ADP, thrombin, thromboxan A2), fibronectin, von Willebrand factor, and vitronectin.

Table 2.8. Vascular integrins (Sources: [123, 131, 132, 141]).

Molecule	Ligands	Distribution
$\alpha_1\beta_1$	Ln, Cn	NK, B & T Lφ, FB, EC
$\alpha_2\beta_1$	Ln, Cn	NK, B & T Lφ, TC, FB, EC
$\alpha_3\beta_1$	Ln, Cn, FN	T Lφ, FB, EC
$\alpha_4\beta_1$	VCAM1, ICAM1, FN	NK, B & T Lφ, Eφ, Mo, EC
$\alpha_5\beta_1$	FN	B & T Lφ, TC, FB, EC
$\alpha_6\beta_1$	Ln	WBC, FB, EC
$\alpha_7\beta_1$	Ln	CMC, FB, EC
$\alpha_{11}\beta_1$	Cn	CMC, SMC
$\alpha_L\beta_2$	ICAMs	Nφ, Mo, Mφ, T, B & NK Lφ
$\alpha_M\beta_2$	ICAMs, FX, Fng	NK & B Lφ, Nφ, Mφ
$\alpha_X\beta_2$	Fng	B Lφ, Mφ
$\alpha_{IIb}\beta_3$	FN, VN, vWF, TSP	TC
$\alpha_v\beta_3$	FN, VN, Cn, PECAM1,vWF, Fng, TSP	B & T Lφ, Mo, EC
$\alpha_6\beta_4$	Ln	FB, EC
$\alpha_v\beta_5$	FN, VN, Fng	FB, Mo, Mφ
$\alpha_4\beta_7$	FN, VCAM1, MadCAM	NK, B & T Lφ
$\alpha_{IEL}\beta_7$	E-cadherin	T Lφ

functions are regulated by: (1) the level of integrin expression,[30] (2) divalent cations,[31] (3) by cellular environment,[32] and (4) affinity modulation[33] [138].

Various proteins link the integrins to the cytoskeleton, such as tensin and filamin. Among these proteins, certain ones have several binding sites; therefore cross-linking actin filaments. They include α-actinin, fimbrin, ezrin–radixin–moesin (ERM). ERMs coordinate signals triggered by cytokine-induced adhesion molecules with extracellular adhesive functions [142]. Moreover, ERM is a marker of Rho-kinase activity.

Integrins switch between active and inactive conformations. In the inactive state, integrins have a low affinity for ligands. Signaling events induce a conformational change with exposure of the ligand-binding site. Ligand-integrin binding connects the actin cytoskeleton to the extracellular matrix, via the assembly of a proteic complex, and interacts with signaling pathways. Mechanical stresses modify the conformational state of integrins, which form

[30] The transcription control of integrin expression promotes a given cell type adhesion while preventing others from interacting negatively, like in angiogenesis.

[31] Integrin–ligand binding require divalent cations, such as Ca^{++}, Mg^{++}, Mn^{++}. This binding thus depends on the content of the extracellular medium. Different divalent cations can have opposite effects on a given integrin.

[32] The ligand specificities vary according to the cell type ($\alpha_2\beta_1$ on platelets binds to collagen and on endothelial cells to collagen and laminin). This cell-type–specific binding depends on the cell environment.

[33] Integrin function can be regulated by both the number of expressed receptors and the environment.

new connections with their specific ECM ligands and cytosolic molecules to relay the shear stress signaling to intracellular pathways and increase cell binding [143].

Integrin-linked kinase (ILK), isoforms of particularly interesting Cys-His-rich protein (PINCH), and parvin form the IPP complex in the cytosol [144].[34] The IPP complex is recruited at focal adhesions with paxillin, vinculin, and focal adhesion kinases and builds an interface between integrins on the one hand and the actin cytoskeleton and signaling pathways on the other. IPP complex participates to the regulation of endothelial cell and cardiomyocyte migration, leukocyte recruitment and platelet aggregation.

The non-receptor tyrosine kinase Ack (Sect. 4.3.1) is ubiquitously expressed. Ack is phosphorylated (activated) by activated integrins linked to cell adhesion on fibronectin by growth factors, such as epidermal growth factor and platelet-derived growth factor (Sect. 10.1). Ack is an early transducer of extracellular stimuli. Ack interacts with adaptors and Cdc42 guanosine triphosphatase. Ack can also play a role in vesicle dynamics.

2.2.4 Immunoglobulin-Like Cell Adhesion Molecules

Certain members of the immunoglobulin (Ig) superfamily, the *Ig cell adhesion molecules* (IgCAM), are involved in calcium-independent cell to cell binding (Table 2.9). Among them, *intercellular adhesion molecules* (ICAM) are expressed on activated endothelial cells, being the ligand for integrins expressed by leukocytes. ICAM1 (CD54) induce reversible adhesion, stabilizing leukocyte for possible extravasation. *Platelet–endothelial cell adhesion molecule*-1 (PECAM1 or CD31) belongs to leukocytes, platelets, and intercellular junctions of endothelial cells. *Vascular cell adhesion molecule*-1 (VCAM1), once binds to $\alpha_4\beta_1$ integrin, induces firm adhesion of leukocytes on endothelium.

Junctional adhesion molecules (JAM) belong to the set of Ca^{++}-independent immunoglobulin-like cell adhesion molecules of the immunoglobulin family. Junctional adhesion molecules are expressed by endothelial cells, leukocytes,

[34] Integrin-linked kinase binds: (1) PINCH and ILK-associated phosphatase on its ankyrin domain; (2) possibly phosphatidylinositol(3,4,5)trisphosphate on its plekstrin homology domain; (3) parvin, paxillin, MIG2/kindlin-2, β1 and β3 integrins, protein kinase B, phosphatidylinositol-3-kinase-dependent kinase-1 on its kinase domain. MIG2/kindlin-2 binds to migfilin, which links to filamin and interacts with filamentous actin and integrins. PINCH, an adaptor protein, binds to receptor tyrosine kinases. PINCH1 binds to Ras-suppressor protein RSU1, a negative regulator of Jun N-terminal kinase, and to receptor-tyrosine-kinase adaptor protein NCK2. α-Parvin binds to F-actin, and kinase TESK1, which phosphorylates cofilin. β-Parvin binds to F-actin, α-actinin, and guanine nucleotide–exchange factor α-PIX. α-PIX binds to Rac1/Cdc42 effector PAK1, and calpain-4, which cleaves talin. When talin is recruited to the plasmalemma and activated in association with phosphatidylinositol phosphate kinase-1γ (PIPK1γ), it binds to β-integrins.

and platelets. JAMs support both homophilic and heterophilic interactions.[35]

Junctional adhesion molecules include JAM1 (or JAM-A), JAM2 (or JAM-B), JAM3 (or JAM-C), JAM4, and JAM-like [145]. JAM1 is located in the apical region of tight junctions, where it links cingulin, occludin, protein zonula occludens-1, etc. JAM1 exists on endothelial cells, circulating cells, and antigen-presenting cells. JAM1 can interact with $\alpha_L\beta_2$-integrins. JAM1 is highly expressed in the brain vasculature, where it stabilizes cell junctions, thereby reducing the vascular permeability. JAM2 and JAM3 are expressed by endothelial cells. JAM2 can interact with $\alpha_4\beta_1$-integrins. JAM3 is also expressed by platelets, monocytes, natural killer lymphocytes, dendritic cells, B lymphocytes, and a subset of T lymphocytes. JAM3 is strongly expressed in lymph nodes where it destabilizes cadherin junctions, thus increasing the vascular permeability. JAM3 is also found in high endothelial venules. JAM3 binds to leukocyte $\alpha_M\beta_2$-integrins and $\alpha_X\beta_2$-integrins in order to link thrombocytes to leukocytes.

2.2.5 Focal Adhesions

Focal adhesions (2–10 µm long, 0.25–0.5 µm wide, 10–15 nm gap) are complexes of clustered integrins and associated proteins that link the components of the extracellular matrix (fibronectin, collagen, laminin, vitronectin) with the cytoskeleton of cultured cells and mediate cell adhesion [146]. These cell–matrix junctions, also defined as focal adhesion plaques or focal contacts, differ from the hemidesmosomes. Actin filaments attach to specific membrane proteins (rather than intermediate filaments).

Table 2.9. Vascular IgCAMs (Sources: [123, 131, 132]).

Molecule	Ligands	Distribution
CD166	CD6, CD166	WBC
CD147		WBC, RBC, TC, EC
CD22	CD45	B Lφ
CD44	Ankyrin, FN, etc.	Lφ
ICAM1 (CD54)	$\alpha_4\beta_1$, $\alpha_L\beta_2$, $\alpha_M\beta_2$	WBC, EC
ICAM2 (CD102)	$\alpha_L\beta_2$	Lφ, EC, Mo
ICAM3 (CD50)	$\alpha_L\beta_2$	WBC
PECAM (CD31)	CD31,$\alpha_v\beta_3$	WBC, EC
VCAM1 (CD106)	$\alpha_4\beta_1$, $\alpha_4\beta_7$	Mo, EC

[35] JAM1 homophilic interactions are used for platelet adhesion to the endothelium [145]. JAM3 has a higher affinity for heterodimerization with JAM2 than for homodimerization. JAM3 heterophilic interactions also exist with integrins for platelet adhesion to leukocytes and for extravasation of leukocytes.

Focal adhesion proteins (FAP) include talin, vinculin, tensin, paxillin, and focal adhesion kinase, among others[36] [147]. Talin and vinculin are cytoskeletal proteins that bind integrins and F-actin cytoskeleton [148]. *Talin* possesses actin-binding and vinculin-binding sites and also binds to β integrin cytoplasmic tails [149]. *Vinculin* binds F-actin and may cross-link talin and actin, thereby stabilizing the interaction [150]. Talin, Vinc, and Pax regulate the formation of focal adhesions and stress fibers.

2.3 Cellular Junctions

Cellular junctions are tiny specialized regions of the plasmalemma. Several histological and functional categories include: (1) impermeable junctions, which maintain an internal area chemically distinct from surroundings; (2) adhering junctions, which reinforce tissue integrity; and (3) communicating junctions for exchange of nutrients and signals with the environment (Fig. 2.4, Table 2.10). Within the junctions, membrane proteins have specific configurations. Integrins and cadherins are associated with signaling molecules, leading either to cell proliferation or apoptosis (Sect. 2.2). Cell junctions can remodel.

2.3.1 Desmosomes

The desmosomes, or macula adherens, are large bundles that anchor one cell to its neighbors. Desmosomes contain two classes of desmosomal cadherins, the *desmocollins* and the *desmogleins*, each having several subtypes, that are specific to differentiation status and cell type. The intercellular space is filled with filaments that bridge not only membranes, but also cytoskeletons of adjacent cells. Two main desmosome types exist: belt and spot desmosomes. *Belt desmosomes* contain actin filament susceptible to contract in presence of ATP, Ca^{++}, Mg^{++}, to close the gap during cell apoptosis. *Spot desmosomes* contain filaments and transmembrane linkers which connect cytoplasmic networks of tonofilaments bundles for mechanical coupling between adjacent cells. *Hemidesmosome*[37] allows adhesion of cells to basement membranes for tissue

[36] The components of focal adhesions are: (1) cytoskeletal proteins, with direct or indirect association with actin, without enzymatic activity, such as tensin, vinculin, paxillin, α-actinin, parvin/actopaxin and talin; (2) tyrosine kinases, such as members of the Src family, FAK, PYK2, CSK and Abl; (3) serine/threonine kinases, such as ILK, PKC and PAK; (4) modulators of small GTPases, such as ASAP1, Graf and PSGAP; (5) phosphatases, such as SHP2 and LAR PTP; and (6) other enzymes, such as PI3K and calpain-2. However, all these components are not necessarily constitutively located in all focal adhesions [146].

[37] Hemidesmosomes are small multiprotein complexes in the basal cell surface of endothelial cells, which resemble half of a desmosome. Hemidesmosomes connect the elements of the cellular cytoskeleton to the underlying basal lamina. They are present at least in the basal layer of the stratified and compound epithelia.

mechanical integration via links between integrin and collagen-7. Hemidesmosomes also serve as anchoring sites for tonofilaments bundles. Cells subjected to mechanical stresses have numerous spot desmosomes and hemidesmosomes, which limit cell distensibility and distribute stresses among layer cells and to the underlying tissues to minimize disruptive effects.

2.3.2 Zonula Adherens

The zonula adherens, or adherens junction, is defined by cell-to-cell adhesion via cadherin–calcium dependent bridging [152]. It forms adhesion belts that link adjacent epithelial or endothelial cells.[38] These cadherin-based adhesive contacts link not only the cytoskeletal proteins of a given cell to the cytoskeleton of its neighboring cells, but also to the proteins of the extracellular matrix.

Table 2.10. Cellular junction features.

Junction	Function	Attachment	Intermembrane space (nm)	Associated molecules
Desmosome	Cell–cell anchor	Cadherin	25–35 Iintermediate	filaments
Hemidesmosome	Cell–BM adhesion	Integrin	25–35 Iintermediate	filaments
Adhesion belt	Cell–cell adhesion	Cadherin Integrin	10–25	Actin filaments
Focal contact	Cell–ECM adhesion	Cadherin Integrin	20–25	Actin filaments
Tight junction	Occlusion of between-cell space	Membrane-joining strands	<1	Membrane proteins
Gap junction	Cell–cell communication	Connecting channel	2–3	Connexin

Hemidesmosomes act in signal transduction via integrin and regulate cellular activities. Hemidesmosomes do not link adjacent cells.

[38] Epithelial and endothelial cells are able to tightly join, via intercellular adhesion, often with an asymmetric architecture of protein and lipid constituents of the plasmalemma, leading to an apical–basal polarity. Adherens junctions contain E-cadherins. Localization of E-cadherins is stabilized via the recruitment of actin filaments either by E-cadherin or synaptotagmin-like protein bitesize independently of E-cadherin [153]. F-actin is required to stabilize E-cadherin–β-catenin–α-catenin complexes. Bitesize is mainly required to organize F-actin in the apical junctional region. Bitesize binds to phosphatidylinositol(4,5)bisphosphate, to Par3, and to F-actin-binding protein moesin of the ezrin–radixin–moesin complex.

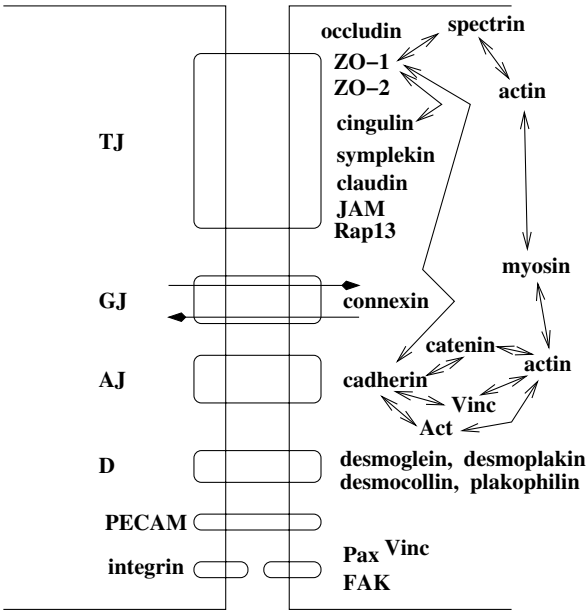

Figure 2.4. Endothelial cell junctions. Tight junction (TJ) is formed by occludin and peripheral membrane proteins, such as cingulin, GTP-binding Rap-13. ZO1 binds to spectrin and cingulin. ZO1 cross-links catenin/cadherin complex. Cadherins (VE-, P-, and N-cadherins) are found in adherens junction (AJ), as well as clusters of VE-cadherin (predominant cadherin) that bind to actin. Cadherins bind to the cytoskeleton via catenins and plakoglobin. β-Catenins bind to α-actinin (Act), vinculin (Vinc), and actin and to VE-cadherin with γ-catenin. Tight junction protein occludin and adherens junction proteins β-catenin and VE-cadherin are co-localized in endothelial cells. Platelet endothelial cell adhesion molecule (PECAM1) maintains junction integrity. Connexins form gap junctions (GJ). Integrins bridge the cells. Desmosomes (D) are made of desmoplakins. Vinculin is located along junctions (Source: [151]).

The adherens junction then is a third kind of anchoring junctions, with its cytoskeletal protein[39] anchors and transmembrane linker proteins.

Actin filaments are associated to the adherens junctions through catenins. Three types of catenins exist, α, β, and γ-catenin (or plakoglobin). β-catenin and plakoglobin bind directly to the cadherin, whereas α-catenin links β-catenin or plakoglobin and then the cadherin–catenin complex to the actin cytoskeleton.

[39] The actin filaments are linked to the adherens junctions rather than the intermediate filaments, which are mainly associated with the desmosome and the hemidesmosome.

2.3.3 Tight Junctions

Tight junctions (TJ), or *zonula occludens*, form protein junctions that leave tiny between-cell space (<1 nm).[40] Membrane of adjacent cells fuse at a point set.[41] They selectively modulate paracellular permeability and act as a boundary between apical and basolateral plasma membrane. Several proteins are involved: *cingulin*, *claudin*,[42] *occludin*,[43] *junctional adhesion molecules* (JAM), *symplekin*, *zonula occludens proteins* (ZO),[44] etc. Two rows of integral membrane proteins, each adjacent membrane contributing for one row, holds the two membranes close together by reciprocal contacts. Sealing strands, which form attachment lines between adjacent cells, are organized into a flexible network that modifies the junction tightness according to local physiological needs and maintains the cell sealing under stress. E-cadherin is specifically required for tight junction formation and is involved in signaling rather than cell contact [156].

The guanosine triphosphatase RhoA (Sect. 4.5.3) regulates the tight junction assembly and cell polarity. The polarity protein Par6 interacts with the transforming growth factor-β receptors (Par6 is a substrate of receptor-2) and with E3 ubiquitin ligase Smurf1, which degrades RhoA, leading to a loss in tight junctions [157].

The plasma membrane of epithelial cells is divided by tight junctions into distinct apical and basolateral regions with different protein and lipid compositions. In polarized epithelial cells, phosphatidylinositol(3,4,5)trisphosphate, which is stably localized at the basolateral plasmalemma, regulates the formation of this domain of the cell surface [158].

Whereas a cell apico-basal polarity complex located in tight junctions of any epithelial cell consists of partitioning defective proteins PAR3 and PAR6, as well as atypical protein kinase C (aPKC) and Rac1 guanine nucleotide exchange factor Tiam1, another proteic complex, which associates PAR3 and PAR6 with VE-cadherin, but lacks aPKC, exists in adherens junctions of endothelial cells [159].

In the endothelium, JAMs, as well as occludins and claudins, are integral membrane proteins located at tight junctions. Junctional adhesion molecules thus control the vascular permeability. JAMs are also involved in angiogenesis in association with $\alpha_V\beta_3$-integrins, as well as in inflammation, being required for leukocyte recruitment and transendothelial diapedesis.

JAMs redistributes from the cell junctions to the apical surface of stimulated endothelial cells. Nectins, immunoglobulin-like cell adhesion molecules,

[40] The tight junction dimensions average 27.4 nm in width and 1.1 μm in length.

[41] The membranes are not cemented together. Such intimate contact blocks molecule passages through the obliterated intercellular space.

[42] Claudin assembly modes increase the diversity of the structure and functions of tight junction strands [154].

[43] Occludin brings opposite external cell leaflets into contact.

[44] There are several zonula occludens proteins: ZO1, ZO2, and ZO3 [155].

recruit cadherins to form adherens junctions, and afterward, claudins, occludin, and junctional adhesion molecules, owing to peripheral membrane proteins (among which are many actin filament-binding proteins), to the apical side of adherens junctions to build tight junctions.

2.3.4 Gap Junctions

Gap junctions, or *nexus* or *macula communicans*, build between-cell channels, which bridges adjacent membranes and thus connect the cytosol of neighboring cells (bore of 1.2–2 nm). These intercellular proteic channels allow low molecular weight molecules ($< \sim 1$ kDa), small signaling molecules, such as second messengers inositol(1,4,5)trisphosphate and cyclic AMP, and ions to diffuse between neighboring cells (metabolic and signaling coupling).[45] These clusters of membrane channels transmit electrochemical current between adjoining cells for coordinated activity (electrical coupling).

At a gap junction, each cell provides hemichannels or *connexons* that dock one to one with hemichannels in the other cell. Hemichannels are hexamers, homomeric or heteromeric according to the *connexin* kind number. Connexon–containing vesicles are transported to the cell surface and fuse with the cell membrane. Inserted hemichannels diffuse in the membrane until they dock with a hemichannel in an apposed membrane to form a cell–cell channel. Pairs of connexons, made by a hexameric array of connexins, thus form intercellular membrane channel.

Phosphorylation of connexin-43 in gap junctions by PKC reduces the permeability to large hydrophilic solutes (ATP, cAMP, IP3, NAD^+), but not to ions. Connexin-43 phosphorylation thus does not affect the propagation of the electrochemical wave.

Gap junctions of the cardiomyocyte mainly consist of two connexons made from six connexins.[46] Various connexins are involved in gap junctions. Connexins, encoded by a gene family, are commonly named by their molecular mass (Cx·). Different connexins assemble to form junctions, which differ in channel conductance, gating, permeability depending on both size and charge, as well as temporal and spatial patterns of expression. Another class of proteins, pannexins, can form gap junctions.

Conformational changes close the channel. Gap junction permeability is controlled by multiple factors, calcium concentration [Ca^{++}], mediated by calmodulin, pH, membrane potential, protein phosphorylation, etc..

[45] Gap junctions mediate between-cell transfer of small molecules like amino acids, nucleotids, vitamins, hormones, cAMP, glucids, etc.

[46] Many kinds of connexins are expressed by the myocardium with possible heterotypic (heterogeneous connexons, each made from a single connexin type), heteromeric (connexons made from different connexins) and homotypic junctions.

2.3.5 Mechanical Effects

Forces are applied to the cell by the cell environment, and conversely, forces are produced by the cell and transmitted via the cytoskeleton and the adhesion sites to the surrounding medium. The interface between the cell and extracellular space is characterized by submembrane plaques composed of integrins, adaptor, and anchor proteins. Numerous stress-induced responses of cells, particularly endothelial cells, which must withdraw shear stress exerted by the flowing blood on its wetted surface, require integrins.

Focal adhesions sense loadings undergone by the cell. The size of focal adhesions increases when any loading is applied to the cell, the number of molecular interactions rising due to conformational changes of proteins involved in adhesion sites or molecule recruitment. Conversely, the size of focal adhesions decreases when loading is removed [160].

Stretch-dependent growth of cell adhesion sites is mediated by Rho GTPases via Rho kinases and Dia1 protein (Chap. 4). Rho kinases activate myosin-2, Dia1 induces focal adhesion assembly and regulates microtubule dynamics. Microtubules stop focal adhesion growth and suppress cellular contractibility via microtubule-associated motors, such as kinesin, and microtubule-associated signaling molecules, such as GEF-H. The guanine nucleotide exchange factor GEF-H stimulates Rho GTPase when it is not bound to microtubules.

2.4 Extracellular Matrix

The extracellular matrix (ECM) support cell functions during development, division, differentiation, and apoptosis, as well as tissue formation and remodeling. The extracellular matrix especially provides a scaffold for appropriate three-dimensional cell assembly. The extracellular matrix also behaves as a reservoir of signaling molecules.

The structure and function of the extracellular matrix varies between different tissues. It is particularly abundant in connective tissues. Cells interact and communicate with other cells and the extracellular matrix. ECM signaling influences regulation of gene expression. The extracellular matrix is synthesized by fibroblasts and smooth muscle cells to serve as a binding material. Imaging techniques have been developed for the structural analysis of soft tissues, in particular to study the crimp of collagen fibers within the bundles [161].

Transforming growth factor-β regulates the deposition of proteins of the extracellular matrix and their interactions with the cells using integrins. TGFβ stimulates the synthesis of collagens and fibronectin, as well as the balance between matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases. TGFβ and its downstream effector Smad are involved in the contraction of collagen gels by fibroblasts.

2.4.1 Structural Proteins

The extracellular matrix is composed of three molecule classes: (1) *structural proteins*, collagen (Cn, κολλαω: to glue), and elastin (En, ελασισ, ελαστρεω: to push); (2) *specialized proteins*, such as fibrillin, laminin (Ln), fibronectin (FN); and (3) *proteoglycans* (PoG), like chondroitin sulfate, heparan sulfate[47] (HS), keratan sulfate, hyaluronic acid (the ground substance).[48] Proteoglycans (or mucopolysaccharides) are composed of a protein to which are attached glycosaminoglycans[49] (GAG). The gel-like ground substance has an important water-binding capacity, which amplify the volume occupied by the macromolecules.

Heparan sulfates can be divided into three different groups according to protein backbones: syndecans (the largest amount), glypicans, and perlecans (the smallest amount). Syndecans have an extended extracellular domain, which can sense applied shears, and a cytoplasmic sequence for signal transmission, possibly linked to G-protein-coupled receptors, especially those bound with endothelial nitric oxide synthase, and to cytoskeletal components, such as actin. Syndecan-1, the most represented heparan sulfate in the vascular endothelium, is also involved in inflammation, binding *chemokines* (chemotactic cytokines; Sect. 10.1).

Without anchorage to the extracellular matrix, the cells cannot survive. The fibronectins attach cells to the extracellular matrix, except in basal laminae (lamina: plate, stands for a thin layer of connective tissue), of endothelia, which involve laminin as adhesive molecule. The laminin is tightly associated with *entactin/nidogen*, which also binds to collagen-4.

The cell life depends on the ECM microstructure. Fibroblasts grown in high-fibril–density extracellular matrices have decreased length-to-height ratios, increased surface areas, and a greater number of projections [164]. Fibroblasts in low-fibril–density extracellular matrices reorganize their extracellular matrix to a greater extent. Furthermore, β_1-integrins are localized according to the local strain field and ECM remodeling events. Furthermore, fibroblast proliferation is enhanced in low-fibril–density extracellular matrices.

Catenins are involved in adhesion and signaling. Distinct forms of catenins could mean that adhesion and signaling are not always coupled [165]. β-Catenins interact with E-cadherin and with α-catenin, anchoring the cadherin complex to the actin cytoskeleton. β-Catenins are mediators of Wnt

[47] Heparan sulfate proteoglycans regulates the distribution of the extracellular signaling molecules, such as Hedgehog (Hh; Sect. 3.2.7), Wingless (Wnt; Sect. 3.2.8), or Decapentaplegic (Dpp) [162].

[48] Altered contents of hyaluronan in the arterial wall disturb the wall functions. Hyaluronan overproduction in the aorta is associated with a thinning of the elastic lamellae [163]. It can lead to increased wall stiffness and promote atherosclerosis.

[49] Glycosaminoglycans with their negative charges act as buffer for cations in excess.

signaling (Sect. 3.2.8).[50] β-Catenin activity is controlled by binding partners. Plakoglobin (γ-catenin) binds E-cadherin, α-catenin, and transcription factors. It is involved in cell adhesion as well as Wnt signaling. *Plakophilins* (Pkp) are proteins located in desmosomes. Like γ- and β-catenins, they are members of the armadillo protein family. They acts in cytoskeleton–plasmalemma interactions. In addition, they can be involved in signal transduction. Plakophilin 2 (Pkp2) deficiency can induce arrhythmogenic right ventricular cardiomyopathy,[51] ventricular tachyarrhythmias, and sudden death [168]. Plakophilin 4 (Pkp4) is observed in the desmosomes and adherens junctions of endothelia.

Cell anchorage and migration are due to glycoproteins, in particular *fibronectins* (FN; nectere: to connect). These connecting elements are fixed to collagen and elastin of the extracellular matrix on the one hand and the cell membrane on the other hand [169]. Fibronectin yields an important substrate for cell adhesion. Fibronectin, linked to actin filaments, is associated with the cell skeleton.

Clotting factor XIII bridges fibronectin to fibrin. The fibronectin enhances platelet adhesion. Activated platelets have specific fibronectin adhesion sites composed of two glycoproteins. Fibroblasts also have such sites, but with distinct affinity glycoproteins. Whereas platelets must be strongly moored whatever the stress field (strong affinity), fibroblasts travel through the fibronectin network (weak affinity). The fibronectin then acts on clotting and healing. The fibronectin also promotes chemotaxis [170] and activates integrin signaling [171]. The fibronectin has indeed several sites that can bind to plasmalemmal integrins [172]. The plasma fibronectin is synthesized by the liver.

2.4.2 Matrix Metalloproteinases

Proteolytic degradation and remodeling of the extracellular matrix is controlled by matrix metalloproteinases (MMP) and the *tissue inhibitors of metalloproteinases* (TIMP). Matrix metalloproteinases are very slow enzymes, most often targeting many substrates (Table 2.11). They are secreted as latent enzymes, which are activated by reactive oxygen species and proteinases among other factors.

[50] The Wingless (Wnt) signaling pathway is involved in many biological processes. The Wnt signaling pathway is either β-catenin–dependent or β-catenin–independent [166]. Diversin is a protein functioning as a molecular switch between these two Wnt pathways. Inversin (Inv) can associate with Dishevelled (Dvl). It inhibits β-catenin–dependent Wnt signaling upstream of the β-catenin degradation complex [167]. Flow over the ciliated MDCK cells, the ciliae being the sensing element of the urinary tract, increases the expression of inversin and slightly reduces β-catenin levels.

[51] Arrhythmogenic right ventricular cardiomyopathies are associated with fibrofatty replacement of cardiomyocytes.

Table 2.11. Examples of matrix metalloproteinases and their substrates (Source: [173]).

Type	Substrates
MMP1	Collagen-1/2/3/7/8/11, glycoproteins (entactin), proteoglycans (aggrecan), L-selectin, IL1β, MMP2, MMP9
MMP2	Collagen-4/5/6/10, elastin,
MMP3	Collagen-3/4/5/9, laminin, elastin, fibronectin, entactin, aggrecan, perlecan, decorin, plasminogen, IL1β, MMP2/TIMP2, MMP7, MMP8, MMP9, MMP13
MMP7	Collagens-4/10, elastin, fibronectin, laminin, aggrecan, decorin, transferrin, plasminogen, β4-integrin, MMP1, MMP2, MMP9/TIMP1
MMP8	Collagens-1/2/3/5/7/8/10, fibronectin aggrecan
MMP9	Collagens-4/5/7/10/14, elastin, fibronectin, entactin, aggrecan, plasminogen, IL1β,
MMP10	Collagens-3/5, elastin, aggrecan, MMP1, MMP8
MMP12	Collagen-4, elastin, fibronectin, vitronectin, laminin, entactin, fibrinogen, fibrin, plasminogen
MMP13	Collagens-1/2/3/4/9/10/14, fibronectin, aggrecan, perlecan plasminogen, MMP9
MMP14	Collagens-1/3, fibronectin, laminin, vitronectin, entactin, aggrecan, perlecan, decorin, MMP2, MMP13
MMP15	Fibronectin, entactin, laminin, aggrecan, perlecan, MMP2
MMP16	Collagen-3, fibronectin, MMP2
MMP18	Collagen-1
MMP19	Collagen-1

The family of matrix metalloproteinases comprises several subsets: (1) collagenases (MMP1, MMP8, and MMP13), (2) gelatinases (MMP2 and MMP9), which digest denatured collagen and other ECM components including basement membranes, (3) stromelysins (MMP3, MMP10, and MMP11), and (4) membrane-type MMPs (mtMMP). Gelatinases, matrilysins (MMP7), and macrophage elastase (MMP12), which are overexpressed in aortic aneurisms (Part II), degrade elastin. Increased levels of MMP14 activate proMMP2. Other proteinases, such as plasminogen activators, serine elastases, and cathepsins, expressed in atheromatous plaques (Part II), contribute to the vessel-wall degeneration. Matrix metalloproteinases are involved in the evolution of atherosclerosis and aneurisms. Matrix metalloproteinases are naturally inhibited by TIMPs. Tissue inhibitors of metalloproteinases also have mitogenic and cell growth–promoting activity. Furthermore, TIMP3 is a constituent of the extracellular matrix and some basement membranes.

Matrix metalloproteinases not only degrade the extracellular matrix, but also have other functions. Matrix metalloproteinase membrane type-1 (mt1MMP) can regulate the development of the microvasculature via platelet-derived growth factor-B (PDGFB) and its receptor PDGFRβ (Sect. 10.1) [174]. PDGFB regulates the function of intramural cells. mt1MMP is associated with the signaling based on PDGFB-PDGFRβ.

Matrix metalloproteinases decrease the endothelial barrier by proteolysis of cell junctions. However, in normal conditions, MMP stimulation is counterbalanced by barrier upregulation by cyclic stresses. Plasmalemmal proteases "a disintegrin and metalloprotease" ADAM10 and ADAM15 also disassemble cell junctions. Endothelial MMP9 is involved in cyclic strain–induced angiogenesis and endothelial MMP2 in hypoxia-generated angiogenesis and migration of smooth muscle cells [173].

Many molecules act on MMPs. nitric oxide inhibits MMP2 via repressor activating transcription factor ATF3 but stimulates MMP13. Inducible cyclooxygenase Cox2 activated by cyclic strain upregulates MMP9 and MM14. Prostaglandin PGJ2 upregulates MMP1. Tissue inhibitors of metalloproteinases are synthesized by endothelial cells, smooth muscle cells, and fibroblasts among other cells. TIMP activity of endothelial cells is sensitive to stresses. According to its active terminal domain, TIMP2 either activates MMP2 or inhibits MMPs. Interaction between MMP14 and TIMP2 is required to activate proMMP2. The fibrinolytic system, also sensitive to stresses, which include plasminogen activators (tPA, and uPA) and inhibitors (PAI1, and PAI2),[52] regulates the conversion of plasminogen into plasmin. Plasmin participates to MMP activation.

Cyclic strains and shear stresses regulate the expression of matrix metalloproteinases by endothelial and vascular smooth muscle cells, as well as the production of proteins of the basement membrane and the interstitial matrix (Fig. 2.5).

[52] Endothelial PAI1 serves also as an antimigratory molecule.

The extracellular matrix is much stiffer in tumors than normal tissues, due to a high content in collagen fibers. Stiff extracellular matrices enhance Rho GTPase activity [175]. Matrix metalloproteinases frequently have high levels in tumors. Matrix metalloproteinases not only are involved in local cancer invasion by degrading the structural components of the extracellular matrix, but also release cell-bound inactive precursors of growth factors and activate survival factors. They also may be involved in cancer initiation, especially in the epithelial–mesenchymal transition. In mammary tissue, MMP3 acts via Rac1b, which raises the production of reactive oxygen species, particularly the mitochondrial superoxide, leading to DNA damage and genomic instability, and eventually to tumorigenesis. MMP3, Rac1b and H_2O_2 enhance the expression of Snail, a transcription repressor of E-cadherin [176].

2.4.3 Basement Membrane

The basement membrane (BM), or basal lamina, is a specialized extracellular matrix sheet at the interface between the connective tissue and the endothelium. Both basement membranes and extracellular matrices are major regulators of embryogenesis and tissue integrity. The basement membrane influences the functions of contacting cells (regulation of cell shape, gene expression, proliferation, migration, and apoptosis) [177].

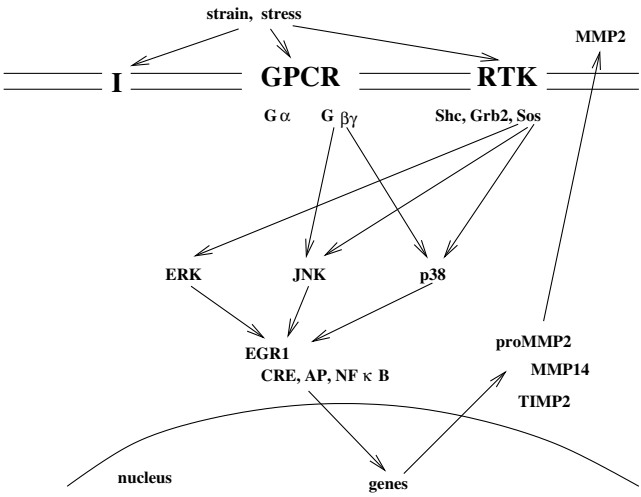

Figure 2.5. Strain promotes the synthesis of matrix metalloproteinase MMP2 in endothelial cells. Cyclic strains are detected by G-protein-coupled receptors (GPCR; Sect. 3.2.1), receptor tyrosine kinases (RTK; Sect. 3.2.2), and integrins (I). Signaling cascades activate ERK1/2, p38, and JNK (Chap. 4), then transcription factors, early growth response protein EGR1, cAMP response element (CRE), activator protein AP1, and nuclear factor-κB (NFκB), leading to the production of MMP/TIMP (Source: [173]).

The basement membranes contain laminins, members of the entactin/nidogen family, collagen-4, and proteoglycans [178, 179]. The laminin receptors are integrins and *dystroglycan*.[53] The laminin type affects receptor interaction. The laminin and collagen-4 networks are linked by *nidogens* [180]. This combined network associates other glycoproteins and proteoglycans, which act as both structural elements and receptor ligands. Proteoglycans bind, via their charged carbohydrate chains, soluble molecules, such as growth factors and ions, and regulate the diffusion of macromolecules.

Basal lamina synthesis is spatially and temporally regulated by neighboring cells. Regulations, mediated by receptors on the cell membrane, depends on the distribution of ligands and receptors [181, 182]. The basement membrane binds a variety of growth factors [183] (Fig. 2.6). This binding regulates the accessibility of these ligands via retention or release from the basement

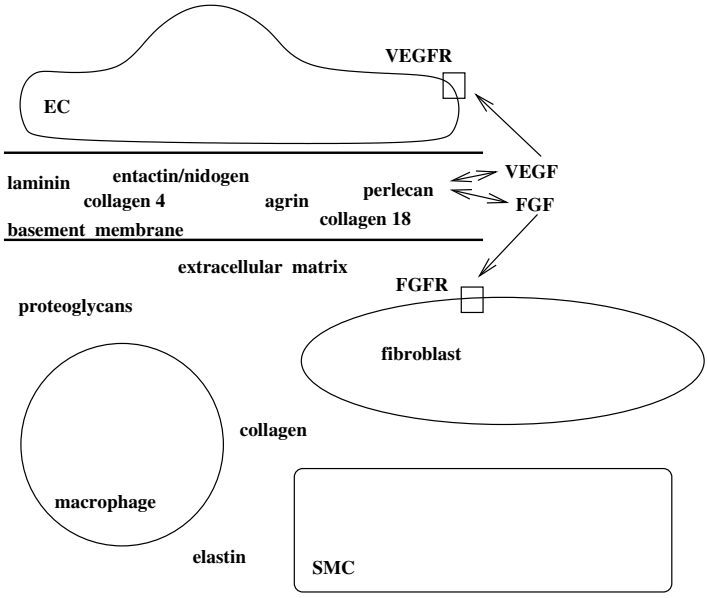

Figure 2.6. The endothelial cell (EC) lying on the basement membrane. The interstitial matrix contains cells (resident macrophages, fibroblasts, smooth muscle cells [SMC], pericytes), as well as fibers and proteoglycans. Heparan sulfate proteoglycans of the basement membrane, such as perlecan, form complexes with growth factors (Sect. 10.1), here the vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF). The growth factors can then bind to their respective receptors VEGFR and FGFR. Perlecan can induce growth factor–receptor interactions and angiogenesis.

[53] Dystroglycan associated with other proteins into complexes are linked to the cytoskeleton.

membrane. Binding to BM components can alter the local ion composition, proteoglycans linking divalent cations [184].

The basal lamina has manifold structural roles. The basal lamina gives a stable surface for endothelium firm anchorage which protects from shearing and detachment. Basal laminae act as selective barriers for macromolecular diffusion.

2.4.4 Interstitial Matrix

The interstitial matrix[54] influence the functions of contacting cells. The interstitial matrix has a fibrillar structure, with a large amount of collagens. The structure of the interstitial matrix depends mainly on the type of fibrils and the type and amount of proteoglycans. Proteoglycans not only act as gel formers by water adsorption, but also have structural and cellular interactions [185]. Osmolarity of interstitial fluid affects the repulsive forces of the negatively charged GAGs, inducing collapse or inflation, which can affect fiber stretching and folding.

Collagen and elastin control the rheology of the connective tissue. The ground substance with glycosoaminoglycans stabilizes the fiber network. Proteoglycans control the level of hydration of connective tissues, and thus partially determine the physical properties of connective tissues.

2.4.4.1 Elastin

A first type of major fibers is given by *elastin fibers* (EnF), with elastin and fibrillin. The elastic fibers, of thickness 0.5 to 1 μm, can ramify and form elastic networks and fenestrated membranes. Tropoelastin is a precursor of elastic fibers synthesized by fibroblasts and vascular smooth muscle cells and secreted in the extracellular space. These cells form a microfibrillar glycoprotein scaffold with fibrillin on which are deposited tropoelastin monomers. Microfibrils are grouped aligned in infoldings of the cell surface. Tropoelastin bridges, as well as connections between elastin and collagen, need lysyloxydase. The *desmosine* cross-links elastin to form elastin fibers [186]. Elastin makes long-chain molecules tied together in a 3D network by scattered cross-links with a degree of order influenced by its environment. Elastin also binds to cells via *elastonectine*. The G-protein-coupled receptor of elastin recognizes also laminin. The ligand-bound receptor induces Ca^{++} influx. Subsequent efflux is much slower with aging.

Elastic fibers hence consist of two components: microfibrils and polymerized elastin. Microfibrils (10–12 nm) in the extracellular matrix are composed of fibrillins-1 and -2, and associated proteins, such as latent transforming

[54] The interstitial matrix is the extracellular matrix part without basement membrane. The interstitial matrix, with the cells, forms the connective tissue.

growth factor-β-binding protein[55] (LTBP1–LTBP4) and microfibril-associated glycoproteins. Microfibrils can form aggregates devoid of elastin.

Deposition and crosslinking of elastin along microfibrils depend on fibrillin-1, fibulin-5,[56] and latent transforming growth factor-β-binding protein LTBP2 [187].[57]

Fibulin-5[58] is a calcium-dependent, elastin-binding protein located at the surface of elastic fibers. Fibulin-5 acts as a scaffold protein that links elastic fibers to cell integrins[59] [188]. Fibulin-5–deficient mice have disorganized elastic fibers and fragmented elastin. Consequently, aortas in these mice are tortuous and softer.

Elastin fibers are the most elastic biomaterials, at least up to a stretch ratio of 1.6 [190], the loading and unloading cycles being nearly superimposed. Strained elastic fibers produce recoil forces. The elastic modulus of elastin fibers ranges from 0.1 to 1.2 MPa[60] [191–193].

2.4.4.2 Collagens

The collagens, the second type of major fibers, are structural proteins that form fibrils, characterized by triplet of helical chains and stabilized by covalent cross-links.[61] The triple helix domain is common for all collagens; the heterogeneity resides in the assembling mode and in the resulting structure (Table 2.12). Certain microfibrils, which contain five rows of basic collagen molecule (300 nm long) forming a helix, are packed together to form a fibril (caliber 50–200 nm), cross-links providing stability.[62] Collagen fibrils can vary

[55] Latent transforming growth factor-β-binding proteins not only are structural components of the extracellular matrix but also (except LTBP2) bind the latent form of transforming growth factor TGFβ and modulate TGFβactivity.

[56] Fibulin-5 is also known as developing arteries and neural crest EGF-like protein Dance/Evec. There are several Dance-binding proteins, such as elastin, emilin, superoxide dismutase, ... Dance interacts with fibrillin-1. Fibrillin-1 microfibrils with fibulin-5 deposits can lead to mature elastic fibers.

[57] LTBP2 located in elastin-associated microfibrils interacts with Dance and can promote deposition of fibulin-5 onto fibrillin-1 microfibrils to form elastic fibers.

[58] Fibulin-5 is an extracellular matrix protein abundantly expressed in large vessels and cardiac valves during embryogenesis, as well as in many adult tissues that contain abundant elastic fibers, such as the aorta.

[59] Fibulin-5 is a ligand for plasmalemmal integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_9\beta_1$ [189].

[60] A value of elastic modulus of elastin from 0.4 to 0.6 MPa is often considered.

[61] Helical structures is made from three separate collagen chains by intertwining a coiled structure. Possible periodic striated aspects of collagen fibrils are due to its trellis arrangement.

[62] Neighboring collagen molecules are arranged to form a right-handed twist. The resulting microfibril, the basic building block of the collagen fibril, interdigitates with adjoining microfibrils. The quasihexagonal arrangement of collagen molecules is continuous. Each microfibril contains at least two to three intermicrofib-

Table 2.12. Selected types of collagens.

Collagen	Location
1	Connective tissue, vessel wall
3	Distensible connective tissue
4	Basement membrane
5	All tissues
6	All tissues
8	Endothelia
12	Interacts with collagens-1 and 3

in thickness and in association with each other[63]. Among collagen groups are the hexagonal-like network building collagens (types 8, and 10), beaded filament collagens (type 6), anchoring fibril collagens (type 7), transmembrane collagens (types 13, and 17), and the multiplexin collagens (types 15, and 18).

Collagen fibers (length of $\sim 10\,\mu m$) consists of collagen fibrils (length of $\sim 1\,\mu m$) composed of a staggered array of long tropocollagens (length of $\sim 300\,nm$, bore of $\sim 1.5\,nm$). Collagen design maximizes the strength with large energy dissipation during deformation.

The collagen is surrounded by extensible glycoproteins and proteoglycans. Proteoglycans are attached to collagen. The rheological properties of pure collagen are thus difficult to assess. Collagen fibers and fiber bundles have sheath with collagens-3 and 4. Collagen-1, the main constituent of arterial wall, is a tensile fiber, of 1 to 20 μm thickness, usually forming wavy bundles, which does not ramify. It is located between elastic lamellae of elastic arteries. Collagen-3 strengthens the walls of blood vessels. Its ultrastructure is similar to collagen-1 but its composition differs, with higher molecular stability. Collagen-3 determines vessel compliance [195]. Collagen-4 provides the basal lamina of the endothelium and the filter for the blood capillaries. Transmembrane collagen-13 is found in intercalated discs of the myocardium.

Procollagens are synthesized by fibroblasts and vascular smooth muscle cells and then cleaved to collagen after secretion from the cell. Extracellular procollagen is combined to tropocollagen, which spontaneously self-assembles into collagen fibrils [196]. The collagen is a load-bearing material that deforms differently in different directions and exhibits viscoelastic behavior. The collagen is stiff (high elastic modulus).[64] Due to their configuration, collagen fibers (CnF) can be stretched by strong stresses up to 16.3 kPa. The mechanical properties of blood vessels depend on the interaction between elastic and collagenous elements. Fibroblasts remodel collagen to fit the tissue to the

rillar cross-linkages, and one intramicrofibrillar bond [194]. Decorin regulates collagen-1 fibrillogenesis.

[63] Collagen-3, 5, and 11 regulate fibril size by incorporation into fibrils-1 and 2.

[64] Collagen is about a thousand times stiffer than elastin. Collagen stiffness depends on fiber arrangement.

environment requirements, in particular to regulate the cell growth and tissue development.

Collagen remodeling by fibroblasts has a crucial role in organizing tissue structures. Fibroblast lamellipodia extend along the collagen fibers, and work on collagen by a mechanism requiring $\alpha_2\beta_1$ integrins as well as myosin 2B assembly and contraction in lamellipodia [197]. The fibronectin provides a separate structural network in interstitial matrices, which interacts with cells.

Animal models of essential hypertension show that the number of connections between elastin and smooth muscle cell increases and fenestrations of the internal elastic lamina are smaller [198]. Elastin and collagen not only intervene in the vessel wall rheology, via their mechanical properties, density, and spatial organization, but also control the function of smooth muscle cells.

3

Ion Carriers and Receptors

The cell is enclosed by a plasma membrane impermeable to many life-sustaining substances. The cell membrane thus incorporates rafts, calveolae, adaptors, transporters, etc. The cell membrane being a major barrier to ion flux, proteic ion carriers, such as ion channels, transporters, exchangers, and pumps, transport ions across it. Besides, signal transduction relies on plasmalemmal receptors and ion carriers.

The motion of ions across cell membranes ensures various physiological processes, such as hormone secretion, action-potential generation, muscle contraction, regulation of blood pressure, salt and water balance, cell proliferation and apoptosis.[1] Moreover, differences in ion concentrations or electrical potential between both sides of the plasmalemma, created by proteic ion carriers produce electrical signals in excitable cells. Changes in membrane polarization occur when ion channels open. The opening and closing of ion channels can be disturbed by gene mutations, leading to alterations in ion conductance through the central pore and possible ion leakage (channelopathies).

3.1 Membrane Carriers

Membrane transport is done via multiple carriers (Table 3.1).[2] Ion channels are gated pores that allow passive ion flux according to their electrochemical gradients. Ion pumps use the energy from ATP hydrolysis to transport ions against their electrochemical gradient. Coupled transporters (antiporters and symporters) switch on the flux of one ion species against its electrochemical gradient with the reverse motion of another.

[1] There are other activities associated with ion fluxes, such as sensory transduction, learning and memory, etc.

[2] Carrier here defines any transmembrane transport process. The terminology is given by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology.

The *antiporter* moves two ions in opposite directions across the membrane. The *channel* has two conformational states, open and closed. It is involved in specific transport of ions or uncharged molecules down their chemical or electrochemical potential gradient.[3] Channels include: (1) voltage-gated channels (electric-field mediation), (2) chemically gated channels (ligand binding), (3) mechanically gated channels (stress or strain activation). The *exporter* functions in the outward direction, the *importer* inwardly. The *pore* is a membrane protein allowing non-specific passage of solutes of different sizes. The *symporter* simultaneously moves two chemical species in the same direction, at least one of them being ionic and driven by its electrochemical potential gradient. The *transporter* is a membrane protein for a specific chemical species. Its binding site opens alternately to the one and the other membrane side. Transporters can act by diffusion, or be driven by electrochemical potential gradients of ions (secondary active transport) or chemical reactions (primary active transport). Transporters carry solutes against their chemical or electrochemical potential gradient. The *uniporter* displaces a single chemical species across the membrane.

Steroids diffuse across the plasmalemma to reach their intracellular receptors, most of them belonging to the nucleus membrane (Sect. 3.3). Except the receptors of steroid hormones, the receptors of multiple messengers are located in the plasmalemma. Attachment of the ligand on its corresponding receptor induces a reaction cascade. The messengers can be hormones secreted by specialized cells and transported by the blood, neurotransmitters released by nerve endings, and substances produced locally. Both agonists and antagonists can fix to receptors with or without effect, respectively. Antagonists block agonist fixation. The messengers generally have specific receptors; but,

Table 3.1. Membrane carrier proteins bind a substance and transport it across the membrane, undergoing conformational changes.

Transporter	Specific active carrier
Uniporter	Transport of a single molecule
Exporter	Cellular efflux
Importer	Cellular influx
Antiporter	Coupled transport in the opposite direction (ions or small molecules, simultaneously or sequentially).
Symporter	Coupled transport in the same direction
Channel	Water-filled proteic pore (for a specific ion or a small molecule)
Pump	Energy-consuming carrier (of ion or small molecule)

[3] Electric potential differences exist across the membrane, the negative side being on the cytosolic face of the membrane. They are expressed in mV, with a negative sign.

a given messenger can have several receptors: the adrenaline has α1, α2, β1, and β2 receptors (Sect. 3.2.1.1); the acetylcholine has nicotinic and muscarinic receptors.

3.1.1 Ion Carriers

Hydrophilic charged elements cross the hydrophobic cell lipid bilayer using ion channels, with a transmembrane hydrophilic pore. Ion channels control the electrical state of the cell by allowing certain ions to cross the membrane and excluding others. For instance, channels selective for sodium ions allow Na^+ to enter the cell. Channels selective for potassium ions allow K^+ to exit the cell, thus generating membrane hyperpolarization and reducing the cell electrical excitability.

Relative selective permeability describes the ability of an ion channel to allow passage of a single ion type or a set of ions. Ion channels are characterized by: (1) gating kinetics, (2) possible modulation by intracellular ions and molecules, and (3) channel conductance.[4]

Ion channels are not confined to the plasmalemma. Intracellular ion channels are found in the membranes of intracellular organelles, such as secretory and endocytotic vesicles, synaptic vesicles, lysozymes, mitochondria, and the endoplasmic/sarcoplasmic reticulum.

The ion channel has a typical diameter of about 8 nm, with a central pore of about 2 nm, and a length of about 12 nm, hence exceeding the lipid bilayer thickness. The central pore has a narrow part. Ion channels are composed of one or more pore-forming subunits, often in association with accessory subunits.[5] The basic structure contains four (tetrameric channel) or five (pentameric channel) transmembrane helices fitted together, which edge the central pore. For instance, the four subunits of Na^+ and K^+ tetrameric channels surround a central pore with a selectivity filter formed by P-loops[6] from each of the subunits [199]. However, ion channels can use a single unit with various domains (monomeric channel) like certain voltage-gated channels. Others are dimers, with similar (homodimeric channel) or different (heterodimeric channel) subunits.

Three functional states of ion channels can be defined: resting (closed), open (conducting), and inactivated. Ion channels share similar features, relative selective permeability and gating (Table 3.2). Gating refers to the channel opening, depending on the presence of external signal, like a ligand (ligand-gated channels) or an ionic flux (voltage-gated channels). Gating is characterized by transitions between conductive and non-conductive conformations

[4] The channel conductance is a measure of the current crossing a single open channel at a given membrane potential.

[5] Accessory subunits contribute to the diversity of ion channels.

[6] Each subunit of ion channel can have two membrane-spanning helices, outer helix M1 and inner helix M2, with an intervening P-loop.

via structural rearrangements of the flexible selectivity filter. Channel opening can be due to a rotation of a cytoplasmic domain of the membrane protein into its cytoplasmic end. The rotation can be due to a change in transmembrane potential. In voltage-dependent channels, a voltage-sensing domain, linked to the pore gate, is able to transduce the energy provided by the transmembrane voltage into gate motion. The opening and closing of ion channels can also be regulated by changes in temperature or mechanical stress. The gating kinetics deals with the onset and duration of the conductive state. Gating can be modulated by biochemical reactions such as phosphorylation.

Intracellular signaling proteins can modulate the ion channel activity. Ion channels are regulated by various mechanisms, such as phosphorylation by protein kinases (Sect. 4.3). Ion channels can be substrates not only for protein kinases, but also for phosphoprotein phosphatases (Sect. 4.4). Phosphorylation and dephosphorylation modulate channel gating. Kinases and phosphatases can bind especially voltage- and calcium-gated ion channels, either directly or via anchoring proteins, such as A-kinase anchoring proteins (Sect. 4.10.1), to form regulatory protein complexes. Calmodulin, a calcium sensor, can also bind to ion channels in the absence of calcium. Calcium–calmodulin complex modulates the channel activity.

The ion flux through the cell membrane across the corresponding channels depends mainly on three factors: (1) the difference in ion concentrations on both sides of the membrane, (2) the voltage across the membrane, and (3) the membrane permeability, which depends on ion channel activity. The membrane permeability depends on: (1) the number of plasmalemmal channels, (2) the opening time duration, and (3) the channel conductance.

Cells are made of about 80% water. The osmotic pressure, fundamental to cell survival, induces stretch force on the cell membrane. Mechanosensitive ion channels (MSC) or stretch-activated ion channels (SAC) detect and respond to forces undergone by the cell. They open and close in response to mechanical stimuli. Two major classes of ubiquitous stretch-activated channels exist [200]: (1) the ion channels that require force transmission from the cytoskeleton; and (2) the ion channels that respond to stress mediated via the lipid bilayer. Several MSCs belong to the transient receptor–potential (TRP) proteins [201]. TRP channels are polymodal, being activated by several types of stimuli. TRP channels can have enzymatic activity. Volume-regulated anion channels (VRAC) are found in epithelial cells. Stretch-activated calcium and inward rectifying potassium channels drive an ion influx leading to cell depolarization [202].

Voltage-gated ion channels, activated for a given membrane polarity threshold, generate electrical impulses in excitable cells. In particular, voltage-dependent ion channels generate and propagate the action potential for excitation-contraction coupling in the heart. The molecular mechanisms of voltage sensing, such as transmembrane motions of voltage-sensing elements, remain controversial. Voltage-gated ion channel tetramers have six transmem-

brane components (S1–S6). Certain cyclic nucleotide-gated channels are similar to voltage-gated ion channels.

S1 to S4 subunits constitute the voltage-sensor domain, S5 and S6 form the hydrophilic pore. In the case of most voltage-dependent channels, when

Table 3.2. Classification of ion channels.

Channel family	Examples
Voltage-gated channels	Voltage-gated sodium channel Voltage-gated potassium channel Voltage-gated sodium/potassium channel Voltage-gated proton channel Voltage-gated calcium channel Voltage-gated chloride channel (ClC) Mitochondrial voltage-gated anion channel
Extracellular ligand-gated channels	GABA-gated Cl^- channel Glycine-gated Cl^- channel Glutamate-gated Ca^{++} channel P2X Nicotine acetylcholine receptor Serotonin-gated cation channel ATP-gated outward rectifying Cl^- channel
Intracellular ligand-gated channels	CFTR Cl^- channels ATP-binding cassette proteins (ABC) IP3R–TRP TRPM7 Nucleotide receptor ATP-sensitive inward rectifying K^+ channel (ROMK, Kir, IRK/Kir2, BIR, etc.) Calcium-activated Cl^- channel (ClCa) Calcium-activated K^+ channels (SK, BK) ATP-gated K^+ channel (K(ATP)) cGMP-gated ion channels (CNG) G-protein–activated inward rectifying K^+ channel (GIRK/Kir3) Calcium-release Ca^{++} channels Ryanodine (Ca^{++} channel) receptor Epithelial Na^+ channel (ENaC)
Mechanosensory channels	TRP Stretch-activated ion channels
Volume-regulated channels channels	VRAC
Miscellaneous	Gap junctions Peptide ion channels

the membrane is depolarized from a resting hyperpolarized state, the voltage sensor undergoes conformational changes leading to pore opening. Sensor S4 has periodically aligned positively charged amino acids. The voltage-sensor domain, without the pore domain, generates depolarization-induced outward currents and inward tail currents during repolarization. The voltage-sensor domain is characterized by pH-dependent gating,[7] zinc ion sensitivity, and proton selectivity [203]. The voltage-sensor domain works not only for voltage sensing but also constitutes a voltage-gated proton channels. Proton efflux through these Hv channels leads to membrane depolarization. Hv channels have been observed in blood cells and alveolar epithelium cells, among others.

Ion channels can form molecular complexes between them, such as large-conductance voltage-gated calcium channels (CaV) and BK-type Ca^{++}-activated voltage-gated potassium channels (BKCa or KCa1.1), for a fast and localized control of neuronal firing and release of hormones and transmitters in the central nervous system [204].

Ligand-gated ion channels open when they are bound with their corresponding ligand. Ion channels can be gated either directly by ligand binding, or indirectly via a cascade of molecular reactions leading to channel opening. There are extracellular ligand-activated channels and intracellular ligand-gated channels. The later are often activated by G-protein-coupled receptors (GPCR). Usual intracellular ligands include calcium ions, ATP, cyclic AMP and GMP, phosphatidyl inositol. Stored calcium is the main source of Ca^{++} influx in non-excitable cells. The cytosolic influx can require calcium-release–activated calcium channels. Intracellular ligand-gated ion channels modify the intracellular concentration of second messengers and can indirectly act on coupling proteins and cofactors, especially those involved in long-lasting processes in the smooth muscle cell. Glutamate ion channels, which are activated by glutamate, do not share sequence similarity with any other ligand-gated ion channels.

Ion channels can be either specific for a given ion or non-selective, such as serotonin-gated ion channel. Nonselective stretch-activated cation channels let cross calcium, sodium, and potassium, whereas others are selective for potassium. Nicotine acetylcholine ion channels in nerves and myocytes are permeable to all cations, including sodium, potassium, and calcium ions. Purinogenic receptors P2X, sensitive to ATP, are sodium and calcium channels. Certain voltage-activated cation channels in the cardiac pacemaker and Purkinje fibers are permeable to both sodium and potassium ions. Voltage-dependent anion channels in the mitochondrial outer membrane is slightly selective for anions. Anion channels usually are less selective than cation channels.

[7] The voltage triggered opening strongly depends on both the intracellular and extracellular pH. Decrease in extracellular pH induces a shift of the current-voltage relationship in the positive direction, whereas intracellular pH changes generate shift in the opposite direction.

3.1.1.1 Transient Receptor Potential Channels

Transient receptor potential (TRP) channels constitute a large family of ubiquitously expressed proteins. Channel activity is affected by several physical parameters such as osmolarity, pH, mechanical force, as well as interactions with external ligands or cellular proteins. They have a diverse permeability to ions, although in general, they are non-selective cation channels. Howver, certain TRPs are highly selective for calcium ions. Transient receptor potential cation channels are, indeed responsible for calcium influx into nonexcitable cells. TRPC (canonical), TRPV (vanilloid receptor), TRPM (melastatin), and TRPP (polycystin) subfamilies differ in selectivity and activation mechanisms. Osmosensor TRPV4, a non-selective cation channel with a slight preference for calcium ions, is highly expressed in the kidney and paraventricular regions of the central nervous system. Ubiquitination and subsequent endocytosis control TRPV4 density at the plasmalemma [205].

TRPs mediate responses to growth factors, pheromones, odorants, mechanical quantities, chemical agents, physical factors, and metabolic stresses [206]. TRPs are activated by the phosphoinositide cascade. The transduction cascade is mediated by G-protein–activated phospholipase-C and produced second messengers: diacylglycerol and inositol trisphosphate. Deactivation of channel activity is regulated by protein kinase-C. PLC, PKC, and the TRP ion channel form a molecular complex with scaffold proteins. TRPs drives the inositide-mediated calcium influx channels, which include both store-operated (SOC; via coupling between TRP and inositol triphosphate receptor IP3R) and store-independent channels (via PLC activation).

Heteromultimeric calcium store-operated TRPs and store depletion-independent PI3- and DAG-activated TRP-like channels (TRPL), with calmodulin binding sites, are light-activated channels. Seven components (TRPC1–TRPC7) form the TRP group of mammalian homologs TRPC of TRP ("TRP homolog"). TRPC nonselective cation channels are activated by PLC-coupled receptors. The store-operated TRPC1 channel is abundantly expressed in endothelial cells, among others. TRPC2 channel is tissue specific. TRPC3, TRPC6, and TRPC7 channels are closely related in molecular structure and function. TRPC3, TRPC6, and TRPC7 channels are activated by diacylglycerol independently of protein kinase-C. TRPC3 channel interacts with IP3R. Receptor-activated non-selective cation TRPC6 channels are highly expressed in manifold cell types, particularly the vascular smooth muscle cells. TRPC6 channels are involved in the depolarization of smooth muscle cells. TRPC6 channels, which are activated by PLC-coupled receptors, also sense the membrane stretch independently of phospholipase-C activity [207]. TRPC6 channels can thus regulate the myogenic tone according to the applied pressure in blood vessels. TRPC4 and TRPC5 are store-independent nonspecific cation channels. TRPCs are able to assemble between them.

TRP-related group ("TRP related") is associated with olfaction and osmolarity transduction pathways or specific genetic diseases. TRP-related

group is subdivided into subfamilies: (1) vanilloid subfamily (TRPV), with epithelial calcium channel[8] (ECaC), calcium transporter-1[9] (CaT1), with osmoreceptor TRPV3,[10] and mammalian vanilloid receptor-1[11] (VR1) and its homolog VRL1; (2) no mechanoreceptor potential-C subfamily (TRPN), with no mechanoreceptor potential C channel (NOMPC); (3) polycystin subfamily (TRPP) with polycystin-1[12] and polycystin-2; (4) melastatin subfamily (TRPM), having a potential role in cell cycle regulation and in regulation of calcium influx in lymphocytes, with TRPM7,[13] a phospholipase-C–interacting kinase, and TRPM2[14]; (5) mucolipin subfamily (TRPML) with mucolipin-1[15]; (6) canonical subfamily (TRPC), and (7) ankyrin-like subfamily (TRPA).

TRP homolog channels are involved in the control of vascular smooth muscle tone via calcium influx. TRPC1 is involved in store-operated activity. TRPC6 is a component of α1-adrenoreceptor activator calcium-permeable non-selective cation channel. TRPC4, expressed in vascular endothelial cells, mediates vasorelaxation of blood vessels by synthesis and release of vasoactive effectors.

Several transient receptor potential canonical channels are plasmalemmal store-operated channels activated by receptor stimulation. Calcium sensor stromal interacting molecule-1 binds TRPC1, TRPC4 and TRPC5 [208]. STIM1 does not bind TRPC3, TRPC6, and TRPC7. However, it intervenes in heteromultimerization of TRPC3 with TRPC1 and of TRPC6 with TRPC4.

3.1.1.2 Calcium Carriers

There are multiple types of calcium channels, including certain TRP channels. Calcium ions regulate protein–protein interactions, transcription, exo-

[8] ECaC is also designated ECaC1, CaT2, or TRPV5. It is highly selective for Ca^{++}.

[9] CaT1 is also named ECaC2 or TRPV6. CaT1 is permeable to Na^{+}, K^{+}, and Ca^{++}.

[10] TRPV3, or OTRPC4, is expressed in kidney (distal nephron), heart, and liver. It is a non-selective cation channel.

[11] Vanilloid receptor-1 is the heat-gated ion channel.

[12] Polycystin-1 and -2 are encoded by PKD1 and PKD2, respectively. A non-selective cation channel results from coassembly of polycystin-1 and -2. Polycystin-L (PCL) is a calcium-regulated cation channel permeable to Na^{+}, K^{+}, and Ca^{++}.

[13] MgATP-regulated, intracellular ligand-gated TRPM7 is also called LTRPC7 or TRP-PLIK (transient receptor potential-phospholipase-C interacting kinase). It is permeable to (in decreasing order of permeability) K^{+}, Na^{+}, and Ca^{++}. It also permeates Mg^{++}.

[14] TRPM2, or LTRPC2, is a cation channel activated by ADP ribose and a specific ADP-ribose pyrophosphatase. It is permeable to both monovalent and divalent cations.

[15] Mucolipin-1, encoded by the MCOLN1 gene, is also called mucolipidin.

cytosis, endocytosis,[16] of apoptosis (calcium being potentially cytotoxic),[17] fertilization, embryonic signaling, and synaptic plasticity. Its concentration is regulated by various channels, pumps, and exchangers. The intracellular free calcium concentration fluctuates between 10 nM and 1 μM, whereas the extracellular free calcium concentration is approximately equal to 1 mM. Within a single contraction–relaxation cycle, the free calcium level in the cardiomyocyte cytosol increases and decreases 100-fold.

Plasmalemmal non-excitable calcium influx channels are classified into two categories: store-operated channels (SOC), responsible for the calcium release activated current (ICRAC), and store-independent channels. The activation of both channels requires phospholipase-C. SOCs are activated by the reduction in stored calcium ions. Store-independent channels are activated either by elevation in the cytosolic calcium level or by DAG.[18]

The divalent cation calcium controls either fast responses such as secretion and contraction, or long-time scale phenomena such as transcription, growth, and cell division. Two kinds of plasmalemmal ion channels control calcium influx. One channel allows transient calcium entry and the other slower, more sustained flux.

Acute rise in intracellular Ca^{++} concentration, either by a release from intracellular stores, or by entry across the plasmalemma, is used in particular by T lymphocytes, endothelial cells, smooth muscle cells and cardiomyocytes. Store-operated calcium release-activated calcium (CRAC) channels mediate calcium influx across the plasmalemma when intracellular stores of calcium are depleted.[19] The endoplasmic reticulum informs the plasmalemma of Ca^{++} depletion. In many cell types, Ca^{++} is released by the

[16] Exocytosis and endocytosis are composed of calcium-dependent and -independent steps. Calcium can act as a co-factor in the regulation of certain membrane fusion, such as fusion of secretory granules and synaptic vesicles with the plasmalemma.

[17] Intracellular calcium compartmentation and transport between the endoplasmic reticulum and mitochondria are modulated by Bcl-2 family proteins. Moreover, Ca^{++}-regulated processes are also involved in clearance of apoptotic cells and cell debris by phagocytes [209]. B-type Ca^{++} channels, which interact with PMCAs, modulate apoptosis of cardiomyocytes [210]. Na^{+}/Ca^{++} exchanger NCX3 is cleaved by Ca^{++}-dependent calpains, leading to calcium overload and cell death [211]. Conversely, upregulation of NCX1 and NCX3 protect against ischemia [212].

[18] DAG can be recycled to PIP2 by the phosphatidylinositol cycle or converted to phosphatidic acid by DAG kinase and CDP-DAG by CD synthetase.

[19] One of the main Ca^{++}-regulated transcription factors is cytoplasmic NFAT. Sustained Ca^{++} influx activates calmodulin-dependent protein phosphatase PP3, which dephosphorylates NFAT, leading to NFAT translocation to the nucleus. The stromal interaction molecule (STIM) is required with plasmalemmal Orai1 for the activation of Ca^{++} release-activated Ca^{++} channels [213]. When calcium is depleted from its stores, STIM1 translocates to vesicular structures near the plasmalemma to activate calcium release-activated calcium channels.

inositol(1,4,5)trisphosphate receptor attached to IP3 from the endoplasmic reticulum (intracellular store). A very small number of IP3Rs exists in the plasmalemma (especially of B lymphocytes) [214]. However, another calcium channel exists. Plasmalemmal Orai1 channel[20] is associated with calcium sensors inside the endoplasmic reticulum. IP3Rs and Orai1s have large and small conductance, and are poorly and highly selective for calcium, respectively. Calcium release from the endoplasmic reticulum indeed leads to aggregation of Ca^{++}-sensing proteins, the stromal interaction molecule-1 (STIM1) in areas of the endoplasmic reticulum close to the plasmalemma, where they can interact with plasmalemma Orai1 (Fig. 3.1).[21] The coupling between Orai1 and STIM1 explains the store-operated Ca^{++} release-activated Ca^{++} influx [215]. STIM1 and Orai1 are coordinated.[22]

Stimulated Ca^{++} release can load mitochondria with a fraction of cytosolic Ca^{++}. Ca^{++} delivery from mitochondria by Na^{+}/Ca^{++} exchanger generates a gradual increase in cytoplasmic Ca^{++} concentration, which induces a second Ca^{++} release from the endoplasmic reticulum [216]. Therefore, a second peak in Ca^{++} cytosolic concentration occurs, which allows partial reloading of mitochondria (Sect. 4.14 with Fig. 4.17).

At least four types of voltage-gated calcium channels exist: L (long-lasting), T (transient), P (Purkinje cells), and N (related to noradrenaline). In the myocardium, L-type calcium channels are involved in excitation–contraction coupling. Slow calcium channels induce the slow depolarization necessary for the normal functioning of heart nodal tissue. Calcium release channels cause calcium delivery from the sarcoplasmic reticulum to the cytosol of cardiomyocytes or smooth muscle cells.

Voltage-gated calcium channels are classified into three main sets: (1) high-voltage activated dihydropyridine-sensitive channels (CaV1.x, L-type), (2) high-voltage activated dihydropyridine-insensitive channels (CaV2.x),[23] and (3) low-voltage activated channels (T-type, CaV3.x). Many CaV2.2 Ca^{++} channel (N-type) isoforms are ubiquitously expressed. CaV2.2 Ca^{++} chan-

[20] Orai1 channel is also called CRACM1. The Orai1 channel forms a multimer that ensures the selectivity for the calcium ion.

[21] STIM1, which is distributed throughout the endoplasmic reticulum, moves and forms clusters as soon as the calcium store empties, close to the plasmalemma (within 25 nm of the plasmalemma), immediately before the activation of CRAC currents. CRAC currents follow Orai1 displacements, the coordinated redistribution of both STIM1 and Orai1 in the endoplasmic reticulum and plasmalemma, respectively, being required for their interaction.

[22] Overexpression of either protein fails to significantly increase the calcium influx. Overexpression of both proteins greatly amplifies store-operated currents.

[23] Alternative names for P/Q-type Ca^{++} channels, N-type, and R-type are CaV2.1, CaV2.2, and CaV2.3, respectively.

nel are inhibited by G-protein-coupled receptors (Sect. 3.2.1) with either a voltage-dependent[24] or -independent manner.[25]

The endoplasmic reticulum in non-muscle cells and smooth muscle cells and the sarcoplasmic reticulum of striated myocytes store calcium ions bound to calreticulin and calsequestrin, respectively. Calcium is released through ryanodine receptors (ryanodine channels) and IP3 receptors. Ryanodine channels (RC) combine with regulatory proteins FKBP. Three RC isoforms exist: RC1 in skeletal muscle, RC2 in cardiomyocytes and RC3 in non-muscle cells. RC2 binds several molecules, such as calmodulin, PKA, phosphatases-1 and -2, triadin, and calsequestrin. Three IP3R isoforms include IP3R2 in cardiomyocytes. IP3R2 releases calcium with a much smaller rate and extent than RC2. Both RC2 and IP3R2 are inhibited by high calcium level and calmodulin and are activated by PKA, PKC or calmodulin-dependent protein kinase-2. Ryanodine channels of the sarcoplasmic reticulum are activated by a positive rising cell potential. Calstabin-2 is a subunit that stabilizes the closed state of the ryanodine receptor, avoiding diastolic Ca^{++} leak from the sarcoplasmic reticulum. Calcium-release activated calcium channel (CRAC) is strongly selective after stimulation by depletion of intracellular calcium levels and depletion of intracellular calcium stores. Store-operated calcium channels are found in arteriolar smooth muscle cells.

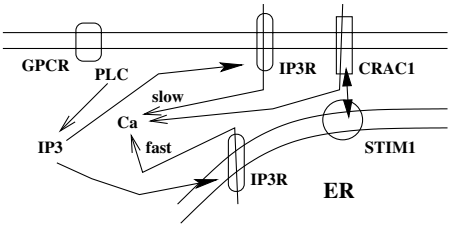

Figure 3.1. Receptor-mediated calcium transports in the cytosol (Source: [215]). Ligand-bound G-protein-coupled receptors stimulate phospholipase-C, which generate inositol(1,4,5)trisphosphate (IP3). The latter interacts with IP3 receptors (IP3R) of the endoplasmic reticulum membrane. IP3Rs, quickly gated by IP3, release calcium from its store, leading to a fast initial rise in cytosolic calcium concentration and a rapid calcium store depletion. In many cell types, the decrease in stored calcium triggers store-operated channels in the plasmalemma, leading to the additional Ca^{++} release-activated Ca^{++} (CRAC) current. Long-term response involves a slower and more persistent calcium entry through a small amount of plasmalemmal IP3Rs, slowly gated by IP3, and a large quantity of Orai1 (or CRACM1). Orai1 couples to calcium sensors STIM1 for store-operated Ca^{++} release-activated Ca^{++} influx.

[24] Inhibition by binding of $G\beta\gamma$, released from Gi/o, to CaV2.2 decreases during depolarization, probably due to dissociation of $G\beta\gamma$.

[25] Inhibition is mediated by several mechanisms. The first one involves Gq and phospholipase-C, the second one Gi/o acting via tyrosine kinase.

The sarco(endo)plasmic reticulum Ca^{++}-ATPase (SERCA) is a member of the P-type ATPase cation pump family. SERCAs maintain a steep calcium level difference between the lumen of the sarcoplasmic reticulum and the cytosol so that fast calcium-mediated signaling can occur. P-type ATPases actively transport cytoplasmic Ca^{++} with a countertransport of luminal H^+ to the cytoplasm. ATP is not only a catalytic substrate but also a stimulatory cofactor.

There are three main isoforms, SERCA1, SERCA2, and SERCA3. Calcium channel SERCA1a is mainly expressed in fast skeletal muscle, SERCA1b in fetal and neonatal tissues, SERCA2a in the heart and slow skeletal muscle, as well as in smooth muscle cells and endothelial cells, and SERCA2b in smooth muscle cells (Table 3.3). Other variants are observed in non-muscle cells and in neuronal cells. SERCA2 is found in all brain regions. SERCA3a and SERCA3b are expressed in endothelial cells, among other cells. In the cardiomyocyte, reuptake of calcium ions in the sarcoplasmic reticulum is done through Ca^{++}-Mg^{++}-ATPase SERCA2a, which has high affinity but low transport capacity. SERCA2a pumps are able to lower the calcium concentration to about 70%. SERCA2a transports two Ca^{++} per ATP. SERCA2a is connected to its regulatory protein phospholamban. Phospholamban binding to SERCA hampers calcium flux. Sarcolipin, expressed in fast-twitch skeletal muscle and atrial myocardium inhibits SERCA1a and SERCA2a.

Plasma membrane Ca^{++}-ATPase belongs to the P-type family of ATPases.[26] Plasma membrane Ca^{++}-ATPase contains an autoinhibitory domain that binds calcium–calmodulin for activation. Calcium–calmodulin-dependent PMCA has a high affinity but a low capacity for Ca^{++}. It is involved in the spatial and temporal control of Ca^{++} level.

Table 3.3. SERCA types and locations.

Type	Locations
SERCA1a	Adult fast-twitch muscle
SERCA1b	Neonatal fast-twitch muscle
SERCA2a	Heart, slow-twitch muscle
SERCA2b	Vascular and mesenteric SMCs
SERCA3a	Platelet, lymphocytes, endothelial cells, Purkinje neurons, pancreas, salivary glands
SERCA3b	Endothelial cells, kidney, pancreas
SERCA3c	Kidney, pancreas

[26] There are five main sets of P-type ATPases: (1) heavy metal ATPases, (2) Ca^{++}-ATPases, (3) H^+-ATPases, (4) amino-phospholipid ATPases, and (5) a set with unknown specificity.

The slow sarcolemmal Ca^{++}-ATPase PMCA contributes to about 1 to 2% of the calcium efflux in the cardiomyocyte. Its activity is much more important in smooth and non-muscle cells. The sarcolemmal Ca^{++} pump transports one Ca^{++} per ATP. All isoforms (PMCA1–PMCA4) are expressed in cardiomyocytes, PMCA1 being the main cardiac type. PMCA activity depends on calmodulin binding and phosphorylation by PKA and/or PKC.

Plasma membrane Ca^{++}-ATPase is the single carrier for calcium extrusion in the majority of cells. At the opposite, in cardiomyocytes, PMCAs play only a minor role in calcium level, being mainly involved in signaling [217]. PMCAs, indeed, do not significantly modify CMC contractile performance. Overexpression of neuronal nitric oxide synthase-associated isoform PMCA4b in arterial smooth muscle cells of mice increases the blood pressure [218], the pump being mainly involved in signal transduction rather than muscle relaxation. PMCA are located in caveolae, nodes of cell signaling. Calcium efflux by PMCA regulates calcium–calmodulin-dependent NOS1 activity (Sect. 4.6); NOS1 is also located in caveolae. PMCA4 is ubiquitously expressed, but its function is tissue specific, due to its interactions with other molecules [210]. There are many PMCA-interacting proteins, such as Ras-associated factor-1, Ca^{++}–calmodulin-dependent protein phosphatase-3, and cytoskeletal syntrophin. PMCAs are tethered to dystrophin complex via syntrophin. Certain PMCA partners recruit PMCA to complexes involved in nitric oxide signaling, presynaptic and postsynaptic Ca^{++} signaling, and actin-cytoskeleton remodeling.

Plasma membrane Ca^{++}-ATPase pumps and Na^+/Ca^{++} exchangers extrude calcium ions. The set of Na^+/Ca^{++}-exchanger (antiporter NCX) comprises the cardiac isoform NCX1, NCX2, which is preferentially expressed in the brain, and NCX3 in skeletal muscle. Na^+/Ca^{++} exchangers can lead to Ca^{++} efflux against Ca^{++} electrochemical gradient without additional energy consumption, because Na^+ entry along the Na^+ gradient yields the necessary energy.

Na^+/Ca^{++} exchangers, which are expressed at high levels in cardiomyocytes (Sect. 7.7.9), exchange (extrude or import) one Ca^{++} ion for three Na^+ ions (entering or leaving the cell, according to the functioning mode, forward or reverse mode, respectively). The Na^+/Ca^{++} exchanger is voltage sensitive and contribute to Ca^{++} influx during cell activation. The Na^+/Ca^{++} exchanger has a relatively low affinity but a high capacity for Ca^{++}. In the heart, NCX is the main source of Ca^{++} extrusion. NCX1 mainly eliminates calcium ions from the cell during diastole. NCX1 extrudes less than 30% of calcium out of the cytosol of cardiomyocytes. NCX1 corrects Na^+ gradient determined by Na^+/K^+ ATPase, which is phosphorylated by protein kinase PKA and/or PKC (Sect. 4.3).

In the absence of NCX1, cardiomyocytes limit Ca^{++} influx through L-type Ca^{++} channels by 50% [219]. Cardiomyocyte adaption hence does not increase other Ca^{++}-efflux mechanisms. NCX1 is activated by the binding of intracellular Ca^{++}, which removes Na^+-dependent inactivation. Ca^{++}-binding domain

of NCX is able to detect fast changes in intracellular Ca^{++} level during the cardiomyocyte functioning [220]. Elevated concentrations of cytosolic Na^+ either inactivates or activates NCX according to phosphatidyl(4,5)bisphosphate levels. In the heart, NCX transport 10 to 15 times more calcium than PMCA.

3.1.1.3 Sodium Carriers

Sodium channels deactivate quickly compared with calcium channels. Fast sodium channels play a key role for rapid communication between cells. Sodium channels in the nephron control the natriuresis, and subsequently the blood volume. Voltage-gated sodium channels yield the action potential of neurons, and voltage-gated potassium channels drive the repolarization. During the propagation of the action potential in the heart, sodium channels also first activate.

Voltage-gated sodium channels are made of one pore-forming α-subunit associated with one or two β-subunit. There are at least nine types of voltage-gated sodium channels (NaV1.1–NaV1.9); NaV1.5 is also known as the cardiac isoform.

Sodium channels have a cytoplasmic activation gate and an inactivation component that diffuses toward the corresponding receptor in the channel to inactivate the sodium channel. The inactivation component is inhibited when the activation gate is open.

The ENaC family of sodium selective channels is composed of many proteins. Acid-sensing ion channels (ASIC) are gated by a decrease in extracellular pH. The Na^+–K^+-ATPase is made of the association of α- and β-subunits, each one having multiple isoforms.[27] The α-isoforms confer different plasmalemmal distributions and kinetic properties. Plasmalemmal α2-Na^+ pumps are confined to *plasmerosomes*,[28] whereas α1-Na^+ pumps are more uniformly distributed [221]. Na^+ affinity depending on the α-isoform, the ionic composition of the cytoplasmic microdomains near the different Na^+ pumps can differ. Besides, α2 Na^+ pumps are colocalized with Na^+/Ca^{++} exchangers for a coupling activity. Cardiac heterozygous α2 Na^+ pumps increase calcium transients during the contractile cycle. Heterozygous α1 Na^+-K^+-ATPase have opposite effects [222].

3.1.1.4 Potassium Carriers

Potassium channels have diverse structures and functions, such as maintenance of the resting cell membrane potential and repolarization in excitable

[27] There are at least four different α-polypeptides (α1, α2, α3, and α4) and three distinct β-isoforms (β1, β2, and β3).

[28] The plasmerosome is the unit made by the plasmalemma microdomain and the adjoining junctional endoplasmic reticulum, separated by a tiny (12–20 nm) junctional space.

cells. Potassium channels, indeed, are ion-selective cation channels that have an equilibrium potential close to the cell resting potential. Potassium channels determine the shape and the duration of action potentials, the cell firing rates, and the cell excitability (Sects. 7.7.9 and 7.8). The potassium channel set is composed of voltage- and ligand-gated channels (Table 3.4).

High-conductance potassium channels,[29] encoded by the Slo gene family (Slo channels), are sensitive to different ions (Ca^{++} and Na^{+}) and second messengers activated by G-protein-coupled receptors. Slo channels, indeed, contain both voltage- and ligand-gating domains. Voltage-sensitive Slo channels have a structure similar to the one of voltage-gated K^{+} channels. Ubiquitously expressed Slo1 channels have the largest single-channel conductance of all K^{+}-selective channels. Slo1 channel isoforms arise from many sources, especially distinct β-subunits, which confer variable Ca^{++}-sensitivity and inactivation rate.

KCNK potassium channels, responsible for a leak current, are strongly regulated. Voltage-gated K^{+} (Kv) channels in excitable cells open in response to changes in voltage difference between the fluids separated by the plasmalemma. Fast-inactivating A-type potassium currents inhibit back-propagation of dendritic action potentials. They are also implicated in synaptic plasticity and regulate heart excitability.[30]

Table 3.4. Example of a set of ion channels: the potassium channel family and associated currents (usual abbreviation).

K^{+} channel	Current
Voltage-gated channels	
Transient outward	Ito
Outward rectifier delayed	IK
Inward rectifying	IK1
Ligand-gated channels	
ATP-sensitive	IK(ATP)
Acetylcholine-activated	IK(ACh)
Calcium-activated	
Sodium-activated	
Arachidonic acid-activated	
Cyclic nucleotide-gated	
Adenosine-sensitive	

[29] This K^{+} channel is also called the big potassium (BK) channel, or maxi-K channel, owing to large single-channel currents.

[30] Four voltage-gated potassium channel-interacting proteins (KChIP1–KChIP4), with several spliced KChIP isoforms, regulate the functioning of K^{+} channels of the Kv4 family [223].

Voltage-gated potassium channels Kv1.5 generate the ultrarapid repolarizing current in atrial myocytes and regulates with oxygen the vascular tone. Kv1.5 is targeted by ubiquitin-like SUMO (SUMO1–SUMO3) and SUMO-conjugating enzyme Ubc9 for adaptive tuning of the electrical excitability of cells [224]. This interaction reversibly modulates Kv1.5 activity, because Kv1.5 is liberated from SUMO by SUMO-specific proteases SENP2. Kv1.5 sumoylation can change the excitability of atrial myocytes and vascular smooth muscle cells, in particular in the pulmonary vasculature according to oxygen level.

In non-excitable cells, voltage-gated K^+ channels can be activated by substances with change lipid charges of the adjoining plasmalemma [225]. Voltage- and calcium-activated K^+ (BKCa) tetrameric channels regulate the vascular smooth muscle tone. The pore-forming α-subunit contains the voltage and Ca^{++} sensors. Modifications in membrane potential displace the voltage sensor. The resulting conformational change then drives the channel opening.

At least certain voltage-gated potassium channels have a component at the external face of the channel with positively charged amino acids, which senses the change in voltage across the cell membrane. These voltage-gated potassium channels open due to the presence of suitable plasmalemmal phospholipids, with their negatively charged phosphate group [226]. Voltage changes generate sensor motions within the lipid bilayer, leading to opening of the channel central pore.

Voltage-gated potassium channels are used for cell repolarization, such as in the myocardium. Transient outward K^+ channels rapidly activate and inactivate. Delayed rectifier K^+ channels have slower activity than transient-outward K^+ channels. Inward rectifier Kir channels, which lack steep voltage-gating, modulate the cell excitability. G-protein-coupled inward rectifier channels are bound to and modified by integrins. They are inactivated by stretch. G-protein–activated inward rectifier K^+ channels are found in atrial myocytes, but not in the sinoatrial node. Potassium channel BK, located at the plasmalemma of smooth muscle cells, is activated by a localized calcium spark produced by the ryanodine channel. Acetylcholine-activated K^+ channels slow the heart frequency. Calcium-activated K^+ channels have been discovered in red blood cells. cGMP channels (CNG) associate changes in intracellular cAMP and cGMP with excitability. ATP-sensitive K^+ channels, members of inward rectifier channels, reduce the action potential duration.

ATP-sensitive potassium channel (KATP) links electrical excitability to cellular energetics. KATP channels are formed by two different proteins, Kir6 and sulphonylurea receptor (SuR), a member of the ATP-binding cassette protein family [227]. Four Kir6.2 subunits generate the channel pore, each Kir6.2 subunit being associated with one SuR1 protein.[31] SuR contains one nucleotide-binding site. KATP channels is gated by two mechanisms: (1) KATP channels show fast, ligand-independent gating, and (2) slow, ligand-dependent gating. KATP channel is inhibited by ATP binding and activated

[31] SuR1 protein corresponds to ABC subfamily C type 8 (ABC-C8).

by Mg^{++}-bound nucleotides. Phosphatidylinositol(4,5)bisphosphate activates, interacting with Kir6.2 subunits. Kir6.2 subunits have binding sites for ATP (with or without Mg^{++}) and PIP2 (mutual exclusion), which stabilize closure and opening of the channel, respectively. Mg^{++}-dependent ATP hydrolysis on SuR overcomes the inhibitory effect of ATP on Kir6.2. However, channel opening by MgADP is more efficient. The state of KATP channel, thereby depends on the metabolic activity of the cell. The function of KATP channels especially depends on actin binding in myocytes.

Conductance increase with respect to steady-state opening of the potassium channel KcsA is induced by protons acting on the intracellular side [228]. Gating is modulated by transmembrane voltage. Gating is followed by inactivation which depends on external K^+ concentration.

According to the number of transmembrane (TM) domains, potassium channels are classified into three main sets: the 2TM, 4TM, and 6TM classes. The 2TM set is composed of inward rectifier potassium channels, including several subsets: Kir1.x to Kir7.x. Kir2.x are strong inward rectifier potassium channels of the heart, Kir3.x G-protein–activated inward rectifier potassium channels, and Kir6.x ATP-sensitive inward rectifier potassium channels. The 4TM set is made of potassium channels responsible for background currents, with subsets TWIK, TREK, TASK, TALK, THIK, and TRESK. The 6TM set comprises Kv, including KCNQ (including cardiac IKs),[32] EAG (including cardiac IKr),[33] Slo, and SK.

Potassium channel subunits can contain two, four, or six/seven transmembrane segments. Subunits with either two or six/seven transmembrane segments form a single pore-forming domain. Subunits with four transmembrane segments contain two pore-forming domains arranged in tandem. One-pore-domain potassium channels (K1P) are made of homo- or heterotetramers. Background potassium channels are composed of K2P channel subunits, also called KCNKx subunits or tandem of P domains in a weak inwardly rectifying K^+ channel (TWIK) subunits. Dimeric two-pore-domain potassium channels (K2P) have unusual gating properties, allowing background activity and membrane-stretch sensitivity. The K2P channel subunits are subdivided into six main structural sets: set 1 with TWIK and KCNK7 channels, set 2 with TWIK-related K^+ (TREK) and TWIK-related arachidonic acid-stimulated (TRAAK) channels, set 3 with TASK channels, set 4 with tandem-pore-domain halothane-inhibited K^+ (THIK) channels, set 5 with TWIK-related alkaline-pH-activated K^+ (TALK) channels, and set 6 with the TWIK-related spinal cord K^+ (TRESK) channels [231].

[32] Decrease in KCNQ potassium channel, coded by KCNQ1 gene and involved in myocardial slow repolarization (IKs, in opposition to rapid phase of cardiac repolarization IKr), are associated with cardiac arrhythmias [229]. Reduced cardiac repolarization and prolonged cardiac action potentials increase the risk of early after-depolarization and torsades de pointes.

[33] The rapid phase of cardiac repolarization is ensured by K^+ channels encoded by human ether-a-go-go related gene (hERG).

TREK channels are two-pore-domain K+ channels belonging to the set of K2P channels, which produce outwardly rectifying currents. They are stimulated by mechanical stretch, intracellular acidification, temperature, polyunsaturated fatty acids (arachidonic acid, lysophospholipids, phosphatidylinositol(4,5)bisphosphate) [230]. They are inhibited by G-protein-coupled receptors activated by neurotransmitters and hormones. A-kinase–anchoring protein AKAP150, a constituent of TREK1 channels,[34] transforms low-activity outwardly rectifying current into robust leak conductance insensitive to stimulators but hampered by GPCRs.[35]

Background potassium channel TREK1 of endothelial cells in small mesenteric arteries and skin vessels is regulated by multiple chemical (hormones and neurotransmitters acting via GPCRs, such as serotonin, and intracellular pH) and physical stimuli [231].[36] Vasodilators acetylcholine and bradykinin induce the production by endothelial cells of nitric oxide via TREK1 [232]. The latter acts on underlying smooth muscle cells, inducing relaxation.

3.1.1.5 Chloride Carriers

Chloride channels contribute to membrane excitability, transepithelial transport, regulation of cell volume, pH control of intracellular organelles, cell cycle, and apoptosis. Three main types of chloride channels exist: voltage-gated,

[34] TREK1 is highly expressed in the brain, particularly in γ-aminobutyric acid–containing neurons of the caudate nucleus and putamen. TREK1 is also found in the prefrontal cortex, hippocampus, hypothalamus, midbrain serotonergic neurons of the dorsal raphé nucleus, and sensory neurons of the dorsal root ganglia. TREK1 is also observed in peripheral tissues.

[35] Inhibition of TREK1–AKAP150 complex by Gs-coupled receptors is faster but have the same magnitude than inhibition of TREK1. Inhibition of TREK1–AKAP150 by Gq-coupled receptors is much reduced than inhibition of TREK1.

[36] TREK1 is activated by intracellular acidosis, depolarization, high temperature, and stretch. TREK1 is closed by protein kinases PKA and PKC pathways. TREK1 is inhibited by stimulated GPCRs (Gs and Gq). TREK1 activity is modulated by polyunsaturated fatty acids, such as arachidonic acid, which reversibly open TREK1 channels. PtdIns(4,5)P2 hydrolysis by PLC shifts TREK1 voltage-dependent activation toward more depolarized voltages. Mechanical and lipid activations of TREK1 can be functionally linked. The C-terminal domain of TREK1 regulates the voltage- and time-dependent gating. A-kinase anchoring protein AKAP150 interacts with TREK1 and modifies its regulation. AKAP150 augments the inhibition of TREK1 by Gs-coupled receptors and reverses the downmodulation of TREK1 by Gq-coupled receptors [231].

ligand-gated,[37] and cystic fibrosis transmembrane conductance regulator[38] (CFTR). In the heart, CFTR mediates protein kinase-A–stimulated Cl^- currents. Chloride channels are also classified as (1) voltage-gated CLC set (with its known members CLC1/2, CLC-Ka/Kb, CLC3/5/6), (2) calcium-activated channels (CaCC), (3) high-conductance channels (maxiCl), (4) CFTR, and (5) volume-regulated channels (VRAC).

CLC channels belong to the largest chloride channel family. CLC chloride channels are activated by strong hyperpolarization or cell volume changes (but not ligand binding), comprises at least nine mammalian members (CLCO, CLC1, CLC2, etc.).[39] Slightly outward rectifying chloride channel CLCK1 is a kidney-specific channel involved in transepithelial chloride transport in the inner medullary thin ascending limb of Henle loop (Part II). Chloride channels CLCNKA and CLCNKB are also found in the kidney, as well as calcium-activated Cl^- channel (CLCAn). Intracellular chloride channels (CLIC) are located in the nuclear membrane. These channels could be involved in cell cycle regulation. Eicosanoid-modulated chloride channels are directly gated by EET.

3.1.1.6 Ion Channel Enzymes

Certain ion channels can have enzymatic activity. The cation channels TRPM6 and TRPM7 are associated with an α-kinase. TRPM7 is involved in cellular Mg^{++} homeostasis. TRPM7 channels are highly permeable to Ca^{++}. It is activated by phospholipase C. TRPM7 is also a receptor for signal transduction. It indeed modulates the function of the actomyosin cytoskeleton. TRPM7 phosphorylates the myosin-2A heavy chain. Myosin-2 is the major motor protein responsible for cell contractility.[40] TRPM7, activated by bradykinin, a

[37] γ-aminobuteryic acid (GABA) and glycine channels in the postsynaptic neuron and skeletal muscles belong to the family of chloride channels. GABA is synthezised from glutamate. Glycine channels are mainly located in the brain. Other types include calcium-activated chloride channel, volume-regulated chloride channel, and calcium-dependent ATP/UTP-activated chloride channel.

[38] The ion channel CFTR also functions as a regulator of other ion channels. CFTR particularly regulates Cl^-/HCO_3^- exchanger. CFTR also transports transport ATP, sodium, bicarbonate ions, and water. CFTR is activated by protein kinase-A, cAMP, and ATP binding. CFTR is phosphorylated by cGMP-dependent protein kinases-1a and -2. CFTR is expressed in endothelial cells, in cardiomyocytes, in erythrocytes, in lung epithelia (for mucus formation), among others. CFTR permeability depends on animal species. CFTR genes belong to gene family of ATP-binding cassette transporters.

[39] CLC1 is expressed in the sarcoplasmic reticulum of skeletal myocytes. CLC2, ubiquitously synthetized, particularly in lung epithelia, is activated by cell swelling or strong hyperpolarization.

[40] Cell contractility associated with myosin-2 is regulated by Rho GTPases and their effectors, myosin light chain kinases and phosphatases, as well as inhibitor

Gq-PLC-coupled receptor agonist, initiates a Ca^{++}-dependent phosphorylation of myosin heavy chains and disassembly of myosin-2A at the cell cortex, promoting cell spreading and augmented adhesion to the extracellular matrix [233].

3.1.2 Molecule Carriers

The membrane channels *aquaporins* regulate the water flux across the plasmalemma. Aquaporin-1 not only leads to water flux but also is a cGMP-gated ion channel. Aquaporin-7, the expression of which is inhibited by insulin, also carries glycerol[41] and thereby regulate fat accumulation. Binding sites for plasma albumin are gp60 or albondin, gp30, and gp18, expressed in particular in the endothelium.

Copper is a cofactor for several enzymes, able to accept and donate electrons as it changes its oxidation state between Cu^{+} and Cu^{++}. It participates in the formation of reactive oxygen species when unbound to proteins within the cell. Cu^{+}-binding metallochaperones, such as antioxidant protein Atox1, transport copper to different intracellular sites for storage or assembly of cupro-enzymes. The intracellular copper concentration is controlled by the balance between the activity of copper-efflux ATPases (ATP7A and ATP7B effluxers) and copper permeases (Ctr1 and Ctr2 importers). Cells use copper toxicity to rid of pathogens. Activated macrophages and neutrophils release ROS using NADPH oxidase and copper for storage in the phagosome.

Transcellular and paracellular (through the tight junction complex) transports of water and electrolytes maintain homeostasis at a given time (owing to biological cycles). Claudin-7, claudin-3, and occludin regulate the permeability of tight junctions, whereas aquaporin-5 is the major transcellular water transporter in salivary acinar cells. Aquaporin-5 in salivary gland is required for water transport not only across the plasmalemma but also through tight junctions [234]. Aquaporins, claudins, and occludins are involved in the cross-talk between both transcellular and paracellular transports, which are coordinated rather than compensatory.

Glucose transporters (GluT) are responsible for the uptake of several monosaccharides, such as glucose, fructose, mannose, galactose, and glucosamine. Insulin regulates glucose homeostasis by increasing glucose-uptake rate, especially into myocytes and adipocytes, using *glucose transporter* GluT4. Adipokine serum retinol binding protein 4 (RBP4) is secreted by adipose tissues in the absence of glucose uptake by GluT4. RBP4 restricts glucose uptake in the liver and skeletal muscle, inhibiting insulin signaling.

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myosin heavy chain kinases, which belong to the α-kinase family. Myosin-2 assembles into bipolar thick filaments and generate a tension of the cell cortex by pulling together oppositely oriented actin filaments.

[41] Glycerol binds three fatty acids to form triglycerides. Glycerol released from fat cells into the blood is taken up mainly by the liver and converted to glucose.

RBP4 increases glucose output by the liver, stimulating the gluconeogenic enzyme phosphoenolpyruvate carboxykinase in the liver [235].

ATP binding cassette transporters belong to a family of membrane proteins involved in the cellular export or import of various substances (ions, phosphate esters, inorganic phosphate and sulfate, glucids, lipids, cyclic nucleotides, iron-chelator complexes, vitamins, peptides, and proteins), including rare elements and drugs. Most of ATP-binding cassette transporters, importers and exporters, are composed of two transmembrane domains for substrate binding and carrying substrates across cell membranes, and two cytoplasmic nucleotide-binding (ATP-hydrolysis) sites. Transmembrane domains contain a variable number of transmembrane helices. Exporters have a set of 12 transmembrane helices, importers are made of 10 to 20 transmembrane helices according to the mass and chemical nature of their substrate. ABC importers, but not ABC exporters, require a binding protein to carry the substrate. In certain types of ABC transporters with two ATP binding sites,[42] ATP binding seems to cause unidirectional transport because it promotes an outward-facing conformation, whereas dissociation of ATP-hydrolysis products elicits an inward-facing state [236]. The exporter cleft, only accessible from the cytoplasm, is separated from the extracellular medium by a closed gate. Binding of two ATP molecules on ATP-binding sites closes the gap. In ATP-free ABC importers, the attached binding protein appears to play the gating role. In the presence of apolipoprotein-A1, ABC-A1 promotes cellular efflux of phosphatidylcholine, sphingomyelin, and cholesterol.

3.2 Plasmalemmal Receptors

There are two main kinds of proteic receptors, plasmalemmal and intracellular, which bind specific ligands. At the cell surface, many different kinds of receptors are expressed so the cell can respond to manifold environmental signals.

Plasmalemmal receptors are mostly integral membrane proteins. They have three basic domains: (1) an extracellular domain, the ligand-binding domain, (2) a transmembrane domain, anchoring the receptor in the membrane, and (3) a cytoplasmic domain, which activates the effector. In some cases, other membrane proteins interact with the receptor to modulate its activity. Certain receptors cluster together in the membrane after binding the ligand.

The plasmalemmal receptors can be regrouped in several main families (Table 3.5): (1) receptor tyrosine kinases (insulin and growth factor receptors), (2) receptor tyrosine phosphatases (cluster determinant protein CD45 of T lymphocytes and macrophages), (3) receptor serine/threonine kinases (activins and TGFβ receptors), (4) receptor serine/threonine phosphatases, and (5) G-protein-coupled receptors (i.e., adrenaline, angiotensin, bradykinin,

[42] Vitamin B12 transporter has a nucleotide-free conformation.

Table 3.5. Receptor families. Heterotrimeric G proteins (Gαβγ) coupled to receptors transduce signals in various pathways. Both receptor tyrosine kinases (RTK) and receptor protein tyrosine phosphatases (RPTP) include several subsets. There are also receptor serine/threonine kinases (RSTK). Steroid receptors reside within the nucleus except the glucocorticoid receptor located in the cytosol until it binds its ligand. T-cell receptor is targeted by a combination of antigen fragment with a glycoprotein of the major histocompatibility complex. Toll-like receptors are members of pattern-recognition receptors.

Receptor type	Features
GPCRs	Activation of GTPases and protein kinases Ion transport
RTKs	Cell growth and differentiation Activation of GTPases and protein kinases
RPTPs	Opposition to RTK
RSTKs	Cell growth and differentiation TGFβ family
Nucleotide receptors	Flow-stress sensing
Steroid receptors	Location in nucleus or cytosol Ligands: glucocorticoids, mineralocorticoids, sex hormones
Cytokine receptors	GTPase activation NRTK activation
Nitric oxide receptors	Association with guanylyl cyclase Effectors: protein kinases B and G
Frizzled receptors	Wnt signaling Activation of cytosolic Disheveled β-catenin nuclear translocation
Notch receptors	Gene transcription Jagged/Serrate and Delta ligands NFκB activation
T-cell receptor for antigen	Dimeric receptor Rise in intracellular calcium level Activation of calcineurin
Toll-like receptors	Detection of pathogen molecules Activation of immune cells

vasopressin receptors; Table 3.6). Cell growth and differentiation are regulated by the opposing activities of protein tyrosine kinases (PTK) and protein tyrosine phosphatases (PTP). Both enzyme sets contain transmembrane or receptor kinase/phosphatase families.

Receptor ligands include hormones (App. A), neurotransmitters (Table 3.7), growth factors (Table 3.8), antigens, immunoglobulins, complement fragments, lipoproteins, etc.

The cytokine receptors are classified into several groups, which include: (1) immunoglobulin superfamily receptors (e.g., IL1 receptor), (2) hematopoietic receptors (class 1; e.g., high-affinity, trimeric IL-2 receptor, hematopoietic growth factor receptors), (3) interferon receptors (class 2; e.g., receptors for IFN1s, IFN2s and tissue factor), (4) tumor necrosis factor receptors (class 3), and (5) G-protein-coupled receptors for hormones and neurotransmitters. Prolactin receptors (PrlR) and growth hormone receptors (GHR) are members of the cytokine receptor superfamily.

3.2.1 G-Protein-coupled Receptors

G-protein-coupled receptors represent the largest group of plasmalemmal receptors. GPCRs are implicated in diverse physiological processes, such as neurotransmission, photoreception (vision), chemosensation, olfaction, cognition, pain perception, metabolism, cell growth, cell differentiation, cell migration, inflammation, immunity, and cardiac function, because GPCRs are activated by multiple ligands and stimulators (Table 3.9). Responses resulting from GPCR activation can integrate several intracellular signaling pathways.

G-protein-coupled receptors interact with guanine-nucleotide–binding proteins in cardiovascular signal transduction.[43] Ligand-activated receptors

Table 3.6. Examples of G-protein-coupled receptors. A collection of distinct GDP/GTP-bound Gα subunits confer pathway specificity (Sect. 4.5). The activation of Gα reduces its affinity for Gβγ with respect to inactive Gα(GDP). The active Gα(GTP) then dissociates from the plasmalemmal complex and binds effectors to trigger the corresponding signaling pathway. Intrinsic GTPase activity of Gα allows its rebinding to Gβγ (G-protein cycle).

Ligand	Activity
Glutamate	Neurotransmission
(metabotropic)	Voltage-sensitive (some)
Cathecholamine	Neurotransmission
Acetylcholine	Neurotransmission
Angiotensin-2	Blood pressure regulation
Endothelin-1	Vasomotor tone
Adrenomedullin	Water and salt balance
Vasopressin	Water and salt balance
Leukotriene	Chemotaxis, inflammation
Calcium	Hematopoiesis

[43] On the cardiomyocyte membrane, angiotensin-2, endothelin-1, norepinephrine, and prostaglandin F2α activate receptors coupled to Gq.

Table 3.7. Receptors of the autonomic nervous system.

Type	Location
Adrenergic receptors	
α1	Vascular smooth muscle cell (skin and splanchnic regions)
α2	Presynaptic nerve endings Platelets Adipocytes
β1	Nodal tissue Myocardium
β2	Vascular smooth muscle cell (skeletal muscles) Bronchial smooth muscle cell Digestive tract
Dopamine receptor	Smooth muscle cells, neurons
Glutamate receptor	Astrocyte
Cholinergic receptors	
Nicotinic	Autonomic ganglia (sympathetic and parasympathetic) Adrenal medulla Neuromuscular junction
Muscarinic	Sinoatrial node Non-vascular smooth muscle cell Glands

catalyze the GDP–GTP exchange at a coupled G protein, and thereby promote the activity of effectors (second messenger–producing enzymes and

Table 3.8. Types of cytokine receptors.

Type	Ligand
Class 1	
GM-CSF subset	Granulocyte–monocyte colony-stimulating factor Interleukins IL3, IL5
IL6 subset	Interleukins IL6, IL11, IL12
IL2 subset	Interleukins IL2, IL4, IL7, IL9, IL15
Hormone receptors	Growth hormone, prolactin
Class 2	Interferons IFNα, IFNβ, IFNγ
TNF receptors	Tumor-necrosis factor
Immunoglobulin superfamily	Macrophage colony-stimulating factor Interleukins IL1

ion channels). G-protein-coupled receptors thus act as guanine nucleotide-exchange factors (GEF) for the subunits of G proteins. GDP release is the rate-limiting step in G-protein activation. After receptor activation, G protein binds to the receptor, the subsequent conformational change decreasing the affinity of the G protein for guanosine diphosphate.

G-protein-coupled receptors signal via heterotrimeric G proteins and via various small monomeric GTPases to regulate the activity of effectors. Activated $G\alpha$ and $G\beta\gamma$ proteins positively or negatively regulate various effectors such as phospholipases (PLA, PLC, and PLD),[44] adenylyl and guanylyl cyclases, phosphoinositide 3-kinases, phosphodiesterases, protein kinase-C, and ion channels (Table 3.10, Fig. 3.2). Subsequently, these effectors activate or inhibit the production of second messengers (cAMP, cGMP, diacylglycerol, inositol trisphosphate, phosphatidylinositol(3,4,5)trisphosphate, and arachidonic and phosphatidic acids), and promote calcium influx, and opening or closure of various ion channels.

Receptor signaling is regulated by both endogenous and exogenous actions. G-protein-coupled receptors also act as scaffolds for the formation and location of signaling complexes in the cell. The response properties are determined by the relationship among the ligand, receptor, G protein, and associated proteins. Activation of G-protein-coupled receptors can be sensitive to the

Table 3.9. Various GPCR ligands and stimulators activate cytoplasmic and nuclear molecules using G-protein– and –independent pathways (Source: [237]).

Biogenic amines	Noradrenaline, dopamine, serotonin, Acetylchonine, histamine
Amino acids	Glutamate, GABA
Proteins and peptides	Angiotensin, bradykinin, Thrombin, bombesin, endorphins, Follicle-stimulating hormone, leuteinizing hormone, Thyroid-stimulating hormone Gastrin-releasing peptide, cholecystokinin, Neuromedin-B, neurotensin, vasopressin, galanin
Lipids	Lysophosphatidic acid, sphingosine-1-phosphate Prostaglandins, leukotrienes Platelet-activating factor, anandamine
Ions	Calcium
Miscellaneous	Light, odorants, pheronomes, Nucleotides, endorphins

[44] PLD1/2 regulate cytoskeletal organization and endocytosis and exocytosis. G-proteincoupled receptor signaling leads to direct stimulation of PLD1 by PKC.

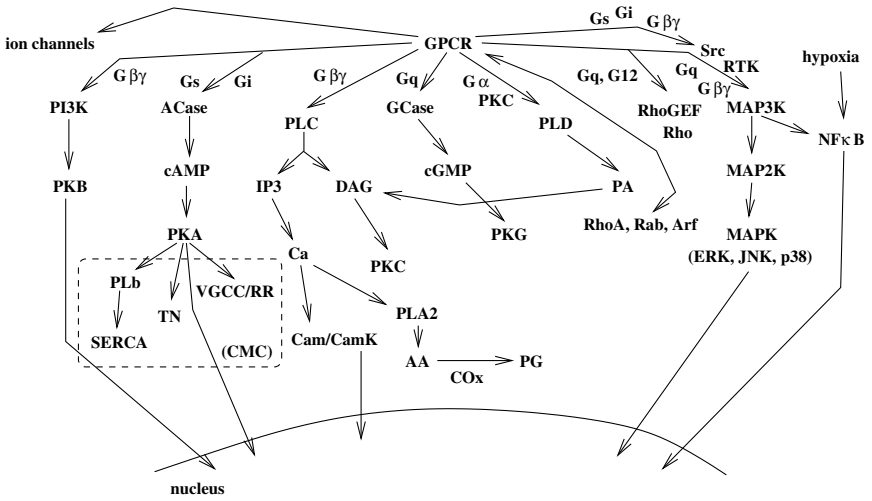

Figure 3.2. GPCR effectors with their associated molecular triggers: Gα (Gs, Gi, Gq, and G12), Gβγ, PKC, Src, and RTK.

plasmalemma lipid composition. Local cholesterol and sphingolipid concentrations affect the ligand binding and receptor transport.

Many G-protein-coupled receptors also exhibit ligand-independent constitutive activity, changing their conformation to an active state upon stimulation, owing to interactions with the plasmalemmal lipids. The plasmalemma tension can then modulate the GPCR activity. The GPCR stimulation then catalyzes G-protein activation.

Table 3.10. Gα effectors. Trimer G proteins are made of Gα and Gβγ. Gα are grouped into four main families: Gs, Gi, Gq, and G12. Activated Gα proteins positively or negatively regulate various effectors (phospholipases PLA2, PLCβ, PLD, adenylyl [ACase] and guanylyl [GCase] cyclases, phosphoinositide 3-kinases [PI3K], protein kinase-C [PKC], phosphodiesterases [PDE]), Rho GTPases, and ion channels. GPCR can signal via either G proteins or NRTK Src, according to ligand level. Besides, cross-talks exist between GPCRs and small GTPases (Source: [237]).

Gα subset	effectors
Gαs	ACase, PKA
Gαi	ACase, phospholipases, PDEs, ion channels
Gαq	PLCβ, diacylglycerol, PKC, calcium ions
Gα12	Rho, RhoGEFs

G-protein-coupled receptors can form homodimers or heterodimers. Two subunits of a receptor dimer coupled to G protein differ in their conformation [239]. A receptor dimer with a single ligand-bound subunit can trigger G-protein activation. The interaction of G protein with the receptor dimer prevents a symmetrical functioning of the dimer and explains the negative cooperativity in ligand binding on GPCR dimers.

Ligand binding on G-protein-coupled receptors can be voltage sensitive, GPCRs serving as sensors for both transmembrane potential and external compounds [240]. Voltage-sensitive G-protein-coupled receptors are triggered by the voltage across the plasmalemma.[45] Gating currents act on structural GPCR components.

3.2.1.1 Examples of G-Protein-coupled Receptors

Acetylcholine Muscarinic Receptors

Main G proteins (Sect. 4.5.1, Fig. 4.7) targeted by acetylcholine muscarinic receptors are given in Table 3.11. M1 and M2 muscarinic receptors couple voltage sensing to acetylcholine binding. Conformational changes are associated with charge motions.[46] Ligand binding of GPCRs can then be modulated by voltage, amplifying or attenuating the signaling.

The activation of muscarinic receptors in cardiomyocytes depends on plasmalemmal potential. Depolarization reversibly induces changes in affinity state of the receptor [242]. Depolarization reduces and increases the affinity of M2 and M1 muscarinic receptors, respectively [243]. The activity of P2Y1 receptors also depends on the plasmalemmal potential. P2Y receptor–Ca^{++} fluxes are larger for a given depolarization than for equivalent amplitude hyperpolarization [244].

Table 3.11. Acetylcholine muscarinic receptors and their main targeted G proteins (Source: [241]).

Type	Main transducer
ACh muscarinic receptors	
M1/M3/M5	Gq/11
M2/M4	Gi/o
ACh nicotinic receptors	
$\alpha 1$–$\alpha 7$	Ligand-gated ion channels

[45] The resting potential difference across the 3 nm-thick plasmalemma is equal to about 70mV. It can affect the conformation of membrane proteins.

[46] Voltage sensitivity is detected not only in voltage-gated ion channels, but also in ion transporters, such as sodium/glucose co-transporter, in voltage-dependent phosphoinositide phosphatase, and glutamate G-protein-coupled receptors mGluR1 and mGluR3.

Adenosine and Nucleotide (Purinergic) Receptors

Adenosine receptors inhibits ATP-using enzymes. Nucleotide receptors P2Y are activated by adenosine diphosphate (ADP), adenosine triphosphate (ATP), uridine triphosphate (UTP), and uridine diphosphate-glucose (UDP-glucose) (Table 3.12).

Ubiquitously expressed nucleotide receptors or purinergic receptors include P1 receptors and P2 receptors. P1 receptors bind adenosine, P2 receptors extracellular adenosine and purine nucleosides and nucleotides (Fig. 3.3) [300]. There are four subtypes of P1 receptors (A1, A2a, A2b, and A3). P1A2 is coupled to Gs and stimulates adenylyl cyclase, whereas P1A1 and P1A3 are coupled to Go/Gi and inhibit adenylyl cyclase. P2 receptors P2X are ion channels (ionotropic receptors)[47] and P2Y are G-protein-coupled receptors (metabotropic receptors). Both P2 receptor types comprise many members. There are at least seven P2X receptor types (P2X1–P2X7) and fourteen P2Y receptor types (P2Y1–P2Y14). Most P2X channels are cation-selective pores that discriminate more or less efficiently different cations.

Purinergic receptors regulate the cardiac function. Receptors P2Y (especially P2Y6, P2Y7, P2Y9, and P2Y14) are found in the heart. In the blood vessel wall, purinergic signaling controls the vascular tone, the wall remodeling (smooth muscle cell proliferation), and the platelet aggregation. The differ-

Table 3.12. Adenosine and nucleotide receptors P2Y, their main targeted G proteins, and order of ligand potency. Receptors P2X are ATP-gated ion channels for Na^+, K^+, and Ca^{++} (Source: [241]).

Type	Main transducer	Potency order
Adenosine receptors		
AR1/3	Gi/o	
AR2A/B	Gs	
Nucleotide receptors		
P2Y1	Gq/11	ADP > ATP
P2Y2	Gq/11	UTP ∼ ATP
P2Y4	Gq/11	UTP > ATP
P2Y6	Gq/11	UDP > UTP > ATP
P2Y11	Gs, Gq/11	ATP > UTP
P2Y12	Gi/o	ADP > ATP
P2Y13	Gi/o	ADP > ATP
P2Y14	Gq/11	UDP-glucose

[47] Purinergic receptors P2X are fast-acting channels that are selective for calcium. When ATP binds to P2X plasmalemmal receptors, extracellular Ca^{++} enters and activates signal transduction pathways. These channels contain two transmembrane domains (hetero- or homo-oligomers).

ent types of cells in the cardiovascular system selectivity express purinergic receptor types (Fig. 3.4). Endothelial cells express purinergic receptors P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11,[48] as well as P2X1,[49] P2X2, P2X3, P2X4, P2X5, P2X6, and P2X7.[50] P2X4 is the most abundant P2X receptor type in vascular endothelial cells. Thereby, P2X4 receptor contributes to ATP- and flow-induced Ca^{++} influx in endothelial cells. P2 receptors (particularly P2X4) on endothelial cells bind ATP, which triggers secretion of nitric oxide and causes vasodilation. P2X receptors are implicated in autocrine loops in endothelial cells. Vascular smooth muscle cells abundantly express receptors P2X1, P2Y2, and P2Y6. P2 receptors are also located in the vessel wall adventitia. Perivascular adrenergic and cholinergic nerves have purinergic receptors.

P2X1 receptors are abundantly expressed in platelets. P2Y1 acts in platelet aggregation and mediates the adenine nucleotide–induced release of nitric oxide. P2Y12 has also been observed in platelets. P2Y13 can be detected in peripheral blood leukocytes. P2 receptors are found in every part of the nephron (Table 3.13).

Adrenergic Receptors

α- and β-adrenergic receptors (AR) belong to the family of G-protein-coupled receptors (Table 3.14). β1-Adrenergic receptors strongly increase cAMP levels, whereas β2- and β3-adrenergic receptors can couple to Gi. In rat ventricular cardiomyocytes, β2-adrenoceptors couple to Gs (preferentially) and Gi-protein, activating either Gs or both Gs and Gi protein [245]. Adenylyl cyclase activation by β2-adrenoceptors coupled to Gαi2 can be done by preventing Gαi2 to interact with the inhibitory Gαi site of adenylyl cyclase [246].

In cardiomyocytes, β1 and β2-adrenergic receptors activate p38 and ERK mitogen-activated protein kinase, respectively (Sect. 4.3.2.4). α1-Adrenergic

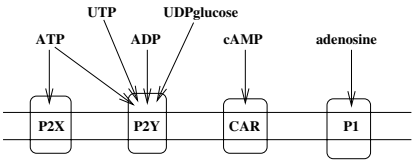

Figure 3.3. Nucleotide receptor types and their ligands.

[48] P2Y4 receptors are slightly expressed in endothelial cells, whereas P2X4, P2Y1, P2Y2, and P2Y11 are the most abundant among the endothelial P2 receptors [300].

[49] P2X1 receptors are abundantly expressed by the endothelium of rat mesenteric arteries [301].

[50] P2X1, P2X2, P2X3, and P2X7 have similar levels in the endothelial cell layer of internal mammary arteries, radial arteries and saphenous veins, whereas levels of P2X5 and P2X6 are lower [302]. P2X4 expression differs between arteries and veins. Both radial and internal mammary arteries have very low P2X4 levels.

receptors can activate the phospholipase-C–protein kinase-C pathway, stress-activated protein kinases (p38 and c-Jun N-terminal kinase), Rho and Rac GTPases (Sect. 4.5), and Ca^{++}–calmodulin-dependent protein kinase [247].

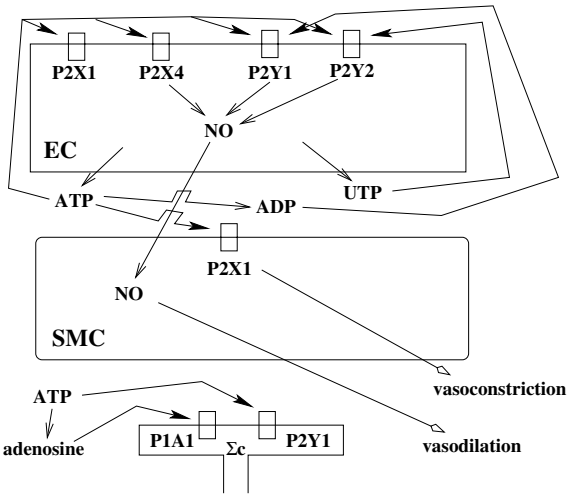

Figure 3.4. Purinergic receptors in the vascular wall (adapted from [303]). ATP, co-transmitter of noradrenaline and neuropeptide-Y, released from perivascular sympathetic nerves bind receptors P2X1, P2X2, and P2X4 of smooth muscle cells and induce vasoconstriction. Adenosine from ATP degradation binds to receptors P1A1 of sympathetic nerves and inhibits transmitter release. Stressed endothelial cells release: (1) ATP, which binds receptors P2Y1 (with a selective agonist action from ADP) and P2X4; and (2) UTP, a ligand for P2Y2. Both ATP and UTP trigger nitric oxide delivery to the smooth muscle cells with subsequent vasodilation.

Table 3.13. Purinergic receptors in the nephron (PCT: proximal convoluted tubule, tDL: thin descending limb, TDL: thick descending limb of the Henle loop, tAL: thin ascending limb, TAL: thick ascending limb of the Henle loop, DCT: distal convoluted tubule, CD: collecting duct, Source: [304]).

P2X1	Afferent arteriole, glomerulus,
P2X2	Afferent arteriole
P2X4	PCT, tDL, tAL, TAL, DCT, CD
P2X5	TAL, DCT, CD
P2X6	PCT, TDL, tDL, tAL, TAL, DCT, CD
P2Y1	Afferent and efferent arterioles, glomerulus, TDL
P2Y2	Glomerulus, TAL, CD
P2Y4	PCT, TDL

Several transcriptional targets of α1-adrenergic receptors have been identified in cardiomyocytes, particularly α-actin, β-myosin heavy chain, atrial natriuretic factor.[51]

Angiotensin Receptors

The action of angiotensins (ATn1–ATn4) is mediated by angiotensin receptors ATR1 and ATR2 (Table 3.15). ATR2 counteracts several growth responses initiated by ATR1. Angiotensin-2 stimulates the vascular cells after binding to the angiotensin-2 type 1 G-protein-coupled receptor. The angiotensin-2 receptor type 1 (ATR1) activates: (1) Gq, Gi/o, and G12/13 proteins, (2) tyrosine or serine/threonine kinases, (3) phospholipases PLC, PLD, and PLA2, and (4) ion channels. Angiotensin-2 acts via AT1 G-protein-coupled receptors on mitogen-activated protein kinase (Sect. 4.3.2.4). The targeted kinases include receptor tyrosine kinases (PDGFR, EGFR, and IRS1; Sect. 3.2.2) and non-tyrosine receptor kinases (Src, JaK/STAT, and FAK; Sect. 4.3.1). Angiotensin-2 also promotes the association of scaffold proteins (paxillin, and talin), leading to focal adhesion. Furthermore, angiotensin-2 stimulates vascular NADPH oxidase via ATR1.

Phospholipase-Cβ1 (Sect. 4.2.1), then PLCγ1, are activated by corresponding stimulated G proteins, within 5 s [249]. Diacylglycerol and inositol

Table 3.14. Adrenoceptors, their main targeted G proteins, and order of ligand potency (α1C-adrenoceptor is derived from α1A-adrenoceptor; Source: [241]).

Type	Main transducer	Ligand potency order
α1A	Gq/11	Ad ∼ NAd
α1B	Gq/11	Ad ∼ NAd
α1D	Gq/11	Ad ∼ NAd
α2A	Gi/o	Ad > NAd
α2B	Gi/o	Ad > NAd
α2C	Gi/o	Ad > NAd
β1	Gs	Ad < NAd
β2	Gs, Gi	Ad > NAd
β3	Gs, Gi/o	Ad ∼ NAd

[51] α1ARs target transcription factors, such as cJun (cJun stands for avian sarcoma virus-17 oncogene; Jun comes from japanese ju-nana: 10 [Ju] + 7 [Nana]), cFos (cellular Finkel Biskis Jinkins murine osteosarcoma virus sarcoma oncogene), and EGR1 (Early growth response factor-1), as well as transcriptional corepressor CARP. The zinc finger-only protein Zfp260 of the Kruppel family of transcriptional regulators, highly expressed in the embryonic heart, is downregulated during postnatal development. Zfp260 is a nuclear effector of α1-adrenergic receptors. Zfp260 also is a transcriptional activator of atrial natriuretic factor and a co-factor for cardiac regulator GATA4, used in MAPK signaling [248].

trisphosphate are thus generated, the production ending within minutes. The transient PLC activation is followed by a sustained activation of phospholipase D via G proteins. Concomitant stimulation of phospholipase A2 initiates arachidonic acid signaling. Effectors are kinases and GTPases, JaK, Src, Ras, and MAPK (Sect. 4.3). Reactive oxygen species via activation of NADPH oxidase are components of ATn2-mediated signal transduction in vascular smooth muscle cells.

The vasculature is characterized by a low density of type 1 angiotensin-2 receptors in endothelial cells (ecAT1R) and the relative abundance in vascular smooth muscle cells (smcAT1R). Endothelial cell activation by angiotensin-2 via ecAT1R reduces the tone of vascular smooth muscle cells, via elevated expression of endothelial nitric oxide synthase [250]. Angiotensin-2 stimulation of ecAT1R thereby reduces smcAT1R-mediated vasoconstriction.

Angiotensin-2 also acts as a proinflammatory agent and growth factor. After injury to the liver, ATn2 assists in tissue repair by stimulating: (1) hepatocytes and hepatic stellate cells to synthesize extracellular matrix proteins and secrete cytokines; and (2) myofibroblasts to proliferate. ATn2 activates NFκB via ATR1 and a protein set including CARMA3, Bcl10, and MALT1 [251].

Bradykinin Receptors

Bradykinin receptors are activated by bradykinin and its derived peptides kinin and kallidin (Table 3.16). Bradykinin, synthetized by tissue kallikrein from kininogen, stimulates endothelial β2-adrenergic receptors, subsequently releasing nitric oxide[52] and prostacyclin to control the vascular tone. Besides, tissue kallikrein deficiency does not disturb uterine artery remodeling during and after pregnancy [252].

Calcium-Sensing Receptors

Calcium-sensing receptors (CaR), types of G-protein-coupled receptors (Table 3.17), are used by cells to sense the concentration of extracellular calcium ions. There are seven transmembrane calcium-sensing receptors, especially on hematopoietic stem cells. Calcium-sensing receptors retain hematopoietic

Table 3.15. Angiotensin receptors and their main targeted transducer, either G proteins or protein phosphatases (Source: [241]).

Type	Main transducer
ATR1	Gq/11
ATR2	PTP, PSTP

[52] Endothelial nitric oxide synthase expression and, consequently, nitric oxide production, increase during pregnancy.

stem cells close to the endosteal surface of the bone marrow. CaRs interact with the extracellular matrix components, particularly collagen-1. CaR is also found in parathyroids, participating in parathyroid hormone response to $[Ca^{++}]_e$.

Endothelin Receptors

Endothelin is secreted by endothelial cells and targets the neighboring smooth muscle cells to regulate the vasomotor tone (Sect. 9.5.4; Table 3.18). Two

Table 3.16. Receptors of the bradykinin/kinin family, of histamine (5HTR3 are ionotropic), serotonin, complement system, free fatty acids, and of lysophosphatidic acid, with their main targeted G proteins (Source: [241]).

Type	Main transducer
Bradykinin/kinin receptors	
BR1, BR2	Gq/11
Histamine receptors	
HR1	Gq/11
HR2	Gs
HR3/4	Gi/o
Serotonin receptors	
5HTR1/5	Gi/o
5HTR2	Gq/11
5HTR4/6/7	Gs
Complement receptors	
C3aR	Gi/o, Gi/z
C5aR	Gi/o, Gi/z, Gq/16
Free fatty acid receptors	
FFAR-A1	Gq/11
FFAR-A2/A3	Gq/11, Gi/o
Lysophosphatidic acid receptors	
LPAR1/2	Gi/o, Gq/11, G12/13
LPAR3	Gi/o, Gq/11, Gs
LPAR4	Gq/11, Gs
LPAR5	Gq, G12/13

Table 3.17. Calcium-sensing receptor, targeted G proteins, and order of potency between extracellular concentrations of Ca^{++} and Mg^{++} (Source: [241]).

G-Protein transducers	Potency order
Gq/11, Gi/o, G12/13	$[Ca^{++}]_{ec} > [Mg^{++}]_{ec}$

endothelin-1 receptors (ETRA and ETRB), that belong to the family of G-protein-coupled receptors, bind ET1 with equal affinity to regulate gene expression for cell contraction, proliferation, and survival. Endothelin-1 activates extracellular signal-regulated kinase ERK1 and ERK2 (Sect. 4.3.2.4) by GPCR-induced activation of the receptor tyrosine kinase EGFR (Sect. 3.2.2.1). GPCRs can activate Src tyrosine kinases (Sect. 4.3.1.2) with subsequent RTK phosphorylation. Once activated, Src and/or RTK phosphorylate Shc adapter. Both phosphorylated Shc and activated RTK then bind growth factor-receptor–bound protein Grb2 and its associated Ras guanine nucleotide-exchange factor Son-of-sevenless to trigger Ras/MAPK signaling.

The docking protein growth factor-receptor–bound protein-2–associated binder-1 (Gab1) is phosphorylated by: (1) receptor tyrosine kinases and cytokine receptors to regulate cell survival and proliferation; and (2) G-protein-coupled receptors. Type A endothelin-1 receptor phosphorylates Gab1 and activates ERK1 [253]. ET1-induced Gab1 phosphorylation is stimulated by phosphoinositide 3-kinase and Src tyrosine kinases and inhibited by PP1. Tyrosine phosphatase SHP2 potentiates ET1-induced ERK1 activation.

Glutamate Receptors

Metabotropic glutamate receptors are activated by L-glutamate, and L-aspartate, among others ligands (Table 3.19). Ionotropic glutamate receptors include members of NMDA/AMPA receptor channel family, which have a high relative permeability to calcium ions and are blocked by magnesium at rest potential. Protons and zinc ions also inhibit NMDA.

Neuronal stimulation and activation of astrocytic metabotropic glutamate receptors (mGluR) induce an astrocytic calcium wave that propagates to astrocytic endfeet close to brain arterioles. Active neurons then rapidly (latency $< 2\,\mathrm{s}$) trigger dilation of intracerebral arterioles. Astrocytic calcium signaling activates astrocytic large-conductance, Ca^{++}-sensitive K^+ channels (BK) for local release of potassium into the perivascular space. A modest rise in extracellular potassium activates inward rectifier K^+ channels (Kir2.1) of brain arteriolar smooth muscle cells, inducing membrane potential hyperpolarization and relaxation [254].[53] Increased neuronal activity thus increases the

Table 3.18. Endothelin receptors, their main targeted G proteins, and order of ligand potency (Source: [241]).

Type	Main transducer	Potency order
ETRA	Gq/11, Gs	ET1 ∼ ET2 > ET3
ETRB	Gq/11, Gi/o	ET1 ∼ ET2 ∼ ET3

[53] Elevations of the concentration of external potassium ions that depolarize the vascular smooth muscle cells induces vasoconstriction of cerebral arteries and arterioles.

local cerebral blood flow to supply sufficient amounts of glucose and oxygen (functional hyperemia).

Leukotriene Receptors

Leukotriene receptors are activated by leukotrienes LTB4, LTC4, LTD4, LTE4, 12S-HETE, and 12R-HETE (Table 3.20). Leukotriene B4, a chemotactic and immune modulating agent implicated in allergic and inflammatory reactions, acts via two G-protein-coupled receptors BLT1 and BLT2. BLT1 and BLT2 signal through three classes of G proteins, Gi, Gq-like, and Gz.[54] LTB4[55] is a chemoattractant produced by neutrophils, macrophages, and mast cells for polymorphonuclear leukocytes and T lymphocytes. Leukotrienes are involved in arteriosclerosis.

Table 3.19. Glutamate metabotropic receptors, GABA receptors, and their main transducers. Glutamate ionotropic receptors include members of N-methyl-D-aspartate (NMDA) and α-amino 3-hydroxy 5-methyl 4-isoxasole propionic acid (AMPA), and kainate receptor families. $GABA_A$ are ligand-gated ion channels. $GABA_B$ receptors can couple to ion channels CaV2.1/2.2 and Kir3 (Source: [241]).

Type	Main transducer
Glutamate metabotropic receptors	
mGluR1/R5	Gq/11
mGluR2/R3/R4/R6/R7/R8	Gi/o
γ-Aminobutyric acid receptors	
$GABA_B$	Gi/o

Table 3.20. Leukotriene receptors, their main targeted G proteins, and order of ligand potency (Source: [241]).

Type	Main transducer	Potency order
BLT1	Gq/11, Gi/o	LTB4 > 12R-HETE
BLT2	Gq/11, Gi/o	LTB4 > 12S-HETE > 12R-HETE
CysLT1	Gq/11	LTD4 > LTC4 > LTE4
CysLT2	Gq/11	LTC4 ∼ LTD4 > LTE4

Prostanoid Receptors

Inflammatory cytokines stimulate the production from arachidonic acid of lipid mediators, such as prostanoids. Rate-limiting enzymes in prostanoid production include cyclooxygenases,[56] in particular constitutively expressed COx1 and inducible COx2. COx2 induced in macrophages and endothelial cells by inflammation stimuli, such as oxidized low-density lipoprotein and interleukin-1, produces eicosanoids that enhance vascular permeability and promote cell chemotaxis.

The effects of prostacyclin (PGI2) and prostaglandin-E2 (PGE2) are transduced by the I prostanoid receptor (IPR) and four E prostanoid receptors (EPR), respectively (Table 3.21; Sect. 8.4). EPR2 and EPR4 are linked to Gs activation of adenylyl cyclase, and EPR1 and EPR3 to Gq and/or Gi. EPR1 and EPR3 mediate PGE2-induced vasoconstriction. PGE2 can activate platelets by EPR3. EPR4 mediates anti-inflammatory effects and activation of metalloproteinase MMP9. Deletion of EPR2 and IPR causes salt-sensitive hypertension.

Prostaglandin-E2 EPR1, an effector of cyclooxygenase-2, impair Na^+-Ca^{++} exchanges and lead to neurotoxicity [256]. Membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) affect the expression of endogenous PGE2 and PGI2. Microsomal prostaglandin-E synthase-1 (mPGES1), a member of the MAPEG family, is a major source of PGE2 formation. Deletion of mPGES1 does not increase blood pressure and

Table 3.21. Prostanoid receptors, their main targeted G proteins, and order of ligand potency (Source: [241]).

Type	Main transducer	Potency order
DP1	Gs	PGD2 > PGE2 > PGF2α > PGI2, TXA2
DP2	Gi/o	PGD2 > PGE2, PGF2α > PGI2, TXA2
FP	Gq/11	PGF2α > PGD2 > PGE2 > PGI2, TXA2
IP	Gs	PGI2 > PGD2, PGE2, PGF2α > TXA2
TP	Gq/11	TXA2, PGH2 > PGD2, PGE2, PGF2α, PGI2
EP1	Gq/11	PGE2 > PGF2α, PGI2 > PGD2, TXA2
EP2	Gs	PGE2 > PGF2α, PGI2 > PGD2, TXA2
EP3	Gi/o	PGE2 > PGF2α, PGI2 > PGD2, TXA2
EP4	Gs	PGE2 > PGF2α, PGI2 > PGD2, TXA2

[54] The expression of BLT receptors can be enhanced in endothelial cells by lipopolysacharides, cytokines, such as tumor necrosis factor-α and interleukin-1β, and LTB4 [255].

[55] LTB4 is synthesized from arachidonic acid by the concerted action of 5-lipoxygenase, assisted by 5-lipoxygenase-activating protein, and of LTA4 hydrolase.

[56] There are three isoforms of cyclooxygenases, COx1, COx2, and COx3.

retards atherogenesis, whereas mPGES1-derived PGE2 accelerates atherogenesis [257]. At the opposite, prostacyclin protects the cardiovascular function.

Tissue Factor and Protease-Activated Receptors

Protease-activated receptor are activated by proteolytic cleavage. Thrombin and trypsin can be agonist proteases for certain protease-activated receptors in vivo. Tissue factor can bind protease-activated receptor, a G-protein-coupled receptor (Table 3.22). Platelet-activating factor (PAF) also acts via a single G-protein-coupled receptor.

Tissue factor can have two distinct structural and functional modes, being either an initiator of blood clotting[57], or a co-factor for cell signaling, unable to promote coagulation.[58] In other words, plasmalemmal tissue factor either: (1) binds the serine protease factor VIIa to activate the coagulation; or (2) binds the G-protein-coupled protease-activated receptor-2 (PAR2), a type of protease-activated receptors, to trigger signaling for inflammation and angiogenesis, the tissue factor belonging to the cytokine receptor family.[59]

Table 3.22. Protease-activated and platelet-activating factor receptors and their main targeted G proteins (Source: [241]).

Type	Main transducer
Protease-activated receptors	
PAR1	Gq/11, Gi/o, G12/13
PAR2/3/4	Gq/11, Gi/o
Platelet-activating factor receptor	
PAFR	Gq/11, Gi/o

[57] Tissue factor, an integral membrane protein, is normally excluded from the endothelial wetted surface. Tissue factor also circulates and becomes active only with a growing thrombus. Coagulation (Sect. 9.4) is triggered when tissue factor is exposed to zymogen coagulation proteases in plasma after vessel wall damage. It binds and activates factor VII. The complex made of coagulant tissue factor and factor VIIa binds and activates factor X. Factor Xa acts with its cellular cofactor Va for thrombin production. Thrombin cleaves fibrinogen to generate fibrin and drives platelet aggregation.

[58] Tissue factor is also activated in inflammation, vascular development, and cancer.

[59] The noncoagulant form of tissue factor bound to factor VIIa (binary signaling complex) activates receptor PAR2. Factor Xa signals via protease-activated receptors PAR1 or PAR2 either as a monomer or as a ternary complex with tissue factor and factor VIIa. Factor Xa is inhibited by antithrombin-3 and tissue factor pathway inhibitor. Factor VIIa is also inhibited by tissue factor pathway inhibitor bound to factor Xa. Thrombin is a potent activator of protease-activated receptors, except PAR2. It activates PAR1 and PAR4 for platelet aggregation. Like Factor Xa, thrombin is inhibited by antithrombin-3. Thrombomodulin hampers the binding of thrombin with PAR1 on the endothelial cell surface.

TF–PAR2 complex formation can be inhibited without preventing the coagulation activity of the tissue factor.

Protein disulfide isomerase (PDI)[60] stabilizes a distinct tissue factor–factor VIIa complex which does not bind factor X [258]. PDI inhibits the coagulation activity of the tissue factor, and switches tissue factor to cell signaling.[61]

Sphingosine-1-phosphate Receptors

Sphingosine-1-phosphate is a lipid growth factor (Sect. 10.1) that acts via a G-protein-coupled receptor (Table 3.23). Sphingosylphosphorylcholine also activates this receptor with equal, smaller, or greater potency than sphingosine-1-phosphate according to the receptor type.

3.2.1.2 Crosstalks and Transactivations

G-protein-coupled receptors act via heterotrimeric G proteins. They could also function in a G-protein–independent manner. β2-Adrenergic receptors signal via either Gα or via tyrosine kinase Src according to the ligand concentration, low or high, respectively [259].

Certain GPCR effectors do not depend on G proteins. Cross-talks between GPCRs and small GTPases exist.[62] GPCR signaling through G proteins can activate Ras and Rho GTPases. Activated Ras triggers the mitogen-activated protein kinase cascade. RhoA, Rab, Arf and ArfGEF can directly associate

Table 3.23. Sphingosine-1-phosphate receptors and their main targeted G proteins (Source: [241]).

Type	Main transducer
S1PR1	Gi/o
S1PR2	Gq, G12/13, Gs
S1PR3	Gq, Gi/o, Gs
S1PR4	Gi/o, G12/13, Gs
S1PR5	Gi/o, G12/13

[60] Protein disulfide isomerase cleaves disulfide bonds in the extracellular domains of certain receptors to regulate protein activity. Protein disulfide isomerase breaks a disulfide bond that is required to activate the coagulation. Nitric oxide regulates PDI activity, hence, suppresses the coagulant activity of tissue factor.

[61] Disulfide/thiol exchange is required for the formation of TF–PAR2 complex.

[62] The small GTPase superfamily includes at least five families: Ras GTPase subfamily (Ras, Rap, and Ral) of regulators of cell signaling, Rho GTPase subfamily (Rho, Rac, and Cdc42) of regulators of actin cytoskeleton, Rab GTPase subfamily of regulators of vesicular transport, Arf GTPase subfamily (Arf, Arl, and Sar) of regulators of vesicular transport, and Ran GTPase subfamily of regulators of microtubule organization and nucleocytoplasmic transport.

with GPCRs. GPCRs can also function as guanine nucleotide-exchange factors for small GTPases.

The interactions between GPCRs and small GTPases is required for cellular transport and cell migration. GPCRs are synthesized and modified in the endoplasmic reticulum, then transported to the Golgi apparatus for additional changes, lastly to the plasmalemma. Rab GTPases are implicated in exocytosis. Rab GTPases also regulate GPCR endocytosis into early endosomes, GPCR targeting to lysosomes for degradation or GPCR recycling from early endosomes. Arf1 and Arf6, with Arf GEFs (ADP ribosylation factor nucleotide–binding site opener ARNO) and β-arrestin, also control GPCR endocytosis [260].

Activated Gi-coupled chemoattractant receptor, the N-formylmethionylleucylphenylalanine receptor (fMLPR), leads to both Ras-dependent and -independent activation of Ral GTPase. Ral GEF RalGDS interacts with Ras, which activates Ral, hence, actin cytoskeleton reorganization (Ras-dependent mechanism). Plasmalemmal translocation of complexes made by β-arrestin and RalGDS allows binding to fMLPR and uncoupling from G protein, thus activates membrane-bound Ral (Ras-independent mechanism) [260].

Ligand binding to GPCRs activates small GTPases either directly or indirectly.[63] Rho-dependent responses can be activated by Gq and G12 (Fig. 3.5). Furthermore, G12 can bind and activate Rho-specific guanine nucleotide-exchange factors [261]. Mutant Gs-induced cAMP can activate small GTPases Rap1 and B-Raf, which in turn stimulate MAP2K and MAPK. $G\beta\gamma$ activates the MAPK pathway using a Ras-dependent process [262]. Gq-coupled receptors use both PKC-dependent and -independent pathways to stimulate the MAPK pathway.

The transactivation of receptor tyrosine kinases by GPCRs includes: (1) the activation of RTKs via non-receptor tyrosine kinases; (2) the formation of complexes between GPCRs and RTKs; and (3) the release of RTK ligands. GPCR stimulation can stimulate receptor tyrosine kinases, such as epidermal growth factor receptors, via proteolytic cleavage due to metalloprotease ADAM and release of EGF-like ligands, such as heparin-binding epidermal growth factor, and via NRTK activation that can phosphorylate EGFR tyrosine residues. β2-Adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multireceptor complex with the epidermal growth factor receptor following cointernalization of both receptors into clathrin-coated vesicles [263]. Several NRTKs (Csk, Lyn, Btk,[64] proline-rich tyrosine kinase-2, and focal adhesion kinase) could mediate MAPK activation by Gi and Gq [237]. Src or Src-like kinases can mediate the phosphorylation of Shc caused by $G\beta\gamma$ subunits and by α-adrenoceptors, after recruitment of

[63] GPCRs for lysophosphatidic acid and thrombin induce stress fibers, focal adhesions, and cell rounding through Rho-dependent pathways [261].

[64] Btk stands for Bruton tyrosine kinase.

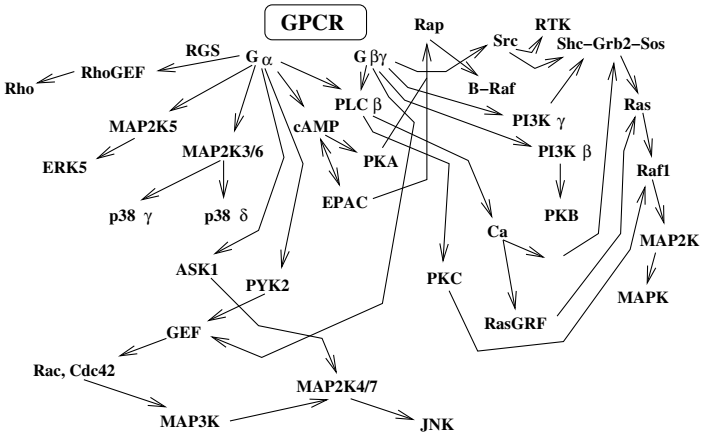

Figure 3.5. Interactions between GPCRs, small GTPAses and MAPKs. G-protein-coupled receptors (GPCR) activate mitogen-activated protein kinase (MAPK) using many pathways. Gβγ can stimulate Ras by the activation of receptor and non-receptor tyrosine kinases, thereby recruiting Sos to the plasmalemma. Activated Gαq can stimulate: (1) Raf1 via phospholipase-Cβ (PLCβ) and protein kinase-C (PKC); (2) Ras via PLCβ and activation of (2a) Ca^{++}-dependent Ras guanine-nucleotide releasing factor (RasGRF), and (2b) Ca^{++}- and PKC-regulated proline-rich tyrosine kinase-2 (PYK2) of the focal adhesion kinase family. GPCR stimulation also leads to phosphorylation of Src homology and collagen (Shc), which subsequently forms complexes with growth factor receptor-bound-2 (Grb2), and activation of tyrosine kinases acting on Son-of-sevenless (Sos). PI3Kγ can then act on the Shc–Grb2–Sos–Ras pathway. PI3Kβ stimulated by GPCRs drives activation of Rac and p21-activated kinase to enhance Raf activity. Gi and Gs, via the binding of cAMP to exchange protein activated by cAMP (EPAC) Rap1GEF EPAC and phosphorylation of Rap1 by PKA, can use the Rap1 pathway, which stimulates B-Raf. Gq, via PYK2, with adaptor Crk and paxillin, can activate GEFs for Rac and Cdc42, leading to the activation of MAP2K4/7 and JNK. G12 can stimulate MAP2K4/7 via apoptosis signal-regulating kinase-1 (ASK1). (8) G12/13, with regulators of G-protein signaling (RGS) also targets RhoGEFs for Rho activation. G12/13 and Gq can stimulate ERK5 (or big mitogen-activated kinase BMK1) via MAP2K5. G12, Gq, and Gβγ can activate p38αin cooperation with Btk and Src. G12/13 and Gq can also activate p38γ and p38δ via MAP2K3/6 (Sources: [237, 260]).

β-arrestin and GPCR kinase GRK2. Src can be activated also by interaction with Gi and Gs or β3-adrenoceptors.

Gq can stimulate MAPK: (1) via a PKC-dependent, Ras-independent process, (2) PKC- and Ras-dependent mechanism, or (3) PKC-independent, Ras-dependent manner, depending on the cell type and the receptor expression levels.

PI3K isoforms can be required for GPCR-induced MAPK activation (Fig. 3.5). PI3Kγ is activated by direct interaction with Gβγ-subunit. PI3Kγ

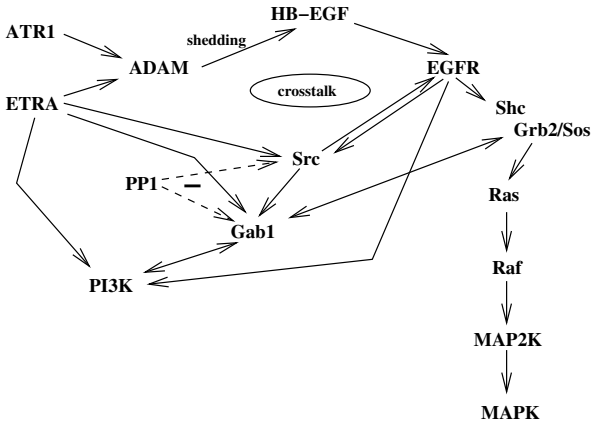

Figure 3.6. Cross-talks between receptors of angiotensin-2 (ATR1) and endothelin-1 (ETRA) and epidermal growth factor receptor (EGFR) in the cardiomyocyte. EGFR transactivation requires metalloproteases of the ADAM family, particularly ADAM12, heparin-binding epidermal growth factor, docking molecule Grb2-binding protein Gab1, Src tyrosine kinase, and phosphoinositide 3-kinase. EGFR transactivation leads to MAPK activation.

can then act on the Shc-Grb2-Sos-Ras pathway. G$\beta\gamma$ dimers activate PI3Kβ, which then stimulates PKB [264].

G-protein-coupled receptors interact with manifold intracellular molecules. The adaptors arrestins bind many phosphorylated GPCRs for endocytosis. β2-adrenoceptors can associate with the Na^+-H^+ exchanger regulatory factor, angiotensin receptors ATR1A with Janus-activated kinase JAK2, metabotropic glutamate receptors with Homers, which can form complexes with IP3 receptors, muscarinic acetylcholine M3 receptors and ATR1A with GTPases Rho and Arf [237].

GPCR ligands, such as lysophosphatidic acid, endothelin-1, platelet-activating factor and thrombin can induce a rapid transient phosphorylation of epidermal growth factor receptors in fibroblasts and in vascular smooth muscle cells. Catecholamines, angiotensin-2 and endothelin-1 act in cardiomyocytes where they can activate heparin-binding epidermal growth factor with members of a disintegrin and metalloprotease (ADAM) family and cause GPCR-induced cardiac hypertrophy (Fig. 3.6). Different types of G proteins (Gi, Gq, G12), different metalloproteases of the ADAM family (ADAM10, ADAM12, ADAM17), and different EGF-like ligands (heparin-binding epidermal growth factor, transforming growth factor-α, amphiregulin) are involved in inter-receptor cross-talk (or transactivation) [265].

3.2.1.3 Arrestins

β-Arrestins-1 and -2 desensitize certain plasmalemmal receptors in conjunction with G-protein-coupled receptor kinases (GRK).[65] They serve as endocytic and signaling adaptors. They act on receptor tyrosine kinases, such as insulin-like growth factor 1 receptor. β-Arrestins act as a scaffold for intracellular assembly of signaling complexes. Dissociation of β-arrestins allows recycling of G-protein-coupled receptors via Rab-dependent mechanisms. β-Arrestins target active kinases to specific locations within the cell and can regulate the lifetime of G-protein-coupled receptors in endosomes [266].

β-Arrestins associate with β2-adrenergic receptor, with angiotensin-2 type-1A receptor (ATR1A; Sect. 4.5), with V2 vasopressin receptor (V2R), with protease-activated receptor-2, and with parathyroid hormone receptor, to activate the ERK branch of the mitogen-activated protein kinase pathway (Sect. 4.3.2.4). In the case of ATR1A and V2R, G-protein-coupled receptor kinases are required. G-protein-coupled receptor kinases and β-arrestins switch off G-protein-dependent signaling, but transduce another kind of signaling from receptors, such as the angiotensin-2 type-1A receptor.[66] Such a signaling causes positive inotropy and lusitropy (Chap. 7), possibly either by regulating cytosolic Ca^{++} level or Ca^{++} sensitivity of troponin and myosin-binding protein-C [267]. β2-Adrenergic receptors cooperate with Gi-protein and β-arrestin in the cardiomyocyte, in addition to major β1-adrenergic signaling, to stimulate CaV1.2 calcium channels and increase the intracellular calcium level.

The association of G-protein-coupled receptor component of sonic Hedgehog receptor, smoothened (Sect. 3.2.7), with β-arrestin-2 and its phosphorylation by G-protein-coupled receptor kinase-2 promote smoothened endocytosis in clathrin-coated pits [268]. β-Arrestins are implicated in endocytosis not only of G-protein-coupled receptors, but also of VE-cadherin and other receptors. β-arrestin-2 binds to low-density lipoprotein receptor and enhances LDLR endocytosis [269]. β-Arrestin-2 also binds type 3 transforming growth factor-β receptor[67] and triggers the receptor phosphorylation by the kinase type 2 TGFβ receptor, thereby down-regulating TGFβ signaling [270]. After binding of Wnt proteins (Sect. 3.2.8) to Frizzled receptors, cytoplasmic Dishevelled is recruited to the plasmalemma and phosphorylated. The adaptor β-arrestin-2, also recruited by Frizzled-4, binds to phosphorylated Dishevelled and allows Frizzled-4 endocytosis [271].

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[65] β-Arrestins bind to activated G-protein-coupled receptors, after receptor phosphorylation by G-protein-coupled receptor kinases. They thus regulate receptor endocytosis and signaling.

[66] This signaling is thus carried out independently from Gαq/PKC pathway. β-Arrestins interacts with the receptor and prevents the coupling between the receptor and G proteins.

[67] Type 3 transforming growth factor-β is also called β-glycan.

3.2.2 Receptor Tyrosine Kinases

Proximal regulators of receptor tyrosine kinase signaling are assigned to different pathways (Fig. 3.7). They can specifically act according to the receptor type or cell type. They include Raf (Sect. 4.3.2.4) and protein Ser/Thr phosphatases (Sect. 4.4).

The tyrosine kinase family includes growth factor receptors, such as vascular endothelial growth factor (Sect. 10.1) and angiopoietin receptors, Tie1 and Tie2 (Sect. 10.4.1 and 10.4.2). Tyrosine kinase signaling is counteracted by tyrosine phosphatases (Sect. 4.4), such as vascular endothelial protein tyrosine phosphatase. Activated receptor tyrosine kinases, including receptors for epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor, are inhibited by glycosphingolipids, such as ganglioside GM3 [272].[68] GM3 binds to and inhibits EGFR. It also interacts with fibroblast growth factor receptor and prevents receptor endocytosis. Although the growth factor receptor is a typical example of RTK, receptor tyrosine kinases have other ligands. The insulin also triggers a receptor tyrosine kinase.

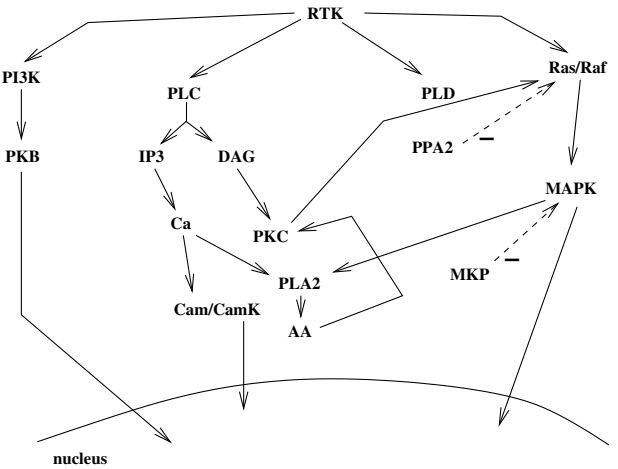

Figure 3.7. RTKs intervene in cell growth, apoptosis, differentiation, and metabolisms. Main RTK effectors include phosphatidylinositol 3-kinase (PI3K), phospholipase-C (PLC), phospholipase-D (PLD), phospholipase-A2 (PLA2), calmodulin-dependent kinases (CamK), protein kinase-C (PKC), protein phosphatase-2A (PPA2), and Ras GTPase–Raf mitogen-activated protein kinase kinase kinase (MKP: mitogen-activated protein kinase phosphatase).

[68] Plasmalemmal glycosphingolipids are implicated in nanodomains with signal transducers, tetraspanins, growth factor receptors, and integrins.

3.2.2.1 Growth Factor Receptors

Signaling cascades start with binding of the signaling molecule with its plasmalemmal receptor, which initiates interactions with proteins, such as the adapters Grb2 and Shc, and the guanine nucleotide-exchange factor Sos. Signaling components are often preassembled into complexes. Scaffold proteins help to maintain these complexes and can contribute to specificity in various signaling systems.

Growth factors (Sect. 10.1) are major ligands of RTKs [273]. The stem cell factor (SCF) exerts its effects by binding to the tyrosine kinase cKit,[69] involved in the hematopoiesis (Sect. 6.3) [274]. Axl and its homologs, Sky and Mer, constitute a RTK subclass. Axl signaling is particularly implicated in platelet functions. Interactions between Axl and $\alpha_{IIb}\beta_3$ integrins, participate in platelet activation and thrombus stabilization, via phosphatidylinositol 3-kinase and protein kinase-B [275]. Axl receptor tyrosine kinases are activated by the vitamin-K–dependent[70] growth arrest-specific gene 6 proteic product Gas6[71] [276].

The binding of the ligand to two adjacent receptors forms an active dimer (receptor dimerization), which is a tyrosine kinase (cross-phosphorylation). This activating autophosphorylation[72] leads to a phosphorylation cascade within the cytosol, using the activated cytosolic tyrosine kinases. Signaling proteins that bind to the intracellular domain of receptor tyrosine kinases include RasGAP, PI3K, phospholipase-Cγ, phosphotyrosine phosphatase SHP,[73] and adaptor proteins.[74]

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[69] Like other hematopoietic growth factor receptors of the cell membrane, cKit is internalized after ligand binding.

[70] Vitamin-K–dependent plasma proteins (prothrombin, coagulation factors VII, IX, and X, protein-C, protein-Z, protein-S, and Gas6) bind to negatively charged phospholipid membranes via a γ-carboxyglutamic acid (Gla)-containing module.

[71] Gas6, a platelet-response amplifier, is structurally similar to the anticoagulant protein S, a cofactor for activated protein C; but Gas6 lacks the element of the anticoagulant activity of protein S. Gas6 inactivation prevents venous and arterial thrombosis in mice with normal bleeding.

[72] Kinases need to be phosphorylated, often by autophosphorylation, to trigger their phosphorylating activity. Dual-specificity Tyr-phosphorylation–regulated kinases (DYRK) phosphorylate themselves on tyrosine (Tyr), and subsequently phosphorylate their exogenous substrates on serine and threonine (Ser/Thr) [277].

[73] SHP1 is a negative regulator of several second messengers implicated in nitric oxide generation (Sect. 9), such as JaK2 and ERK1/2.

[74] The recruitment of transmembrane proteins to the clathrin coat involves adaptor protein complexes (AP), which interact directly with both clathrin and the membrane receptors (Sect. 5.1).

3.2.2.2 EGFR/ErbB/HER Family

Epidermal growth factor receptor regulates cellular processes and is implicated in hyperproliferative diseases. Direct stimulation of the receptor tyrosine kinase by ligand binding to the receptor extracellular domain yields formation of receptor dimers (dimerization) and subsequent EGFR autophosphorylation of two receptor molecules in several Tyr residues. Phosphorylated tyrosines provide docking sites for effectors and regulators, such as Shc, Grb2, and c-Cbl, to activate intracellular signaling cascades and ubiquitination. Widely expressed EGFR is activated by epidermal growth factor, heparin-binding EGF, transforming growth factor-α, amphiregulin, betacellulin, epiregulin, epigen and crypto.[75] Indirect EGFR phosphorylation (activation) occurs with hyperosmolarity, oxidative stress, and mechanical stress, due to possible inactivation of phosphatases that antagonize the intrinsic receptor kinase activity. EGFR can also be stimulated by chemokines, cell-adhesion molecules and G-protein-coupled receptors.

After EGFR phosphorylation, Cbl ubiquitin ligase is phosphorylated by EGFR and ubiquitinates EGFR. Recruitment of adapters (Cin85, and endophilins) leads to EGFR endocytosis. Endocytosis and proteolytic degradation of the epidermal growth factor receptor after ligand binding regulate EGF signaling (signaling termination by negative feedback). p38 Kinase is required for destruction of ligand-stimulated Cbl-bound EGFR [284].

The erythroblastoma viral gene product B (ErbB) proteins include four receptor tyrosine kinases (ErbB1–ErbB4) and many extracellular ligands with an epidermal growth factor domain. Whereas neuregulin Nrg1 is expressed in the endocardium, ErbB2 and ErbB4 are expressed in the myocardium [285]. ErbB systems are hierarchically configured into redundant modules to generate an output despite disturbances. Phosphorylated ErbB1 recruit signal transducers such as adapter growth factor receptor bound Grb2 and Src-homology-2-containing (Shc), which recruit Ras and activate the mitogen-activated protein kinase cascades. ErbB1 also recruits STAT5. ErbB2 does not bind EGF-like ligands, but is a partner of the three other ErbBs, favoring their heterodimerization. Heterodimer ErbB3 activates PI3K. ErbB4 shares recognition and signaling features with ErbB1.

The member of the EGF receptor–ErbB family, ErbB2/HER2, is a ligandless receptor kinase, which has pleiotropic effects. ErbB2 is a coreceptor for GPCRs, which activate extracellular signal-regulated kinase ERK1/2 in the heart.[76] This tyrosine kinase receptor can form a RTK–GPCR complex in cardiomyocytes [286].

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[75] Except crypto, these EGFR ligands are synthesized as membrane precursors that undergo proteolysis to become active.

[76] ERK1/2 acts in the recruitment of β-arrestin, in the activation of the non-receptor tyrosine kinase Src, and in the transactivation of tyrosine kinase receptors.

G-protein-coupled receptors can use EGFR as a downstream signaling partner to generate mitogenic signals [265].[77] This inter-receptor cross-talk targets various GPCRs. Subsequently, GPCR-stimulated EGFR activates downstream effectors, such as mitogen-activated protein kinases, phosphoinositide 3-kinase[78] (and PKB), and modulates ion channel activity, for cell survival, proliferation, and migration, especially in cardiac hypertrophy, and cancer cells (Fig. 3.8).

GPCR-induced EGFR transactivation depends on the cell type and the signaling context. The cross-talk can involve Src tyrosine kinases. Protein kinase-C and calcium ions can be required in cardiomyocytes, cardiac fibroblasts, and vascular smooth muscle cells. MAPK activation by Gq-coupled bradykinin receptor depends on phosphoinositide 3-kinase-β and protein kinase-Cϵ [289]

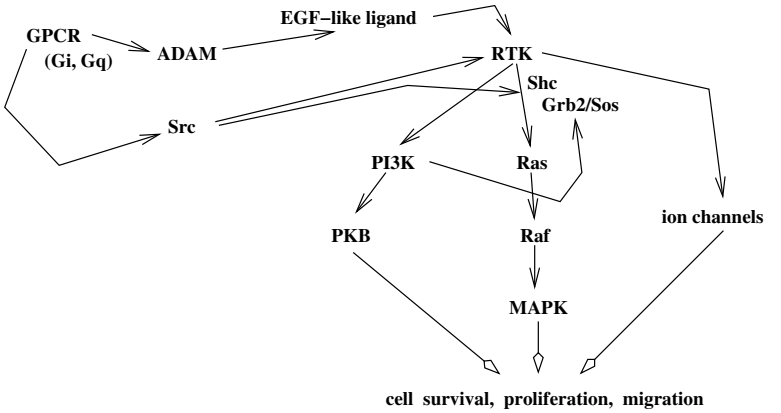

Figure 3.8. Inter-receptor cross-talk. Receptor tyrosine kinase (RTK), such as epidermal growth factor receptor, can be a downstream signaling partner of G-protein-coupled receptors, especially for the activation of mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K), which leads to cell survival, proliferation, and migration. The cross-talk involves a disintegrin and metalloprotease (ADAM) and EGF-like ligand. Such pathways are implicated in hyperproliferative diseases.

[77] Transduction of a mitogenic signal from the plasmalemma to the nucleus either involves receptor tyrosine kinases, adapters Shc and Grb2, and the Ras/MAPK pathway or Ras/MAPK activation by G-proteincoupled receptors and Gab1 [287]. RTKs can act as downstream mediators in GPCR mitogenic signaling through intracellular cross-talk. The epidermal growth factor receptor is quickly phosphorylated upon stimulation of fibroblasts with GPCR ligands, such as endothelin-1, lysophosphatic acid, and thrombin.

[78] Inhibition of phosphoinositide 3-kinase does not affect GPCR-induced EGFR phosphorylation, but prevents MAPK stimulation in the presence of both GPCR ligands and low doses of EGF [288]. PI3K plays an important role upstream from Ras at Grb2 level in GPCR-mediated MAPK stimulation via both Gq and Gi proteins.

(Fig. 3.9).[79] MAPK activation by Gi-coupled lysophosphatidic acid receptor is regulated by PI3Kγ and PKCζ [290] (Fig. 3.9).[80]

Angiotensin-2–induced phosphorylation of platelet-derived growth factor receptor-β and thrombin-stimulated phosphorylation of insulin-like growth factor-1 receptor is observed in smooth muscle cells. Angiotensin-2–induced activation of mitogen-activated protein kinase mainly results from calcium-dependent activation of receptor tyrosine kinase via Gq-coupled ATR1 in vascular smooth muscle cells. Angiotensin-2 rapidly induces the phosphorylation of the epidermal growth factor receptor and its association with Shc and Grb2 [291]. Angiotensin-2 induces Ca^{++}-dependent transactivation of EGFR which serves as a scaffold for preactivated c-Src and for downstream adapters, leading to MAPK activation in vascular smooth muscle cells.

Multisubstrate docking proteins, such as Grb2-associated binder-1 (Gab1) and insulin receptor substrate IRS1/2, regulate RTK-induced PI3K activity.[81]

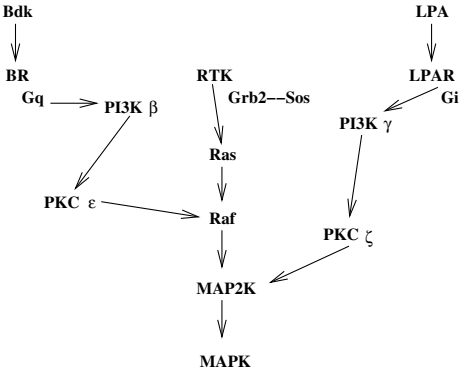

Figure 3.9. MAPK activation by bradykinin and lysophosphatidic acid via their GPCRs coupled to Gq (BR) and Gi (LPAR), respectively. Protein kinase-C isoforms (PKCε and PKCζ) are stimulated by corresponding phosphatidylinositol 3-kinase isoforms (PI3Kε and PI3Kγ) to activate MAPK pathway either at Raf or MAP2K level.

[79] Gq (Gα11) coupled to the bradykinin receptor increases the production of phosphatidylinositol 3-kinase-β and activates PI3Kβ, which then associates with protein kinase-Cε and induces the translocation of PKCε from the cytosol to the plasmalemma to transmit the mitogenic signal to the MAPK pathway.

[80] Lysophosphatidic acid receptor coupled to the inhibitory G protein Gi activate MAPK using either a Ras-dependent pathway or -independent cascade associated with phosphatidylinositol 3-kinase-γ and protein kinase-Cζ. LPA also induced the activation of MAP2K, but not of Raf1.

[81] Docking molecules Grb2-binding protein Gab1 and intracellular substrate for insulin IRS1, also targeted by growth factors, and IRS2 are used by numerous signaling pathways. These substances form the family of IRS1-like multisubstrate docking proteins. Gab1 is phosphorylated following activation of RTKs and cytokine receptor. Gab1 is a substrate for EGF and insulin receptors and a docking

Zinc-dependent metalloproteases of a disintegrin and metalloprotease family (ADAM) are involved in EGFR transactivation. G-proteincoupled receptor ligands activate metalloproteinase and induce the shedding of heparin-binding epidermal growth factor (HB-EGF) and subsequent transactivation of the epidermal growth factor receptor in cardiomyocytes, leading to cardiac hypertrophy [294]. The enzyme that sheds HB-EGF from the plasmalemma of cardiomyocytes is a disintegrin and metalloprotease-12 (ADAM12). Stimulations by ATn2 or ET1 of cultured rat neonatal cardiomyocytes activate EGFR after shedding of HB-EGF caused by ADAM12. ADAM12 shedding of HB-EGF in cardiomyocytes thus represents a major signal transduction pathway leading to cardiac hypertrophy resulting from pressure overload.

PI3K/PKB pathway is predominantly driven via phosphorylation of epidermal growth factor receptors. The HER family consists of EGFR, HER2, HER3, and HER4. Heterodimerization and phosphorylation of HER family members drive signaling. Human epidermal growth factor receptor[82] HER3 is particularly involved. Once phosphorylated by the epidermal growth factor receptor, ErbB3 associates with phosphatidylinositol 3-kinase and couples growth factor receptor tyrosine kinases to phosphatidylinositol 3-kinase [295]. Similarly, ErbB3 can undergo a cross-phosphorylation by the receptor tyrosine kinase ErbB2 (HER2). The receptor tyrosine kinase ErbB2 is activated by EGFR family members, but does not bind any known ligand. ErbB2 efficiently interacts with EGFR and ErbB3, whereas ErbB3 and EGFR do not form stable complexes [296]. Downstream effectors, mitogen-activated protein kinase and Jun N-terminal kinase are durably inhibited when both EGFR and ErbB2 activities are prevented.

3.2.2.3 Ephrin Receptors

The ephrin receptors (Eph) is the largest group of receptor tyrosine kinases. They interact with ligands, the *ephrins*, to transmit bidirectional signaling via between-cell contacts. The classification of the ephrin family members

protein for several proteins, including PI3K. Overexpression of Gab1 enhances cell growth [292]. Stimulation of the hepatocyte growth factor receptor tyrosine kinase induces mitosis, motility, and branching tubulogenesis of epithelial and endothelial cell lines in culture via Gab1. Following stimulation of epithelial cells with HGF, Gab1 associates with phosphatidylinositol 3-kinase and tyrosine phosphatase SHP2 [293].

[82] HER3, also known as ErbB3, is a type 1 receptor tyrosine kinase similar in sequence to the epidermal growth factor receptor. ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog-3 is the gene that encodes the ErbB3 member of the epidermal growth factor receptor family of receptor tyrosine kinases. ErbB3 does not have a kinase domain, but does form heterodimers with other EGFR family members to get a kinase activity and activate signaling pathways, especially via PI3K. ErbB3 mediates the EGF responses in cells expressing both ErbB3 and EGFR.

is mainly based on the composition and cell-surface binding. There are two classes of Eph receptors and ephrins, with EphA1–A8 and EphB1–B6. Ephrin-A has a glycosylphosphatidyl inositol anchor, whereas ephrin-B is a transmembrane ligand.

Eph receptor tyrosine kinases, like other RTKs, initiate signal transduction via autophosphorylation after ephrin binding (forward signaling). Moreover, ephrins are plasmalemmal compounds with signaling capability (reverse signaling). Ephrins hence act in bidirectional communications, to and from a given cell. Ligands-A bind the receptors-A on the extracellular part of the membrane. Ligands-B link to receptors-B, homo- and heterodimers, which possess a cytoplasmic tail with a kinase signaling activity and an extracellular part. Eph receptors are inhibited by protein tyrosine phosphatases. Tyrosine phosphatase receptor-O specifically dephosphorylates both EphA and EphB receptors for termination of Eph signaling [278].

Eph receptors and ephrins control cell migration and adhesion. EphrinB2 and EphB4 regulate vasculature morphogenesis and blood vessel permeability during vasculogenesis as well as in postnatal angiogenesis. EphrinB1 and ephrinB2 are expressed by endothelial cells in most arteries, ephrinB1, EphB3, and EphB4 in veins[83] [279–281]. Pericytes and smooth muscle cells adjacent to endothelial cells express ephrinB2 and EphB2. Interactions of Eph receptors with ephrins require cell–cell contact because both the receptor and the ligand are membrane-bound. Eph receptors and ephrins mediate a cell repulsive response in migrating cells, destabilizing between-cell contacts.

The binding capacities lead to several pathways regulated by Eph receptors, including Src, Ras, and c-Jun N-terminal kinases (JNK). Signaling mediated by ephrins and Eph receptors regulates a variety of processes, including cell shape, adhesion, separation, and motion (attraction and repulsion). Ephrins and Eph receptors, indeed, modulate the activity of the actin cytoskeleton, regulating the actin reorganization. Once EphA2, a downstream target of the mitogen-activated protein kinase (MAPK) pathway (Sect. 4.3.2.4), is transported to the cell membrane, it binds to EphA1, and downregulates MAPK signaling (negative feedback loop). EphB2 and its ligands are involved in the growth of the vascular smooth muscle cells [282]. Furthermore, EphB2 and its ligands regulate interactions between endothelial and either mesenchymal cells in developing arteries or smooth muscle cells in adult arteries.

The tyrosine phosphorylation of ephrin-B1 induced by the receptor bond recruits adapter Grb4. From the three SH3 domains of Grb4, ephrin-B1 can be linked to numerous signaling molecules. Moreover, ephrin-B1 also contains PDZ-binding motif for additional association with proteins, such as phosphatases and GTPase-activating proteins. The extracellular region of ephrin-

[83] Whereas ephrinB1 is expressed by arterial and venous endothelial cells, ephrinB2 is expressed on arteries but not veins. EphrinB2 interacts with multiple EphB receptor classes, such as EphB4 preferentially expressed on veins.

B1 also binds to the extracellular domains of adjacent claudin-1 or -4, which are major constituents of the tight junctions [283] (Sect. 2.3). The tyrosine phosphorylation of the cytoplasmic domain of ephrin-B1 depends on claudin. Phosphorylated ephrin-B1 stimulates the intercellular permeability, the formation of the tight adhesion between adjacent cells being hindered by interactions of claudins with ephrin-B1.

3.2.2.4 Angiopoietins

Angiopoietin-2 (Ang2) activates receptor tyrosine kinase Tie2 during lymphatic vessel development. Angiopoietin-2 either acts as a Tie2 antagonist or as a Tie2 activator [297].[84] Angiopoietin-2 is also expressed by stressed endothelial cells, acting as an autocrine regulator of the receptor tyrosine kinase Tie2 and the protein kinase-B pathway to protect the cell.

3.2.3 Receptor Serine/Threonine Kinases

A set of transmembrane receptors is characterized by intracellular serine/threonine kinase domains. Receptor serine/threonine kinases (RSTK) include the transforming growth factor-β (TGFβ) receptors. The TGFβ family is composed of activins, inhibins, and bone morphogenetic proteins (BMP). They are implicated in cell proliferation and differentiation Fig. 3.10). They also regulate migration and adhesion of various cell types.

Receptor serine/threonine kinases can be divided into subsets. TGFβ acts via both type 1 and type 2 receptors. Ligands first bind to type 2 receptors, which then phosphorylates type 1 receptors, thus initiating the signaling cascade. TGFβ regulates the cell cycle via proto-oncogene cMyc. The expression of multiple type 1 receptors within different cell types can drive cell-specific responses to TGFβ. TGFβ type 3 receptor and endoglin present ligands to the receptors.

3.2.4 Receptor Tyrosine Phosphatases

The cytosolic part of these transmembrane enzymes contains the phosphatase domain. Receptor tyrosine phosphatases form symmetric dimers, each

[84] Rather than antagonizing angiopoietin-1 activity, angiopoietin-2 could instead compensate for Ang1 signaling defect. When protein kinase-B activity is high, after strong Ang1 or Ang2 signaling, Ang2 expression could be stopped to prevent overstimulation of the PKB pathway. Angiopoietin-2 expression by endothelial cells, which is strong during angiogenesis, is indeed induced by the transcription factor FOXO1 after inhibition of the PI3K/PKB pathway. FOXO1 could also regulates the production of proteins that modulates Ang2 responsiveness. Therefore, Ang2 administration to endothelial cells, shunting FOXO1 activity, would not lead to efficient Tie2 activation.

monomer blocking the active site of its partner. Ligand-binding to receptor tyrosine phosphatases leads to dephosphorylation of target proteins.

Cell adhesions provide structural support and communication paths. Cell-adhesion molecules are specialized plasmalemmal receptors, which form clusters for intercellular contacts via their ectodomains. These dynamic molecular clusters act in signaling via phosphorylation.

There are at least six subsets of transmembrane PTPs. Plasmalemmal receptor tyrosine phosphatases RTP2B[85] mediate cell adhesion. Their intracellular catalytic regions dephosphorylate cadherin–catenin complexes, thereby stabilizing the intercellular contacts [298].

Heparan sulphates regulate RTPs. Reactive oxygen species, such as hydrogen peroxide, as well as post-translational reversible oxidation, affect RTP functioning. Many RTPs are thus transiently oxidized during signaling. Reactive oxygen species produced by NADPH oxidase are controlled, since NADPH oxidase subunits must assemble into a multiprotein complex with scaffold and regulators and bind to inositol phospholipids and GTPase Rac, the latter acting as a molecular switches [299].

3.2.5 Semaphorins and Plexins

Certain transmembrane semaphorins (Sect. 5.2) can function as both ligand and receptor (reverse or bidirectional signaling). The main receptors for semaphorins are plexins, which regulate Rho GTPases. Plexin-A and semaphorins-3 are expressed by endothelial cells. Cardiomyocytes synthesize plexin-A1 and plexin-D1. Plexins interacts with both semaphorins on adjacent cells and with semaphorins in the extracellular medium. Plexins can function as both signaling receptors and as ligand-binding receptors in co-receptor complexes for both plexins and vascular endothelial growth factor receptors

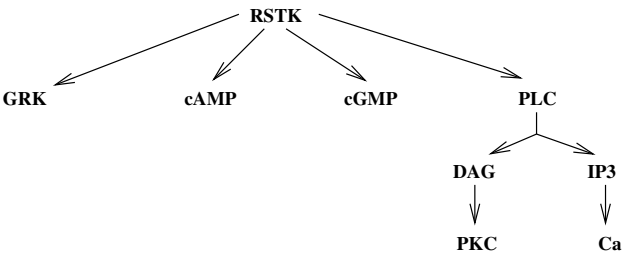

Figure 3.10. Receptor Ser/Thr kinases (RSTK) comprises transforming growth factor-β family receptors. RSTKs are implicated in cell growth inhibition. Main effectors are cAMP, cGMP, phospholipase-C (PLC), and GPCR kinases (GRK).

[85] RTP2B family include RTPμ, which interacts with cadherin in the nervous system, in the vascular endothelia and the intercalated discs of cardiomyocytes, RTPκ, RTPρ (PTPRT), and PCP2 (RTPψ, RTPπ, or RTPλ).

in particular. Phosphorylated plexins stimulate kinases. Plexins A regulate kinases Fes and Fyn. Plexin-B1 binds to and stimulates receptor tyrosine kinases Met21 and ERBB2. Semaphorin kinase signaling activates extracellular signal-regulated kinase.

Semaphorin-6 yields an example of reverse signaling in heart development. Semaphorin-3 reduces endothelial cell motility, inhibiting angiogenesis. At the opposite, semaphorin-4 promotes endothelial cell migration and angiogenesis, its receptor plexin-B1 being expressed in endothelial cells.[86] Semaphorin-4D may act both remotely and locally like vascular endothelial growth factor VEGF-A.

3.2.6 Notch Receptors

Notch proteins are a family of large transmembrane glycoproteic receptors. They act both as plasmalemmal receptors and as regulators of gene transcription. Ligand binding to Notch receptors triggers Notch proteolysis, releasing the intracellular Notch parts (icNotch), i.e. the Notch transcription factors, which migrate to the nucleus. There are four Notch genes, that encode receptors (Notch1–Notch4), which have at least five different Notch ligands (Jagged1/Serrate1, Jagged2/Serrate2, Delta1, Delta2, and Delta3).[87]

Notch is maintained in a resting autoinhibited conformation in the absence of ligand. Notch signaling starts from Notch cleavages and the release of an intracellular fragment. Because most Notch ligands are transmembrane proteins, Notch signaling is associated with between-cell contacts, being restricted to neighboring cells. Ligand-receptor binding induces two successive Notch proteolytic cleavages. The cleavage of the ectodomain of ligand-bound Notch receptor by ADAM-type metalloproteases followed by a cutting catalyzed by γ-secretase initiates Notch signaling. The extracellular first cleavage requires the tumor necrosis factor-α–converting enzyme (TACE). The cleaved extracellular domain undergoes endocytosis by the ligand-expressing neighboring cell. The second cleavage is done by a proteic complex (presenilins, nicastrin, APH1, and PEN2 proteins). The cleaved cytoplasmic domain of the Notch receptor is released and translocates into the nucleus, using the endosomal transport. Arrestin kurtz form a trimeric complex with Notch receptor, leading to Notch ubiquitination and Notch signaling attenuation. The basic Notch pathway is the same in most Notch-dependent processes. But, the pathway regulations are different [306]. Lethal giant disc regulates Notch endosomal transport and prevents Notch delivery to a compartment where it

[86] Semaphorin-4D produced by malignant cells, in breast and lung neoplasms among others, stimulates endothelial cell migration for angiogenesis [305].

[87] Notch ligands are characterized by an N-terminal Delta, Serrate and LAG2 (DSL) domain for interactions with the Notch receptor. The ligands are subdivided into two classes, (1) Delta or Delta-like (Dll) and (2) Jagged. The location of both ligands and receptors within the cell affects Notch signaling.

can be aberrantly activated. Moreover, Notch regulators are themselves controlled for efficient interactions with other signaling pathways. E3 ubiquitin ligases, which interact with Notch ligands, are required for ligand activation, thereby for controlling the activity of the Notch pathway.

In the nucleus, the Notch-signaling pathway leads to DNA-binding protein CSL (transcription factor CBF1/RBP-Jκ, Su(h), and LAG1). The intracellular fragment displaces a repression complex and, with CSL, recruits the co-activator Mastermind (Mam). Mastermind is required to activate the transcription. Together with co-factors, kinases, and ubiquitin ligases, Notch then activates gene expression. Notch receptors regulate cell growth and death in various cell types, as well as differentiation in embryo and tissue development in adults. Notch receptor activation induces the expression of the specific target genes hairy enhancer of split-3 (HES3) and related transcription factors (HRT), as well as sonic Hedgehog via PKB and the transcription factor STAT3,[88] and promotes regenerative responses following hypoxia [307].

The Hairy-related transcription factor family of basic helix–loop–helix (bHLH) proteins, includes three members, Hrt1, Hrt2, and Hrt3.[89] The transcription of Hrt genes is activated by Notch in cooperation with CSL/RBP-Jκ. Hrt proteins interact with GATA factors and repress the transcriptional activity of GATA-dependent genes. Hrt2 regulates cardiomyocyte identity. The transcriptional repressor Hairy-related transcription factor-2 is expressed in ventricular cardiomyocytes, in endothelial cells, and vascular smooth muscle cells, but not in atrial cardiomyocytes. Hrt2 prevents the activation of atrial genes in ventricular cardiomyocytes [308].

Notch is required in somitogenesis.[90] Notch is also involved in cardiac development, especially for cardiac valve and trabecula formation, and in self-renewal of adult cells, particularly in hematopoiesis. Notch-signaling pathway is also involved in many cancers, particularly human lung cancers.

In the immune system, B and T lymphocytes develop from common bone marrow lymphoid-restricted progenitors. Notch is involved in the T-cell development, T lymphocytes being formed once progenitors have left the bone marrow and reached the thymus. The thymus produces multiple Notch activators. At the opposite, Notch repression leads progenitors toward B-cell development, B lymphocytes being essentially produced in the bone marrow.

Notch1 upregulates expression of interferon-γ in peripheral T lymphocytes via activation of nuclear factor-κB (NFκB). The intracellular domain of Notch1 directly interacts with NFκB and competes with IκBα, leading to NFκB retention in the nucleus and activation of IFNγ promoter [309]. At the

[88] STAT3 is activated at the plasmalemma.

[89] They are also called Hey, Hesr, CHF, and HERP. Hrt proteins hinder Notch-dependent activation of their genes.

[90] Somites are clusters of mesodermal cells that give birth to segmental body structures, such as blood vessels during embryonesis.

opposite, IκBα can enter in the nucleus and bind to NFκB, exporting the latter to the cytosol.

3.2.7 Hedgehog Receptors

The Hedgehog family of secreted proteins is involved in various developmental processes, especially in embryogenesis. Hedgehog signaling remains active in adult life. Hedgehog signaling regulates adult stem cells for the maintenance and regeneration of adult tissues. Hedgehog signaling is particularly implicated in maintenance and self-renewal of neural progenitors in stem cell niches of the postnatal brain. Hedgehog signaling is implicated in certain cancers. This family of morphogenic signals comprises three types of secreted proteins: sonic Hedgehog (Shh), indian Hedgehog (Ihh), and desert Hedgehog (Dhh).

Hedgehog receptor is composed of at least two proteins, Patched and Smoothened. Patched is an integral membrane protein, smoothened a G-protein-coupled receptor. Patched is an inhibitor of smoothened activation. Binding of Hedgehog (Shh) to Patched (Ptc) relieves Patched inhibition of Smoothened. There are two Hedgehog receptor PtcH1 and PtcH2. The three mammalian Hedgehogs bind both receptors with similar affinity. PtcH2 is mainly expressed in the testis, where it binds desert Hedgehog.

Hedgehog is translated into a protein, that is subsequently cleaved into two proteins. One Hh end is responsible for its activity and can be palmitoylated; the other acts as a cholesterol transferase, as the cholesterol modification of Hh is involved for its transport. In particular, Sonic Hedgehog is synthesized from a precursor, undergoing cholesterol-mediated cleavage.

Sonic Hedgehog can serve as an autocrine signal. Paracrine Hedgehog signaling requires Dispatched protein. Sonic Hedgehog induces proliferation of neuronal precursor cells. Sonic Hedgehog is also involved in the growth of various tissues (prostate, gastrointestinal tract, lungs, skeleton, and muscles). Shh is implicated not only in embryonic development of the vasculature, but also adult angiogenesis. Hh upregulates Notch target genes.

The pathway downstream from Smoothened involves the Gli family of transcription factors, with the activator Gli1 and the repressors Gli2 and Gli3. Many molecules affect Hedgehog signaling in the extracellular space, at the plasmalemma, or in the cytoplasma. The epidermal growth factor and insulin-like growth factor pathways modulate Hh signaling acting on Gli via Ras, MAPK, and PKB pathways in certain cell types. Protein kinase-A, glycogen synthase kinase GSK3β, and casein kinase CK1 phosphorylate Gli and prevent activation of Shh pathway. Other protein kinases (casein kinase CK2, cyclin-dependent kinase CDK9, CDK11, and protein phosphatase PP2A) positively regulate Hh signaling. Hh-interacting protein-1 sequesters Hedgehog. The developmental protein Numb is an inhibitor of the Hedgehog pathway, which is downregulated in cell progenitors and cancer cells. Numb not only inhibits Notch1 pathway by E3-ligase–dependent ubiquitination, but also suppresses

Hedgehog signaling by Itch-dependent ubiquitination of Gli1 [310]. Cell adhesion molecule-related/downregulated by oncogenes (CDO) and brother of CDO (BOC) modulate Hh signaling in space and time, acting either directly as coreceptors or indirectly downstream of Patched. 3β-Hydroxysteroids regulate Smoothened.

Hedgehog signaling responds to mechanical loading. In particular, Hedgehog signaling is affected by equibiaxial cyclic strain (10% stretch, 1 Hz, 24 h duration) and pulsatile flow (either 0.3 ml/mn, 0.2 Hz, pressure amplitude of ~ 0.8 kPa, or 25 ml/mn, 2 Hz, pressure amplitude of ~ 6.65 kPa) in cultured adult rat vascular smooth muscle cells [311]. Cyclic strains reduce the expression of sonic Hedgehog and Patched-1, reducing Gli2 activity and SMC proliferation, and increasing SMC apoptosis. Although Notch signaling also decays when smooth muscle cells bear cyclic strains, constitutive icNotch-3, which acts on Hh signaling, is overexpressed in loaded cells. icNotch-3 can thus compensate strain-induced inhibition of Hedgehog signaling. Pulsatile flow exposure also decreases Hh signaling, with subsequent reduction in cell proliferation and increase in apoptosis. Hh expression in vitro and in vivo decays in cells subjected to chronic exposure of time-dependent wall stresses.

3.2.8 Wnt Receptors

Signaling by ligands of the Wnt family is characterized by two main pathways: (1) a β-catenin-dependent branch, the so-called canonical pathway, and (2) β-catenin–independent non-canonical pathways. The non-canonical pathways function with downstream effectors Disheveled, GTPases Rho and Rac (Sect. 4.5), and JNK (Sect. 4.3.2.4).

Activated β-catenins are required for canonical Wnt signaling, which transmits signals from the extracellular environment and the Wnt receptor to the nucleus. β-Catenin resides in the cytoplasm. β-Catenin is either destroyed or activated. In the latter case, it can relay the Wnt signal into the nucleus.

In the absence of Wnt stimulation, cytosolic β-catenin and β-catenin–binding proteins, scaffold axin, adenomatous polyposis coli protein (APC), casein kinase CK1α, and glycogen-synthase kinase GSK3 form a complex, that phosphorylates and degrades β-catenin.[91] Consequently, the cytosolic β-catenin level remains low, and Wnt-responsive genes are repressed.

When a cell receives a Wnt signal, stimulated Frizzled receptor and the low-density lipoprotein-receptor–related protein receptor LRP6 becomes activated and forms a complex.[92] The scaffold protein Disheveled is required for the Frizzled-LRP6 aggregation and LRP6 phosphorylation. GSK3, which

[91] Within the destruction complex, β-catenin is sequentially phosphorylated by CK1α and GSK3. Phosphorylated β-catenin is then polyubiquitinated by E3 ubiquitin ligase and destroyed by the proteasome.

[92] The low-density lipoprotein receptor (LDLR) family includes the low-density lipoprotein-receptor–related proteins (LRP), apoliprotein-E receptor-2, and very low-density lipoprotein receptors.

phosphorylates (activates) LRP6, is recruited to the receptor complex (signalosome), as well as casein kinase CK1γ, which also phosphorylates LRP6. LRP6 phosphorylation within the signalosome allows axin recruitment to the receptor complex [312–314]. Thereby, β-catenin avoids axin-mediated degradation and translocates to the nucleus.

In fact, two mechanisms eliminate axin-based degradation of β-catenin. Stimulated plasmalemmal Frizzled receptors recruit the cytoplasmic adaptor Disheveled, which exist as both monomers and polymers. The Wnt signaling proteins Disheveled transduces both canonical and non-canonical Wnt signals. Disheveled forms polymers required for signal transduction [315]. Disheveled polymers serve as a scaffold for axin recruitment and inactivation (Fig. 3.11). Frizzled receptors also function with LRP5–LRP6 coreceptors. Phosphorylated LRP5–LRP6 by CK1 and GSK3 can interact with Axin, thus potentiating Axin inactivation at the plasmalemma.

β-Catenins activate transcription factors T-cell factor (TCF) and/or lymphoid enchancer factor, which stimulate target genes. Many Wnts promotes the nuclear accumulation of β-catenin, which then binds to TCF family members, thus relieving TCF repression and activating target genes. In the nucleus, histone acetyltransferase CREB binding protein (CBP) and p300 promotes Wnt signaling.

Multiple molecules modulate Wnt/β-catenin signaling. Wnt morphogens, synthesized in the endoplasmic reticulum, undergo various modifications (glycosylation, palmitoylation) for transport and functioning. Many factors are involved in Wnt protein secretion: lipoproteins, the retromer complex, and

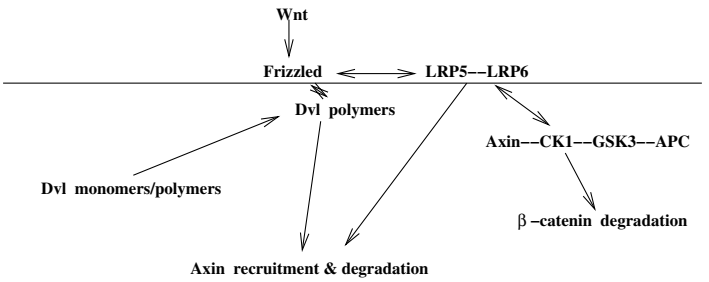

Figure 3.11. Wnt signaling and β-catenin degradation. Wnt stimulates Frizzled receptors, which recruit Disheveled (Dvl). Disheveled transduces signals from the Wnt receptor Frizzled, leading to the cytosolic accumulation and stabilization of β-catenins, and subsequently to the activation of transcription factors. Disheveled forms polymers to transduce Wnt signals. Moreover, Disheveled polymers recruit and inactivate Axin. A complex made by Axin, casein kinase CK1, glycogen synthase kinase GSK3, and adenomatous polyposis coli protein (APC) leads to β-catenin degration. LRP5–LRP6 associated with Frizzled receptors prevents β-catenin degradation by inactivating this complex. Once the degradation complex is inactivated, β-catenins accumulate in the cytosol and translocate into the nucleus to regulate the cell fate and regeneration (Source: [315]).

the multipass transmembrane protein Wntless (Wls) [316].[93] Wntless (Wls), specifically found in Wnt-secreting cells, promote the secretion of Wnt proteins. Heparan sulfate proteoglycans affect the transport of extracellular Wnt proteins. Syndecan-4, a plasmalemmal heparan sulfate that binds fibronectin, regulates Wnt signaling. It activates protein kinase-Cα and GTPase RhoA. With integrins, it stabilizes focal adhesions. Nucleoredoxin interacts with Disheveled, and inhibits the Wnt–β-catenin pathway [317]. The redox-dependent regulation of Wnt–β-catenin signaling contributes to cell proliferation induced by oxidative stress.

Hypoxia activates the heterodimeric transcription factor hypoxia-inducible factor-1 (Sect. 4.8.2). HIF1 is composed of two subunits: oxygen-sensitive HIF1α and constitutive HIF1β. HIF1 activates genes associated with glycolysis, angiogenesis, and pH regulation; HIF1 particularly targeting glucose transporters, vascular endothelial growth factor, carbonic anhydrase-9, and cyclooxygenase-2.

In normoxia, β-catenin, a transducer of the Wnt pathway and a coactivator of T-cell factor and leukocyte enhancer factor activity, promotes cell proliferation (Fig. 3.12). In hypoxia, β-catenin potentiates HIF1 activity and favors adaptation to hypoxia and cell survival [318]. β-Catenin binds HIF1. The complex then binds to DNA via HIF1. Hypoxia inhibits the formation of complexes between β-catenin and T-cell factor-4, inducing cell-cycle arrest, because HIF1α competes with T-cell factor-4 with HIF1α for binding to β-catenin. In normoxia, the complex formed by β-catenin and T-cell factor-4 stimulates von Hippel Lindau ligase for degradation of hydroxylated HIF1α [319].

Wnt signaling regulate cell polarity. Wnt signaling also determines the cell fate via the association of β-catenin with T-cell factor and leukocyte enhancer factor. Wnt–β-catenin signaling regulates cell fate during embryogenesis as well as regeneration in adults. Inappropriate activation of the Wnt pathway can lead to cancer.

Embryonic stem cells implicated in cardiogenesis, both in cell differentiation and spatial organization, are subjected to a biphasic pattern of Wnt signaling, being first activated then inhibited, with a possible cross-regulation between the canonical and non-canonical Wnt pathways [320].[94] The canonical Wnt–β-catenin signaling is required in early events that involve the fate of embryonic stem cells and mesoderm generation, driving the evolution of meso-

[93] Wntless, which works for Wnt proteins like the multipass transmembrane protein Dispatched in the Hedgehog pathway for the Hh release from the producing cell, is also known as evenness interrupted (Evi) or sprinter (Srt).

[94] Wnt–β-catenin signaling before gastrulation promotes cardiac differentiation, whereas signaling during gastrulation inhibits heart formation in both the mouse and zebrafish embryos. Wnt3A stimulates mesoderm formation and activates a negative feedback that represses Wnt pathway and increases cardiogenesis. However, the kinetics of the expression and the concentrations of effectors of Wnt signaling depend on the animal species.

derm cells of the cardiac compartment into the cardiac lineage. Conversely, during later stages of cardiac development, i.e., maturation of settled cardiac cells in suitable regions of the embryo, Wnt signaling is inhibited, thus limiting the size of the heart-forming field.

Cells of the first and the second heart fields contribute to the left ventricular myocardium and the outflow tract, right ventricular myocardium, endocardium, and smooth muscle of the great vessels, respectively. The pluripotent progenitors of the second heart field, which are initially located outside the heart, migrate into the heart and give birth to cardiomyocyte, endothelial, and smooth muscle lineages. The transcription factor Islet1 intervenes in the fate and migration of second heart field progenitors. Islet1 expression in cardiac progenitors requires β-catenin via the canonical Wnt signaling [321]. Wnt signaling promotes right ventricular and interventricular myocardial expansion [322]. In opposition, the canonical Wnt signaling hinders the first heart field specification, the cells of which are characterized by Hand1 and Tbx5 transcriptional regulators.

Wnt proteins are not only implicated during development, but also in the evolution of stem and progenitor cells during aging. Wnt signaling is more active in aged cells [323]. Moreover, the activation of the canonical Wnt pathway in aged myogenic progenitors leads to the conversion from myogenic to fibrogenic lineages.

3.2.9 Receptors of the Immune System

T-cell receptor (TCR), targeted by a combination of antigen fragments with a glycoprotein of the major histocompatibility complex, leads to activation

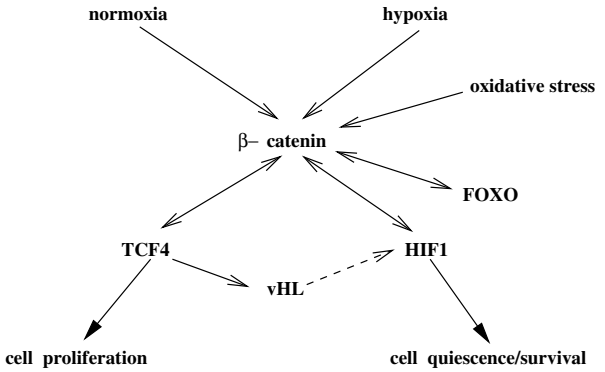

Figure 3.12. Schift in β-catenin signaling according to oxygen availability. During normoxia, β-catenin–T-cell factor-4 (TCF4) complex stimulates von Hippel-Lindau ligase (vHL), which degrades HIF1, thereby, avoiding cell-cycle arrest. In contrast, during hypoxia, β-catenin potentiates HIF1 activity leading to cell-cycle arrest (Source: [319]).

of protein phosphatase PP3 for nuclear factor of activated T cells (NFAT). Dephosphorylated NFAT enters the nucleus, and with accessory transcription factors, binds to the gene promoters. Pattern-recognition receptors distinguish microbial molecules from hosts. Toll-like receptors (TLR), members of pattern-recognition receptors, are involved in the early detection of pathogen molecules, which drive the activation of the adaptive immune response. Toll-like receptors can also activate innate immune cells.

The differentiation and activation of granulocytes, monocytes/macrophages, microglial cells, dendritic cells, osteoclasts, and platelets are regulated by signals that activate plasmalemmal and intracellular receptors. These receptors are implicated either in specific tasks associated with chemical pathways, such as Toll-like receptors and growth factor receptors, or in modulating the response magnitude (setting thresholds for cell responses to specific stimuli), without inducing pathway activation, such as macrophage interferon-γ receptors.

The family of plasmalemmal triggering receptors expressed on myeloid cells (TREM) are members of the immunoglobulin superfamily [324]. The TREM family is encoded by the TREM cluster on chromosome 6p21, which includes TREM1 and TREM2 genes, as well as the TREM-like subset, with TREML1 and TREML2 genes. The TREM-like genes encode TREM-like transcripts TLT1 and TLT2.

TREM substances participate in particular to inflammation and coagulation, among other functions. TREM1 and TREM2 forms a complex with the signaling adaptor DAP12. Ligand binding to the receptor activates Src kinase, which phosphorylates DAP12. The latter then binds to protein tyrosine kinase Syk. The downstream effectors, such as protein kinase PKB and PKCθ, mitogen-activated protein kinases, are then activated. TREM1 amplifies inflammatory signaling, acting synergistically with Toll-like receptors and Nod-like receptors. TREM2 is involved in osteoclastogenesis. TREM2 downregulates the expression of inflammatory cytokines by microglial cells. TREM2 is also implicated in clearing apoptotic neurons. TREM2 inhibits TLR-induced cytokine production by macrophages.

TREM-like transcript-1 is expressed by platelets. In the absence of cell stimulation, TLT1 is sequestered in α-granules of circulating platelets. After activation, TLT1 translocates to the plasmalemma and contributes to thrombin-mediated platelet aggregation. TLT1 recruits Src homology protein tyrosine phosphatases SHP1 and SHP2 (Sect. 4.4). The TLT2 expression by B lymphocytes, granulocytes, and tissue-resident macrophages is upregulated by inflammation. TREM2 binds plexin-A1, particularly after plexin activation by semaphorin-6D (Sect. 3.2.5).

3.3 Intracellular Hormone Receptors

The receptors of steroid and thyroid hormones (App. A) reside either in the cytoplasm, or in the nucleus. The cytoplasmic hormone–receptor complex translocates to the nucleus. The nuclear hormone–receptor complex binds to specific hormone response elements (HRE) of DNA for gene transcription.[95] These hormone receptors thus are bi-functional, binding hormone and directly activating gene transcription, hence behaving like ligand-activated transcription factors for responsive genes. Steroid and thyroid hormone receptors are, indeed, made of distinct domains. Among these domains of the single polypeptide chain, there are the transcription-activator domain, the DNA-binding domain, and the hormone-binding domain. Thyroid hormones receptors are nuclear receptors that bind DNA in the absence of hormone, usually repressing gene transcription. Thyroid hormone binding causes the receptor to behave as a transcriptional activator.

The steroid/thyroid hormone receptors can be decomposed into two sets. Type 1 receptors is targeted by sex hormones. They include androgen receptors, estrogen receptors, progesterone receptors, glucocorticoid receptors, and mineralocorticoid receptors. The type 2 receptor set is composed of vitamin-A receptors, vitamin-D receptors (for calcitriol, active form of vitamin-D), retinoid receptors (RARs, RXRs), and thyroid hormone receptors.

Sex steroid hormone receptors regulate gene expression in vascular cells. Cardiovascular coregulators include an estrogen receptor coactivator, the steroid receptor coactivator-3 (SRC3), and myocardial androgen receptor coactivator FHL2 [325].[96] Estrogen receptors, androgen receptors and two progesterone receptor isoforms (PR-A, PR-B) are expressed in the vasculature. Estrogen receptors and progesterone receptors in vascular cells can be activated in the absence of ligand by growth factor pathways. Sex steroid hormone receptors, particularly estrogen receptors ERα and ERβexist in cardiomyocytes, as well as aromatase.

Sex steroid hormone receptors can regulate other nuclear receptors, such as the peroxisome proliferator-activated receptor PPARα and the liver X receptors, which govern metabolic pathways [325]. Sex steroid hormone receptors

[95] Steroid hormones bind and activate their nuclear hormone receptors, which then dimerize and bind to specific DNA response elements, interacting with coregulators (coactivators and corepressors). Hormone response elements are DNA sequences of promoters of hormone-responsive genes.

[96] SRC3, also known as RAC3, AIB1, TRAM1, and pCIP, is a member of the p160 family of nuclear hormone receptor coactivators, with SRC1 and thyroid hormone receptor interacting factor SRC2 (or TIF2). SRC proteins recruit protein acetyltransferases, such as cAMP response element-binding protein CBP/p300 and its associated factor P/CAF, and protein methytransferases, such as coactivator-associated arginine methyltransferase CARM1 and protein arginine methyltransferase PRMT1, to nuclear receptor target gene promoters to modify chromatin structure and/or assemble transcription initiation complex.

stimulate endothelial nitric oxide synthase and also cause vascular dilatation by activated estrogen receptors. Estrogen receptors-α have protective effects on injured blood vessels by promoting reendothelialization and inhibiting smooth muscle cell proliferation. Estradiol acts on ERα to upregulate the production of atheroprotective prostacyclin by activation of cyclooxygenase-2 [326]. Conversion of testosterone to estrogen in males by aromatase allows the maintenance of vascular relaxation. Testosterone thus is able to activate both androgen receptors and estrogen receptors. Progesterone lowers the blood pressure. Estrogen receptors-β are required for vasodilatation and normal blood pressure in both males and females [325].

Nuclear steroid receptor coactivators, which enhance the transcription initiation mediated by nuclear receptors and other transcription factors, shuttle between the cytoplasma and nucleus where they act. Steroid receptor coactivator-interacting proteins (SIP) sequester steroid receptor coactivators in the cytoplasma [327]. Extracellular stimuli induce SIP phosphorylation by casein kinase-2, thereby, dissociating from SIPs the steroid receptor coactivators, which can move to the nucleus.

Steroid receptor coactivator-3 is also a coactivator for transcription factor myocardin in vascular smooth muscle cells [328]. Myocardin, a serum response factor cofactor exclusively expressed in cardiac and smooth muscle cell lineages, is involved in SMC differentiation to the nonproliferative, contractile phenotype.[97] Estrogens by acting on SRC3 can limit/prevent abnormal proliferation of vascular smooth muscle cells.

The retinoids (retinoic acid, or vitamin A, and its derivatives) bind to retinoic acid receptors (RAR). There are at least three RAR subtypes (RARα, RARβ, and RARγ). The retinoid X receptors (RXR) are retinoid-responsive transcription factors, that enhance the DNA-binding activity of RARs and of thyroid hormone receptors (Sect. 2.1). There are also three RXR subsets (RXRα, RXRβ, and RXRγ).

Peroxisome proliferator-activated receptors, similarly to RAR/RXR complexes, form PPAR/RXR heterodimers to specify the transcriptional response. In particular, ligand-dependent transcription factor peroxisome proliferator-activated receptor PPARδ, a member of the nuclear hormone receptor superfamily, binds to response elements. PPARs can act as lipid sensors in the regulation of nutrient metabolism, upregulates VEGF (Sect. 10.1) in colon carcinoma cells [329]. PPARδ thus favors angiogenesis (Sect. 10.4.2) and cancer development. Moreover, PPARδ inhibits epithelial tumor cell apoptosis via VEGF autocrine positive signaling loop, using PI3K and antiapoptotic factor PKB. PPARδ is induced by PGE2. It is also stimulated by Wnt–APC–β-catenin.

[97] Steroid receptor coactivators regulate signaling by transcription factors such as AP1, NFκB, HNF1α, MEF2C, E2F1, and TEF.

4

Signaling Pathways

Signaling pathways can be defined by an *initiation stage* and an *effector stage*. The initiation phase is triggered by ligand binding to its receptor. This biochemical event generates the effector stage with more or less numerous chemical reactions leading to specific cell responses. Signal propagation can be achieved by proteic complex assemblings, protein activation cascades, protein turnovers, and post-translational modifications of proteins, such as phosphorylation/dephosphorylation, glycosylation, ubiquitination, acetylation,[1] which reversibly modify the properties of involved proteic effectors.

Transducers can involve: (1) mechanosensitive ion channels such as large conductance, large pore, low ion selectivity carriers; (2) conformational changes of molecules; and (3) molecular switches in the plasmalemma or cytosol. Signaling pathways contain between-protein interactions that occur in nodes of signaling pathways. Similarly to lumped parameter models for blood flow in the vasculature, pathway modeling can focus on effector interactions and signaling networks can be considered to mimic electrical analogs, the effectors being represented as nodes linked via electrical resistances. Mathematical modeling of cell behavior are, indeed, based on molecular interactions inside and between cells involved in cell signaling.

Protein–protein interactions act as simple nodes for cue transmission or as hubs (multiple connection nodes). Molecular interaction forces of ligand–receptor complexes can be measured using atomic force microscopy. The binding kinetics between plasmalemmal receptors and their extracellular ligands, as well as the distribution of receptors, can also be assessed by mechanical imaging [330].[2]

[1] Acetylation affects the functions of various proteins, such as histones, many transcription factors, enzymes (histone acetyltransferases, histone deacetylases, kinases, phosphatases, etc.), as well as cytoskeleton-associated proteins.

[2] The heterogeneous distribution of vascular endothelial growth factor receptor-2 (Sect. 10.1) in microvascular endothelial cells, as well as linkages between VEGFR2 and the cortical cytoskeleton, has thus been demonstrated.

Moreover, cells can differentially respond to a given substance according to the location and kinetics of ligand binding and subsequent clustering of molecules. The activation of gene transcription by transcription factors is achieved via the recruitment of coregulators. Such between-protein interactions not only connect transcription factors to the transcription machinery, but also allow chromatin remodeling that favors the assembly of the transcription initiation complex.

Lipid–lipid and lipid–protein interactions are also required to form signaling complexes. For example, phospholipase-D2 hydrolyzes plasmalemmal phosphatidylcholine into phosphatidic acid, which recruits GEF Son-of-sevenless. Sos activates Ras in epidermal growth factor signaling (Sect. 4.3) [331].[3]

Ubiquitination also remodels proteins and affects their location and interactions with other proteins. Ubiquitination modulates the endocytosis route (caveolin or clathrin association, degradation or signaling from endosome) [332]. Signals transmitted by the receptor from the endosome could differ from those initiated at the plasmalemma.

Regulated proteolysis is required in many cellular processes, especially when the cell must react quickly. Gene expression and signaling particularly undergo quality control. Irreversible proteolysis is initiated after a specific post-translational cleavage using specific adapters. Proteases cleave targeted substrates of the cytoplasma, plasmalemma, and extracellular matrix, therefore release reactants, convert structural matrix proteins to signaling molecules, regulate growth factors, and modify signaling (Table 4.1).

Proteolysis at the cell surface includes two kinds of enzymes: (1) membrane-tethered proteases, which are soluble enzymes; and (2) integral membrane proteins responsible for the regulated intramembrane proteolysis. The transmembrane serine proteases are involved in cell signaling and interaction with its environment. Proteases of the ADAM and BACE families cleave plasmalemmal proteins.[4]

Intramembrane-cleaving proteases (ICliP) form a family of enzymes that cleave hydrophobic substrates within the lipid bilayer. This family includes: (1) the site-2 protease zinc metalloproteases, presenilin and presenilin-like aspartyl proteases; and (2) the rhomboid set of serine proteases. Presenilin is the catalytic component of γ-secretase complex.

Environmental signals can be efficiently processed by the cell using a pathway coordinating the activity of multiple functionally related mRNAs. The coordination of gene expression, indeed, depends on coordinated transcription, translational activation, and mRNA stability, as well as post-transcriptional

[3] Grb2–Sos interaction afterward promotes Sos recruitment to activated receptors. Phosphatidic acid can be converted to diacylglycerol by phosphatidic-acid phosphatase, which recruits RasGRP1.

[4] ADAM10 targets E-cadherin; BACE1 processes P-selectin ligand-1, neuregulin-1 and voltage-gated sodium channels; γ-secretase cleaves Notch receptor; and SPPL2 degrades tumor necrosis factor-α.

events controlled by RNA-binding proteins (RNABP) and small non-coding RNAs [334]. The components of the ribonucleoproteic process (RNA-binding proteins, non-coding RNAs, and metabolites) interact with mRNA regulatory elements, the untranslated sequence elements for regulation (USER). Such RNA operons/regulons guarantee the coregulated expression of a set of proteins acting in a given pathway. RNA operons can be activated or repressed by RNABP phosphorylation.

4.1 Signaling Triggered by Ligand-Bound Receptor

The cell signaling pathways, once the receptor is activated by its ligand, involve: (1) molecular transformations with a given reaction rate, (2) intermolecular associations due to changing binding affinity, and (3) intracellular translocations for optimized reaction compartmentation (Fig. 4.1).

Signaling pathways can lead to phosphorylation of transcriptions factors, which in turn control the expression of target genes. Furthermore, protein kinase-A (Sect. 4.3.2.2) and most mitogen-activated protein kinases (Sect. 4.3.2.4) can link to target genes, either at the promoter, or at the transcribed region of the genes [335].

Table 4.1. Protein cleavage and signaling. For example, proteolysis by matrix metalloproteinases creates space for cell migration, produces substrate fragments with biological activity, releases ECM-bound growth factors, regulates tissue architecture, activates or deactivates the activity of signaling effectors. MMP substrates include growth factors, receptor tyrosine kinases, cell-adhesion molecules, cytokines and chemokines, other MMPs, and proteases. In particular, MMPs release stored VEGF for angiogenesis and also cleave VEGF. Truncated VEGF increases vessel diameter; uncleaved VEGF increases vessel sprouting (Source: [333]).

Substrate	Role/Cleaved fragment effect
Cleavage of structural matrix proteins	
Collagen-1	Cell migration
Collagen-2	Bone morphogenetic protein antagonist fragment
Collagen-4	Anti-angiogenic fragment
Collagen-18	Anti-angiogenic fragment
Cleavage of chemokines	
Interleukin-8	Increase in chemokine activity
Monocyte chemotactic protein-1	Decrease in chemokine activity
Cleavage of signaling molecules	
Insulin growth factor-binding protein	Release IGF
Latent transforming growth factor-β	TGFβ
Vascular endothelial growth factor	Truncated VEGF

Plasmalemmal ligand-bound receptors are removed from the cell membrane and incorporated into endocytic vesicles, which fuse with early endosomes. Ubiquitin drives the vesicle incorporation. Early endosomes contain phosphatidylinositol 3-phosphate, early endosome antigen-1, and small GTPase Rab5. Rab5 is replaced by Rab7 in late endosome. The endosomal sorting complexes required for transport (ESCRT0–ESCRT3) are used for transport of ubiquinated proteins from endosomes to lysosomes via multivesicular bodies. ESCRTs interact between them, and with clathrin and 3-phosphoinosides. ESCRT0 recruits ESCRT1, which connects ESCRT2; the latter links ESCRT3 [336]. In opposition to other ESCRTs, ESCRT3 does not recognize ubiquitin; instead it recruits deubiquitinases to remove ubiquitin before incorporation into multivesicular bodies.

4.1.1 Signaling Initiation

Plasmalemmal receptor types include: (1) receptors coupled to *G proteins*[5] which activate or inactivate enzymes to modulate the formation of second messengers (Table 4.2, Sect. 4.5); (2) receptors, the cytoplasmic domain of which[6] is activated when the receptor is linked to its ligand and activates

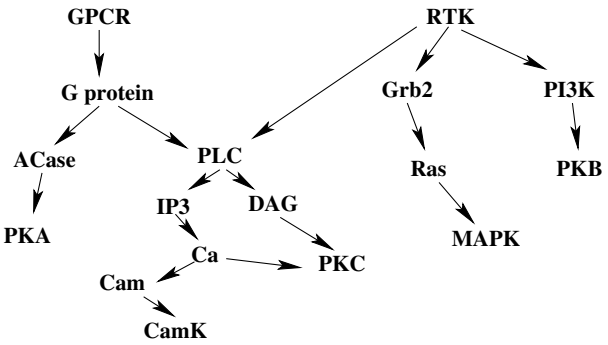

Figure 4.1. Examples of pathways activated by G-protein-coupled receptors (GPCR) and receptor tyrosine kinases (RTK). Several reaction cascades can be triggered by each receptor. The pathways are selected according to the context. The same modules can be used by different receptors, specific responses being achieved by different processes (Source: [6]).

[5] Subfamilies of G proteins include Gs and Gi, which respectively activate and inhibit adenylyl cyclase to regulate the intracellular cAMP level. The receptor-associated Gq family of G proteins stimulates enzymes that activate phospholipases to release second messengers derived from membrane lipids. The activated phospholipase-C releases IP3 and DAG, and phospholipase-A2 and phospholipase-D initiate arachidonic and phosphatidic acid pathways.

[6] Once the receptor cytoplasmic domain is activated by phosphorylation, the receptor links to effectors, such as tyrosine kinases, guanylyl cyclase,

one or more specific enzymes to simultaneously stimulate multiple signaling pathways (Table 4.3);[7] and (3) receptors linked directly or indirectly to ion channels.

Enzyme-linked plasmalemmal receptors have cytoplasmic domains that either have an enzymatic activity or are associated with an intracellular enzyme. In both cases, enzymatic activity is stimulated by ligand binding to the receptor. Transmembrane receptors with intrinsic enzymatic activity include adenylyl/guanylyl cyclases, tyrosine phosphatases, tyrosine and serine/threonine kinases, and plexins.

4.1.2 Molecule Transformations and Multicomponent Complexes

Signal transduction allows the cell to adapt to the changing environment, using various procedures at the molecular scale. Information is often transmitted via protein modifications, especially reversible protein *phosphorylation*. The reversible phosphorylation targets not only the receptors and effectors of the signaling set, including many transcriptional regulators, but also the associated components of the signaling pathway (ubiquitin ligases, and guanine nucleotide-exchange factors). Whatever the signaling substance, the ligand-bound receptor undergoes a conformational change. The receptor is then usually phosphorylated by an associated protein kinase. The majority of proteins contain multiple phosphorylation loci with different kinetics. The phosphorylation loci depend on the stimulus type, time dynamics, and subcellular location. Owing to several phosphorylation loci, the proteins can serve as hubs for signal integration.

Proteins participating in a shared cellular process, especially in cellular transport and signaling pathways, can transiently interact between them or form more or less stable proteic *complexes*. Furthermore, additional protein interactions can occur within proteic complexes, between complexes, and between free proteins and complexes.

The *location* of signaling complexes at plasmalemmal microenvironments modulates signaling outputs. Membrane rafts (Chaps. 2 and 5), which contain a set of proteins, can change their size and composition in response to stimuli for protein interactions [119]. Specific anchors allow to orientate non-specific enzymes toward specific targets and remove them from undesirable substrates.

Most regulated processes in the cell, characterized by a great sensitivity and a high specificity, especially the signaling pathways, with its triggers, switches, transducers (activators and inhibitors), and adapters, require the assembly and disassembly of multicomponent functional complexes made of proteins and other molecules. For example, weak interactions between growth factors and certain receptor domains are enhanced by cooperative interactions

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phospholipase-C, and stimulators or inhibitors of GTPases involved in the cytoskeleton arrangement and endothelial permeability (Rho family).

[7] VEGFR2 receptors act on the PLC and Ras pathways.

with additional receptor domains and/or other compounds, such as heparan sulfate, to form specific complexes, the weak interactions being replaced by stronger and specific dockings [337]. The assembling dynamic starts with non-specific, transient associations. Assembling the first stages involve electrostatic interactions between encounters. The non-specific complexes quickly separate. Partners of specific complexes reorient and, after desolvation (water molecules move away from protein surfaces), form much stronger links between them.

4.1.3 Molecule Translocations and Compartmental Organization

Signaling has a compartmental organization, the successive events acutely occurring in spatially restricted domains. Signal transduction starts with interac-

Table 4.2. Examples of second messengers and corresponding receptor ligands. Antidiuretic hormone receptors interact with specific kinases. Atrial natriuretic peptide, brain natriuretic peptide, C-type natriuretic peptides, produced by endothelial cells and monocytes, are vasorelaxants, which also inhibit the proliferation of vascular smooth muscle cells, via guanylyl cyclases coupled to the plasmalemmal natriuretic peptide receptors. β1- and β2-adrenergic, as well as D1- and D5-dopamine receptors interact with Gs and Gi subunits of G proteins coupled to adenylyl cyclase, whereas α1- and α2-adrenergic receptors function with Gq coupled to phospholipase-C and Gi, respectively (Sect. 4.5). Erythropoietin regulates the proliferation, differentiation, and maturation of erythroid cells via Epo receptors, members of class 1 cytokine receptors, which use the JaK–STAT pathway. The parathyroid hormone receptor is a G-protein-coupled receptor leading to cAMP pathway. The calcitonin G-protein-coupled receptor is also associated with adenylyl cyclase. Whereas the insulin receptor is a receptor tyrosine kinase, the glucagon receptor belongs to the G-protein-coupled receptor family, which activates adenylyl cyclase. Somatostatin acts on insulin and glucagon secretion via two somatostatin-receptor subtypes. Somatostatin, produced by several body tissues, inhibits growth hormone release (negative feedback loop). The growth hormone receptor is a receptor tyrosine kinase that activates receptor-associated tyrosine kinase JaK2. The prolactin receptor belongs to the cytokine receptor superfamily. The oxytocin receptor is a G-protein-coupled receptor that requires Mg^{++}.

Second messenger	Activators
cAMP	Catecholamines (α2, β), dopamine (D1–D5), glucagon, calcitonin, oxytocin, adrenomedullin, parathyroid hormone
cGMP	Naturetic peptide, nitric oxide
Protein kinase	Insulin, growth hormone, prolactin, erythropoietin, growth factors, antidiuretic hormone angiopoietins, ephrins
Phosphoinositides and calcium	Catecholamines (α1), angiotensin-2, vasopressin

tions between multiple types of proteins at the cell surface. Besides, cells connected by gap junctions communicate rapidly with each other, either by ionic current or diffusion of low molecular weight second messengers (calcium ions, cAMP, cGMP, diacylglycerol, and inositol(1,4,5)trisphosphate). Cells then use chemical communication, releasing stimulating molecules, which diffuse to a target cell with suitable receptors.

Receptor excitation triggers the displacement of cytosolic adapters and enzymes to the plasmalemma. These *translocations* correspond to effective control mechanisms aimed at switching on the signaling pathway. A signaling complex with scaffold proteins, possible membrane ion channels, and possible cytoskeletal components is assembled. The receptor complexes recruit cytoplasmic signaling components, which initiate a biochemical cascade that finally causes changes in gene expression.

Pathway cycles are formed by convertible types of signaling proteins, resulting from activity of either kinase or phosphatase on phosphoproteins, or activity of either guanine nucleotide-exchange factor or GTPase-activating protein on small GTPases. Multisite phosphorylation increases the sensitivity of the signaling dynamics. The activity of a cycle with two opposing enzymes depends on the location of the involved molecules within the cell. This location can depend on the time and space concentration gradient of the target protein, hence on the protein diffusivity and the signaling dynamics with its given response rates.

Table 4.3. Main receptors and effectors (GPCR: G-protein-coupled receptor; RTK: receptor tyrosine kinase; RSTK: receptor serine/threonine kinase; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; PK(A,B,C): protein kinase-A, -B, -C; PL(A,C,D): phospholipase-A, -C, -D; MAPK: mitogen-activated protein kinase; PI3K: phosphatidylinositol 3-kinase; AA: arachidonic acid; Lkt: leukotriene; PG: prostaglandin; GRK: GPCR kinase).

Receptor	Effectors
GPCR	cAMP/PKA
	PLC/PKC
	PLA, PLD
	MAPK
RTK	PI3K/PKB
	PI3K/PLC
	PLA/AA/Lkt,PG
	GTPases/MAPK
RSTK	GRK
	cAMP, cGMP
	PLC/PKC/Ca^{++}

Signaling molecules commonly do not spread by diffusion. They can instead use endocytic vesicles (signaling endosomes) or other cell transport means driven by given molecular motors [338]. Endocytosis indeed is not only aimed at ending signaling via degradation of activated receptor complexes after internalization from the plasmalemma. The signal transduction can exploit the compartmentation of endocytosis. Signaling factors (Shc, Grb2, and Sos) can be found on early endosomes [339]. Endocytosis provides signaling temporal regulation according to the transport kinetics. Endocytosis can either lead to quick signal transduction, or inversely slow down the process.

4.1.4 Complicated/Complex Pathways

Signaling pathways can correspond to either linear or complex cascades (Fig. 4.2). In linear cascades, a ligand binds to the receptor, which activates an effector. This effector either carries out the action or stimulates another single downstream effector, which in turn does the same activity until the effector leads to the signaling response, whether the pathway requires a single stage or is multi-tiered. Complicated pathways are composed of multiple steps characterized by linear interactions.

Complex networks are characterized by nodes and hubs formed by effectors. These nodes and hubs act as mesh nodes in spatial discretizations of any explored organ for computational purposes, the node values of involved physical quantities depending on the adjoining node ones and conversely, or as nodes of a communication network, receiving and providing information from and to neighboring ones. They usually are constituted by protein isoforms. These related proteins cause a pathway divergence with multiple routes. The node is positively and negatively regulated. Furthermore, the node can be the biochemical site for cross-talk with other signaling networks.

The concept of stimulus-specific interconnected signaling pathways, which includes timing, amplitude, and duration of signaling responses for coordinated behavior in time and space, as well as subcellular locations and specific protein recruitments, relate extracellular signals to the expression of nuclear transcription factors of specific genes more efficiently than linear pathways [340]. Signaling networks have various kinetics, activation being either transient or sustained [341]. Brief stimuli can cause sustained kinase activation at low concentrations of corresponding phosphatase (bistable behavior) [342]. This state can be eliminated by kinase-induced increase in related phosphatase. The signaling network can then respond proportionally (monostable state).

A single transduction pathway can be rather complex due to its numerous inputs, outputs, and regulatory nodes. Moreover, different signaling pathways interact. For example, synergistic activity results from co-stimulation with ligands that mobilize calcium ions and from ligands that trigger cAMP production. Co-stimulation of receptors causes a peculiar pattern of production and suppression of members of a set of targeted molecules, whereas a single

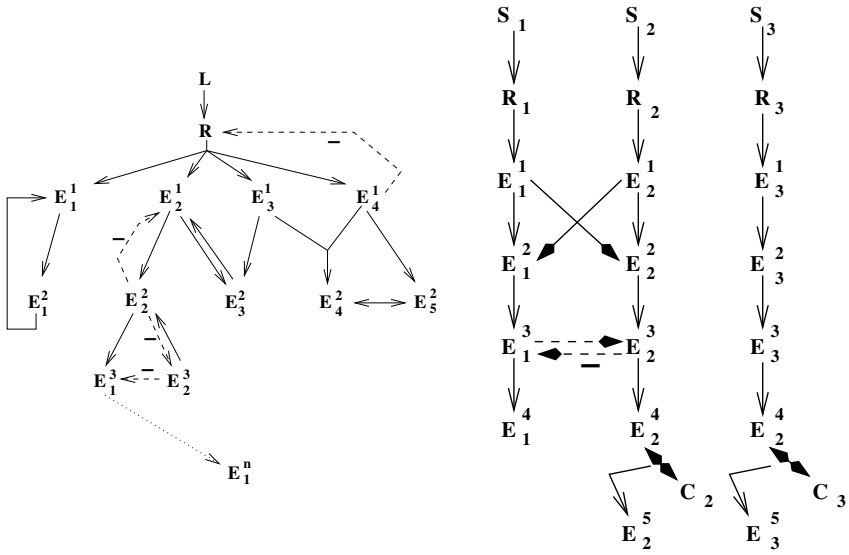

Figure 4.2. (**Left**) Complex pathway with non-linear interactions between effectors (L: ligand; R: receptor; E_i^s: effector i in pathway stage s). Diagrams are useful for representing reactions of a signaling process. The binding of a ligand to its receptor triggers the activation of signaling pathways through effectors and intracellular second messengers, which finally lead to biological effects. Complex signaling pathways are characterized by cooperation, reciprocal effects, interaction, cross-talk, and positive and negative feedback. (**Right**) Parallel interacting signaling pathways activated by their respective signals (S_1, S_2, and S_3), with targeted components (effectors E_1, E_2, and E_3) are actived when they simultaneously receive their signals. Specificity is lost when both pathways are activated, although they are exposed to a single signal due to mutual activation or shared component. They achieve different responses when they are exposed to their own signals and either cross-inhibition or insulation. Cross-activation between pathways triggered by S_1 and S_2 downstream from tier 1 is associated with mutual inhibition downstream from tier 2 (downstream from the cross-activation node, here between E_1^3 and E_2^3) to eliminate unwanted interactions between pathways. Insulation between pathways triggered by S_2 and S_3 is achieved when shared component E_2^4 is incorporated into different molecular complexes (C_2 or C_3, made of different adapters and scaffold proteins), each one being specific for each signal that must be processed.

receptor stimulation can either favor or prevent the production of the entire set of molecules. Input ligands can modulate signaling pathways rather than control a given output.

Coupled positive and negative feedback loops induce complex dynamics, favoring the occurrence of instabilities and oscillatory behavior. Positive feedback amplifies the signal, whereas negative feedback attenuates it. A given effector can not only stimulate its activator, but also inactivate its inhibitors. Signaling cascade steps with positive feedbacks are endowed with bistability,

characterized by two stable activities, basal and stimulated. Negative feedback can stabilize the cascade outputs (monostable state) and adapt the pathway to parameter variations.

Information processing by cells requires the coordinated activity of a network of signaling pathways. Cross-talk between pathways allows combinations of stimuli. The architecture of the signaling network in which the majority of inputs modulates signaling (only a few inputs independently control cellular outputs) has been proposed in macrophages, selecting activation of toll-like receptors, G-protein-coupled receptors, cytokine receptors, and receptor tyrosine kinases [343]. Cells can distinguish between adaptive and maladaptive signals that share the same pathway.

Spatial and temporal signaling dynamics after receptor stimulation determine the specificity of cellular responses via activation of kinase and phosphatase cascades. Distinct spatio-temporal activation of a given signaling pathway, according to temporal control and spatial distribution of activated transducers, causes different responses. The magnitude of kinase activation depends on the stimulus amount (activation differs from an all-or-none response) [344]; but gene expression, which depends on the nature of the inductive signal, can be independent of the amplitude of kinase activation.

The mitogen-activated protein kinase pathway (Sect. 4.3.2.4) governs cell fate by transducing and processing multiple growth factor signalings. The Raf–MAP2K–ERK cascade, controlled by Ras GTPase, particularly regulates cell proliferation and differentiation. Ras recruits Raf MAP3K from the cytosol to the plasmalemma for activation. In turn, MAP2K, ERK, and scaffold proteins translocate to the plasmalemma to be activated also. The signal sensitivity of the module is determined by its subcellular location [345]. The activation threshold is low at the plasmalemma and high in the cytosol. The MAPK module with a greater complex lifetime at the plasmalemma than in the cytosol transmits signals with a larger efficiency. MAP2K and ERK dephosphorylation occurs about two and five times faster, respectively, in the cytosol than at the plasmalemma. Cells can hence respond appropriately to physiological stimuli, with maximal outputs for low inputs.

The Raf–MAP2K–ERK cascade is a module of three nodes[8] activated by many external cues from growth-factor receptors, which hence must coordinate these signals whereas keeping specific responses. Different effectors or modules, upstream or downstream from the Raf–MAP2K–ERK cascade, or their intracellular location can explain distinct regulatory mechanisms. Epidermal growth factor uses the Raf1–MAP2K1/2–ERK1/2 pathway, as well as neuronal growth factor, but they have different effects. EGF transiently activates ERK, leading to cell proliferation, whereas NGF generates sustained ERK activation, causing cell differentiation [346]. EGF stimulation promotes a negative feedback loop from ERK to Raf1, whereas NGF elicits a positive feed-

[8] A pathway node represents an effector, i.e., a single protein, proteic complex, or gene, with a given task.

back loop from ERK to Raf1, with a persistent response (Fig. 4.3). Another short negative feedback from ERK1/2 to MEK1/2 exists for integrin signaling. The cell response can be altered by pathway changes. Blockage of protein kinase-C in NGF-stimulated cells transiently activates ERK (NGF-stimulated cells proliferate rather than differentiate). Therefore, PKCδ is involved in the NGF-induced positive feedback. Conversely, concomitant PKC activation in EGF-stimulated cells yields sustained ERK signaling, transforming negative feedback from ERK into positive feedback (PC12 cells differentiate rather than proliferate). Moreover, the response feature varies between EGF and NGF. Augmentation in EGF level (negative feedback) gradually increases ERK phosphorylation, whereas NGF (positive feedback) drives all-or-nothing ERK activation (switch-like, bistable dynamics).

4.1.5 Modeling and Simulation

Mathematical modeling is aimed at describing the macroscopic reactions of cells and tissues from the nanoscale mechanisms. Discrete-state models ignore kinetic properties of the process. Modeling of behavioral networks is mostly described by differential equations of rise or decay in concentration of

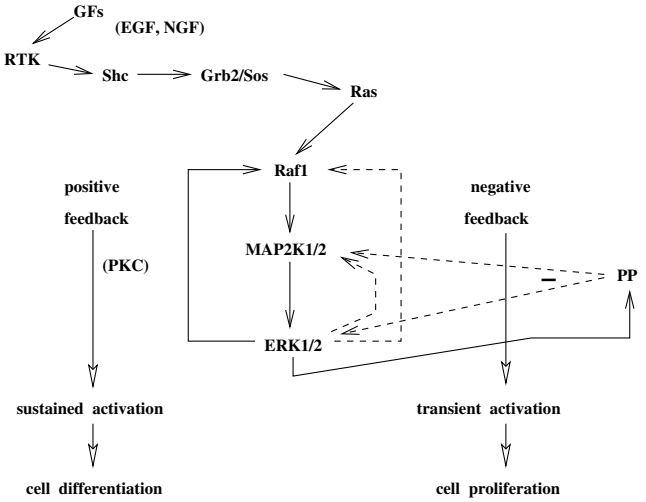

Figure 4.3. The Raf–MAP2K–ERK cascade can be activated by multiple ligand-bound receptors. Different receptors thus activate this common three-node module of the corresponding signaling pathways which generates distinct outputs. Pathway specificity can be determined according to the type of feedback loops. A positive feedback loop from ERK to Raf1 induces persistent activation leading to cell differentiation (PC12 cells subjected to NGF stimulation via TrkA receptor differentiate). A negative feedback loop from ERK to Raf1 explains the occurrence of transient activation generating cell proliferation (PC12 cells subjected to EGF stimulation via EGF receptors proliferate; Source: [346]).

biochemical species of interest. The equation parameters describing molecule synthesis, decay, transformation and transport (rates of reaction and diffusion, activation thresholds, etc.) must be identified using available data.[9] Hybrid systems, which incorporate both continuous and discrete dynamics, model both the logic of interactions and the dynamics of biochemical changes, simplifying the parameter identification problem.

The temporal dynamics of signaling networks are commonly described by chemical kinetics equations (currently ordinary differential equations), one for each time-dependent molecule involved in the chemical transformation. The concentration depends on production and consumption, both having given rates. A set of coupled ordinary differential equations for the set of elementary reactions with forward and reverse rate constants usually gives the rates of production and consumption of individual chemical species, using mass action kinetics.[10] Time integration of equations yields the concentration of each implicated species at given times.

Physicochemical models of cell signaling pathways of short-term vascular regulation and long-term vascular adaptation can be based on deterministic or stochastic form of ordinary and partial differential equations. These models not only describe the set of implicated elementary reactions, following a reductionist strategy (after a selection of the degree of details), but also changes in location[11], as well as other non-enzymatic changes of state, such as assembling into multi-component complexes.

4.2 Phospholipases and Phosphatidylinositol 3-Kinase

Inositol phosphates are intracellular second messengers that regulate various cell processes, from calcium signaling to chromatin remodeling. Inositol(1,4,5) trisphosphate (IP3) is the most well-known messenger. A phospholipase type, phospholipase-C (PLC), splits phosphatidylinositol(4,5)bisphosphate

[9] Parameter identification can be done by solving the model differential equations, starting from a guess for the numerical values of the parameters, and solving the equations using suitable numerical algorithms. The simulation results are then compared with experimental data. If the results do not match, a new guess is made for a new simulation. In the absence of a suitable parameter set, the model must be modified.

[10] The mass action law states that reaction rates are proportional to the concentrations of the reacting species. More precisely, chemical reaction rates are defined as the product of a rate constant and the concentration of the reactants. The calibration stage is aimed at adjusting the model parameters to experimental data.

[11] Between-compartment transport is done with a given rate, whereas the transport is assumed to be instantaneous within a compartment. Otherwise, species concentrations must be described with respect to space using partial differential equations.

(PIP2) into IP3 and diacylglycerol (DAG). IP3 can be phosphorylated by kinases to produce inositol hexakisphosphate IP6, mono- and bis-pyrophosphorylated inositol phosphates PPIP5 and PP2IP4. PPIP5 is implicated in gene expression and protein phosphorylation, among other functions. Inositol-(1,3,4,5)tetrakisphosphate (IP4) is the polar head group of phosphatidylinositol(3,4,5)trisphosphate, which allows the PIP3 effector to distinguish PIP3 from its precursor PIP2. IP3 phosphorylation into IP4 promotes PLCγ phosphorylation [347].

4.2.1 Phospholipases

Phospholipases target phospholipids of the cellular membranes. Phospholipases are involved with phospholipids in the transmission of ligand-bound receptor signaling. Activated phospholipases particularly leads to protein kinase-C, which is maximally active in the presence of diacylglycerol and calcium ions, released from its intracellular stores by inositol trisphosphate. There are four main types of phospholipases, PLA, PLB, PLC, and PLD.

Phospholipase-A hydrolyzes one of the acyl groups of phosphoglycerides or glycerophosphatidates. Phospholipases-A1 and -A2 hydrolyze the acyl group at the 1- and 2-position, respectively. Phospholipase-A2 hydrolyzes phosphatidylcholine, producing free fatty acids and lysophosphatidylcholine, which potentiate diacylglycerol activity. Phospholipase-A2 forms arachidonic acid, which is involved in signaling. Arachidonic acid is the precursor of eicosanoids (Sect. 8.4), such as leukotrienes and prostaglandins. Eicosanoids can also be synthesized from diacylglycerol formed by phospholipase-C.

Phospholipase-B attacks lysolecithin, releasing glycerylphosphorylcholine and fatty acid.[12] Certain phospholipases B hydrolyze phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine.

Phospholipase-Cγ hydrolyzes phosphatidylinositol(4,5)bisphosphate into inositol trisphosphate (inositol(1,4,5)trisphosphate, IP3) and diacylglycerol (DAG). Inositol trisphosphate and diacylglycerol are second messengers. The phospholipase-C family includes many isozymes classified into six subsets: PLCβ, PLCδ, PLCγ, PLCϵ, PLCζ, and PLCη. Each isoform require calcium for catalysis. PLCβ is activated by G protein, PLCδ by high calcium levels, PLCγ by receptor tyrosine kinases, and PLCϵ by Ras guanosine triphosphatase.

Phospholipase-D hydrolyzes phosphatidylcholine, releasing phosphatidic acid which in turn is converted to diacylglycerol by phosphatidic acid phosphomonoesterase. There are two isoforms, PLD1 and PLD2. Phosphatidic acid serves as a signaling effector, being hydrolized to form diacylglycerol.[13]

[12] Phospholipase-B is also known as lysolecithin acyl hydrolase or lysolecithinase.

[13] Phosphatidic acid and diacylglycerol are interconvertable owing to phosphatidic acid phosphohydrolase and diacylglycerol kinase. Fixation of serine, choline, ethanolamine, or inositol on phosphatidic acid leads to phosphatidylserine, phosphatidylcholine, phosphatidyléthanolamine, or phosphatidylinositol.

4.2.2 Phosphatidylinositol 3-Kinase

The family of phosphatidylinositol 3-kinases (PI3K) includes four sets of serine/threonine protein kinases, PI3K1A, PI3K1B, PI3K2, PI3K3 and an additional set, PI3K4. Heterodimeric PI3K contains a subunit that has enzymatic activity. Phosphoinositide 3-kinases-1A is composed of: (1) one regulatory subunit among five isoforms (p50α, p55α, p55γ, p85α, and p85β), which contains SH2 and SH3 domains interacting with other signaling proteins; and (2) a catalytic subunit among three isoforms (p110α, p110β, and p110δ) that phosphorylates inositol. p110αIsoform, but not p110β, acts via the insulin receptor substrate. PI3K catalytic subunits p110α and p110β are ubiquitous, whereas p110γ is expressed primarily in hematopoietic cells, myocytes, and pancreatic cells. PI3K2 is made of three catalytic isoforms (C2α, C2β, and C2γ) without regulatory subunit. PI3K3 of heterodimers, with catalytic (Vps34) and regulatory (p150) subunits, is mainly involved in cell transport. PI3K4 includes related enzymes, such as mammalian target of rapamycin (mTOR).

Phosphatidylinositol 3-kinase is phosphorylated (activated) by various receptor- and receptor-associated protein tyrosine kinases. PI3K is activated by the receptors of hormones (insulin) and growth factors (PDGF, EGF, IGF1, HGF and NGF; Sect. 10.1). PI3K phosphorylates phosphatidylinositols at position 3 of the inositol ring (Sect. 2.1). In response to various stimuli, phosphatidylinositol 3-kinase phosphorylates phosphatidylinositol (4,5)bisphosphate to generate phosphatidylinositol(3,4,5)trisphosphate, to which pleckstrin-homology domains of signaling proteins bind, as well as phox-homology domains of certain proteins. PI3K thus generates substrates for phospholipase-Cγ, which produces second messengers (diacylglycerol and inositol trisphosphate). The effectors also include serine/threonine kinases, protein kinase-B and 3-phosphatidylinositol-dependent protein kinase-1 (PDK1), various isoforms of protein kinase-C (PKC), and members of the TEC family of tyrosine kinases, including TEC, BTK (Bruton tyrosine kinase), ITK (interleukin-2–inducible T-cell kinase), and RLK (resting lymphocyte kinase) [348]. The PKB activation depends on PI3K products and can require a sequential phosphorylation by two phosphoinositide-dependent kinases, PDK1 and PDK2.

4.3 Protein Kinases

Protein phosphorylation is the most widespread post-translational modification in signal transduction.[14] Protein phosphorylation is involved in cell metabolism, transport, growth, division, differentiation, motility, muscle contraction, immunity, learning, and memory.

[14] Many substrates must be phosphorylated at multiple sites to change their function.

A protein kinase is an enzyme that reversibly adds phosphate groups to target proteins to change the substrate activity, location, or binding with other substances and regulate signaling pathways. Protein kinases catalyze the transfer of phosphate from ATP to specific amino acids of proteins.[15] The phosphorylation rate depends on the kinase location. The order of the stages (ATP binding, substrate binding, ADP release, and substrate release) of protein phosphorylation depends on the kinase type. According to the targeted residues, protein kinases are classified into: (1) tyrosine (Tyr) kinases, (2) serine/threonine (Ser/Thr) kinases phosphorylating serine or threonine, and (3) dual specificity kinases taht phosphorylate the three residues. In contrast, protein phosphatases (Sect. 4.4) remove phosphate groups from amino acid residues of proteins phosphorylated by protein kinases.[16]

Any protein kinase must recognize its phosphorylation sites among multiple amini acids that compose the targeted protein. Multiple features allow specific functioning: the structure of the catalytic site, the interactions between the kinase and its substrate due to complementary sequences, hydrogen bonding, surface charge and hydrophobicity, the formation of complexes with scaffolds and adapters,[17] competition between substrates,[18] and error correction.[19]

Tyrosine kinases are mainly associated with growth factor signaling. Serine/threonine kinases are principally related to second messengers, such as cAMP (Sect. 4.11), cGMP (Sect. 4.12), diacylglycerol (Sect. 4.2), inositol trisphosphate, and calmodulin (Sect. 4.14). The protein kinase activity is strongly regulated by phosphorylation, either by the kinase itself (autophosphorylation), or kinase-bound activators or inhibitors. Because enzymes can have different phosphorylation sites, phosphorylation and dephosphorylation either regulate the enzyme activity or directly launch enzyme activation or inhibition.

[15] The number of substrates and phosphorylation sites (from a few sites to hundreds of sites) varies according to the kinase type. The amino-acid composition in the neighborhood of the target residue plays a role. Adjoining proline-directed kinases could regulate a larger number of proteins than non-Pro-directed kinases [349].

[16] The numbers of Tyr kinases and phosphatases are similar; but Ser/Thr phosphatases is much more numerous than Ser/Thr kinases.

[17] Scaffold proteins coordinate interactions of kinases with other kinases and phosphatases. Scaffolds also can recruit regulators of kinases.

[18] Any substrate can act as a competitive inhibitor for other substrates.

[19] Iterative control of multiple phosphorylations on a given targeted protein minimizes erratic phosphorylation. Any wrong phosphorylation (on an inappropriate site) can be corrected by active phosphatase.

4.3.1 Protein Tyrosine Kinases

Protein tyrosine kinases[20] (PTK) modulate multiple cellular events, such as differentiation, growth, metabolism, and apoptosis. PTKs include: (1) transmembrane *receptor tyrosine kinases* (RTK; Sect. 3.2.2); and (2) cytoplasmic *non-receptor tyrosine kinases* (NRTK).

Non-receptor tyrosine kinases recruited in signaling pathways following ligand binding to receptor include Janus, Src, and Tec kinase families.

4.3.1.1 Janus Kinases

The Janus kinases[21] (JaK) are activated by the cytokine receptors. Intracellular Janus kinases are involved in the JaK–STAT pathway. The JaK family includes four members (JaK1, JaK2, JaK3, and tyrosine kinase TyK2). JaK1 and JaK2 are involved in interferons-II (interferon-γ) signaling (Sect. 10.1). JaK1 and TyK2 are associated with interferons-I. JaK2 is an effector of prolactin receptors.

4.3.1.2 Src Kinases

The Src family of intracellular NRTKs are signaling proteins associated with cell migration, proliferation, and adhesion, especially during angiogenesis (Sect. 10.4.2). The Src kinase family includes Src, Yes, Fgr, Fyn, Lck, Lyn, Blk, and Hck, which are either expressed in a wide variety of cells or have a restricted expression pattern (Blk, Hck, Fgr, Lck, and Lyn). The Src family of cytoplasmic PTKs have regions with similar sequences, the so-called Src homology (SH) domains.[22] The Tec family,[23] a subgroup of Src NRTKs, includes Tec, Txk, Btk, Bmx, and Itk/Tsk/Emt.

Src becomes activated during the transit from the perinuclear region to the plasmalemma, which requires the actin cytoskeleton and RhoB-associated endosomes.[24] Disruptions in actin filaments inhibit both the membrane translocation and activity of Src [351]. The active Src colocalizes with RhoB, in the

[20] Tyrosine kinases are enzymes catalyzing the phosphorylation of tyrosine residues of proteins.

[21] The Janus kinases have been initially named just another kinase.

[22] The SH1 domain is a catalytic domain. The SH2 and SH3 domains are protein-binding domains. SH2 usually binds phosphotyrosine-containing proteins and SH3 interacts with cytoskeletal proteins.

[23] They have a pleckstrin- and Tec-homology domain. Lyn protein-tyrosine kinase is a member of Src-related family of protein tyrosine kinases, found in hematopoietic cells, macrophages, platelets, and B lymphocytes. Tec kinases are also expressed in many hematopoietic cells. In fibroblasts in particular, using the Tec-homology domain, Tec binds to Lyn kinase. Lyn then phosphorylates Tec protein, which thus acts downstream of Lyn in intracellular signaling pathways [350]. Tec/Btk tyrosine kinases can be activated in response to cytokines, such as IL3 and IL6, probably via Janus kinases.

[24] RhoB is an endosome-associated Rho GTPase (Sect. 4.5).

perinuclear region. Fibronectin is a main Src-activating extracellular stimulus. Src- and RhoB-containing structures are associated with Src-promoted polymerized actin.

4.3.1.3 Focal Adhesion Kinases

Focal adhesion kinases (FAK) are tyrosine kinases that mediate several integrin signaling pathways. They can perform autophosphorylation on a tyrosine residue. FAKs interact with Src, PI3K, Grb2, p130Cas, and paxillin. Hence, activated FAK controls cell adhesion and motility [352]. Tyrosine-phosphorylated FAK promotes interactions with certain proteins, which allow connections to the Rac and Rho GTPases and extracellular signal-regulated kinase isoform ERK2 cascade. FAK acts on the assembly and maturation of focal contacts. FAK-Src kinase activity promotes phosphorylation of phosphatidylinositols. FAK-Src functions for the disassembly of focal contacts, activating calpain and extracellular matrix metalloproteinases; it also regulates cadherin-mediated cellular junctions. FAK signaling to Rho GTPases regulates changes in actin and microtubules in cell protrusions of migrating cells.

4.3.2 Protein Serine/Threonine Kinases

Numerous serine/threonine kinases function in signal transduction, either as initiating nodes, the *receptor serine/threonine kinases* (Sect. 3.2.3), or as intermediate nodes, the *non-receptor serine/threonine kinases* (NRSTK). Typically, second messengers activate Ser/Thr kinases, whereas extracellular signals stimulate Tyr kinases. Kinases IKK of inhibitory factors IκB of the nuclear factor-κB are serine kinases (Sect. 4.8.1).

Non-receptor serine/threonine kinases include cAMP-dependent protein kinase-A (PKA), protein kinase-C (PKC), Rho-kinases, ribosomal S6 kinase, p21-activating kinase, Bcr proteins, etc. Certain enzymes have been considered to be Ser/Thr kinases, although they have a dual specificity, such as the mitogen-activated protein kinases and glycogen synthase kinase-3.[25]

4.3.2.1 Calmodulin-Dependent Protein Kinases

Calmodulin-dependent Ser/Thr protein kinases (CamK) require activated calmodulin for their activity. Members of the CamK family are CamK1, CamK2, and CamK4. Calcium–calmodulin-dependent protein kinase-2 phosphorylates a large number of substrates, such as ion channels and intracellular proteins of signal transductions. For example, it increases PLA2 activity. It is made of several subunits, especially a catalytic domain, a regulatory domain, and binding domains to form oligomers and act on its targets.

[25] Phosphorylation at different residues controls the enzyme activity. Ser/Thr phosphorylation causes inactivation and Tyr phosphorylation results in increased activity.

4.3.2.2 AGC Protein Kinases

AGC kinases form a set of kinases stimulated by growth factors and insulin. About 70 among the 518 protein kinases encoded by the human genome are members of the AGC family of serine/threonine protein kinases. The set of AGC kinases includes protein kinase-A, several types of protein kinases-C, protein kinases-B and -G, p70 ribosomal S6 kinases (S6K), p90 ribosomal S6 kinases (RSK), mitogen- and stress-activated protein kinases (MSK), and serum- and glucocorticoid-induced protein kinase (SGK) [353]. These kinases regulate cell division, growth, survival, metabolism, motility, and differentiation. Once phosphorylated, PKB and S6K intervene in PI3K pathway, PKC in calcium signaling, RSK and MSK in MAPK pathway, and PRK in Rho signaling.

Nuclear Dbf2-related (NDR) protein kinases, a subclass of the AGC family of protein kinases, control morphological changes, mitosis, cytokinesis, and apoptosis. NDR kinases are regulated by phosphorylation by kinases and dephosphorylation by protein phosphatase-2A. There are four related kinases, NDR1, NDR2, large tumor suppressor-1 LATS1 and LATS2.[26]

Protein kinase-B[27] is activated by ligand-bound growth factor receptors via phosphatidylinositol 3-kinase. Protein kinase-B mediates survival and proliferation signaling via various effectors. Protein kinase PKB1 can block cancer cell migration by inhibition of transcription factor nuclear factor of activated T-cells and extracellular signal-regulated kinase [354]. Hyperproliferation and antiapoptotic activities caused by stimulation of insulin-like growth factor-1 receptor are reversed by PKB2 downregulation [355].

The family of protein kinase-C includes multiple enzymes that have specific tissue expression. PKCs can be divided into three main groups: (1) the calcium-sensitive isoforms (PKCα, PKCβ1, PKCβ2, and PKCγ), (2) the calcium-independent isoforms (PKCδ, PKCϵ, PKCη, and PKCθ), and (3) the atypical PKCs (PKCζ, and PKCλ), which are neither activated by calcium nor diacylglycerol. Activated by diacylglycerol and calcium ions, PKCs are involved in signaling initiated by certain hormones, growth factors, and neurotransmitters. Diacylglycerol causes PKC translocation from the cytosol to the plasmalemma, where it binds calcium ions and plasmalemmal phosphatidylserine. These events relieve PKC autoinhibition. The phosphorylation of various PKC substrates leads to either increased or decreased activity.[28] PKC also moves to the cytoskeleton, perinuclear sites, and the nucleus.

[26] NDR1 is also known as serine/threonine kinase-38 (STK38), and NDR2 as STK38L.

[27] Protein kinase-B is also named Akt.

[28] Phosphorylation of the epidermal growth factor receptor by PKC prevents EGFR activity.

Diacylglycerol Receptors and Kinases

There are many diacylglycerol receptors (DAGR), protein kinase-C (PKC) and -D[29] (PKD), chimaerins,[30] and others. The receptors for activated C-kinase (RACK) bind to and position newly activated PKCs to discrete cellular locations. The peptides associated with RACK–PKC interaction acts as translocation activators or inhibitors. PKCδ and PKCϵ are implicated in the evolution of the cardiac function after myocardial infarction [358]. PKCδ and PKCϵ are also implicated in vasculogenesis. PKCα and PKCϵ control integrin signaling to extracellular signal-regulated protein kinase [359]. Protein serine/threonine kinases-D (PKD) include PKD1/PKCμ, PKD2 and PKD3/PKCν. They are located in the cytosol, nucleus, Golgi complex, and plasmalemma.

Diacylglycerol kinases[31] phosphorylate diacylglycerol to stop its signaling and produce phosphatidic acid. They then modulate PKC signaling because protein kinases-C are activated by diacylglycerol, and Ras signaling, as well as the protein recruitment to membrane domains.

4.3.2.3 AMP-Activated Protein Kinases

AMP-activated protein kinase (AMPK), a serine/threonine protein kinase, is a heterotrimeric αβγ molecule with a catalytic subunit-α and two regulatory subunits, β and γ. The γ-subunit contains two binding domains for regulatory nucleotides (AMP, and ATP) in a mutually exclusive manner. The AMPK activity is modulated by AMP-to-ATP ratio and kinases. AMP-activated protein kinase is activated by leptin and adiponectin.

Under normal conditions, the intracellular concentration of adenosine triphosphate is on the order of 1 millimolar. AMP-activated protein kinase belongs to the main enzyme set that regulates ATP levels, discriminating between the mono- and triphosphate compounds. ATP and AMP competitively bind to the same AMPK site. AMPK then senses the relative ratio of ATP to AMP and controls the cell metabolism according to energy availability.

Activated AMPK promotes ATP-producing catabolisms (fatty acid oxidation, glucose uptake, and glycolysis) and impedes ATP-consuming metabolisms (synthesis of fatty acid, cholesterol, glycogen, and proteins). AMPK is phosphorylated (activated) by tumor suppressor LKB1. Serine/threonine protein kinase LKB1 and AMPK act as cellular energy sensors that regulate cell metabolism, and hence, cell fate. The LKB1–AMPK pathway particularly

[29] PKD function is determined by its intracellular location and cell context [356]. The release of reactive oxygen species from the mitochondria activates PKD.

[30] The chimaerins have a DAG-binding C1 domain and a GTPase-activating protein (GAP) domain that targets Rho GTPases. They block Rac signaling, cell proliferation and cytoskeletal reorganization [357].

[31] Diacylglycerol kinases are classified into five subtypes according to their structure.

phosphorylates acetyl coenzyme-A carboxylase (ACC), which catalyzes the rate-limiting step in lipogenesis and tumor suppressors.

AMP-activated protein kinase increases the cell sensitivity to insulin [28]. In the liver, AMP-activated protein kinase phosphorylates (inhibits): (1) acetyl-CoA carboxylase, which converts acetyl-CoA to malonyl-CoA for fatty acid synthesis in the liver; (2) 3-hydroxy-3-methylglutaryl-CoA reductase, thus decreasing cholesterol synthesis in the liver; and (3) CREB-regulated transcription coactivator-2 (TORC2), which activates gluconeogenesis and upregulates expression of insulin receptor substrate-2, a mediator of insulin signaling. At the opposite, in muscles, activated AMPK promotes fatty acid oxidation and activates PGC1α promoter, thus increasing metabolism.

AMP-activated protein kinase regulates tight junction assembly and disassembly [360]. AMPK is phosphorylated by LKB1, which controls cell polarity. LKB1, indeed, activates PAR1 kinases implicated in cell polarity and microtubule affinity regulating kinases (MARK). LKB1 phosphorylates AMPK during tight junction assembly stimulated by calcium. Moreover, AMPK enhances the stability of tight junction from disassembly induced by calcium depletion. AMPK also phosphorylates the regulatory site of the non-muscle myosin regulatory light chain involved in mitosis and cell polarity [361]. LKB1 regulation of epithelial cell polarity and mitosis is due to MRLC phosphorylation by AMPK.

4.3.2.4 Mitogen-Activated Protein Kinases

Manifold mitogen-activated protein kinases (MAPK) form the MAPK family. Mitogen-activated protein kinases are indeed targeted by kinases in the upstream part of MAPK cascades, MAPK kinase (MAPKK, MAP2K, MEK, or MKK) and MAPKK kinase (MAPKKK, MAP3K, MEKK, or MKKK; Table 4.4). MAP3Ks can be activated by either extracellular signals or by MAPKKK kinase (MAPKKKK, MAP4K, or MKKKK), which can be linked to the plasmalemma.

MAPK cascades (Fig. 4.4, Table 4.5) are composed of specific tiers of signal transmission from environment stimulations. There are up to six activation levels in the cytosol and nucleus. The pathways are characterized by a

Table 4.4. Mitogen-activated protein kinase (MAPK) pathways. Each MAPK cascade consists of main triple-decker part with a MAP kinase kinase kinase, a MAP kinase kinase and a MAP kinase. Pathway effectors differ according to the stimulus type.

Stimulus	Growth factors	Growth factors, stresses
MAP4K	Ras, Rap	Rho, Rac, Cdc42
MAP3K	Raf	ME2K, JN3K (MLK, TAK, ASK)
MAP2K	MEK	MEK
MAPK	ERK	ERK, JNK, p38

three-tiered cascade defined by MAP3Ks, MAP2Ks, and MAPKs. Activated MAPKs can translocate to the nucleus to phosphorylate a number of transcription factors, such as members of the ternary complex factor (TCF) family, which associate with serum response factor and target Fos, and components of the activator protein-1 complexes, such as Jun and activating transcription factor ATF2.

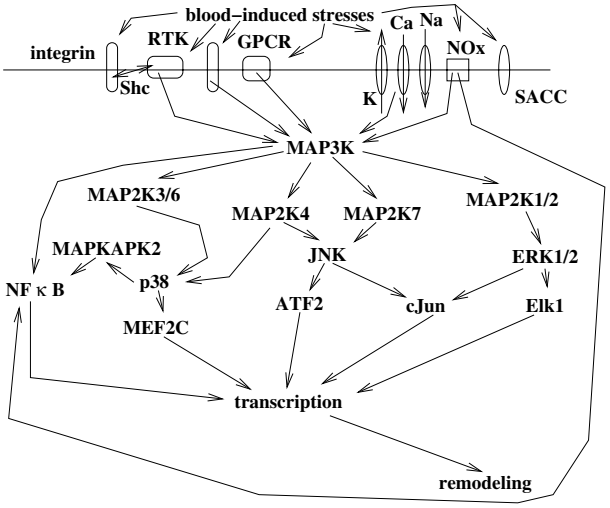

Figure 4.4. Mitogen-activated protein kinase cascades in cells of the vessel wall: a pathway with several steps including the three-tiered part with MAP3K, MAP2K, and MAPK. Mechanical stresses stimulate mediators of the MAPK cascade. Effectors of the MAPK cascade lead to gene transcription for adaptation and, if necessary when subjected to long-duration non-physiological magnitude forces, for remodeling of the vasculature wall (Source: [362]).

Table 4.5. Extracellular stimuli activate the MAPK pathways via small GTPases. Activated MAP3Ks (Raf, MAP3K, TGFβ-activated kinase TAK) phosphorylate MAP2Ks on two serine residues, which in turn phosphorylate MAPKs, extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), and p38 on both threonine and tyrosine residues. Activated MAPKs can translocate to the nucleus and phosphorylate transcription factors (ternary complex factor TCF, activator protein AP1 with JUN, and activating transcription factor ATF2). TCF forms a complex with serum response factor (SRF).

	Cascade 1	Cascade 2	Cascade 3
Tier 1	Ras	MAP3K	TAK
Tier 2	MAP2K1/2	MAP2K4/7	MAP2K3/6
Tier 3	ERK	JNK	p38
Transcription factor	SRF–TCF	SRF–TCF AP1	SRF–TCF ATF2

The set of MAP3Ks is composed of Raf, MAP3K1, MAP3K2, MAP3K3, MAP3K4, mixed lineage kinases (MLK), Mos, Tao proteins, apoptosis signal-regulating kinase ASK1, transforming growth factorβ–activated kinase TAK1, dual leucine-zipper kinase, and tumor progression locus Tpl2 [363, 364]. Regulatory domains of MAP3Ks interact with upstream regulators, such as Rho and Ras GTPases. Activated MAP3K phosphorylates (activates) one or several MAP2Ks. Many MAP3Ks also activate the IκB kinase–nuclear factor κB pathway.

Activated MAP2K phosphorylates a specific MAPK. Activated MAPK phosphorylates cytosolic and nuclear effectors. MAPK members have, indeed, many substrates: (1) other protein kinases belonging to either downstream components (MAPK activating protein kinases MAPKAPK1 and MAPKAPK2, ribosomal S6 serine/threonine kinase RSK, which phosphorylates downstream glycogen synthase kinase GSK3) or upstream elements (Raf1, and Sos); (2) enzymes (phospholipases PLA2 and PLCγ); (3) plasmalemmal proteins, such as growth factor receptors (EGFR); and (4) transcription regulators, such as the serum response factor, proto-oncogenes cFos, cMyc, cJun, and other transcription factors (ATF2, Elk1, and MEF2), as well as members of the steroid/thyroid hormone receptors. MAPKs are inhibited by MAPK phosphatases (tyrosine phosphatases and serine/threonine phosphatases).

Mitogen-activated protein kinases are linked to various subcellular organelles, such as microtubules, endosomes, the endoplasmic reticulum and the actin cytoskeleton. Active MAPKs often translocate from the cytosol to the nucleus to phosphorylate nuclear targets. MAP2Ks can carry MAPK from the cytoplasm to the nucleus and inversely.

The mitogen-activated protein kinase cascades include in their downstream part several intracellular signaling networks. The three main MPAK cascades involved in signal transduction include: (1) extracellular signal-regulated protein kinase, with its two isoforms ERK1 and ERK2, (2) c-Jun N-terminal kinase (JNK), with its three elements JNK1, JNK2 and JNK3, and (3) p38 kinase isozymes. The two other MAPK families are ERK3, ERK4, and ERK5. ERK3 is mainly regulated by autophosphorylation. ERK5,[32] activated by growth factors, is required for angiogenesis and cardiovascular development.

The family of calcium–calmodulin-dependent protein kinases which act downstream from mitogen-activated protein kinases includes: (1) the ribosomal S6-kinase group (RSK1–RSK4), (2) the mitogen- and stress-activated kinase class (MSK1–MSK2), (3) MAPK-interacting kinases (MNK1–MNK2), and (4) MAPK-activated protein kinases (MAPKAPK or MK; MK2, MK3, and MK5). RSKs are exclusively activated by extracellular signal-regulated kinases. MSKs and MNKs are downstream effectors of both ERKs and p38s. RSKs and MSKs regulate gene expression by phosphorylating Fos, as well as cAMP-responsive element–binding proteins for RSKs or histone H3 for MSKs.

[32] ERK5 is also called big MAPK1 (BMK1).

MNKs phosphorylate eukaryotic translation-initiation factor eIF4E and factors which bind to certain mRNAs, to regulate the expression of a specific set of proteins. MNKs are particularly involved in growth control and inflammation. MK2, activated by p38, is required for cytokine synthesis in inflammation and the cell cycle. MK3, also activated by p38, regulates chromatin remodeling. MK5, which particularly interacts with ERK3, is activated by ERK and p38, but not JNKs.

Autoinhibition is a functioning mode of protein kinase. As protein kinases, its catalytic domain binds ATP and substrates phosphoryl transfer. They change their activation region into an autoinhibitory module [365]. The activation region must unlock from another molecule element to bind ATP.

Raf MAP3Ks

Raf activation includes translocation to the plasmalemma, induction of a conformational change by Ras and phosphorylation. Activated Raf1 activates mitogen-activated protein kinase MAP2K, extracellular signal-regulated kinase (ERK). The kinetics (sustained or transient activation) and intensity of Raf–ERK pathway stimulation by growth factors specify the signaling type. Raf1 is a direct effector of Ras GTPase. Its activation involves plasmalemmal recruitment and binding to RasGTP. It is inactivated by protein phosphatase-5 (Sect. 3.2.4) [366].

The Ras–Raf–ERK signal transduction pathway controls multiple processes, such as proliferation, differentiation, senescence, and apoptosis, depending on the duration and strength of the external stimulus and the cell type. The Ras–Raf–ERK pathway is activated in most human tumors, especially tumor cells with BRAF[33] or Ras gene mutations. When the MAPK cascade is inhibited, tumor growth is completely hindered in BRAF mutant and only partially in Ras mutant tumors [367].

The Ras-Raf-PI3K signaling pathway leading to protein kinase-B can act either synergistically with or in opposition to the Ras–Raf–ERK pathway. PKB phosphorylates Raf, which then can inhibit activation of the Ras–Raf–ERK pathway. Cross-action between the Ras–Raf–ERK and the Ras–Raf–PI3K–PKB pathways modulates cell life modes [368] (Sect. 4.3.2.2).

Activated Raf kinase inhibitor protein (RKIP),[34], a serine/threonine kinase, binds to and inhibits Raf1 kinase [369]. PKC-phosphorylated (active)

[33] BRAF is a protein implicated in the growth and survival of cancer cells. It is mutated in the majority of melanoma and a minority of colon, breast, and lung cancers.

[34] RKIP, or phosphatidylethanolamine-binding protein-2, disrupts the Raf1–ERK1/2 and nuclear factor-κB pathways, interacting with associated kinases. RKIP binds to several proteins that activate NFκB (transforming growth factor β-activated kinase-1, IκB kinase-α and -β, and NFκB-inducing kinase), which, after degradation of IκB, translocate to the nucleus to bind to target gene promoters.

RKIP inhibits G-protein-coupled receptor kinase-2. Ser/Thr GRK2 downregulates various G-protein-coupled receptors, particularly those hampering cell locomotion. Moreover, RKIP does not inhibit Raf1, which can then phosphorylates MAPK and ERK. RKIP indeed binds to locostatin and is then unable to inhibit Raf1 kinase, hindering cell migration. RKIP thus performs anticancer actions, particularly decreasing angiogenesis and vascular invasion.

Extracellular Signal-Regulated Kinases

Extracellular signal-regulated kinases ERK1 and ERK2, activated by growth factors, regulate proliferation and differentiation. ERKs are expressed in many tissues and belong to the cascade with Raf MAP3Ks (A-Raf, B-Raf, and C-Raf/Raf1) and MAP2K1/2. Activators bind to receptor tyrosine kinases or G-protein-coupled receptors. ERK pathway activation can be due to stimulation of plasmalemmal proteins Ras via receptor tyrosine kinases in association with adapter Grb2 and guanine nucleotide-exchange factor Sos. Activation of Ras by Son-of-sevenless triggers Raf stimulation, Raf recruitment to the plasmalemma, phosphorylation (activation) of MAP2Ks, and then activation of ERK1 and ERK2. ERK1 and ERK2 have many targets, such as transcription factors (NFκB, cJun, Elk1), kinases (RSK, MAPKAPK2), plasmalemmal receptors (EGFR), upstream tier activators (Raf1, Sos), and paxillin.

PKC can activate Raf1, and hence has a role in MAP4K. ERK1/2[35] shear stress–dependent[36] activation requires PKCϵ [370].[37] Raf1, a ERK1/2 activator, is recruited to the plasmalemma, and activated by Ras, possibly phosphorylated by cSrc on the one hand and by diacylglycerol-regulated PKCα and PKCϵ on the other hand [371].

Extracellular signal-regulated kinase-5, like ERK1/2, is activated by growth factors, especially during the development of the cardiovascular system. ERK5 is required in endothelial cells. MAP3K2 and MAP3K3 activate MAP2K5, a specific kinase for ERK5. The ERK5 signaling pathway leads to ERK5 translocation into the cell nucleus. Activated ERK5 also phosphorylates substrates, such as the myocyte enhancer factor-2 [372]. ERK5 is activated by growth factors as well as oxidative stress and hyperosmolarity. ERK5, as well as ERK1/2, stimulates proto-oncogenes, such as cFos and cJun. ERK5 promotes the expression of cyclin D1, which regulates G1/S transition of the cell cycle, implicating a cAMP response element.

[35] ERK1/2 refers to both ERK1 and ERK2, which are activated by MAP2K1 and MAP2K2.

[36] Wall shear stress applied on endothelial cells activates extracellular signal-regulated kinase isoforms ERK1 and ERK2 in endothelial cells in a time- and force-dependent manner.

[37] PKCϵ seems to be specific for shear ERK1/2 activation, PKCα and PKCζ having no effect on ERK1/2 activity.

Protein kinases contains a site aimed at selectivity binding nucleotides and inhibitors. Inhibitors prevent autophosphorylation, and thus dysregulate signaling. A gatekeeper residue in ERK2 impedes autoactivation [373].[38]

c-Jun N-Terminal Kinases

The family of c-Jun N-terminal kinase regulates stress responses, neural development, inflammation, and apoptosis. JNK1 and JNK2 are expressed in many tissues. JNK3 is a specific compound of the brain. Many involved MAP3Ks are activated by Rho GTPases. MAP3Ks activate MAP2K4 and MAP2K7. JNKs are regulated by multiple scaffolds (JIP1–JIP4, β-arrestin-2, filamin, and Crk2) for kinase activation and/or substrate selection.

p38 Mitogen-Activated Protein Kinases

p38 MAPKs are activated by numerous factors: hormones, UV beams, ischemia, cytokines (interleukin-1, and tumor necrosis factor), osmotic pressure changes and heat stresses.[39] Four p38 isoforms (α,[40] β, γ,[41] and δ[42]) act in a cascade that involves MAP3Ks (MAP3K1–MAP3K4, TAK1, ASK1, MLK2–MLK3, DLK, Tpl2, and Tao1–Tao2). These MAP3Ks activate MAP2K3, MAP2K4, and MAP2K6, which phosphorylate p38 [374]. In the classic pathway (MAP3K–MAP2K cascade), p38 is phosphorylated (activated), downstream of MAP2K3 and MAP2K6. However, in some cells, such as activated T cells, an alternative pathway for p38 activation exists. p38 MAPK is phosphorylated by Zap70, itself activated by Src kinases p56lck (MAPK-independent p38-activation) [375]. p38 MAPKs are linked to scaffold proteins (JIP2, JIP4, and OSM protein).[43]

Stress-Activated Mitogen-Activated Protein Kinases

Stress-activated[44] MAPKs (SAPK) include JNKs and p38s activated by MAP3K1–MAP2K4/7 and TAK1–MAP2K3/6 cascades, respectively. ASK1–MAP3K5 stimulates both JNK and p38. Activated JNK and p38 induce gene expression vie different transcription factors, such as cJun, ATK2, Elk1, and MEF2.

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[38] ERK2, activated by phosphorylations on two residues due to MAP2K1/2, changes its conformation and phosphorylates cytoplasmic and nuclear proteins.
[39] p38 MAPKs are implicated in asthma and autoimmunity.
[40] p38α MAPK, also called CSBP1, is required for angiogenesis and erythropoietin synthesis.
[41] p38γ MAPK is also termed ERK6 or SAPK3.
[42] p38δ MAPK is also named SAPK4.
[43] OSM protein interacts with the actin cytoskeleton.
[44] Various stresses (chemical, physical, and metabolic) are involved.

SAPKs can be more or less implicated in cardiac remodeling associated with overload or ischemia/reperfusion injury, modulating signaling pathways associated with cardiac hypertrophy and cell apoptosis [376]. p38 MAPK can promote remodeling with interstitial fibrosis, leading to a loss in contractibility, but without significant hypertrophy [377]. Cells can withstand relatively high mechanical stresses owing to their dynamic cytoskeleton. p38 Kinases (but not ERK or JNK) are responsible for stretch-induced activation of an eicosanoid pathway that involves E-prostanoid receptor EP4 and cyclooxygenase COx2 (but not EP1 and COx1) via MAPKAPK2 [378]. However, prostaglandin PGE2 induces actin filament depolymerization in cells stretched during a long time (6 hours, 5% elongation, 0.5 Hz).

Downstream of mitogen-activated protein kinase pathways, MAPK-activated protein kinases regulate gene expression at the transcriptional and post-transcriptional levels and control the cell cycle. Moreover, MAPKAPKs are involved in actin remodeling, and consequently, in cell shape and cell motility [379]. Hsp27, an inhibitor of Rho-dependent (stress) actin fiber formation, competes with cofilin for binding to the 14-3-3-protein. Increased release of cofilin from the 14-3-3 protein is followed by cofilin dephosphorylation (inactivation) and actin binding. Rho activation causes phosphorylation of cofilin by LIM kinase and its release from the barbed ends of the actin filaments.

4.3.2.5 Rho-Kinases

Rho-kinase is a main effector of small GTPase Rho, which is implicated in cell adhesion, cell motility, cell growth, cell contraction, and cytokinesis. Rho-kinases are activated by autophosphorylation or RhoA (Sect. 4.5.3). Rho-kinase activation, which depends on integrins, induces interaction with Rho and rho-kinase translocation to the plasmalemma. Rho-kinases enhance the formation of focal adhesions and the assembly of actin stress fibers. Moreover, rho-kinases bind with and phosphorylate (inhibit) myosin light chain phosphatase (MLCP) which dephosphorylates the regulatory light chain of myosin-2 (Fig. 4.5). Hence, rho-kinases regulate the strength and the speed of actin–myosin crossbridging in smooth muscle and non-muscle cells.

There are two rho-kinase isoforms: RoK1 (RoKβ) and RoK2 (RoKα). RoK2 is the main type in muscles and brain. Rho-kinase-2 phosphorylates (inhibits) myosin light chain phosphatase, and hence, increases myosin-2 activity even at a constant intracellular calcium level (*calcium sensitization*), and ensures sustained vasoconstriction. RoK inhibitors suppress coronary artery spasms and bronchospasms.

Activated rho-kinases by lysophosphatidic acid[45] reduce the peak current amplitude of T-type calcium channels Cav3.1 and Cav3.3 without affecting the voltage dependence of activation and inactivation [380]. They induce depolarizing shifts for activation and inactivation in Cav3.2 channels.

4.4 Protein Phosphatases

Protein phosphatases: (1) dephosphorylate phosphoserine (pSer) and phosphothreonine (pThr) in the cell, or (2) dephosphorylate phosphotyrosine (pTyr), either exclusively or with pThr, at the plasmalemma or in the cytoplasma. Both tyrosine and serine/threonine dephosphorylations are associated with cell growth, proliferation and differentiation, and turning off proliferation signaling. Protein phosphatases mainly act on proteins, but certain protein phosphatases are able to remove phosphate from either phospholipids or mRNA. Inositide phosphatases (SHIP) and synaptojanins dephosphorylate inositol phospholipids. The SH2-containing inositol 5-phosphatase SHIP1 binds

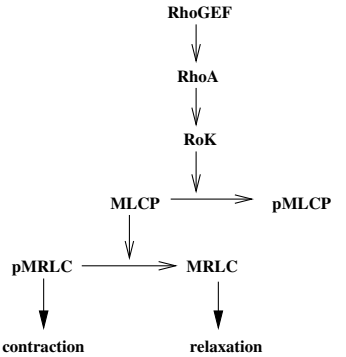

Figure 4.5. Rho-kinase and protein phosphatase regulate the activity of myosin-2, the nanomotor in smooth muscle and non-muscle cells. Myosin-2 is regulated by phosphorylation and dephosphorylation of its myosin regulatory light chain (MRLC) by Ca^{++}–calmodulin-regulated myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP). Phosphorylated MRLC (pMRLC) activates actin–myosin-2 binding. Myosin light chain phosphatase dephosphorylates MRLC, thus inducing relaxation. Activated small GTPase RhoA stimulates rho-kinase, which phosphorylates MLCP (pMLCP), thereby inhibiting MLCP activity and increasing MRLC phosphorylation, favoring contraction. The Rho–rho-kinase pathway contributes to the tonic phase of contraction in smooth muscle cells.

[45] Lysophosphatidic acid (LPA) activates five different G-protein-coupled receptors coupled to Gαq (LPA1–LPA5), which activates phospholipase-C and protein kinase-C; Gαi (LPA1–LPA3), which inhibits protein kinase-A; Gαs (LPA4), which stimulates PKA; or Gα12/13 (LPA1, LPA2, LPA4, and LPA5) which activates RhoA.

to tyrosine residues of numerous substrates. SHIP1 is involved in cytoskeleton rearrangement for cell mobilization.

Protein tyrosine phosphatases (PTP) and protein tyrosine kinases (PTK) act in partnership to coordinate their activities in the control of cell signaling. Kinase–phosphatase cascades are characterized by protein–phosphoprotein cycles that allow negative and positive controls and negative and positive feedback of a signaling pathway. The pathway from the platelet-derived growth factor receptor (PDGFR, Sect. 10.1) to mitogen-activated protein kinases ERK1/2, which involves Ras, Raf, and MAP2K, gives an example of kinase–phosphatase control of signaling (Fig. 4.6).

4.4.1 Protein Serine/Threonine Phosphatases

The serine/threonine protein phosphatases include five known groups: PP1 (α, β, $\gamma 1$, and $\gamma 2$), PP2A, PP3 (PP2B), PP4, and PP5. In general, protein

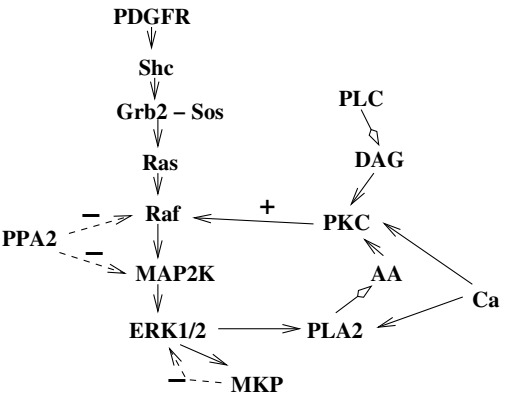

Figure 4.6. Kinase–phosphatase cascade induced by stimulation of the platelet-derived growth factor receptor (PDGFR) associated with a balance between phosphorylation (activation) and dephosphorylation (inhibition). MKP is a flexibility node of the pathway, modulating the cascade effect (Source: [342]). Control of the Ras–ERK cascade needs the plasmalemmal recruitment of proteins serving as adapters, anchors, and scaffolds (Grb, and Shc), and as regulators (Sos, and RasGAP). These complexes can be activated at the plasmalemma or in the endosomes, where they are able to recruit signaling molecules to trigger biological responses. ERK1/2 activates cytoplasmic phospholipase-A2 (PLA2), which produces arachidonic acid (AA). The latter stimulates protein kinase-C (PKC) via diacylglycerol (DAG). PKC in turn activates Raf (positive-feedback loop). Receptor tyrosine kinases activate other effectors such as phospholipase Cγ, which stimulates PKC. Protein phosphatase 2A (PP2A) dephosphorylates both Raf and mitogen-activated protein kinase kinase (MAP2K). Mitogen-activated protein kinase phosphatase (MKP) dephosphorylates ERK1/2. MAPK phosphorylates MKP, reducing MKP degradation (negative feedback).

phosphatases have less substrate specificity than protein kinases. Among Ser/Thr phosphatases, chronophin regulates cofilin phosphorylation.

Protein phosphatase-1 (PP1) regulates the glycogen metabolism. The diverse forms of protein phosphatase-1 result from the association of a catalytic subunit (PP1c) and different regulatory subunits. These regulatory subunits allow the targeting of various subcellular locations, and modulate binding of stimulators and effectors. Protein phosphatase-1 contains binding domains for hepatic and muscle glycogen and smooth muscle myosin, which target the catalytic subunit to these substrates [381]. The hepatic glycogen-binding domain has two binding subdomains, one that suppresses the dephosphorylation of glycogen phosphorylase, another that enhances the dephosphorylation of glycogen synthase. The PP1c target depends on the interaction between the regulatory subunits and catalytic subunit that form the PP1 complex. Protein phosphatases PP1 and PP2A account for more than 90% of all Ser/Thr phosphatase activities in most cells.

Heterotrimeric protein phosphatase-2A (PP2A) is also a strongly regulated phosphatase that fulfills numerous functions. Protein phosphatase-2A is composed of catalytic (PP2Ac), scaffold (structural), and regulatory subunits. PP2A is regulated by one regulatory subunit among the set of regulatory subunits (classified into B/B55, B'/B56, and B" subsets), which control PP2A substrate specificity, cellular location, and enzymatic activity. Protein phosphatase-2A can both positively and negatively influence the Ras–Raf–MAP2K–ERK signaling pathway (Fig. 4.6). Moreover, PP2A regulates G2–M transition of the cell cycle. PP2A is required to keep the Cdc2–cyclin B complex[46] in its inactive precursor form. PP2A inhibits complete Cdc25 phosphorylation (activation). It is also positively implicated in the exit from mitosis.

Calcineurin (PP3 or PP2B) activates the transcription of interleukin-2, which stimulates growth and differentiation of T lymphocytes when the intracellular concentration of calcium rises. Calcineurin is activated by the Ca^{++}–calmodulin complex. The Ca^{++}–calmodulin complex activates PP2B by binding to its catalytic subunit and displacing the inhibitory regulatory subunit.

Protein phosphatase-4 is involved in microtubule organization at centrosomes during mitosis and meiosis. PP4 contains a regulatory subunit that interacts with the catalytic subunit [382]. The regulatory subunit target the centrosomal microtubule organizing centers.

Protein phosphatase-5 is located in the cytoplasm, centrosomes and microtubules, or the nucleus. PP5, provided with autoinhibition from its targeting and regulatory domain, acts in microtubule organization and controls the cell cycle. PP5 regulates steroid receptor signaling [383]. PP5 can be activated in vitro by arachidonic acid.

[46] The complex formed by Cdc2 and cyclin B prevents genome re-replication before mitosis. The tyrosine phosphatase Cdc25 dephosphorylates (activates) Cdc2–cyclin B.

4.4.2 Protein Tyrosine Phosphatases

Classical protein tyrosine phosphatases can be classified as receptor-like transmembrane enzymes (rPTP) and cytoplasmic (cPTP) proteins. PTPs can dephosphorylate (inactivate) receptor tyrosine kinases (RTK), such as Eph receptors [384]. A second set of PTPs includes dual specificity phosphatases[47] which catalyze MAPK dephosphorylation (inactivation), such as cell-cycle regulatory phosphatase Cdc25 and tumor-suppressor phosphatase PTEN.

Plasmalemmal phosphatase and tensin homolog PTEN[48] can act as cytoplasmic tumor suppressor and phosphoinositide phosphatase that restricts the activity of phosphatidylinositol 3-kinase by dephosphorylating phosphatidylinositol(3,4,5)trisphosphate (PIP3) to phosphatidylinositol(4,5)bisphosphate. PTEN also dephosphorylates tyrosine-phosphorylated focal adhesion kinases. Phosphatidylinositol 3-kinase interacts with FAK in PKB phosphorylation. Protein phosphatase-1δ is also located in focal adhesions and associates with focal adhesion kinases. PTEN interacts with adapter NHERF and platelet-derived growth factor receptors [385]. Polyubiquination degrades PTEN, whereas nuclear monoubiquitylated stabilizes PTEN. Ubiquination also regulates PTEN stability and its nuclear location, whereas PTEN favors chromosome integrity [386]. PTEN acts on chromatin and regulates expression of Rad51, that reduces the incidence of spontaneous DNA double-strand breaks.

The prototypic tyrosine phosphatase is the plasmalemmal regulator CD45 in leukocytes, nucleated hematopoietic cells and their precursors. Cluster determinant protein CD45 is involved in differentiation of hematopoietic cell lineages and immunity. CD45 positively or negatively regulates Src kinases according to the cell type and context. CD45 also functions as a Janus kinase that negatively controls the cytokine-receptor signaling. After stimulation, cytokine receptors JaK1, JaK2, JaK3, and Tyk2 are phosphorylated. Activated JaKs can phosphorylate transcriptional activators STATs for translocation to the nucleus.

Table 4.6. Examples of signaling substances targeted by some protein tyrosine phosphatases (SHP: Src homology protein tyrosine phosphatase; PTEN: tumor-suppressor phosphatase (phosphatase and tensin homolog deleted on chromosome 10, an antagonist of the PI3K–PKB pathway); MKP: mitogen-activated protein kinase phosphatase; Source: [384]).

Tyrosine phosphatase	Signaling molecules
SHP2	PDGF, endothelin-1
PTEN	PDGF, EGF
MKP (JNK phosphatase)	TNFα

[47] MAPK phosphatases act on both tyrosine and threonine.

[48] PTEN stands for phosphatase and tensin homolog deleted on chromosome 10. It is also called MMAC1 or TEP1.

4.4.3 Dual-Specificity Protein Phosphatases

Dual-specificity mitogen-activated protein kinase phosphatases dephosphorylate (inactivate) both phosphothreonine and phosphotyrosine on activated MAPKs (Sect. 4.3.2.4; Table 4.7). Certain MKPs are inducible, others are stabilized or destabilized by phosphorylation, others display catalytic activation. MKP1, MKP2, MKP3, and MKP7 are regulated by phosphorylation. ERK phosphorylation stabilizes MKP1 and increases its half-life, whereas it elicits MKP3 degradation [387]. However, the interaction of MKP3 with its specific substrate ERK2 enhances MKP3 activity.

Mitogen-activated protein kinase phosphatases are classified into two sets according to intracellular location and transcriptional regulation. The first set is primarily located in the nucleus and encoded by immediate-early genes. It includes MKP1, MKP2, DUSP2, and HVH3. The expression of the nuclear MKPs is induced shortly after cell stimulation by growth factors and stresses. The second set is located either primarily in the cytoplasm or in both the cytosol and nucleus. They are characterized by slower kinetics. Mitogen-activated protein kinase phosphatases, which are specific for MAPKs, could also serve as anchors for MAPKs and control their intracellular location.

4.5 Guanosine Triphosphatases

The receptor activation leads to biochemical events that can involve guanosine triphosphatases. These proteins cycle between two conformations induced by the hydrolytic activation–deactivation cycle, binding either guanosine diphosphate (GDP) or guanosine triphosphate (GTP). These protein switches are flicked off (inactive GDP-bound state) and on (active GTP-bound state; Fig. 4.9). This cycle is regulated to avoid pathological states. Once activated, the switch activates the pathway effector immediately downstream from it.

Table 4.7. Types of mitogen-activated protein kinase phosphatases and their specific substrate with order of potency (Source: [387]).

MKP	Substrate (potency order)
MKP1	ERK < p38 ∼ JNK
MKP2	p38 < ERK ∼ JNK
MKP3	p38 ∼ JNK < ERK
MKP4	JNK < p38 < ERK
MKP5	ERK < p38 ∼ JNK
MKP7	ERK < p38 ∼ JNK
DUSP2	JNK < p38 ∼ ERK
HVH3	ERK
HVH5	ERK < p38 ∼ JNK

The two major kinds of GTPases include: (1) the large guanine nucleotide–binding proteins, the *G proteins*, and (2) the monomeric *small guanosine triphosphatases* (small GTPases).

4.5.1 G Proteins

Heterotrimeric guanine nucleotide–binding proteins (G protein) are transducers of various extracellular signals (hormones, neurotransmitters, chemokines, photons, odorants, tastants, nucleotides, and ions). G-protein heterotrimers are attached to the inner leaflet of the plasmalemma. G proteins regulate the production or influx of second messengers, such as cAMP and calcium. The activation of G proteins is induced by ligand-bound G-protein-coupled receptors[49] (Fig. 4.7).

The G-protein heterotrimer is composed of a GDP-bound Gα-subunit and a GβGγ-dimer (Fig. 4.8). There are many isoforms of each of the three subunits. Genes for 16 Gα-subunits, grouped in four main classes, 5 Gβ-subunits, and 11 Gγ-subunits, are identified, with possible splice variants[50] or post-translational modifications [388].[51] A single receptor can stimulate a single G protein (linear signaling). Some receptors promiscuously interact with G proteins, whereas others specifically interact. A given G protein can be regulated by several receptors, either positively or negatively (convergent receptor signaling). A given receptor can activate several G proteins (divergent receptor signaling), such as the α-adrenergic receptor targeting both Gs and Gi.

The activated G protein is dissociated into GTP-bound Gα and GβGγ (or Gβγ; Fig. 4.8). The isolated GTP-bound Gα acts on a second messenger. However, although certain G-protein heterotrimers dissociate after receptor activation in vivo, other G-protein heterotrimers undergo simple rearrangements, then possibly modulating G-protein effector activity [389].

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[49] Each member of the GPCR family has a similar structure with seven transmembrane helices, an extracellular amino-terminus, an intracellular carboxy-terminus, and three interhelical loops on each side of the plasmalemma. G-protein-coupled receptors form both homo- and heterodimers. When a single element of the dimer bind to its ligand, the dimer cannot fully activate the G protein. Besides, when two receptors are dimerized to form a signaling unit, the signal can differ from the response given by either receptor alone. Furthermore, the heterodimerization of a GPCR with another one affect the functioning of the bound GPCR, modulating its activity.

[50] Splicing is a modification of genetic information prior to translation.

[51] There are several distinct subunit genes [388]: (1) a Gαi subset with Gαi1, Gαi2, Gαi3, Gαo1, Gαo2, Gαt1, Gαt2, Gαgust, and Gαz; (2) a Gαq subset with Gα11, Gαq, Gα14, Gα15, and Gα16; (3) a Gα12 subset with Gα12 and Gα13; (4) a Gαs subset with Gαs and Gαolf; (5) a Gβ subset from Gβ1 to Gβ5; and (6) a Gγ subset with farnesylated isoforms Gγ1, Gγ11, and Gγ8cone, and with geranylgeranylated isoforms Gγ2, Gγ3, Gγ4, Gγ5, Gγ7, Gγ8olf, Gγ10, and Gγ12.

Once the target has been stimulated, Gα fills its GTPase activity. When GTP is hydrolyzed, Gα returns to the GDP-bound conformation, dissociates from its target, and reassembles with Gβγ. The duration of G-protein activation is thus controlled by the intrinsic GTPase activity of Gα. Subclasses of Gα subunits include: (1) Gs, which stimulates adenylyl cyclase and activate calcium channels; (2) Gi, which inhibits adenylyl cyclase and potassium channels; and (3) Gq, which activates phospholipase-C, and protein kinases-C and -D. Gq/11 stimulates the mitogen-activated protein kinase cascade. G12/13 activates Rho GTPases. In dual signaling pathways activated by a single G protein, both activated Gα and Gβγ stimulate effectors. In other pathways, the major regulator is Gβγ, the activity of which can be suppressed by excess Gα liberated by other activated G proteins.

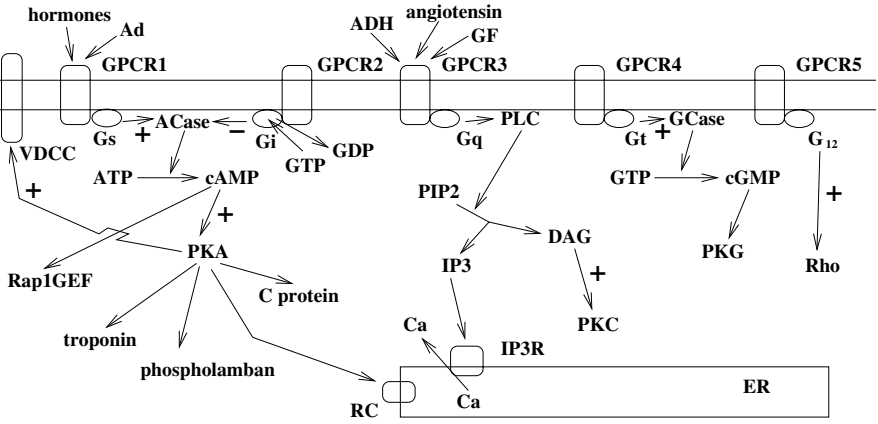

Figure 4.7. G-protein-coupled receptors (GPCR) are associated with three G-protein subunits: Gα, which binds GDP (inactive state) or GTP, after ligand binding and stimulation, Gβ, and Gγ. Activated Gα activates an effector. Several types of Gα include Gαs (stimulatory), Gαq, Gαi (inhibitory), Gαt, and Gα12. Gαs stimulates adenylyl cyclase (ACase), which produces a second messenger, cAMP. Gαq-linked G-protein-coupled receptor activates phospholipase C (PLC) which generates second messengers, inositol trisphosphate (IP3), and diacylglycerol (DAG). Gαi inhibits adenylyl cyclase. Gαt stimulates guanylyl cyclase (GCase), which forms cGMP. Gα12 activates RhoA GTP-binding proteins. Protein kinase-A (PKA) regulates myocardial contraction, acting on contractile proteins, activating ion channels, such as ryanodine receptors (RR) of the endoplasmic reticulum (ER) and voltage-dependent calcium channels (VDCC) and sodium channels of the plasmalemma (GβGγ subunit inhibits voltage-gated calcium channels VDCC), and carriers. PKA also increases flow of metabolic energy, stimulating phosphorylase kinases and hormone-sensitive lipases, and activates gene transcription. cAMP exert direct effects on other effectors, such as Rap1GEF. IP3 binds to its endoplasmic reticulum receptor IP3R and causes release of stored Ca^{++} which rises transiently in the cytosol. PLC also activates plasmalemma Ca^{++} influx channels, the transient receptor potential (TRP) Ca^{++} channels (TRPC) (adapted from [238]).

4.5.2 Regulators of G-Protein Signaling

The interactions of GPCR, G protein, and effectors also involve the fine-tuned regulators of G-protein signaling (RGS) proteins, which accelerate GTP hydrolysis. RGS act as GTPase-activating protein (Fig. 4.8). They are negative regulators of the signal transduction mediated by Gi/o and Gq/11. The family of small RGS proteins is composed of different members, encoded by different genes of the cells of the vascular walls, which share a RGS homology domain. However, their specific activities in GPCR signaling is associated with another structural domain. The smaller RGS proteins likely function as GαGAPs, whereas the larger RGS proteins and RGS-like proteins are Gα effectors. Moreover, certain RGS proteins are involved in the assembly of signaling complexes.

Cardiac RGSs hinder phospholipase-C activity via Gq/11, especially phospholipase-C stimulation of endothelin-1[52] (Sect. 9.5.4). Hence, RGS4 has an antihypertrophic effect. Furthermore, cardiac RGSs regulates the activation and deactivation kinetics of Gβγ-gated K^+ channels, thus the inward rectifier K^+ channel regulated by acetylcholine via Gi/o (RGSs accelerate GTP hydrolysis rate of Gαi/o and regulate ACh-dependent relaxation).[53]

The membrane-attached RGS3, a Gβγ-binding protein [390] strongly expressed in the heart [391], attenuates signaling not only via Gαi and Gαq/11, but also via Gβγ-mediated signaling (through phospholipase-C, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase). RGS6 is relatively abundant in atrial myocytes [392]. RGS-PX1, a Gαs-specific GAP, inhibits adenylyl cyclase stimulation induced by the complex α-adrenoceptor–

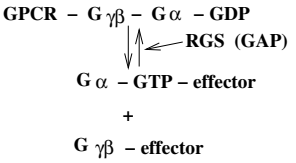

Figure 4.8. G-protein, its components, its activation–desactivation, and regulators of G-protein signaling proteins (RGS).

[52] Endothelin-1 has positive inotropic effects and stimulates heart wall growth via Gq activation.

[53] Binding of ACh to cardiac muscarinic M2-receptors enables GTP to replace GDP at the Gα-subunit of Gi/o-proteins. The subunits dissociate and the Gβγ-subunit activates K^+ channels. Phosphatidylinositol(1,4,5)trisphosphate inhibits RGS activity on Gαi/o-subunits. The rising intracellular Ca^{++} concentration leads to the formation of Ca^{++}–calmodulin complexes. Ca^{++}–calmodulin binds to RGS, then inhibits the effect of phosphatidylinositol trisphosphate and activates RGS. Consequently, K^+ channels are inactivated. With decaying intracellular Ca^{++} concentration, Ca^{++}–calmodulin dissociates and phosphatidylinositol-trisphosphate inhibits RGS again.

Gαs. RGS-PX1 might then modulate the activity of cardiac Ca^{++} channels. RGS-PX1 binds to membranous phosphatidylinositol 3-phosphate, thus participating to early endosomes. RhoGEFs have Dbl homology (DH) and pleckstrin homology (PH) domains. The DH domain is responsible for exchange activity and the PH one is likely involved in subcellular localisation. RGS-like RhoGEFs act on Gα12 and Gα13 [390]. GPCRs coupling to G12/13 in cardiomyocytes is associated with contractility [247], and protein kinase-C–mediated activation of sarcolemmal Na^+/H^+ exchangers [393]. Another RGS-like protein family, GPCR kinases (GRK), phosphorylate activated GPCRs. GRK2, highly expressed in the human heart (as well as GRK5 and GRK6), interacts with Gq/11 (GRK2 sequesters activated Gαq).

The activity of RGS proteins is regulated within a cell. Phosphatidylinositol(1,4,5)trisphosphate inhibits RGS proteins [394]. Ca^{++}–calmodulin restores the GAP activity. Both PIP3 and Ca^{++}–calmodulin bind to the same RGS site. Inhibition and disinhibition of GAP activity of RGS4 by these molecules explain oscillations in the intracellular calcium concentration.[54] Furthermore, RGS proteins are targeted by different protein kinases.[55]

RGS proteins are implicated in interactions involving various signaling molecules associated with G-protein signaling pathways and ion carriers at the plasmalemma, such as Ca^{++}, phospholipids (especially phosphoinositides), and tyrosine kinases.[56] Besides, lipopolysaccharides and angiotensin-2 increase the expression of RGS proteins in vascular cells.

4.5.3 Small GTPases

The small Rho GTPases act in various cell activities, which require changes in the cell cytoskeleton, such as its polarity, shape, adhesion, motion, division, and differentiation.

During cell migration, Rho GTPases drive the membrane protrusion at the leading edge and the contractility of the cell body. Rho GTPases indeed regulate the assembly of filamentous actin (F-actin) in response to signaling. Their effectors induce the assembly of contractile actin–myosin filaments (stress fibers in particular) and integrin-containing focal adhesions. Consequently, the small Rho GTPases act in vascular processes, such as smooth muscle cell contraction, cell adhesion, endothelial permeability (Sect. 9.2),

[54] At low $[Ca^{++}]_i$, GAP activity of RGS4 is hampered by PIP3. Hence, Gq/11 stimulates phospholipase-C, which rise $[Ca^{++}]_i$. Ca^{++} thereby binds to calmodulin. Ca^{++}–calmodulin reduces Gq/11 activation and $[Ca^{++}]_i$ decays. When Ca^{++}–calmodulin complex is dissociated, the PIP3 inhibition of RGS4 is restored.

[55] Protein kinase-C phosphorylates RGS2, decreasing its Gq/11 inhibition. cGMP-dependent protein kinase phosphorylates RGS4, inducing its translocation to the plasmalemma. Protein kinase-A phosphorylates RGS9, inhibiting the GAP activity on Gt.

[56] Receptor tyrosine kinases regulate phosphoinositide activity.

leukocyte extravasation (Sect. 9.3), platelet activation (Sect. 9.4), and migration of smooth muscle cells (SMC) and endothelial cells (EC) involved in angiogenesis (Sect. 10.4.2) and wall remodeling (Sect. 10.7.2) [395]. They are also required in vascular disorders associated with pathological remodeling and altered cell contractility.

The *Rho kinase* (RoK), an effector of the small Rho GTPase, is involved in atherosclerosis as well as in post-stenting restenosis. The phosphorylation of the ezrin–radixin–moesin (ERM) family is significantly suppressed by RoK inhibitor fasudil [396]. Rho GTPases also control other cellular activities [397]. Rho regulates several enzymes involved in phospholipid metabolism (phospholipase D, phosphatidylinositol kinase). It controls delayed rectifier K^+ channels.

The activation–inactivation cycle of Rho GTPases is a regulated process. The activation of GTPases into GTP-bound conformations is controlled by specific *guanine nucleotide-exchange factors* (GEF), which catalyze GDP release to induce association of GTP, thereby activating Rho GTPases (Fig. 4.9). GTP is hydrolyzed to GDP by GTPase in combination with *GTPase-activating* proteins[57] (GAP). The Rho guanine nucleotide-exchange factors (RhoGEF) has DH and PH domains. Other domains[58] are specific to each member.

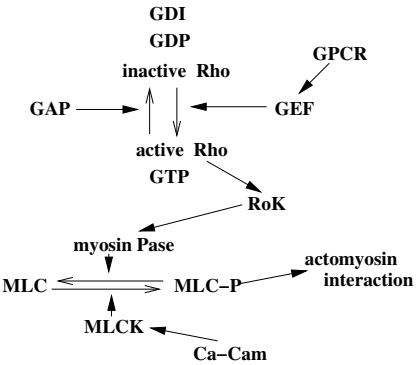

Figure 4.9. Small GTPase states and effectors. Rho GTPases cycle between inactive GDP- and active GTP-bound states. This cycling is controlled by three types of regulatory proteins: (1) guanine nucleotide dissociation inhibitors (GDI), which stabilise the inactive form; (2) guanine nucleotide-exchange factors (GEF), which catalyze the exchange of GDP for GTP; and (3) GTPase-activating proteins (GAP), which enhance the low intrinsic GTPase activity of Rho GTPases.

[57] GTPase-activating proteins regulate the inactivation of small GTPases by accelerating their slow intrinsic GTPase activity.

[58] The regulator of G-protein signaling (RGS) domain acts as a GTPase-activating protein (GAP).

More than 80 Rho guanine nucleotide-exchange factors activate Rho GTPases encoded by 22 genes, which regulate the actin cytoskeleton according to the type of control input. Cdc42, Rac1 and RhoA intervene in the formation of filopodia, lamellipodia, and contractile actin–myosin filaments, respectively. GEFs link specific cytoskeletal responses to corresponding signaling. Reprogrammed GEFs act on unrelated processes [398].

In the absence of signaling, the major fraction of small GDP-bound Rho GTPases is located in the cytosol, and bound to *guanine nucleotide dissociation inhibitors* (GDI sequestration). Guanine dissociation inhibitors slow the rate of GDP dissociation from Rho GTPases, which remain inactive. GDIs prevent the translocation of Rho GTPases from the cytosol to the plasmalemma.

The three members of the VAV family (VAV1, VAV2, and VAV3) are guanine nucleotide-exchange factors for Rho GTPases. They act not only on the immune system, but also regulate angiogenesis [399]. VAV2 and VAV3 bind to EphA2, in response to Ephrin-A1 stimulation, and trigger Rac1 GTPase activity. They also attenuate the blood pressure via a catecholamine-dependent stimulation of the renin–angiotensin system [400]. VAV3-deficient mice exhibit tachycardia and hypertension.

Cloned-out of library (Cool) proteins (p85 Cool1, p90 Cool2, and p50 splice variant of Cool1) are guanine nucleotide-exchange factors.[59] Cool1 regulates degradation of epidermal growth factor receptor. Cool1 acts as a scaffold protein, linking Cdc42 to E3-ligase Cbl. Cool1 is both an activator of Cdc42 and an effector of activated Cdc42 (Table 4.8).

Small Rho GTPases can be activated via G-protein-coupled receptors,[60] tyrosine kinase receptors, and cytokine receptors. In particular, the growth factors recruit Rho, Rac, and ERM.[61] The activation of Rho proteins increases the level of membrane-associated Rho proteins and decreases the cytosolic Rho concentration.

Ras GTPases

Membrane-associated GTPases Ras and Rho activate intracellular pathways in response to extracellular signals for multiple functions (Table 4.9). The Ras activation by receptor tyrosine kinases involves the binding of Ras to Ras-specific GEF Son-of-sevenless, which has been recruited to the plasmalemma. The binding is followed by nucleotide exchange with Sos. G-protein-coupled receptors can also initiate Ras signaling, using a Sos homolog, Ras guanine nucleotide-releasing factor-1, which like Sos catalyzes Ras nucleotide exchange.

[59] Whereas Cool2 exhibits GEF activity, Cool1 is characterized by autoinhibition, containing an element that inhibits its GEF activity [401].

[60] GPCR ligands, thrombin, endothelin, prostaglandin E2, angiotensin, α-adrenergics, sphingolipids, etc., activate Rho GTPases.

[61] Ezrin, radixin, and moesin are involved in membrane recruitment of the Rho GTPases. In endothelial cells, RhoA colocalizes with ERM proteins [402].

The Ras GTPases include Ras, Rap, Ral, and others [403]. Rho, Rac, and Cdc42 are the three best known classes of the Rho protein family. Rho kinases are effectors of Rho GTPases. Rac and Cdc42 GTPases act via p21-activated kinases (PAK). Rho GTPases regulate cytoskeletal activity during cell motility, shape change, and contraction.[62] Each Rho class has specific effects on the actin cytoskeleton. Rho is involved in the formation of cytoplasmic stress fibers, Rac in the formation of membrane ruffles and lamellipodia, and Cdc42 in the formation of filopodia (radial unipolar bundles). All Rho classes also regulate the assembly of integrin-containing adhesion complexes, and thus cell-matrix interactions and cell adhesion.

The components of the GTP-binding protein superfamily have a similar effector domain, which interacts with downstream pathway targets. Both Rap and Ras[63] can bind the same effectors to regulate intracellular signaling events.

Table 4.8. Examples of small GTPases and their corresponding GEFs and regulators. GEFs act both upstream and downstream of a small GTPase. GEF Tiam1, an effector for Ras, also activates Rac for temporal and spatial coordination of Ras and Rac pathways. Activated Ras activates its upstream GEF Sos (stimulatory feedback loop). Autoinhibition of Cool1 GEF activity is relieved by EGF stimulation via Src- and FAK-dependent phosphorylation of Cool1. Src-mediated phosphorylation of Cool1 and Cbl promotes and inhibits Cool1–Cbl binding, respectively. Activated Cdc42 promotes the formation of the Cdc42–Cool1–Cbl complex, which hinders EGFR signaling by sequestring Cbl away from EGFR, thus preventing Cbl-catalyzed EGFR degradation. Cbl negatively regulates EGFR as well as other receptor tyrosine kinases for platelet-derived growth factor, colony-stimulating factor-1, stem cell factor, and fibroblast growth factor. EGFR hyperactivation is associated with various human tumors. Conversely, the Cool1–Cbl–EGFR complex prevents Cbl-catalyzed ubiquitination of EGFR, thus EGFR downregulation. Formation of complexes with Cbl balance EGFR signaling and EGFR degradation. Cool2 binds activated Cdc42 and promotes GEF activity for Rac; but activated Rac inhibits the GEF activity of Cool2 (Source: [401]).

Small GTPase	GEF	Upstream regulator
Cdc42	Cool1–Cbl	Src–FAK on Cool1 (activation)
	Cool1–Cbl	Src on Cbl (inhibition)
Rac	Tiam1	Ras-GTP
	Cool2	Cdc42-GTP
Ras	Sos	Ras-GTP

[62] The regulation of the non-muscle and smooth muscle F-actin cytoskeleton involves phosphorylation of the regulatory myosin light chains, leading to the formation of F-actin filaments.

[63] The Ras GTPases, H-Ras, N-Ras, and K-Ras, have isoform-specific effects, in particular due to their different location at the inner surface of the plasmalemma and possibly in the Golgi complex membranes, both sites that are involved in Ras signaling. H-Ras and N-Ras GTPases are palmitoylated and can be located

Table 4.9. Main functions of small GTP-binding proteins (Arf: ADP-ribosylation factors; Rab: Ras from brain; Ran: Ras-related nuclear proteins; Rap: Ras-related proteins; Ras: superfamily of GTPases; Rho: Ras homology; Cdc42: cell-division cycle-42; Rheb: Ras homolog enriched in brain).

Type	Role
Arf	Control of cell trafficking (vesicle budding and maturation) Phosphoinositide metabolism
Cdc42	Control of cytoskeleton
Rab	Control of vesicle transport (clathrin-coated vesicle formation, endosomal motility)
Rac	Control of cytoskeleton
Ran	Control of nuclear transport
Ras	Control of differentiation and growth
Rheb	Cell growth
Rho	Control of cytoskeleton and integrin activity Gene transcription

In the GTP-bound conformation, Ras binds to and activates effector proteins, members of the Raf kinase family, phosphatidylinositol 3-kinase, and members of the RalGEF family [405].

Oncogenic Ras proteins, H-Ras and K-Ras in particular, are activated by most growth factors as well as integrins. R-Ras[64] antagonizes H-Ras signaling. In vitro, R-Ras enhances integrin-mediated cell adhesion, whereas H-Ras inhibits integrin activities. R-Ras promotes the differentiation of myoblasts, whereas H-Ras inhibits it.

R-Ras regulates cell survival and integrin activity, particularly in the remodeling of blood vessels. In vivo R-Ras is mainly expressed by smooth muscle and endothelial cells [406]. In the absence of R-Ras, neointimal thickening in response to injury and tumor angiogenesis are increased, whereas R-Ras ex-

in the plasmalemma and in the Golgi complex membranes. K-Ras GTPases localize in the plasmalemma. A de- and re-acylation (de- and re-palmitoylation) cycle maintains the specific intracellular distribution [404]. Moreover, the kinetics of H-Ras and N-Ras GTPase trafficking are different. H-Ras GTPases, stimulated by growth factors, are rapidly and transiently activated at the plasmalemma, whereas they have a delayed and sustained activation at the Golgi apparatus membranes. N-Ras GTPases are activated sooner than H-Ras GTPases at the Golgi complex. Inhibition of palmitoylation blocks Ras activation. De-palmitoylation redistributes farnesylated Ras in required membranes. Re-palmitoylation, which occurs at the Golgi complex, enables Golgi membrane anchorage. Ras is then redirected to the plasma membrane by exocytosis.

[64] R-Ras differs from the other members of the Ras family. It contains a proline-rich SH3 domain binding site. It can be phosphorylated by Eph receptors and Src. Both the SH3 binding site and phosphorylation regulate R-Ras activity.

pression is greatly reduced in hyperplastic neointimal smooth muscle cells and angiogenic endothelial cells.

Formation of the GTP-bound Rap1 state (activation) occurs through interaction with the Rap1-specific guanine nucleotide-exchange factor C3G. Under basal conditions, C3G is associated with adapter Crk2. Stretch of the extracellular matrix (ECM) is transduced into intracellular biochemical signals. GTPase Rap1 induce integrin-mediated adhesion and then influence actin dynamics. GTPase Rap1 indeed activates integrins via a Rap1-GTP-interacting adapter (RIAM) [407]. Stretch-mediated Rap1 activation occur in intact cells, as well as in stripped cells[65] [408]. Both cytoskeletons and intact cell stretching induces signaling using Rap1GEF C3G and Crk2 (Rap1–Crk2–C3G pathway). Stretch-induced phosphorylation of Crk-associated substrate (CAS) of the cytoskeleton is due to Src-family kinases (SFK). CrkII binds directly to phosphorylated CAS, especially in cell-ECM adhesion sites.

Arf GTPases

The ADP-ribosylation factor (Arf) family of proteins belongs to the Ras superfamily of small GTPases. Arf proteins regulate vesicular motions and organelle structure by recruiting coat proteins, modulating actin structure at the plasmalemma and regulating phospholipid metabolism. Arf proteins are also involved in various processes, such as secretion, endocytosis, cytokinesis, and cell adhesion. Their cellular distribution (compartmentation)[66] and the interacting molecules dictate the function of Arf GTPases. Several GEFs and GAPs interact with Arf proteins.

The family of Arf GTPases includes Arf isoforms (Arf1–Arf6), Ras-related protein Sar1, Arf-related protein ArfRP1, and Arf-like (Arl) GTPase. Arf GTPases can be classified into three classes: class 1 (Arf1, Arf2, and Arf3) regulate the assembly of coat complexes onto budding vesicles and activate lipid-targeted enzymes, class 2 (Arf4 and Arf5), and class 3 (Arf6).

[65] Extraction using Triton X100 leads to a remaining cell complex that contains mainly cytoskeletal and adhesion proteins and few membrane lipids or cytoplasmic proteins.

[66] Arf1 regulates the early secretory pathway, from the Golgi apparatus to the endoplasmic reticulum and between Golgi cisternae, recruiting coat protein complex 1, clathrin, and adaptor proteins (AP1, AP3, and AP4) [409]. Arf1 also interacts with membrin and SNAREs. Arf6 is located at the plasmalemma and endosomal compartments, where it regulates endocytic membrane trafficking and actin remodeling. It recruits clathrin and AP2. Arf6 acts on phosphatidylinositol(4)phosphate 5-kinase and phospholipase-D, for production of phosphatidylinositol(4,5)bisphosphate.

4.6 Nitric Oxide Synthases

Nitric oxide is synthesized by nitric oxide synthases (NOS), which use NADPH and O_2 as cosubstrates. Nitric oxide activates specific soluble guanylyl cyclase (Sect. 4.12). Active NOS is a homodimer, whatever the isoform. NOSs are hemeproteins; heme irons can bind NO in both ferric or ferrous states within seconds from starting of NO synthesis. NO binding to the heme reversibly inhibits NOS catalysis (negative feedback by the inactive NOS–NO complex).

Nitric oxide is produced by two Ca^{++}-dependent constitutive NOS isoforms: neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3).[67] Transiently-activated constitutive NOS synthesizes NO in response to: (1) increased intracellular calcium concentrations by different activators followed by calmodulin binding; and (2) to other stimuli, such as the wall shear stress.

Endothelial nitric oxide synthase (eNOS) is expressed not only by the vascular endothelium, but also by the airway epithelium and certain other cell types. Calcium-activated calmodulin binds to and transiently activates constitutive NOS dimers. Post-synthesis processing (primarily by acylation) incorporates eNOS to plasmalemmal caveolae. This compartmentation facilitates protein-protein interactions and signal transduction. Within caveolae, eNOS is targeted by G-protein-coupled receptors as well as other receptors (estrogen receptor, and high-density lipoprotein receptor SR-BI) [410]. Binding to caveolin-1 inhibits NO synthesis by eNOS [411]. eNOS can be myristoylated, palmitoylated, farnesylated, acetylated, and phosphorylated. These modifications assist in localizing the enzyme to various cell compartments. Except phosphorylation, these changes do not significantly affect eNOS activity.

Certain protein kinases (PKA, PKB, and AMPK) phosphorylate Ser1177 residue and activate eNOS, whereas others, such as PKC, phosphorylate Thr495 residue[68] and promote dephosphorylation of Ser1177 residue, hence inhibiting eNOS [412] (Tables 4.10 and 4.11).[69] Phosphatases PP1 and PP2A dephosphorylate Thr495 and Ser1177 residues of eNOS, respectively.

nNOS is, in particular, located in synapses. nNOS interacts with postsynaptic density protein PSD95 and PSD93 in the nervous system, as well as α1-syntrophin in the striated muscle [414]. Caveolin-3 binds to nNOS and inhibits NO synthesis.

A cytokine-inducible NOS (iNOS or NOS2) produces NO only in selected tissues, such as lung epithelium, and cells, such as endothelial cells, dermal fibroblasts, and CD8 T lymphocytes. NO plays a role in host defense. It is typically synthesized in response to inflammation, and is implicated in vascular diseases and transplant rejection. iNOS activity appears slowly after

[67] The constitutive NOS are designated after the cell types in which they were originally discovered.

[68] Thr495 corresponds to a calmodulin binding site.

[69] VEGF stimulates PKB and PKC, IGF1 stimulates PKB.

cytokine-exposure (TNF, IL, or IFN), is sustained, and can function independently of calcium and calmodulin. However, calmodulin can be tightly bound to iNOS;[70] therefore, iNOS production is prolonged. Cells regulate inducible NOS via aggresome [415]. The dynein–dynactin complex associates cytokine-induced iNOS to the aggresome at the microtubule-organizing center.

Inducible NOS in non-stimulated T lymphocytes is implicated in atherosclerosis and graft intimal thickening. JaK/STAT signaling, which occurs during T-cell activation, inhibits iNOS expression. Endothelial cells and other

Table 4.10. eNOS phosphorylation and dephosphorylation. Ser1177 phosphorylation leads to eNOS activation, and Thr495 phosphorylation to eNOS inactivation (Source: [412]).

	Ser1177		Thr495	
	Phosphoryl^t (activation)	Dephosphoryl^t (inhibition)	Phosphoryl^t (inhibition)	Dephosphoryl^t (activation)
Phosphatases		PP2A		PP1
Kinases	PKA PKB PKG AMPK CamK2	PKC (via PP2A)	PKC AMPK	PKA (via PP1)

Table 4.11. Stimulators of multisite eNOS phosphorylation and dephosphorylation of specific serine and threonine residues (Source: [413]).

Residue site	Phosphorylation stimulators	Dephosphorylation stimulators
Ser114	Shear stress, HDL	ATP, VEGF
Ser615	ATP, bradykinin, VEGF	
Ser633	Shear stress, ATP, bradykinin, VEGF	
Ser1177	Shear stress, ATP, bradykinin, histamine, thrombin, hydrogen peroxide, insulin, estrogen, adiponectin, leptin, sphingosine 1-phosphate, VEGF, IGF1	
Thr495		Bradykinin, hydrogen peroxide, VEGF

[70] NOS affinity for calmodulin obeys the following order: nNOS $<$ eNOS $\ll$ iNOS.

stromal cells induce iNOS expression in CD8 T cells, at a greater extent than in CD4 T cells. Inducible NOS in resting T cells and low NO concentrations increase T-cell proliferation in response to allogeneic endothelial cells of grafted vessels [416]. iNOS induction depends on NFκB, which is inhibited by STAT.

Asymmetric dimethylarginine and monomethyl arginine inhibit all NOS isoforms.[71] Asymmetric dimethylarginine is a cardiovascular risk factor because it reduces NO signaling, hence eliciting endothelial dysfunction and augmenting systemic and pulmonary blood pressure [418]. Its plasmatic concentration, indeed, rises particularly in pulmonary hypertension.

NO Effects

Nitric oxide acts not only on the vasomotor tone (Sect. 9.5.3), but also on immunity, neurotransmission, ion conductance, glycolysis and apoptosis. Biological effects of NO can be mediated by modifications of proteins. The ubiquitin-dependent N-end rule pathway recognizes degradation signals based on a destabilizing N-terminal residue. Oxidation of N-terminal cysteine (before its arginylation) of regulatory proteins requires nitric oxide. NO regulates particularly the heart proteolysis of RGS4, RGS5, and RGS16 [419].

NO Activity in the Heart

In the cardiomyocyte, the regulation of intracrine nitric oxide signaling depends on the location (sarcolemma, or sarcoplasmic reticulum) of NO synthase isoforms (NOS1, and NOS3) [420]. Nitric oxide inhibits β-adrenergic-induced inotropy after compartmentation of NOS3 in caveolae, hampering L-type Ca^{++} channels. NOS3 binds to caveolin-3 of caveolae, which also incorporate β-adrenergic receptors and L-type Ca^{++} channels. NOS1 associates with ryanodine receptors of the sarcoplasmic reticulum and then stimulates Ca^{++} release. NOS1 and NOS3 thus have opposite effects on Ca^{++} influx.

Table 4.12. NO targets in the cardiovascular system (Source: [417]).

Target	Function
Guanylyl cyclase	cGMP formation
NADPH oxidase	Enzyme inhibition
Tissue plasminogen activator	Vasodilation, platelet inhibition
Ca^{++}-activated K^+ channel	Vasodilation
Cyclooxygenase-2	Prostaglandin synthesis
Cytoskeleton proteins	Dysfunction

[71] Methylarginines are formed by arginine methyltransferases. After proteolysis, methylarginines are released. These endogenous amino acids are degraded by dimethylarginine dimethylaminohydrolase.

In the heart, NO is an important regulator of both perfusion and contractility [421]. Endothelial NOS exerts paracrine and autocrine influences on the cardiomyocyte. In the heart, nitric oxide produced by endothelial cells favors the perfusion via vasodilation and prevention of platelet aggregation. Nitric oxide in endothelial cells subjected to mechanical and chemical stimulators increases the intracellular calcium level, which activates nitric oxide synthase-3 by promoting the binding of Ca^{++}–calmodulin to NOS3 and activation of PI3K and, subsequently, of PKB. Nitric oxide produced by cardiomyocytes regulates the force and rate of contraction. Moreover, noradrenaline and acetylcholine released from autonomic nerve endings stimulate their respective receptors to activate NOS3 in cardiomyocytes (Fig. 4.10).

Both NOS1 and NOS3, constitutively expressed in cardiomyocytes are involved.[72] NO acts on the excitation-contraction coupling. NOS3 facilitates the electromechanical coupling in response to sarcomere stretch. NOS3 mediates the slow rise in calcium transient and force (Anrep effect). NOS1 hinders L-type calcium current and enhances calcium reuptake into the sarcoplasmic reticulum by SERCA (Fig. 4.10). NOS1 and possibly paracrine NO from adjoining endothelial cells promote cardiomyocyte relaxation and thereby ventricular filling, which increases stretch, whereas NOS3 sustains length-dependent increase in calcium transient and force generation in stretched fibers. Moreover, NOS1 and NOS3 attenuate $\beta 1/\beta 2$-adrenergic positive inotropy and chronotropy. NOS3 also potentiates acetylcholine activity. NOS reinforces pre- and post-synaptic vagal control of the cardiac contraction. They thus protect the heart against excessive stimulation by catecholamines. In the ischemic myocardium, NOS1 augments the effect of constituve NOS. Last but not least, NOS modulates oxygen consumption, promotes free fatty acids rather than glucose oxidation, struggles against oxidative stresses, prevents apoptosis at low concentrations, and acts in adaptive and regenerative processes.

Cyclic adenosine monophosphate and cyclic guanosine monophosphate have opposing effects in the cardiomyocyte.[73] A high NO concentration increases the cGMP level leading to negative inotropy by a PKG-dependent reduction in myofilament responsiveness to calcium. (The calcium ions remain

[72] NOS3 is distributed between plasmalemmal and T-tubular caveolae, in associated with myocyte-specific caveolin-3. NOS1 could be located in the sarcoplasmic reticulum, NOS1α in mitochondria. NOS3 also exists abundantly in endothelial and endocardial cells, and NOS1 in both adrenergic and cholinergic nervous fibers.

[73] cAMP/cGMP binding proteins include: (1) cAMP- and cGMP-dependent protein kinases PKA and PKG, (2) several phosphodiesterases, and (3) A/G-kinase anchor proteins. Phosphodiesterase PDE4 and PDE5 selectively degrades cAMP and cGMP, respectively. PDE1 and PDE2 target both cAMP or cGMP. Compartmentalized signaling events involve scaffold proteins AKAPs and GKAPs for PKA and PKG, respectively. Sphingosine kinase-1 interacting protein (SKIP), an anchor for sphingosine kinase-1, can serve as AKAP.

available.) Low NO concentration increases cAMP level by adenylyl cyclase activation, leading to positive inotropy [422]. The negative cGMP effects in cardiomyocytes are mediated by cGMP-gated ion channels, protein kinase-G, and phosphodiesterases. cGMP-stimulated cAMP phosphodiesterase PDE2 and cGMP-inhibited cAMP phosphodiesterase PDE3 are regulated by the intracellular concentration of cGMP. PDE3 is inhibited by an increase in cGMP level, which raises the cAMP level. PKA then activates calcium channels, countering the PKG effects. In contrast, cGMP can stimulate PDE2, thereby reducing the cAMP level and PKA activity. Interaction between cGMP and cAMP signaling pathways are impaired in failing cardiomyocytes [423].

cAMP-dependent protein kinase PKA phosphorylates target proteins, including the L-type calcium channel for increased calcium influx using calcium-induced calcium release, thereby enhancing CMC contraction [417]. cGMP activates PKG, which inhibits the L-type Ca^{++}-channel, and activates BKCa channel and myosin light chain phosphatase.

Exogenous NO acting on soluble guanylyl cyclase triggers the cGMP–PKG pathway. PKG1 then inhibits L-type Ca^{++} channels CaV1.2a [424]. Muscarinic receptor stimulation does not act on this cascade but stimulates calmodulin-dependent cardiac nitric oxide synthase, which produces endogenous NO. Muscarinic inhibition occurs rather via inhibition of cAMP signaling via Gαi2-dependent inhibition of adenylyl cyclase. Stimulation of β1/β2-adrenergic receptor produces positive inotropy via Gαs activation of adenylyl cyclase activating cAMP levels (Fig. 4.10).

Nitric oxide produced by inducible NOS in all cardiac cell types, including interstitial macrophages, under exposure to cytokines and other inflammatory mediators exerts paracrine and autocrine effects. Large NO concentrations decrease the contraction of cardiomyocytes and vascular smooth muscle cells. NO produced by iNOS also induces apoptosis of various cell types. In the cardiomyocyte, NO provokes TNFα synthesis using the cGMP–PKG pathway. TNFα induces iNOS expression and yields a positive feedback inflammatory loop [425].

4.7 NADPH Oxidases

Every cell type of the vessel wall contains NADPH oxidases (NOx), thereby producing reactive oxygen species (Fig. 4.11) [249].[74] These NADPH oxidases have specific features, constitutive and inducible activities, and sub-

[74] NAD(P)H oxidases belong to the small set of superoxide-producing enzymes of the vasculature, which includes xanthine oxidase, cytochrome P-450, and uncoupled nitric oxide synthase. Reactive oxygen species act on cell growth and apoptosis, and cause vasodilation, driving signaling cascades via activation of kinases and inhibition of tyrosine phosphatases and activating various transcription factors.

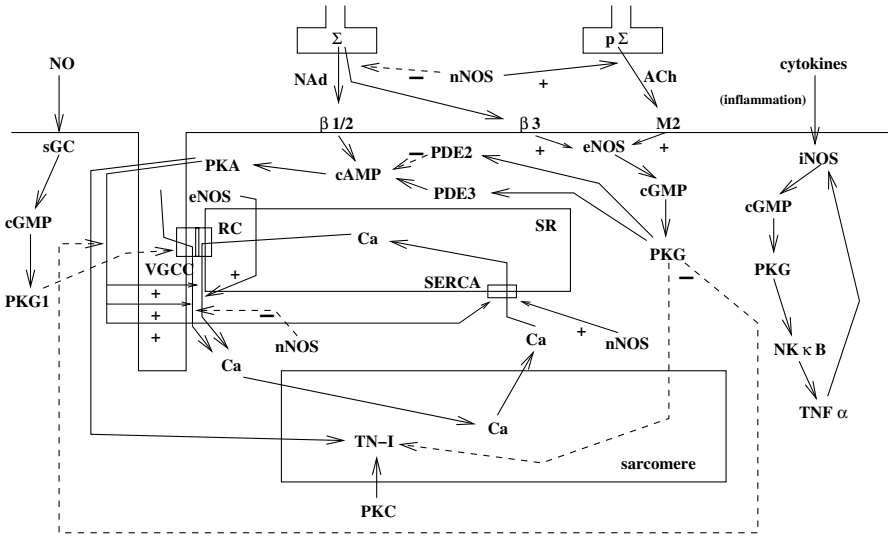

Figure 4.10. eNOS and nNOS cooperate to regulate the sympatho-vagal balance in the heart. nNOS (NOS1) acts in the parasympathetic and sympathetic endings. nNOS potentiates acetylcholine release and hampers noradrenaline release in the synaptic cleft. eNOS (NOS3) is activated in cardiomyocytes by both stimulated muscarinic cholinergic and β3-adrenergic receptors. Activated eNOS in the cardiomyocyte opposes adrenergic activity but reinforces vagal input. Catecholamine stimulation of β3-adrenoceptors of cardiomyocytes activates eNOS via Gαi, which increases cGMP level and leads to negative inotropy. Co-located eNOS and ryanodine channels RC2 in the T-tubule–sarcoplasmic reticulum junction favor calcium influx. cGMP opposes cAMP-positive inotropy because it activates phosphodiesterase PDE2 for cAMP degradation, although cGMP can potentiate cAMP effects via inhibition of PDE3. PKG activation reduces the L-type calcium current. PKG also decreases myofilament sensitivity to calcium, thereby promoting relaxation. nNOS hinders L-type calcium channels (VGCC) but promotes sarcoplasmic reticulum calcium ATPase (SERCA). Exogenous NO acts on soluble guanylyl cyclase (sGC) for cGMP production, and activates PKG1. Activated PKG1 inhibits L-type channels CaV1.2a. iNOS triggered by cytokines and other inflammatory mediators in the cardiomyocyte activates the cGMP–PKG pathway, and hence NFκB, leading to TNFα synthesis. TNFα upregulates iNOS in cardiomyocytes. (Sources: [417, 421, 424, 425]).

strate specificity.[75] Protein kinase-C activates vascular NAD(P)H oxidase. Phospholipase-D and -A2 could also stimulate NAD(P)H oxidase via effectors. Thrombin, several growth factors, and angiotensin-2 stimulate NOx activity in vascular smooth muscle cells and endothelial cells (Table 4.13). Both

[75] Vascular NADPH oxidases are composed of subunits, especially catalytic gp91phox (also called nox2), or nox1 in smooth muscle cells, as well as nox4 and nox5 (phox means phagocyte oxidase, nox NADPH oxidase). Stimulation leads to subunit assembling.

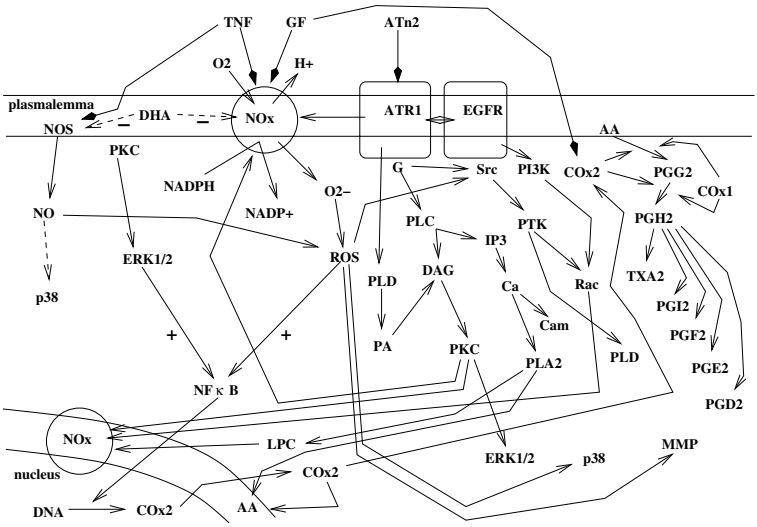

Figure 4.11. NAD(P)H oxidase in vascular cells (Sources: [249, 426]. NOx is upregulated in vascular smooth muscle cells by platelet-derived growth factor (PDGF), transforming growth factor-β (TGF), and tumor necrosis factor-α (TNF), and in endothelial cells by angiotensin-2. Stimulation of the AT1 receptor (AT1R) by angiotensin-2 (ATn2) activates phospholipase C via G protein. PLC produces inositol trisphosphate (IP3) and diacylglycerol (DAG), releasing calcium from intracellular stores and activating protein kinase-C (PKC), respectively. PKC phosphorylates the catalytic NOx subunit. Calcium ions activate phospholipase-A2 (PLA2), which produces lysophosphatidylcholine and arachidonic acid (AA). Arachidonic acid is metabolized into prostaglandins by constitutive (COx1) and inducible (COx2) cyclooxygenases. Angiotensin-2 also activates the Src kinase, which participates to the activation of the EGF receptor (EGFR) and activates protein tyrosine kinases. Activated phospholipase-D produces phosphatidic acid (PA), which is converted to DAG, subsequently enhancing PKC activity. Reactive oxygen species (ROS) activate Src associated with EGFR. Long-duration exposure of vascular endothelial cells to omega-3 fatty acid docosahexaenoate (DHA) causes its incorporation into plasmalemma. DHA inhibits nuclear factor-κB (NFκB) and, subsequently, COx2. Furthermore, it decreases the production of reactive oxygen species by inhibiting NADPH oxidase (NOx), and reduces PKC association to the plasmalemma. Last but not least, NOx is stimulated by pulsatile wall shear stress.

increase Rac GTPase activity. Besides, angiotensin-2 transactivates the EGF receptor with ROS via Src.

4.8 Transcription Factors Involved in Stress Responses

4.8.1 Nuclear Factor-κB

The genes regulated by transcription factor NFκB in coordination with different signaling pathways control cell proliferation, apoptosis, cell adhesion, stress response, tissue remodeling, and innate and adaptive immunity. NFκB activation depends on its induction pattern. For example, NFκB regulation of the Jun N-terminal kinase signaling can have opposing results according to the cell type.

Nuclear factor-κB is inactive in resting cells due to sequestration in the cytosol by its inhibitor. NFκB includes five members: NFκB1 (p105/p50), NFκB2 (p100/p52), RelA (p65), RelB, and Rel (or cRel). NFκB hence corresponds to any dimeric transcriptional factor of the Rel family. The NFκB signaling module is at least composed of: (1) five homo- and hetero-dimeric transcription factors, bound to NFκB dimers to favor cytosolic location in unstimulated cells; (2) five regulatory subunits, NFκB-bound inhibitors of NFκB (IκB); and (3) three IκB kinases, with two catalytic subunits, IKKα (IKK1) and IKKβ (IKK2), and a regulatory subunit, IKKγ, or NFκB essential modulator NEMO. IKKα, a negative regulator, limits NFκB activation. NEMO is essential for NFκB activation.

After degradation of their cytoplasmic inhibitors IκB, the homo- and hetero-dimers composed of the five NFκB/Rel proteins translocate to the nucleus and bind various sites in the genome to interact with associated transcription factors. NFκB thus activates transcription of numerous genes implicated in inflammation, immune response, and antiapoptosis. Transcriptional activation domains exist in RelA, RelB, and cRel, but are lacking in NFκB1 (p50) and NFκB2 (p52). NFκB binding and unbinding on its target sites within transcription gene is characterized by fast dynamics [427].

NFκB activation is triggered by multiple plasmalemmal or intracellular receptors. Cooperative and antagonistic cross-talks exist between NFκB

Table 4.13. Expression of NAD(P)H oxidase in vascular smooth muscle cells depends on numerous compounds that either favor or prevent it.

Upregulation	Downregulation
ATn2	Statins
ET1	Estradiol
Thrombin	
PDGF	
TGFβ	
TNFα	
PGF2α	
oxLDL	
WSS	

and other signaling pathways, with JNK, tumor suppressor and transcription factor p53, and nuclear receptors [428]. Adapter Bcl10 is required for the activation of NFκB by GPCRs stimulated by their ligands, such as endothelin-1 [429].

Nuclear factor-κB signaling uses canonical and non-canonical pathways according to the stimulus type. However, there is cross-talk between inflammatory (*canonical pathway*) and developmental (*non-canonical pathway*) stimuli in the immune system owing to a fourth inhibitor of NFκB. NFκB activation in response to inflammatory stimuli (TNF) occurs via the canonical pathway. The canonical pathway is characterized by phosphorylation using IκB kinases IKKγ and IKKβ and proteolysis of IKKα, IKKβ, and IKKϵ, which release RelA–p50 heterodimers, which translocate to the nucleus and activate target genes (NFκB/RelA pathway). Developmental stimuli, such as the one generated by the lymphotoxin-β receptor (LTβR), induce NFκB-inducing kinase- and IKKα-dependent phosphorylation and partial proteolysis of p100 to generate p52, leading to RelB–p52 heterodimers that translocate to the nucleus. Furthermore, LTβR can also activate RelA–p50 using the canonical pathway in the presence of NFκB2/p100 [430]. Inhibitor p100 sequesters RelA–p50 dimers in the cytoplasm, thus inhibiting translocation to the nucleus. Protein p100 allows cross-talk between canonical and non-canonical signaling pathways. However, inhibitor p100 forms two different inhibitory complexes with RelA for pathway specificity (insulation). One pathway mediates developmental NFκB activation.

NFκB regulates expression of cytokines, chemokines, and adhesion molecules. NFκB is, in particular, activated by inflammatory cytokines (tumor necrosis factor-α, and interleukin-1) and cell stress (UV, and γ-irradiation). The release of NFκB from IκB is stimulated by at least two cytosolic pathways: the classical and alternative pathway. The *classical pathway* is particularly triggered by bacterial and viral infections and inflammatory cytokines (tumor-necrosis factor, and interleukin-1). This IKKκ-dependent pathway leads to activation of the inhibitor of NFκB kinase. The IKK complex phosphorylates IκBs for polyubiquitination and proteasomal degradation. NFκB is then released, predominantly with elements composed of RelA, cRel and p50, which enter the nucleus for transcription. Target genes of the classical pathway encode mediators of inflammation, cytokines (TNF, IL1, IL6, and IL8), chemokines, proteases, and apoptosis inhibitors. The *alternative pathway* is initiated by CD40, lymphotoxin, and B-cell activating factor of the tumor necrosis factor family. It involves NFκB-inducing kinase (NIK), which activates IKKα and processes p100. p52/RelB heterodimers are released. The two pathways mediate different immune functions.

Tumor necrosis factor receptors include TNFR1, TNFR2, CD30, and CD40. Tumor necrosis factor receptor-1, the main TNF receptor, trimerizes and forms a signaling complex with several adapters, including TNF receptor–associating factor-2 (TRAF2) and TNF receptor–interacting protein. Thsi

complexe then triggers nuclear factor-κB[76] and mitogen-activated protein kinase pathways.

In disturbed endoplasmic reticulum due to glucose starvation, perturbation in intracellular calcium stores, or inhibition of protein glycosylation, protein foldings are compromised. An adaptive response is then triggered to avoid alteration of the processing of unfolded proteins in the endoplasmic reticulum. This reaction is mediated by three transmembrane proteins of the endoplasmic reticulum: (1) the protein kinase endoribonuclease IRE1, (2) the protein kinase-R-like endoplasmic reticulum kinase, and (3) the transcriptional activator ATF6. In disturbed endoplasmic reticulum, TNFR1 activates Jun amino-terminal kinase downstream from IRE1, which is linked to TNFR1 in a complex [432, 433].

Hypoxia activates IκB kinase-β, which degrades IκBα and releases transcription factor NFκB. IKKβ also amplifies cellular sensitivity to TNFα. IKKβ hydroxylation by prolyl hydroxylase,[77] mainly PHD1, withdraws the repression of NFκB [434].

NFκB inhibits cell apoptosis induced by TNFα, upregulating the expression of antiapoptotic genes, such as cellular flice-inhibitory protein, BCL2 family proteins, and X-chromosome-linked inhibitor of apoptosis. NFκB activates cellular flice-inhibitory protein[78] (cFlIP). The mitogen-activated protein kinase signaling cascade also regulates cell death and survival, particularly the c-Jun N-terminal kinase pathway. JNK is activated by MAP2K4/7, themselves activated by MAP3Ks (MAP3K1/4, apoptosis signal-regulating kinase ASK1, and TGFβ-activated kinase TAK1). Cytokines, such as TNFα and IL1, relatively quickly induce a transient MAPK activation leading to survival cues; but TNFα generates a prolonged JNK activation and ROS accumulation in the absence of cellular flice-inhibitory protein (cFlIP), a prolonged MAPK activation promoting cell apoptosis (Fig. 4.12). TNFα favors ROS accumulation in the absence of NFκB. At the opposite, the expression of antioxidants is upregulated by TNFα in the presence of NFκB and the long form of cFlIP (cFlIPl). The long form of cFlIP interacts with MAP2K7 and inhibits the interactions of MAP2K7 with MAP2K1, ASK1, and TAK1. cFlIPl binds to MAP2K7 and MAP2K1, cancelling JNK and ERK pathways [435]. TNFα favors the interaction of cFlIP with MAP2K7 during transient activation of JNK activation, but also promotes a quick degradation of cFlIPl during prolonged JNK activation degradation via ubiquitin ligase ITCH.

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[76] Tumor necrosis factor receptor-1 recruits receptor-interacting protein-1 (RIP1). RIP1 is polyubiquitylated and targets the NEMO regulatory subunit of IκB kinase to activate NFκB [431].

[77] There are three known prolyl hydroxylase isoforms: PHD1, PHD2, and PHD3.

[78] Cellular flice-inhibitory protein is also designated as cash or casper. The cellular flice-inhibitory protein gene encodes two splicing variants, the long form of cFlIP (cFlIPl) and the short form of cFlIP (cFlIPs).

4.8.2 Hypoxia-Inducible Factor

Cells adapt to changes in oxygen availability. The cellular response to hypoxia involves two pathways associated with NFκB and the hypoxia-inducible factor. Survival transcription factors stimulated by decreased oxygen levels are mediated by the hypoxia-inducible transcription factor (HIF; Fig. 1.6). Hypoxia-inducible factor-1 regulates the expression of genes producing molecules for angiogenesis, vasodilatation, glycolysis, and erythropoesis. The hypoxia-inducible factor is an α/β heterodimer that binds hypoxia response elements to hypoxia-inducible genes. The concentration of the HIFα subunit (but not HIFβ) is regulated by oxygen.

HIF1 is constitutively synthesized. Two oxygen-dependent repression mechanisms exist. HIF1 is repressed either by prolyl hydroxylases (Sect. 4.8.1), mainly by PHD2, with co-substrate oxygen to undergo E3-ligase-dependent ubiquitination initiated by the binding of von Hipple-Lindau protein and subsequent proteasomal degradation, or by asparagine hydroxylase, the factor inhibiting HIF. HIF proteasomal degradation due to hydroxylation is mainly catalyzed by HIF prolyl hydroxylase, which requires oxygen.[79] Protein hydroxylation by HIF asparagine hydroxylase on manifold proteins (hypoxia-inducible factor, IκB proteins, etc.), also corresponds to an oxygen-sensitive signal for various cellular processes [436].[80] HIF hydroxylases thus inhibit HIF. Reductions in the hydroxylation rate during hypoxia allow HIF to ac-

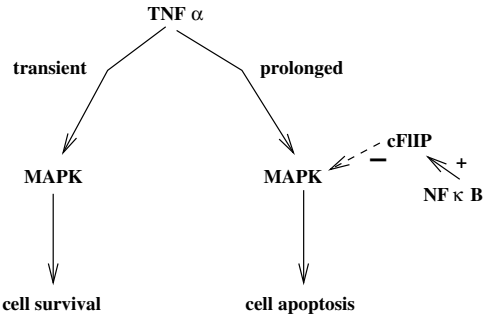

Figure 4.12. TNFα can drive both cell survival and death cues. TNFα stimulates MAP3Ks, which phosphorylate MAP2K7, which in turn activates JNK. NFκB upregulates the expression of antioxidants, antiapoptotic proteins, and cellular flice-inhibitory protein (cFlIP). TNFα favors the interaction of cFlIP with MAP2K7, thereby inhibiting the prolonged activation of the JNK pathway.

[79] Hydroxylation of HIF1α can be catalyzed by four oxoglutarate dioxygenases, the three prolyl hydroxlyases (PHD1, PHD2 and PHD3), and an asparagine hydroxylase.

[80] Prolyl hydroxylation of HIF catalyzed by 2-oxoglutarate (PHD1, PHD2, and PHD3) promotes interaction with the von Hippel-Lindau E3 ligase and VHL-mediated proteolysis, whereas asparagine hydroxylation catalyzed by iron-

tivate transcription. The hypoxia-inducible transcription factor regulates the transcription of various genes, such as those coding for glycolytic enzymes, erythropoietin, and vascular endothelial growth factor.

4.8.3 Forkhead Box Class O

Members of the class O of forkhead box transcription factors (FoxO1, FoxO3, FoxO4, and FoxO6) are implicated in metabolism[81] and cell fate.[82] Forkhead box class O transcription factors are also involved in stress responses, especially from reactive oxygen species. FoxOs upregulate several antioxidant enzymes. FoxOs induce cell-cycle arrest and entry into cellular quiescence.

The FoxO activity is regulated by post-translational reversible modifications (phosphorylation, acetylation, and ubiquitylation). Phosphorylated FoxOs either inhibit via phosphatidylinositol 3-kinase and protein kinase-B or stimulate via c-Jun N-terminal kinase the transcription of target genes. Both PI3K effectors, protein kinase-B and serum- and glucocorticoid-induced kinase (SGK), phosphorylate FoxO in the nucleus, which is then exported to the cytoplasm (Fig. 4.13).[83] The signaling is modulated by Ser/Thr kinase CK1 and the Ras–Ral pathway [438]. FoxO is phosphorylated not only by CK1, but also by Ser/Thr kinase DYRK. PI3K signaling is counterbalanced by PTEN.

Polyubiquitylation leads to subsequent slow proteasomal degradation of FoxOs. Monoubiquitylation stimulates, whereas acetylation could inhibit FoxOs. FoxO monoubiquitylation, indeed, induces FoxO nuclear translocation.

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dependent dioxygenase (HIF asparagine hydroxylase) prevents recruitment of coactivators.

[81] Transcription by FoxOs is regulated by insulin and/or insulin-like growth factor via PKB, which leads to relocalization of PKB-phosphorylated FoxOs from the nucleus to the cytosol [437]. FoxOs favor gene expression of enzymes of gluconeogenesis and suppress the expression of genes of glycolysis. FoxOs increase insulin sensitivity (in normal conditions but not diabetes) by upregulating insulin receptor and IRS2. FoxOs regulate fatty acid oxidation. Fatty acid breakdown and oxidative phosphorylation allow the ATP generation. FoxOs upregulate fatty acyl-CoA carriers and sterol carrier proteins SCPx and SCP2 for suitable fatty acid processing. However, FoxOs oppose other insulin functions. Insulin–PI3K signaling stimulates cell-cycle progression, preventing FoxO-induced cell-cycle inhibition.

[82] FoxO targets genes involved in cell survival (Fas ligand, TGFβ2, etc.) and cell cycle (cyclins).

[83] The transcription factor FoxO3 is targeted by insulin and growth factor (IGF1) signaling. In the absence of stimulation, FoxO is localized in the nucleus for transcription of target genes. Growth or survival signals activate PKB which phosphorylates FoxO. Moreover, PKB inhibits apoptosis signal-regulating kinase ASK1 and glycogen-synthase kinase GSK3, which are implicated in cell apoptosis. SGK is related to PKB via PI3K and phosphoinositide-dependent kinase (PDK).

JNK-mediated phosphorylation, acetylation, and monoubiquitylation of FoxOs can be caused by oxidative stress. In turn, modified FoxOs intervene in cell oxidative stress resistance. Oxidative stresses enhance FoxO binding to histone acetyltransferases and FoxO acetylation by HATs, such as p300, cAMP responsive element binding-binding protein (CBP), and CBP-associated factor. FoxO deacetylation can be done by Sirt1 and other histone deacetylases.

The small GTPase Rheb regulates the cell growth via the PI3K and TOR pathways. The increasing activity of the Rheb-target-of-rapamycin–S6-kinase pathway sensitizes the cell to oxidative stresses and is implicated in decaying locomotor activity with aging [439].

Both FoxOs and p53 regulate cell cycle and apoptosis. Deacetylase Sirt1 and deubiquinase USP7 affect FoxOs and p53 in an opposite manner, whereas JNK stimulates both proteins under stress conditions [437]. Sirt1 stimulates FoxOs, hence increasing cell life duration, and concomitantly inhibits p53, augmenting cancer risk. Conversely, USP7 inhibits FoxOs and stimulates p53. Tumor suppressor p53 controls FoxO regulators, such as SGK, PI3K, and PTEN.

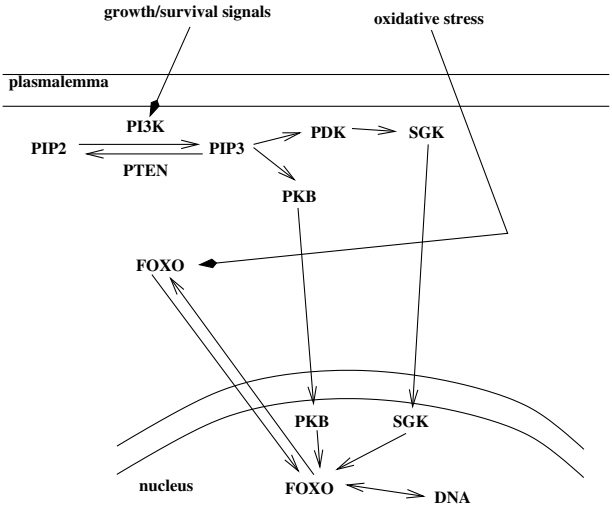

Figure 4.13. FoxO transcription factor and interacting substances. FoxO is phosphorylated by several enzymes, especially protein kinase-B (PKB), and serum- and glucocorticoid-induced kinase (SGK). Both PKB and SGK depend on phosphoinositide 3-kinase (PI3K), which is counterbalanced by phosphatase and the tensin homolog deleted on chromosome 10 (PTEN). Phosphorylate FoxO is translocated to the cytosol. Growth and survival signals activate PKB.

4.8.4 Tumor Suppressor p53

Tumor suppressor p53 accumulates in response to DNA damage, oncogene activation, and hypoxia. However, the p53 level is controlled by Mdm2 ubiquitination, Mdm2 being upregulated by p53. Tumor suppressor p53 activates or represses the transcription of specific genes. Tumor suppressor p53 not only protects against cancer development, but also regulates glycolysis, repair of DNA damage, and cell fate (cell-cycle arrest, cell survival, senescence, autophagy, and apoptosis during ischemia). This transcription factor, indeed, has additional targets independent of transcription. The known p53-interacting molecules include caspase-3 (apoptosis), and 14-3-3σ(cell growth control) among others (p21, Mdm2, Bax, and PUMA). It acts via the Ras–MAPK pathway. eIF5A promotes p53 expression. Tumor suppressor p53 undergoes post-transcriptional modifications (phosphorylation, acetylation, methylation, and ubiquitination), which affect its stability and activity [440]. Most of these modifications can be reversed, owing to phosphatases, deacetylases, and deubiquitylating enzymes. Tumor suppressor p53 can be activated by various signals using different pathways. Tumor-suppressor activities of p53 involve Arf or not.

The AMPK pathway activates cyclin-dependent kinase inhibitor p21 and p53 for short-term survival of cells subjected to transient glucose deprivation. Tumor suppressor p53 regulates the expression of Tp53-inducible glycolysis and apoptosis regulator (TIGAR), decreasing the activity of glycolytic 6-phospho 1-kinase. This diversion in the major glycolytic pathway into the pentose phosphate pathway increases NADPH production, eliciting decay in concentration of reactive oxygen species. However, sustained stresses switch p53 function from cell survival to apoptosis, targeting PUMA. Tumor suppressor p53 induces both the extrinsic pathway via activation of cell-surface receptors, and predominantly the intrinsic pathway associated with cell stresses (Fig. 4.14). Tumor suppressor p53 couples transforming growth factor-β to the RTK–Ras–MAPK cascade to stop cell growth [441]. Kinases CK1ϵ and CK1δ phosphorylate p53 in response to RTK–Ras–MAPK signaling.

Tumor suppressor p53 regulates cell growth only at physiological levels. Therefore, p53 concentration must be strongly controlled in an appropriate range to avoid unwanted effects, such as tumorigenesis in the case of a rising p53 level. The p53-associated cellular protein-testes derived[84] (PACT) binds to p53 and inhibits it via Mdm2 to hamper apoptosis and favor cell growth [442].

Tumor suppressor p53 also prevents cell migration, then tumor cell metastasis. There is, indeed, a dual activity between Ras and p53 on GTPase RhoA activation for cell motility, as p53 restricts Ras stimulation of RhoA [443]. Small GTPase Ras promotes RhoA translocation to the plasmalemma, where RhoA can be activated. Concomitant loss in p53 inhibits p190RhoGAP and thus favors RhoA activation.

[84] PACT is also called P2P-R or RBBP6.

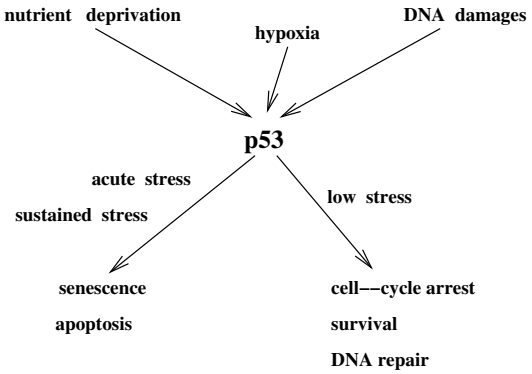

Figure 4.14. p53 function according to stress features, intensity, occurrence, and duration.

4.8.5 Transcription Factor p73

Transcription factor p73 is a structural and functional homolog of the tumor-suppressor p53, which favors cell growth, upregulating AP1. It also promotes cell survival when it functions synergistically with cJun, targeting cyclin-D1. p73 enhances the binding of phosphorylated cJun and Fra1 to AP1 [445]. The synergy between p73 and cJun activities amplifies signaling.

4.9 Hippo Pathway

Cell proliferation during embryogenesis and in childhood becomes restricted in adulthood, cell divisions serving for tissue maintenance. The Hippo pathway promotes apoptosis, particularly when it is primed by *irradiation*, being activated by p53 [444]. The p53 activation of the Hippo pathway is tempered by Ras. The Hippo pathway restrains cell proliferation, possibly preventing activation of the transcriptional coactivator Yes-associated protein (Fig. 4.15). The Hippo pathway can then serve as a tumor suppressor.[85]

4.10 Coregulators

Co-regulators participate in signaling by organizing molecular interactions and determining pathway dynamics. For example, A-kinase anchoring proteins

[85] Hippo signaling could be associated with at least two genes, Fat and Bantam. The protocadherin Fat, which stabilizes large tumor suppressor kinase LATS, might act as a transmembrane protein for ligand binding to prime the Hippo pathway. The protocadherin Dachsous could extracellularly interact with Fat. The Hippo pathway could repress miRNA Bantam, which elicits tissue growth and inhibits apoptosis.

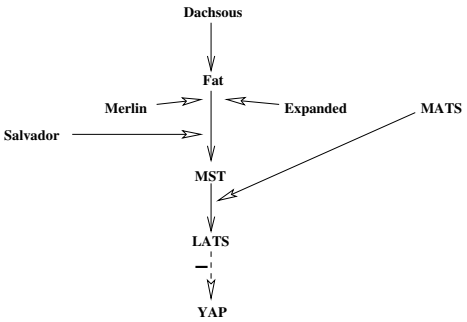

Figure 4.15. The Hippo pathway requires two plasmalemmal proteins, Merlin (observed in particular in endothelial cells and encoded by the NF2 tumor-suppressor gene, its phosphorylation depending on p21-activated kinase and its activator GTPase Rac) and Expanded, which promote the phosphorylation (activation) of large tumor suppressor kinase LATS by activated mammalian sterile-20-like kinase MST (mammalian homolog of Hippo). The scaffold protein Salvador, which binds to LATS and MST, favors MST phosphorylation. LATS activation requires plasmalemmal localization of Mob as tumor suppressor (MATS). LATS activated by MST phosphorylates (inactivates) the transcriptional coactivator Yes-associated protein (YAP). YAP regulates the activity of various transcriptional regulators, such as p73, p53-binding protein-2, etc. The protocadherin Fat might act as a transmembrane protein for protocadherin Dachsous to trigger the Hippo pathway (Source: [444]).

control the location and substrate specificity of protein kinase-A. Inhibition of PKA–AKAP interactions modulates PKA signaling. Annexins act on many partners implicated in signaling cascades and on interactions with the cell membrane and cytoskeleton.

4.10.1 A-Kinase Anchoring Proteins

Manifold cell functions can be specifically and efficiently controlled by a limited number of proteins over various distinct enzymes. Cell activities, particularly effector responses to receptor stimulations, are processed by protein–protein interactions required in signaling molecules. Regulators and targets must be appropriately located to achieve the cell functions, especially in the heart the spatiotemporal regulation of firing action potentials by nodal cells or the secretion of hormones by cardiomyocytes.

Signaling networks are particularly associated with focal cytosolic points of enzyme activity, which transmit the action of the messenger in the cytosol. Anchoring proteins yield a framework that orients these enzymes toward selected substrates to elicit a specific physiological response. A-kinase anchoring proteins[86] (AKAP), signal-organizing molecules, target protein kinases and

[86] A-kinase anchoring proteins are signaling and scaffold proteins. The first described AKAP is microtubule-associated protein-2 (MAP2). All AKAPs share

phosphatases to environments where the enzymes control the phosphorylation state of neighboring substrates [446]. A-kinase anchoring proteins bind to diverse enzymes, such as protein kinases PKA[87] and PKC, protein phosphatases, and phosphodiesterases [447]. AKAP coordinates different signaling enzymes according to the binding partner.[88]

AKAPs contribute to *spatial regulation* of signaling events, targeting the enzyme to specific sites (plasmalemma, mitochondria, cytoskeleton, or centrosome), where they are located.[89] Within a site, a given AKAP can link to diverse substrates. Different AKAPs within a given site can assemble distinct signaling complexes.[90] The displacement of enzymes into and out of these complexes contributes to the *temporal regulation* of signaling events. AKAPs can form complexes, including enzymes for both signal transduction and signal termination. These loci then regulate the forward and backward steps of a given signaling process. The location of an AKAP complex can be modulated by competition between binding partners, for example, between an enzyme and a component of the cytoskeleton. It can also be modulated by AKAP phosphorylation. The recruitment or release of AKAP-binding partners can alter the response to signals or change the location of a signaling complex to yield a dynamic localization and reorganization of AKAP complexes.

The muscle-specific A-kinase anchoring protein (mAKAP) forms complexes with PKA, phosphodiesterase PDE4D3 and guanine nucleotide-exchange factors for GTPase Rap1 Epac1 [451]. In cardiomyocytes, assembling occurs at the perinuclear membrane. PDE4D3 is an adapter for Epac1 and ERK5. Phosphorylation of PDE4D3 by mAKAP-associated ERK favors cAMP production and subsequent PKA and Epac1 activation. Phosphorylation of PDE4D3 by PKA reduces cAMP concentration and Epac1 suppression of mAKAP-associated ERK5 activity, as ERK5 induces cardiomyocyte hypertrophy.

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common properties. AKAPs contain a PKA-anchoring domain. Most mammalian AKAPs, indeed, bind protein kinase-A2. AKAPs are able to form complexes with different signaling molecules.

[87] Protein kinase-A1 in lamellipodia of migrating cells phosphorylates α4-integrins, the cytoplasmic domain of which acts as AKAP specific to PKA1 located in cell protrusions [448].

[88] The same AKAP, AKAP79/150, can bind to different types of ion channels, AMPA-type glutamate receptors and M-type potassium channels, and thus regulate their activities using different enzymes, PKA, PP2B, and PKC, in hippocampal (PKA and PP2B are active, PKC inactive) and superior cervical ganglion neurons (PKA and PP2B remain inactive, PKC active) respectively [449].

[89] Different AKAP types may have specific cytoplasmic distribution.

[90] PKC interacts with AKAP79, AKAP350, AKAP-Lbc108, etc., PKN with AKAP79, and PKD with AKAP-Lbc108 [450].

4.10.2 Annexins

Annexins are Ca^{++}-regulated proteins. Once bound to the cell membrane with calcium ions (Ca^{++}-dependent linkage) or certain membrane phospholipids via membrane-binding domain,[91] annexins provide Ca^{++} signaling, and thus regulate many cellular processes. Annexins indeed organize membrane domains and interact with proteins. They are involved in membrane–cytoskeleton attachments, endocysis, and regulation of ion fluxes across membranes [452].

Annexins bind to many cytosolic proteins, such as Ca^{++}-binding proteins.[92] Annexins not only act inside the cells, but also have extracellular functions. For example, annexin-A1 inhibits leukocyte extravasation. On the vascular endothelium, annexin-A2 is a co-receptor for tissue plasminogen activator and plasminogen, inducing fibrinolysis. Annexin-5 might mask the membrane phospholipids used by coagulant factors in the clotting cascade.

Annexins-2, -4, and -6 modulate plasmalemmal Cl^- channels and sarcoplasmic reticulum Ca^{++} channels. In association with S100A10, annexin-2 interacts with the Na^+ channel, the two-pore acid-sensitive K^+ channel-1 (TASK1), and TRPV Ca^{++} channels.

Annexin-1 might merge opposing membranes of the invaginating bud to close the neck of the nascent vesicle and allow scission from the plasmalemma. Moreover, annexin-1 can act on vesicle fusion in the presence of Ca^{++}. EGF stimulation of EGF receptor tyrosine kinase leads to formation of a class of endosomes,[93] EGFR-containing multivesicular endosomes, which requires phosphatidyl inositol 3-kinase and annexin-1[94] [453].

Annexin-2 is a component of the F-actin end, which propels newly formed endocytic vesicles from the membrane to the cytoplasma (actin-dependent transport). Annexin-2 is also involved in membrane raft formation, especially in the smooth muscle cells. Annexin-2 forms a complex with caveolin-1 and cholesteryl esters.

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[91] Annexin-A2 binds directly to phosphatidylinositol(4,5)bisphosphate. This association stabilizes actin assembly sites on the cell membrane.

[92] Annexins-1 and -2 interact with S100A11 (or S100C) and S100A10 (or p11). Annexin-A7 binds to sorcin, annexin-A11 to S100A6 (calcyclin).

[93] Multivesicular endosomes that are composed of activated EGFR are distinct from endosomes labeled by lyso-bisphosphatidic acid.

[94] Annexin-1 is phosphorylated within EGFR-containing endosomes. Annexin-1 mediates vesicle formation after EGF stimulation (and not protein selection and sorting). EGF or EGFR selection for vesicle inclusion and degradation is done via endosomal sorting complexes required for transport. Annexin-1 can reduce EGF signaling because it can remove the catalytic domain of EGFR.

4.11 cAMP Signaling

Cyclic adenosine monophosphate is a second messenger produced by adenylyl cyclase, which controls numerous cellular functions. The diversity of action of this diffusible second messenger depends upon its location and subsequent metabolism in discrete subcellular compartments. The spatiotemporal regulation of cAMP is indeed associated with nanodomains immediately beneath the plasmalemma. The compartmentation of second messenger cAMP allows the spatial segregation of cAMP signaling events. The subplasmalemmal cAMP signaling module contains the A-kinase anchoring protein gravin, phosphodiesterase-4, and protein kinase-A [454]. This multiprotein complex regulates the activity of ion channels, plasmalemmal receptors, and enzymes. cAMP signaling pathways require many components, as cAMP effectors induce divergent responses. The targets of cAMP include: (1) cAMP-dependent protein kinase-A (PKA), which mediates most of the actions of cAMP; (2) cyclic nucleotide–gated ion channels; and (3) GEFs Epac1 and Epac2, which regulate Rap1 activity. The concentration of cyclic adenosine monophosphate depends on adenylyl cyclases (ACase) and phosphodiesterases (PDE), particularly the PDE4 family. Cyclic adenosine monophosphate also acts at a family of four cAMP receptors (CAR1–CAR4).

cAMP subcellular compartmentation has been particularly described in cardiomyocytes [455]. In cardiomyocytes, the multiproteic complex formed by cAMP, PKAs, and PDEs attracts G proteins, adenylyl cyclases, A-kinase anchoring proteins, and phosphoprotein phosphatases. cAMP cascades are activated by G-protein-coupled receptors, which can lead to opposing effects. Moreover, the coupling between cAMP pathways and other signaling pathways participates in the distinct effects of cAMP signaling.

The three β-adrenergic receptors act via distinct cAMP signaling pathways in neonatal cardiomyocytes of mice. β1- and β2-adrenergic agonists[95] produce different effects on myocardium contractility[96] [456].

cAMP subcellular compartmentation, characterized by co-localization of components of the cAMP cascade, is implicated in β-adrenergic stimulation of voltage-gated L-type calcium channel [457]. A molecular complex composed of ryanodine channel RC2, FK506 binding proteins FKBP12.6, PKA, protein phosphatases PP1 and PP2A, and anchoring protein mAKAP controls the functioning of ryanodine channels of the sarcoplasmic reticulum. In failing

[95] The sympathetic nervous system acts via β-adrenergic receptors of cardiomyocytes and nodal cells. β-Adrenergic receptors are also involved in cardiac remodeling.

[96] Stimulation of β1-adrenergic receptors in β2-adrenergic receptor–knockout cardiomyocytes produces the greatest increase in contraction rate via protein kinase-A. Activation of β2-adrenergic receptors in β1-adrenergic receptor–knockout cardiomyocytes causes an initial increase in contractibility without requiring PKA, followed by a decrease in contraction rate involving coupling to a G protein.

human hearts, these calcium channel tetramers are phosphorylated by PKA and dissociated, hence, inducing defective channels [458].

4.11.1 Adenylyl Cyclases

Nine adenylyl cyclase sets catalyze the synthesis of the second messenger cAMP. Many cell types express more than one adenylyl cyclase species. However, Ca^{++}–calmodulin-stimulated adenylyl cyclases, ACase1, are restricted to neurons and to secretory cells.

Adenylyl cyclases are activated by Gs proteins and inhibited by Gi proteins. Adenylyl cyclases are regulated by different signaling effectors, particularly Ca^{++}. Like ACase1, ACase3 and ACase8 are also activated by Ca^{++}–calmodulin [459]. Ca^{++}-PP3 inhibits ACase9. Ca^{++} inhibits ACase6. Ca^{++}-inhibited ACase5 is mainly located in the striatum and the heart. ACase2, ACase4, and ACase7 are insensitive to Ca^{++}, but stimulated by protein kinase-C. ACase6 is inhibited by protein kinase-C. A soluble adenylyl cyclase (sAC), which is responsive neither to G proteins nor other regulators of membrane-bound enzymes, is stimulated by bicarbonates in a pH-independent process.

Like G-protein-coupled receptors and glycosylphosphatidylinositol-anchored proteins, adenylyl cyclases, especially Ca^{++}-regulated ones, can be found in membrane rafts and caveolae. Certain regulatory complexes are made of G-protein-coupled receptors, like β2-adrenergic receptors, with K^+ channels Kir3 and adenylyl cyclase [460]. These complexes are not disturbed by receptor activation or functioning of $G\alpha$ subunit. However, $G\beta\gamma$ interfere with the formation of the dopamine receptor-Kir3 channel complex, but not with the maintenance of the complex.

4.11.2 Phosphodiesterases

Cyclic nucleotide phosphodiesterases hydrolyze cAMP. Phosphodiesterases bind metal ions (Mg^{++}, Mn^{++}, and Zn^{++}) [461]. Calmodulin is a Ca^{++}-dependent regulator of phosphodiesterases. Certain members of the PDE family induce vasodilation[97] of pulmonary arteries[98] [462] and via aortic

[97] PDE4D also controls cAMP levels in the respiratory conduits. Parasympathetic control of the smooth muscle tone of the airways involves: (1) M1/M3-muscarinic receptors coupled to phospholipase-C and Ca^{++}, and (2) M2-muscarinic receptors inhibitory for adenylyl cyclase. Released acetylcholine from parasympathetic nerves induces smooth muscle contraction, with a control by M2-muscarinic receptors to limit the bronchoconstriction magnitude.

[98] In chronic hypoxia-induced pulmonary hypertensive rats, cAMP and cGMP PDE activities rise. The increased cAMP–PDE activity in the first pulmonary arterial branches and intrapulmonary vessels is due to PDE3, augmented cGMP–PDE in the main pulmonary artery to PDE1. PDE5 activity is higher in first pulmonary branches and intrapulmonary arteries.

endothelial cells[99] [463]. PDE2 are stimulated by cGMP. PDE4, PDE7, and PDE8 are specific for cAMP [464, 465]. Phosphodiesterases limit the diffusion of second messengers, subsequently affecting the activity of cyclic nucleotide-gated ion channels, cAMP-GEFs, and enzymes such as PKA and PKG.

PDE4 isoenzymes (PDE4A, PDE4B, PDE4C, and PDE4D) have multiple promoters and transcription factors [466]. PDE4s are phosphorylated (activated) by PKA. Activation of PDE4D in vascular smooth muscle cells increases its affinity for Mg^{++} and decreases cAMP concentration.

Multiprotein signaling complexes create focal points of enzyme activity that transduce actions of many signaling agents. PDE4D and PKA form a complex coordinated by A-kinase anchoring proteins. In the heart, muscle-selective A-kinase anchoring protein (mAKAP) assembles a cAMP signaling module, which includes PKA and PDE4D3 [467]. PDE4 is recruited by β-arrestin near G-protein-coupled receptors[100] [468].

4.12 cGMP Signaling

Cyclic guanosine monophosphate (cGMP) is a second messenger implicated in cell growth, smooth muscle cell relaxation, cardiovascular homeostasis, and inflammation, among other functions [469]. There are two well-known activators of cGMP signaling: the atrial natriuretic peptide[101] and nitric oxide. Natriuretic peptides bind to a transmembrane receptor, the particulate guanylyl cyclase (pGC).[102] Nitric oxide acts in smooth muscle cells via soluble guanylyl cyclase[103] (sGC).

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[99] Cultured bovine aortic endothelial cells only contain cGMP stimulated PDE2 and rolipram sensitive PDE4. L-arginine/NO/cGMP pathway leads to vasodilation.

[100] β2-Adrenergic receptor stimulation promotes production of cAMP. β-Arrestins desensitize β2-adrenergic receptor by binding to receptors and recruiting phosphodiesterases for cAMP degradation, hence restricting cAMP signaling magnitude.

[101] The atrial natriuretic peptide increases endothelium permeability and lowers arterial blood pressure in hypertension. B-type natriuretic peptide, another cGMP signaling generator, is used as a diagnostic index for heart failure.

[102] Particulate guanylyl cyclases belong to a plasmalemmal receptor family (GC-A—GC-G). pGCs have intracellular cyclase domain. GC-A binds atrial and B-type natriuretic peptides; GC-B links C-type natriuretic peptide.

[103] sGC belongs to the hem nitric oxide/oxygen–binding protein family H-NOx. H-NOx proteins bind both NO and O_2. However, sGC is selective for NO, with a NO-sensitive ferrous (Fe^{++}) state and NO-insensitive oxidized ferric (Fe^{+++}) state. Two sGC isoforms, $\beta1\alpha1$ and $\beta1\alpha2$, have similar enzymatic activities. sGC translocates from the cytosol to the plasmalemma after activation, implicating heat-shock protein-70 and possibly other ions (Ca^{++}, and Mg^{++}) and substances (ATP, and GTP).

At least three types of cGMP-binding proteic effectors: (1) cGMP-modulated cation channels, (2) cGMP-dependent protein kinases[104] (cGK), and (3) cGMP-regulated phosphodiesterases[105] (PDE), transduce the cGMP signal. cGK1 stimulates myosin phosphatase. cGK1 can also interact with inositol(1,4,5)trisphosphate receptor–associated cGK1β (IRAG), inhibiting intracellular Ca^{++} release. IRAG might be involved in the anti-platelet effects of exogene nitric oxide. Vasodilator-stimulated phosphoprotein (VASP) activated by cGK1 inhibits platelet adhesion to endothelial cells. cGK1 stimulates growth of vascular smooth muscle cells, stimulating sumoylation of the transcription factor Elk1.

Nitric oxide is an intercellular signaling molecule in most cell types, having diverse activities (blood flow regulation, neurotransmission, immune response; Sect. 9.5). Guanylyl cyclase-coupled receptor activation by NO binding reversibly triggers a conformational change that transduces the signal with a ligand-concentration dependent intensity. Downstream effectors include kinases, phosphodiesterases, and ion channels. Receptor deactivation follows NO unbinding.

Cyclic guanosine monophosphate is also linked to Rac GTPase for the regulation of actin cytoskeleton during cell migration. Constitutively active Rac increases the activity of transmembrane guanylyl cyclases in a cell subjected to a chemotactic signal [470]. Consequently, concentration in second messenger cGMP rises up to tenfold. Rac effector Ser/Thr p21-activated kinases

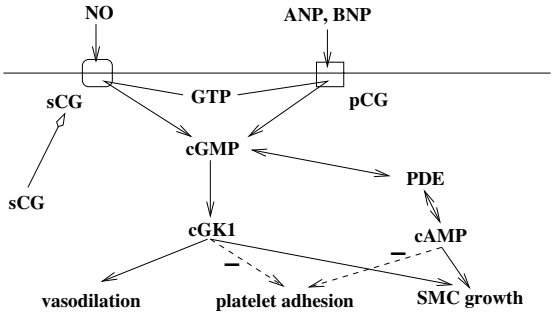

Figure 4.16. cGMP signaling pathways are involved in cellular functions (vasodilation, clotting, angiogenesis, etc.). Signaling is induced by natriuretic peptides and nitric oxide, via particulate guanylyl cyclase (pGC) and soluble guanylyl cyclase (sGC). Effectors are cGMP-dependent protein kinases-1 (cGK1) and phosphodiesterases (PDE; adapted from [469]).

[104] There are three known cGMP-dependent protein kinases: cytosolic cGK1, with two isoforms cGK1α and cGK1β, and membrane-bound cGK2. cGK1 regulates the vasomotor tone and the blood pressure. cGK2 acts in intestinal electrolyte transport and in bone formation.

[105] PDEs hydrolyze cyclic adenosine monophosphate and/or cyclic guanosine monophosphate.

(PAK1, and PAK2) bind and stimulate the activity of guanylyl cyclases. The Rac–PAK–GC–cGMP pathway is involved in fibroblast migration induced by platelet-derived growth factor and lamellipodium formation.

4.13 Signaling via Cell Junctions

4.13.1 Elastin-Laminin Receptor

Certain transmembrane receptors couple the cells to the extracellular matrix and can transduce applied loadings. Elastin strongly increases calcium influx and inhibits calcium efflux in aorta smooth muscle cells [471]. It also increases sodium influx in monocytes. κ-Elastin induces a vasorelaxation mediated by the elastin–laminin receptor (ELR) and endothelial NO production [472]. Cyclic stretch of the matrix of cell culture (30 mn duration), which mimics pressure pulse effects on the vessel wall, inhibits the expression of the proto-oncogene cFos, as well as the proliferation of coronary vascular smooth muscle cells grown on elastin matrices [473]. These effects depend on ELR signaling. In contrast, cells grown on a matrix do not exhibit changes in gene expression or proliferation when the matrix is stretched.

The elastin–laminin receptor is located on both endothelial and smooth muscle cells. Both laminin and elastin bind to cells via the elastin–laminin receptor. The elastin–laminin receptor is a heterotrimeric receptor that recognizes several hydrophobic domains on elastin, laminin, and collagen-4. It forms a transmembrane complex with other proteins.

4.13.2 Adhesion Molecules

Integrins, cadherins, and other adhesion molecules interact with growth factor receptors. Cell adhesion is necessary for activation of growth factor receptors, and growth factors are required to stimulate cell adhesion or motility. However, adhesion molecules can trigger ligand-independent activation of growth factor tyrosine kinase receptors, translating environmental cues into intracellular signals [474]. Conversely, growth factors can act on adhesion molecules for adhesion-independent signaling. The receptors for PDGF and VEGF, among others, are activated by integrins [475, 476]. The EGF receptor associated with E-cadherin can be tyrosine phosphorylated and then activates MAPK and Rac without EGF stimulation [477].

Rap1 GTPase induces the formation of an integrin-activation complex that binds to integrins. Rap1 GTPase can then mediate protein kinase-C activity associated with the integrin activation, using adapter talin, a PKC substrate. GTPase Rap1 provokes the migration of talin toward the plasmalemma. GTPase Rap1 and protein kinase-Cα then induce, via Rap1 effector RIAM and with recruited talin, the formation of an integrin-activation complex that binds to and activates integrins [478].

Integrin-mediated cell adhesion regulates numerous cellular responses. Integrins mediate either activation or inhibition of anchorage-dependent receptors. The integrins are indeed mainly involved in positive signaling via tyrosine receptor pathways. The integrins can also negatively regulate receptor tyrosine kinases [479]. Signaling cooperation exists between integrins and growth factor receptors. For instance, the $\alpha_v\beta_3$ integrin binds to the platelet-derived growth factor receptor and the vascular endothelial growth factor receptor-2. The collagen-activated integrin $\alpha_1\beta_1$ attenuates epidermal growth factor receptor signaling via the activation of a tyrosine phosphatase TCPTP. TCPTP regulates cell proliferation. Cell adhesion to collagen induces TCPTP translocation to the cell cortex, where TCPTP co-localizes with and is activated by α_1 integrin.

The protein tyrosine kinase Syk of the intracellular Src family is a signaling effector of immune receptors, which is also involved in integrin-mediated responses. Cross-talks between integrins and immune-receptor signaling pathways for cooperation then occur from the proximal stage. Both immune pathways and integrin-mediated signaling also work with same effectors, phospholipase PLCγ2, and adapter SLP76. Integrin-triggered signaling during interactions between lymphocytes and antigen-presenting cells implicates not only protein tyrosine kinase Syk, but also adapters such as DAP12. β3-Integrin clusters bound to fibrinogens on the platelet plasmalemma (whereas β1-integrins link to collagen) phosphorylate (activate) Syk.

Overexpressed p120-catenins disrupt stress fibers and focal adhesions, decrease RhoA functioning, and increase the activity of Cdc42 and Rac1, thereby promoting cell migration. p120Ctn binds the Rho exchange factor Vav2. p120Ctn interacts with a transcriptional factor, the protein Kaiso[106] [480]. Both p120Ctn and Kaiso transcripts are increased at the wound border compared with endothelial cells away from the wound border [481]. The C-terminal (COOH-terminal) Src kinase (CSK) is involved in VE-cadherin signaling [482]. The association of VE-cadherin and CSK in endothelial cells is increased with elevated cell density, inhibiting cell growth.

Signaling via Focal Adhesions

Focal adhesions are also important sites of signal transduction. Their components propagate signals arising from the activation of integrins following their association with ECM proteins, such as fibronectin, collagen, and laminin. The interaction of integrins with matrix ligands can either generate or modulate signals for motility, cell division, differentiation, and apoptosis [483]. Integrins and *paxillin* are implicated in signal transduction. Paxillin binds to β integrin cytoplasmic tail, Vinc, or other cytoskeletal and signaling proteins [484]. Pax recognizes integrin sequences distinct from α-actinin binding sites. Pax

[106] The p120Ctn-binding partner Kaiso is a member of the POZ-zinc finger family of transcription factors implicated in development and cancer.

binding is independent of FAK association, although both bind to the same region of β_1 integrin. Pax provides multiple docking sites for activated FAK and Src [485]. Various regulatory proteins, such as calpain-2, protein kinase-C, FAK, and Src, control the assembly of focal adhesion [146]. Focal adhesion disassembly involves microtubules and dynamin, which interacts with the focal adhesion kinase [486].

4.13.3 Signaling via Gap Junctions

Gap junctions allow communications between adjoining cells. The messenger ATP is released by multiple kinds of stimuli (mechanical stresses, osmotic pressure changes, rise in intracellular concentration of inositol trisphosphate, decay in extracellular calcium ion level, etc.). Manifold ATP release mechanisms include vesicular release, active transport via ABC transporters, diffusion via stretch-activated channels, voltage-dependent anion channels, pores opened by P2X7 receptors, and connexin hemichannels.

Connexin hemichannels, normally closed, are paths for ATP, NAD^+, glutamate, prostaglandins, etc. Hemichannels open following membrane depolarization and mechanical stimulation. Inositol trisphosphate activates hemichannel composed of connexin 43. Decreases in extracellular Ca^{++} and Mg^{++} levels potentiate or trigger the opening of hemichannels, releasing particularly ATP and glutamate. Connexin-32 and -43 have two and one calmodulin interaction sites, respectively. A Ca^{++}-binding site exists for hemichannels made of connexin-32. Connexin hemichannels open when the cytosolic calcium concentration rises [487]. However, $[Ca^{++}]_i$ elevation following release from cell storage compartments can close gap junctions.

4.14 Calcium Signaling

Among the second messengers, calcium regulates cellular functions in all cell compartments on timescales ranging from milliseconds to days [488]. The cell response to stimulation of certain receptor tyrosine kinases and G-protein-coupled receptors depends on the amplitude and duration of calcium influx. Calcium entry into the cell can be modulated to ensure the suitable response.

A small calcium proportion binds to effectors, such as annexins, calmodulin, synaptotagmin, S100 proteins, and troponin-C (Sect. 7.7). The calcium-signaling effectors are involved in cellular transport (operating in time of order $\mathcal{O}[10\,\mu s]$), metabolism (operating in time of order $\mathcal{O}[s]$), gene transcription (operating in time of order $\mathcal{O}[mn]$), cell fate and motility (operating in time of order $\mathcal{O}[h]$), and myocyte contraction (operating in time of order $\mathcal{O}[10\,ms]$), according to the location, timing, and calcium-bound molecules. Calcium ions are mostly linked to buffers. Ca^{++} buffers affect both the amplitude and the recovery time of Ca^{++} fluxes. Ca^{++} buffers have different expression patterns, motility, and binding kinetics. Calcium ions mediate or stimulate multiple cellular processes, such as the myocyte contraction.

An Example: Myocyte Contraction

The myocyte contraction illustrates the role of calcium ions in cell functioning. Several protein sets are involved in such a process: (1) plasmalemmal calcium channnels for influx of calcium ions from and efflux to the extracellular space; (2) calcium channnels in the membrane of intracellular calcium stores, mainly the sarcoplasmic reticulum, to ensure a sufficient amount of calcium into the cytosol for suitable activity; (3) second messenger to release calcium from its intracellular stores; (4) the mitochondrial machinery to synthesize the energy source ATP; (5) buffers; (6) sarcomere effectors; and (7) regulators such as kinases and phosphatases (Fig. 4.17). Other molecules participate in myocyte contraction, especially to coordinate the deformation of the cell compartments. Dystrophin associates with cytoplasmic syntrophin and forms the dystrophin–glycoprotein complex (DGC), which links plasmalemmal β-dystroglycan. The latter binds α2-laminin/merosin of the basal lamina. Dystrophin thus stabilizes the sarcolemma especially during contraction.

Instantaneous and Long-Lasting Responses to Calcium

Calcium signaling is characterized by instantaneous and long-lasting responses. Intracellular calcium control various events according to its spatial and temporal distributions and magnitude. Cytosolic and plasmalemmal calcium sensors, with high affinity for Ca^{++} (binding Ca^{++} even at small increment in concentration above resting levels) and distinct targets, transduce calcium signals into functional changes. The best known calcium sensor is ubiquitous calmodulin (Cam), a specific cytosolic receptor of Ca^{++}. Ca^{++}–calmodulin complex activate calmodulin-dependent protein kinases. Calmodulin regulates other targets, such as calcineurin (PP3) and phosphodiesterases.

Once released in the cytosol, Ca^{++} binds to calmodulin. The Ca^{++}–Cam complex then interacts with other proteins for *instantaneous response*. Instantaneous reaction begins once the cytosolic concentration $[Ca^{++}]_i$ rises ($[Ca^{++}]_i \sim 10^{-4}\,[Ca^{++}]_e$), and ends as soon as $[Ca^{++}]_i$ returns to its basic level. The release of cAMP is initiated by the occupancy of *G-protein-coupled receptors* by ligands. cAMP is produced from adenosine triphosphate after the activation of adenylyl cyclase by the receptor-activated *Gs protein* [489] (Fig. 4.18).[107]

cAMP travels to the cytosol where it accumulates at specific sites [491]. AKAPs thus target PKA to locations of cAMP production, where phosphory-

[107] The ligand-bound GPCR catalyzes the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the subunit of the associated G protein, which is activated to initiate or to inhibit signaling cascades. Gs activates adenylyl cyclase, which converts ATP to cAMP [490]. cAMP binds to PKA, the main intracellular effector of cAMP signaling, which is compartmented by A-kinase anchoring proteins. cAMP can also activate cyclic nucleotide-gated ion channels, phosphodiesterases and guanine nucleotide-exchange factors.

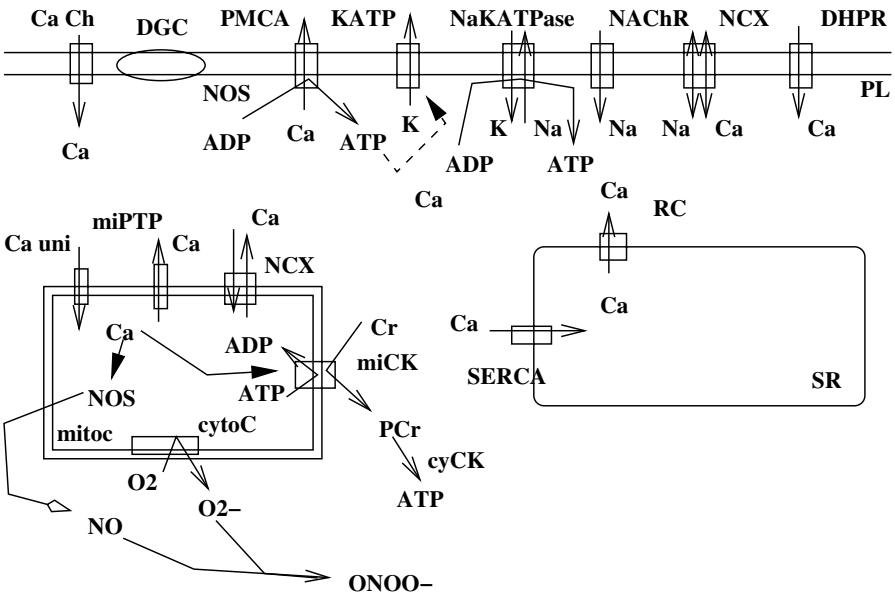

Figure 4.17. Calcium messengers for myocyte contraction. Calcium influxes are due to Ca^{++} channel (Ca Ch), indirectly by nicotinic acetylcholine receptor (NAChR), which drives Na^{+} influx, thereby stimulating Ca^{++} influx by the sodium–calcium exchanger (NCX), to dihydropyridine receptor (DHPR) of the plasmalemma (PL) and ryanodine receptor of the sarcoplasmic reticulum (SR). A fraction of imported calcium enters in mitochondria (mitoc) by Ca^{++} uniporter (Ca uni). It stimulates nitric oxide synthase (NOS) and mitochondrial creatine kinase (miCK), generating NO and ATP. ATP is indirectly exported from phosphocreatine via cytosolic creatine kinase (cyCK) for contraction and ion ATPase activity. Mitochondrial Ca^{++} is exported by NCX and mitochondrial permeability transition pore (miPTP). Cytosolic Ca^{++} efflux is done by Ca^{++} ATPases of the plasma membrane (PMCA) and the sarcoplasmic reticulum (SERCA), and by NCX. Na^{+} is exported by sodium–potassium ATPase. Nitric oxide can form with superoxide (O2−), produced by the respiratory chain, peroxynitrite (ONOO−) (adapted from [492]).

lation is confined to a subset of potential substrates. cAMP controls the flux of cell-entering Ca^{++} and Ca^{++} activates the synthesis of cAMP.

The lasting contraction of the vascular smooth muscle cells, triggered by Ca^{++} influx during the instantaneous response, is due to a *long-lasting response*. When $[Ca^{++}]_i$ increases, the Ca^{++}–Cam complex responsible for the transient reaction interacts with the membrane Ca^{++} pump to augment its functioning. Moreover, Ca^{++}-activated protein kinase-C (PKC) enhances the Ca^{++} pump efficiency. The Ca^{++} efflux thus compensates Ca^{++} influx [493]. Ca^{++} recycling is enhanced with increased submembrane $[Ca^{++}]$, which activates membrane-bound PKC (Fig. 4.18). Furthermore, signal-transduction activation of phospholipase-C (PLC) generates inositol trisphosphate (IP3)

and diacylglycerol (DAG) from phosphatidylinositol diphosphate (PIP2). IP3 induces Ca^{++} release from the endoplasmic reticulum (ER). DAG remains in the membrane and, as long as its membrane concentration is sufficient,[108] PKC is fixed by DAG to the membrane and is activated. PKC thus acts as a transducer during the long-lasting reaction.

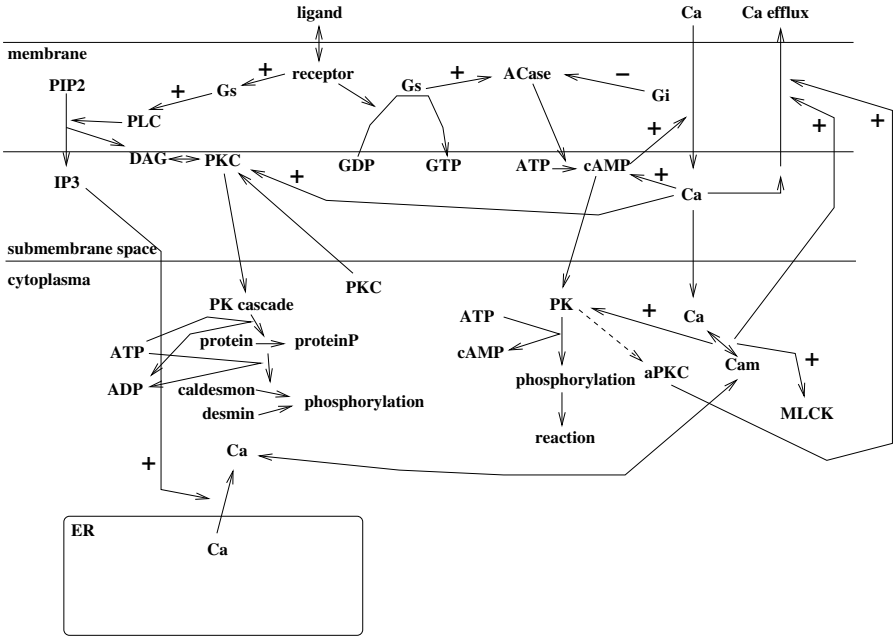

Figure 4.18. Second messengers (adapted from [493]). The activated receptor activates phospholipase C (PLC), which cleaves membrane-bound phosphatidylinositol bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 travels to its receptor on the surface of the endoplasmic reticulum, which is coupled to a calcium channel. Calcium (Ca) is thereby released from its ER stores. Cytosolic calcium is then available for binding to calmodulin (Cam) or various cytoskeletal proteins. Protein phosphorylations are catalyzed by Ca–Cam–dependent protein kinases. ATP is converted into cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (ACase), stimulated by stimulatory G protein (Gs) and inhibited by inhibitory G protein (Gi). cAMP activates cAMP-dependent protein kinases, such as protein kinase-A (PKA). DAG and Ca^{++} activate protein kinase-C (PKC) at the plasmalemma. Phosphorylation of cell proteins by PKC produces cell responses.

[108] Long duration fixation of PKC on the plasma membrane provides a memory effect, for subsequent amplified response. However, the delay between successive stimuli must not be too long.

4.14.1 Calcium Signaling Components

Several Ca^{++}-regulated proteins maintain low intracellular concentration of Ca^{++} ($[Ca^{++}]_i$) and couple changes in $[Ca^{++}]_i$ to physiological responses. They include Ca^{++} membrane carriers and Ca^{++} effectors. According to the Ca^{++}-binding site, Ca^{++} effectors are grouped into different families. EF-hand effectors include calmodulin, troponin-C, and the S100 proteins. Once bound to Ca^{++}, they regulate their cellular targets.

Different cues activate various types of Ca^{++} channels, such as receptor-operated channels, second messenger–operated channels, store-operated channels, thermosensors, and stretch-activated channels (Table 4.14). Several types of Ca^{++} channels belong to the transient receptor protein family, which regulates slow cellular processes (smooth-muscle cell contraction, and cell proliferation). Activated receptors yield entry in the cell of calcium from the extracellular space and the formation of second messengers that release calcium from its intracellular stores (Tables 4.15 and 4.16). Calcium effluxes are then induced by the combined action of buffers, pumps and exchangers (Tables 4.14, 4.16, and 4.17).

A set of substances (inositol trisphosphate, cyclic ADP ribose, nicotinic acid adenine dinucleotide phosphate, and sphingosine-1-phosphate) modulate the calcium release from the endoplasmic reticulum and other organelles (Fig. 4.19). Inositol(1,4,5)trisphosphate is produced from phosphatidylinositol(4,5)bisphosphate (PIP2) by different isoforms of phospholipase C. PLCβ is activated by G-protein-coupled receptors, PLCγ by receptor tyrosine kinases, PLCδ by calcium influx, and PLCϵ by Ras. IP3 stimulates its receptor IP3R at the membrane of the endoplasmic reticulum. IP3R is modulated by phosphorylation by calcium–calmodulin-dependent kinase-2 and protein kinases PKA, PKC, and PKG, after possible recruitment of corresponding scaffold proteins.

Phospholipase-Cγ has dual roles in regulating cellular calcium concentrations. It generates inositol trisphosphate, which releases calcium from intracellular stores. It binds the transient receptor potential channel TRPC3 and promotes its insertion into the plasmalemma for calcium influx. The transcription factor TFII-I outside the nucleus[109] inhibits calcium entry into cells by binding phospholipase-Cγ, antagonizing interaction of phospholipase-Cγ with the calcium channel TRPC3. This competition in favor of TFII-I for binding to PLCγ thereby suppresses surface accumulation of TRPC3 channels and hinders calcium influx across the plasmalemma [494]. Dephosphorylated TFII-I could free PLγ and elevate the density of plasmalemmal TRPC3.

Ubiquitously expressed extended synaptotagmin-like proteins (ESyt) bind Ca^{++} in a phospholipid complex of intracellular membranes (ESyt1) and plasmalemma (ESyt2, and ESyt3) [495]. They then serve as calcium sensors, with

[109] The cytosolic TFII-I fraction remains substantial after growth factor stimulation, although the amount of nuclear TFII-I increases.

multiple C2 domains. C2 domains are protein modules used as calcium and phospholipid-binding sites and/or as protein–protein interaction domains.[110]

Table 4.14. Ca^{++} signaling components: (1) Ca^{++} channels (Source: [488]). Ca^{++} channels are associated with channel regulators, such as triadin, junctin, sorcin, FKBP12, phospholamban, IP3R-associated PKG substrate IRAG, and IRBIT. The ryanosine channel-2 complex, indeed, includes stabilizers, such as FKBP12.6, calmodulin, and phosphatases PP1 and PP2a via scaffold proteins spinophilin and PR130, respectively, kinases attached by A-kinase anchoring proteins, and calsequestrin anchored by membrane-bound junctin and triadin. Ca^{++} pumps are characterized by their affinities (functioning thresholds), transport rates, and opening duration. Secretory-pathway Ca^{++}-ATPases could be responsible for Ca^{++} sequestration into Golgi compartments.

Voltage-gated channels	CaV1.1–CaV1.4 CaV2.1–CaV2.3 CaV3.1–CaV3.3
Receptor-operated channels	NMDA receptors (NR1, NR2A, NR2B, NR2C, NR2D) ATP receptor (P2X7) nACh receptor
Second-messenger–operated channels	Cyclic nucleotide–gated channels (CNGA1-CNGA4, CNGB 1, CNGB 3) Arachidonate-regulated Ca^{++} channel (IARC)
Transient receptor potential ion channels	TRPC1–TRPC7 TRPV1–TRPV16 TRPM1–TRPM8 TRPML, TRPNI
Inositol trisphophate receptors	IP3R1–IP3R3
Ryanodine receptors	RR1-RR3
Polycystins	PC1–PC2
Calcium pumps	Plasma membrane Ca^{++}-ATPases (PMCA1–PMCA4) Sarco(endo)plasmic reticulum Ca^{++}-ATPases (SERCA1–SERCA3) Golgi pumps (SPCA1–SPCA2)
Plasmalemmal Na^{+}/Ca^{++} exchangers	NCX1–NCX3
Mitochondrial Ca^{++} channels	Na^{+}/Ca^{++} exchangers Ca^{++} uniporter H^{+}/Ca^{++} exchanger Permeability transition pore

[110] Synaptotagmins, ferlins, multiple C2 domains and transmembrane regions proteins (MCTP) are components of three other families of proteins that contain several C2 domains.

4.14.2 Types of Calcium Signalings

Signaling pathways are characterized by their spatial distribution (celllular compartmentation), associated with involved molecular complexes, and temporal dynamics to keep the specificity of calcium signaling. Quick, brief, localized calcium transients are associated with fast responses, transient, repetitive, distributed calcium oscillations, which can generate calcium waves, trigger slow responses. Calcium flux oscillations of given amplitude and frequency are determined by the intensity of the stimulus. Furthermore, calcium is able to regulate its own signaling pathways by affecting the functioning of Ca^{++} channels.

Ca^{++} influx for long-term effect can be triggered by inositol trisphosphate on plasmalemmal IP3Rs and Orai1 channels (store-operated Ca^{++} release–activated Ca^{++} influx, Sect. 3.1.1).

Table 4.15. Ca^{++} signaling components: (2) receptors (Source: [488]). G-protein-coupled receptors are associated with G protein component subtype Gα Gq, G11, G14, and G16. They are controlled by regulators of G-protein signaling RGS1, RGS2, RGS4, and RGS16.

Components	Types
G-protein-coupled receptors	Muscarinic receptors (M1–M3)
	α1-Adrenoceptors (A–C)
	Endothelin receptors (ETA, ETB)
	Angiotensin receptor (AT1)
	Bradykinin receptors (B1, B2)
	Histamine receptor (H1)
	Serotonin receptors (5HT2A, 5HT2B, 5HT2C)
	Leukotrine receptors (BLT, CysLT1, CysLT2)
	Ca^{++}-sensing receptor (CaR)
	Prostanoid receptor (PGF2alpha)
	Thrombin receptor (PAR1)
	Bombesin receptors (BRS1, BRS2)
	Cholecystokinin receptors (CCK1, CCK2)
	Metabotropic glutamate receptors (mGlu1, mGlu5)
	Luteinizing receptor (LSH)
	Neurotensin receptor (NTS1)
	Oxytocin receptor (OT)
	Substance-P receptor (NK1)
	Substance-K receptor (NK2)
	Substance-B receptor (NK3)
	Thyrotropin-releasing hormone receptor (TRHR)
	Vasopressin receptors (V1A, V1B)
Receptor tyrosine-kinases	Platelet-derived growth factor receptors (PDGFRα, PDGFRβ)
	Epidermal growth factor receptors (ERBB1–ERBB4)

Certain activated G-protein-coupled receptors trigger calcium influx (Table 4.15). Both metabotropic glutamate receptor mGluR1 coupled to Gαq and mGluR5 coupled to Gα11 in the nervous system activate PLCβ, but trigger different types of calcium signaling via IP3R, generating a single Ca^{++} transient and an oscillatory pattern, respectively [488].[111]

Table 4.16. Ca^{++} signaling components: (3) Ca^{++} effectors (Source: [488]).

Phospholipases-C and inositol trisphosphate	PLCβ (1–4), PLCγ(1, 2), PLCδ (1–4), PLCϵ, PLCζ
Ca^{++}-binding proteins	Calmodulin Troponin-C Synaptotagmin S100A1–S100A12, S100B, S100C, S100P Annexin-1–annexin-10 Neuronal Ca^{++} sensor Visinin-like proteins (VILIP-1–VILIP-3) Hippocalcin, recoverin Kv-channel–interacting proteins (KchIP1–KchIP3) Guanylate-cyclase-activating proteins (GCAP1–GCAP3)
Ca^{++}-regulated enzymes	Ca^{++}–Cam-dependent protein kinases (CamK1–CamK4) Myosin light chain kinase (MLCK) Protein kinase-C (PKCα, PKCβ1, PKCβ2, PKCγ) Phosphorylase kinase IP3 3-kinase Protein phosphatase (calcineurin) cAMP phosphodiesterase (PDE1A–PDE1C) Adenylyl cyclases (ACase1, ACase3, ACase5, ACase6, ACase8) Nitric oxide synthase (eNOS, nNOS) Ca^{++}-activated proteases (calpain-1, calpain-2)
Ca^{++}-sensitive ion channels	Ca^{++}-activated K^+ channels (small SK, intermediate IK, Large-conductance BK channels) Cl^+ channel (HClCA1)

[111] In pancreatic acini, muscarinic receptors, which are more sensitive to the inhibition of RGS, induce small, localized Ca^{++} transients, and cholecystokinin receptors large, distributed Ca^{++} transients. Among other GPCRs, bradykinin receptors and neurokinin-A receptors give large, rapid calcium influxes, lysophosphatidic acid receptors, thrombin receptors, histamine receptors trigger small, slow, persistant calcium fluxes.

Scaffold spinophilin binds actin, regulators of G-protein signaling and G-protein-coupled receptors. Bound to GPCRs, it reduces the intensity of calcium signaling by GPCRs, such as α1B-adrenergic receptors [496]. In opposition, scaffold protein neurabin, which does not bind to α1B-adrenergic receptors, increases the intensity of calcium signaling by α1B-adrenergic receptors. Spinophilin prevents binding of RGS2 to cytosolic neurabin. Neurabin binds RGS2, which is thus removed from GPCR. RGS2 inhibits calcium signaling by GPCRs, especially α1B-adrenergic receptors. Conversely, neurabin hinders binding of RGS2 to spinophilin associated with GPCR. Therefore, spinophilin and neurabin act as a functional pair of antagonist regulators that tune the intensity of calcium influx by GPCRs (Fig. 4.20).

4.14.3 Calcium and Nervous Control of Blood Circulation

The activity of the cardiovascular system is controlled by the nervous system. Neuron activities depend on the temporal feature of the calcium signal (Table 4.18). Local increases in intracellular calcium trigger neurotransmitter release less than 100 μs after Ca^{++} influx. Ca^{++}-binding synaptotagmin acts as a Ca^{++} sensor for fast neurotransmission [223]. Members of the neuronal calcium sensor family are involved in manifold neuronal signaling pathways. The mammalian set of neuronal calcium sensors contains NCS1,[112] three visinin-like proteins (VILIP), neurocalcin, hippocalcin,[113] recoverin,[114] three guanylyl cyclase-activating proteins (GCAPs),[115] and four voltage-gated potassium

Table 4.17. Ca^{++} signaling components: (4) transcription factors and buffers (Source: [488]).

Transcription factors	Nuclear factor of activated T-cells (NFATc1–NFATc4) cAMP response element-binding protein (CREB) Downstream regulatory element modulator (DREAM) CREB-binding protein (CBP)
Cytosolic buffers	Calbindin, calretinin, parvalbumin
Endoplasmic reticulum buffers	Calnexin, calreticulin, calsequestrin, GRP

[112] NCS-1 regulates phosphatidylinositol 4-kinase-3β, as well as many other proteins.

[113] Hippocalcin is expressed at high levels in hippocampal pyramidal neurons and moderately in certain other neuron types.

[114] Recoverin is expressed only in the retina.

[115] GCAPs exist only in the retina. They are involved in light adaptation during phototransduction.

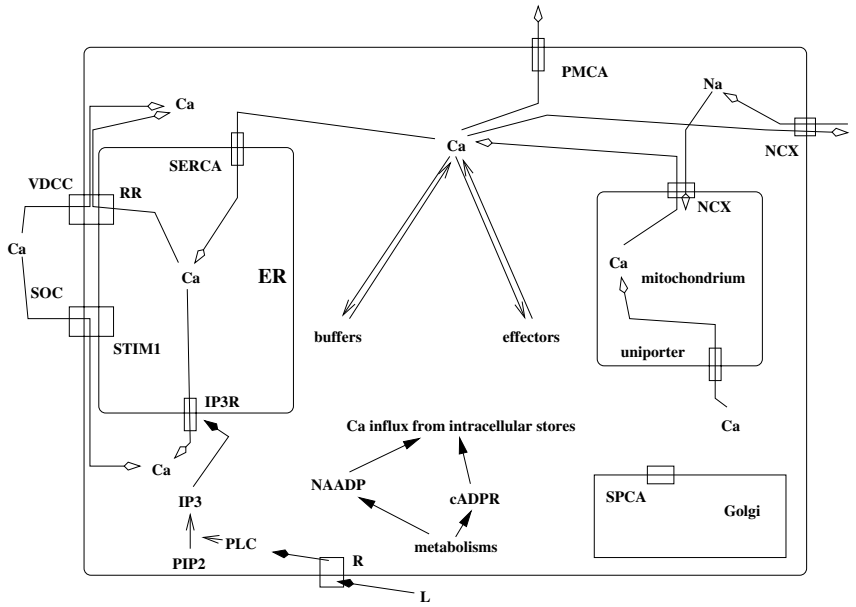

Figure 4.19. Calcium activities in cells. Ligand (L)-bound receptor (R) causes the entry of calcium from the extracellular space and the formation of second messengers, such as inositol trisphosphate (IP3), cyclic ADP ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP), and sphingosine-1-phosphate (S1P). The second messenger releases calcium from its intracellular stores, the endoplasmic reticulum (ER; sarcoplasmic reticulum in the myocyte). IP3 is formed from phosphatidylinositol bisphosphate (PIP2) by different isoforms of phospholipase C, and targets its receptor IP3R. Metabolism-linked messengers cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), generated by ADP ribosyl cyclase from NAD and NADP, respectively, favors Ca^{++} release from its stores. Ca^{++} can also be transported into the cytosol via either ryanodine receptor (RR) associated with plasmalemmal voltage-dependent calcium channels, or by plasmalemmal, non-excitable store-operated channels (SOC; Orai1 channel) coupled to Ca^{++} sensor stromal interaction molecule-1 (STIM1) in regions of the endoplasmic reticulum close to the plasmalemma. Most of the intracellular calcium binds to buffers; the remaining part targets effectors. Afterward, calcium ions leave its effectors and buffers and is removed from the cell by various exchangers and pumps. Na^+/Ca^{++} exchanger (NCX) and plasma-membrane Ca^{++}-ATPase (PMCA) expell Ca^{++} in the extracellular medium, and sarco(endo)plasmic reticulum Ca^{++}-ATPase (SERCA) pumps fill the stores. Mitochondria participate to the recovery. They quickly sequester Ca^{++} via uniporters; Ca^{++} is then slowly released into the cytosol to be extruded by SERCAs and PMCAs. (Source: [488]).

channel-interacting proteins (KChIP1–KchIP4), with several spliced KChIP isoforms.[116]

[116] Members of the KChIP family are characterized by: (1) differential expression according to the neuron type and regions for a given neuron type (hippocampus,

The cardiomyocyte permanently bears calcium influxes and effluxes for contraction and relaxation. Adaptative responses associated with modifications in gene transcription then require changes in the calcium signaling pathway. Calcium ions act indirectly on transcription factors. For example, the effector PP3 dephosphorylates the transcription factor nuclear factor of activated T cells NFAT3, which can enter into the nucleus to induce gene transcription, in opposition to the PI3K pathway which inhibits glycogen synthase kinase-3, thus inactivating NFAT3 (Sect. 7.7.7). Elevation of cytosolic

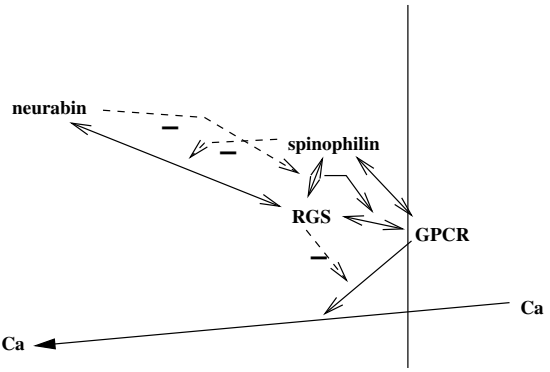

Figure 4.20. A functional pair of antagonist regulators of GPCR-triggered calcium influx. Both spinophilin and neurabin bind regulators of G-protein signaling RGS2, which hampers calcium influx by G-protein-coupled receptors. GPCR-bound spinophilin prevents RGS2 binding to cytosolic neurabin, thus inhibiting calcium influx. Conversely, neurabin hinders RGS2 binding to spinophilin, removing RGS2 away from GPCR, and thereby favoring GPCR-driven calcium influx.

Table 4.18. Time scales of calcium-regulated processes in neurons (Source: [223]).

Neurotransmission	< 1 ms
Channel activity	> 1 ms
Short-term plasticity	> 100 ms
Long-term potentiation	< 10 s
Long-term depression	> 10 s
Gene expression	> 10 s

cortex striatum, and cerebellum); and (2) differences in target and location. They augment Kv4 expression, shift the activation voltage threshold, slow the channel inactivation, and accelerate the recovery rate from inactivation. KChIP3, also called calsenilin, interacts with presenilin, like KChIP4, and amyloid precursor protein. It alters Ca^{++} release from the endoplasmic reticulum. KChIP3 is a transcriptional repressor, known as a downstream regulatory element antagonistic modulator (DREAM) at low Ca^{++} concentration. Ca^{++} binding to KChIP3 hampers KChIP3 binding to the downstream regulator element (DRE) of the gene promoter.

calcium levels can be caused by angiotensin-2 and endothelin-1 via GPCR (Gq), and phospholipase-Cβ which generates inositol trisphosphate. Diacylglycerol can target, via protein kinase-D, histone deacetylases and could recruit the mitogen-activated protein kinase pathway to activate the cAMP response element-binding protein.

The adrenergic pathway enhances calcium signaling via adenylyl cyclase, which produces cAMP. The latter leads to phosphorylation by PKA of calcium channels CaV1.2 and ryanodine receptors-2, as well as phospholamban which inhibits SERCA channels. Calcium ions are involved in cardiac hypertrophy and congestive heart failure. Decay in SERCA activity, at least partially due to enhanced inhibition by phospholamban, is associated with a decline in β-adrenergic signaling.

4.15 Mechanotransduction

Mechanotransduction occurs at the endothelium plasmalemma, as well as within the blood vessel and cardiac wall (Sect. 9.5; Fig. 4.21). Nowadays, numerical simulations deal with fluid-structure interaction because of the strong coupling between the blood dynamics and the compliant wall mechanics.[117] Although the blood flow simulations in any explored segment of the vasculature are carried out in a deformable fluid domain, the numerical results remain questionable because: (1) the material constants are most often not known in vivo, and (2) the vessel wall is assumed to be a more or less passive material.[118] The blood vessel wall is a living tissue that is able to quickly react to the load applied on it by the flowing blood. In a given region of a blood vessel segment, the endothelial and smooth muscle cells sense the large-amplitude space and time variations in small-magnitude wall shear stress and

[117] Blood vessel wall deformability allows the transient storage of blood in the elastic arteries (close to the heart) during the ventricular ejection (Chap. 8). Consequently, the blood permanently flows in the arterial tree downstream from the heart during the cardiac cycle. The highly deformable venous compartment plays the role of blood reservoir, adapting the operative blood volume to the body needs. Artery collapsibility is used to measure the arterial blood pressure in the systemic circulation (Part II). Vein collapsibility is targeted by treatment of dysfunctional superficial veins of the lower limbs. The artery distensibility allows the propagation of the pressure wave, which runs from the heart toward the microvessels. The wall displacements determine the local time-dependent size of the blood vessel lumen.

[118] The size of the computational domain depends on the controled motions of the blood vessel wall. In addition to the flow governing equations (Conservation Equations in Concluding Remarks) coupled to the equations of the wall mechanics, the set of equations to be solved can incorporate the equations that describe the biochemical reaction cascades triggered by stresses imposed by the flowing blood on the flexible wall.

wall stretch generated by the large-magnitude blood pressure. These cells respond with a short-time scale to adapt the vessel caliber according to the loading, especially when changes exceed the limits of the usual stress range. The mechanotransduction pathways determine the local vasomotor tone, i.e., the lumen bore of the reacting blood vessel, hence affect the wall deformation, taking into account the short-term adaptation to stresses applied at its wetted surface.

Various cell types react to loading with a cell production that depends on the stress direction. Several investigator teams have developed bioreactors (Sect. 10.3.3) that comprise microgrooved silicone cell-growing surfaces, which are subjected to stretching. Cultures of confluent, elongated, aligned cells are then obtained. The expression of atrial natriuretic peptide, connexin-43, and N-cadherin in cardiomyocytes increases when the cell is subjected to strain perpendicular to the main cell axis imposed by microgrooves [497]. However, the expression of these proteins do not significantly vary with respect to unstretched cells when the loading direction is parallel to the main cell axis. When the stretching direction is similar to the microgroove axes, tendon fibroblasts growing on microgrooves take on elongated shape according to the microgroove direction. When the stretching direction is oriented at 45 and 90 degrees with respect to microgrooves, tendon fibroblasts do not change shape. Whatever the stretching direction, α-actin expression by tendon fibroblasts is upregulated in response to 8% cyclic uniaxial stretching [498]. However, the closer the stretching direction to the microgroove axes, the higher the α-actin production. The longer the stretching duration, the greater the α-actin synthesis. Mesenchymal stem cells differentiate and adapt their protein synthesis according to the mechanical loading in bioreactors [499].

Mechanical stretching of cultured cardiomyocytes, which leads to cardiomyocyte hypertrophy, activate angiotensin-2 type 1 G-protein-coupled receptor AT1R [500],[119] The release of angiotensin-2 by the stimulated cardiomyocytes can mediate a ligand-dependent stretch-induced response (autocrine control) [501]. Angiotensin-2 acts as an initial mediator of the stretch-induced hypertrophic response.

Mechanotransduction at the endothelial plasmalemma drives many cell responses (Tables 4.19 and 4.20; Sect. 9.5). Heterotrimeric G protein subunits Gαq/α11 and Gαi3/αo are activated within 1 second of flow onset, providing the earliest mechanochemical signal transduction events in sheared endothelium [202]. Certain amino acids of transmembrane helices interact to lock the receptor in an inactive state. The receptor activation results from motion of its transmembrane helices embedded in the lipid bilayer solvent.

Mechanical stress across the plasmalemma of endothelial cells can directly activate G-protein-coupled receptors. Hemodynamic shear stresses applied to the plasmalemma of endothelial cells change the conformation of bradykinin-

[119] Mechanical stretch induces association of the AT1 receptor with Janus kinase-2, and translocation of G proteins into the cytosol.

B2 G-protein-coupled receptor, independently of ligand binding [502]. The response time to stimulation by shear stress is about 80 s. Hypotonic stress

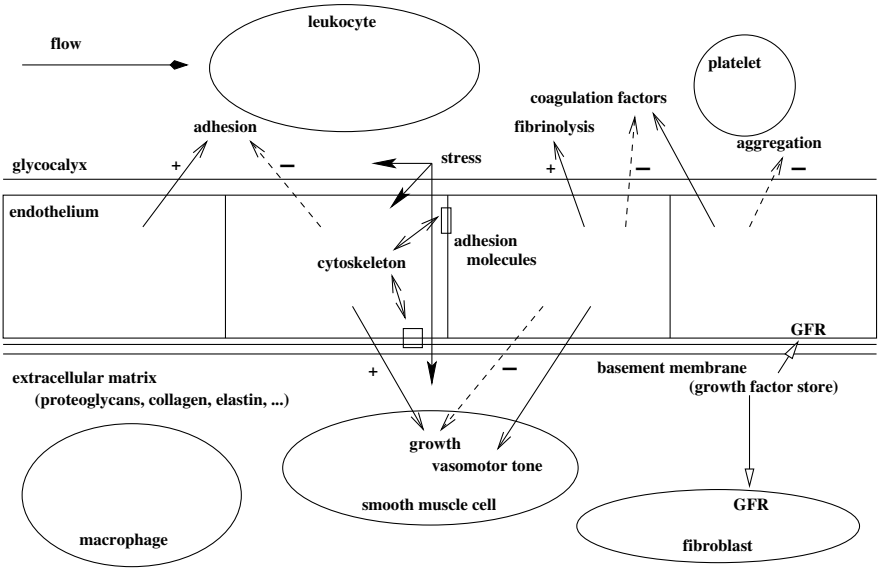

Figure 4.21. Mechanotransduction and interactions between vascular cells. Time-dependent three-dimensional shearing (torque), stretching (tension), and bending result from loading applied by the flowing blood on the vasculature walls. Shear stress at the wall has a low magnitude but strongly varies both in magnitude and direction during the cardiac cycle (Part II). Pressure applied on the vessel wall by the blood has high magnitude and undergoes large amplitude variations during the cardiac cycle. The mechanical loading sensed by the endothelium is transduced into chemical signals that allows the endothelial cell to adapt. Furthermore, cues are sent to adjoining cells, especially smooth muscle cells, to regulate the vasomotor tone. Interactions between flowing molecules (clotting factors) and cells (leukocyte, or thrombocyte) and the endothelium to regulate blood coagulation, cell extravasation and aggregation on the one hand (Chap. 9), and between the extracellular matrix, mural cells and the endothelium required for cell growth and tissue maintainance on the other hand are also summarized. Endothelial cells detect hemodynamic stresses via mechanosensors (adhesion molecules, mainly integrins, ion channels, and plasmalemmal receptors such as GPCRs and RTKs). Signaling pathways augment the activity of transcription factors. Time-dependent hemodynamic stresses on the endothelium as well as within the wall are implicated in: (1) the vascular tone (vessel bore) regulation; (2) wall adaptation (short-term) and remodeling (long-term); (3) tissue evolution (angiogenesis); and (4) vasculature diseases and tumors (Chap. 10 and Part II).

and membrane fluidizing agents also lead to a significant increase in receptor activity.[120]

Table 4.19. Elements of endothelial cell responses to blood flow.

Receptors	G proteins (Gs), RTK
Ion channels	Ca^{++}, K^+, Na^+, and Cl^- channels
Adhesion molecules	VE-Cadherin, PECAM1, integrin
Transport substances	Caveolin
Second messengers	IP3, cGMP
Vasoactive compounds	PGI2, NO, ET1, adrenomedullin
Growth factors	PDGF
MAPK production	ERK1/2
Gene expression	cFos, cJun

Table 4.20. Endothelial cell signaling pathways triggered by blood stresses (RTK: receptor tyrosine kinase; ET: endothelin; NO: nitric oxide; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated protein kinase; PI3K: phosphatidylinositol 3-kinase; FAK: focal adhesion kinase; AKAP: A-kinase anchoring protein; NOx: NAD(P)H oxidase; HSPG: heparan sulfate proteoglycan; LKlF: lung Kruppel-like factor; TxnIP: thioredoxin-interacting protein; ROS: reactive oxygen species; Sect. 9.5).

G proteins	Ras, PKC–ET, ERK
RTK	PI3K, MAPK
VEGFR2	Cbl–PLC–PI3K–ERK, PECAM1, VE-cadherin
Ca^{++} channels	NO
MSK^+ channels	NOx, TGFβ
VGK^+ channels	PKA/C–AKAP–SAP–Pyk
Na^+ channels	ERK1/2
VE-cadherin	PI3K–PKB (with VEGFR2, β-catenin, and SHP2)
PECAM1	ERK; NKκB; PI3K (VEGFR2)
Integrin	Rho–csk, VEGFR2–Shc, IKK–NKκB, Cdc42, Rac
Occludin	cell junction
FAK	Grb2–Src–Rho–MAPK
Caveolin	NOS, COx, PGI2; ERK1
HSPG	NO
NOx	ERK
elastin-laminin receptor	cFos
LKlF	NO, ET, adrenomedullin
TNFα	ASK1
ROS	ERK1/2
TxnIP (ASK1)	MAPK, VCAM1
NO	cGMP, Ca^{++} (MLCK), ERK1/2
ET	collagen, Ca^{++}

[120] B2 bradykinin G-protein-coupled receptor can be stimulated by ligand binding, shear stresses generated by fluid flow, plasmalemma stretches caused by osmolarity changes, or plasmalemmal fluidity variations switches from inactive to active conformation.

5

Transport and Cell Motion

Molecule transports regulate not only the composition of the plasmalemma, but also the interaction between the cell and its environment. Cell motility allows tissue adaptation to environmental cues.

5.1 Endocytosis and Secretion

Intracellular trafficking pathways can be described by compartments that exchange their membranes and cargo proteins in an organized sequence of events between cell constituents, the material fluxes being strongly controlled owing to various sources of signaling, especially signaling molecules in trafficking membranes.

5.1.1 Cell Transport Features

5.1.1.1 Membrane Curvature

Membrane curvature is an important event in the generation of vesicular or tubular structure in the plasmalemma. Endophilin, with its membrane-binding domain, such as the BAR domain, is required in the formation of highly curved membrane patches. These domains can interact with dynamin and actin regulatory proteins, forming narrow tubular invaginations of the plasmalemma.

The molecule endocytosis, as well as secretory transfer, occur by the transport of vesicles between donor and acceptor membranes. Although transport between cellular membranes is mostly due to spherical vesicles (diameter of 60–100 nm), tubules and larger transport structures exist, either from membrane budding or vesicle merging (e.g., fusion between vesicles, endosomes, and/or lysosomes, to form multivesicular body vesicles).

Membrane fusion is usual process in cell endo- and exocytosis. It is regulated by many proteins, such as membrane-anchored soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors (SNARE) and protein phosphatases. Conformational changes of these proteins at least could assist the formation of a fusion neck delineating a pore between the two membranes in contact. A fusion neck can be formed quickly, with a time scale of an order of magnitude of $\mathcal{O}(100\,\mathrm{ns})$ [503].

Endocytosis[1] is the uptake of nutrients and vesicle-mediated internalization of plasmalemmal lipids and associated proteins, especially receptors. Exocytosis corresponds to the transport of newly synthesized proteins from the endoplasmic reticulum, through the Golgi apparatus to the plasmalemma.

5.1.1.2 Exocytosis

Proteins destined for secretion or the plasmalemma are synthesized and inserted in the endoplasmic reticulum. In the endoplasmic reticulum, proteins fold properly owing to chaperones such as protein disulfide isomerase. However, misfolding can occur. Misfolded proteins are either retained in the endoplasmic reticulum or carried back into the cytosol for degradation by the proteasome.

Certain proteins are secreted owing to a specific stimulation using different export routes.[2] Molecule transport is easier through transmembrane channels and pores, the molecules interacting with their carriers. Specific binding sites can exist inside the channel, apparently leading to an increase in transmembrane transport.

5.1.1.3 Endocytosis

Endocytosis defines the internalization of molecules from the cell surface into membrane compartments, and then into vesicules for cellular trafficking. Endocytosis is required in a wide range of cellular functions, including nutrient uptake, regulation of growth factors, cell-surface homeostasis, synaptic transmission, and immune defense. Endocytosis is regulated by extracellular molecular signals.

Internalized material is transported from the cell cortex by the early endosome. Substances either are recycled to the plasmalemma by recycling endosomes, or conveyed by late endosomes and subsequently by lysosomes for degradation. The vesicles are delivered to target membrane, using molecular motors along microtubules or actin filaments.

[1] At the end of the nineteenth century, Metchnikoff observed particle ingestion by cells.

[2] They include proangiogenic lectins FGF1 and FGF2 (expelled by heat shock and shear stress, respectively), interleukin-1β (secreted by activated monocytes), macrophage migration inhibitory factor (released by monocytes in the presence of bacterial lipopolysaccharides), and galectin (during differentiation).

Early endosomes move from the cell periphery to the cell core, where they can mature.[3] However, endosomes do not necessarily mature. The mature endosomes fuse with late endosomes and can be targeted for degradation.

Endocytosis includes various transport types. Phagocytosis (φαγειν: to eat) refers to uptake of big particles, especially by macrophages; pinocytosis (πινω: to drink) refers to uptake of liquid and certain macromolecules. Fluid-phase pinocytosis deals with dilution of the molecule in the absorbed medium. Adsorptive pinocytosis refers to adsorption of the substance on the membrane with or without the receptor. *Endocytic pits* (membrane indentations) and vesicles (membrane invaginations) correspond to micropinosomes, macropinosomes, and phagosomes. Coated vesicles lose their coat when they form endosomes (larger vesicles), which mature before interacting by fusion with lysosomes. Lysosomes are vesicles with hydrolytic enzymes digesting the molecules conveyed by endocytosis. Lysosomes recycle both produced and incorporated molecules. Transcytosis from apical to basal membranes is an endocytosis followed by an exocytosis.

The endocytosis uses several mechanisms. The two major paths include clathrin- and membrane-raft endocytosis. Clathrin-mediated receptor endocytosis promotes signaling, whereas caveolar internalization is required for receptor turnover.

Molecule internalization can also be done by via structures that contain glycosyl-phosphatidylinositol–anchored proteins and fluid-phase markers[4] [504]. Internalization, after binding to the plasma membrane, can require membrane rafts and caveosome-like structures (devoided of caveolin)[5] [505].

Endocytosis and signaling are strongly coupled. Removal of receptors from the plasmalemma and subsequent internalization determines the signaling competence of receptors. Receptors are then either recycled to the cell membrane or transported to lysosomes for degradation. Reversible protein changes, such as phosphorylation and ubiquitination, drive this process. In the endosome, ubiquitin serves as a molecular tag on trafficking cargoes.

5.1.1.4 Endocytosis Events

Endocytosis begins at the plasmalemma with the binding between a molecule and its corresponding surface receptor (Sect. 9.2). The receptors are concentrated in membrane microdomains in association with a network of endocytic adaptor proteins. Ligand–receptor interactions, indeed, often need aggregation of numerous ligand–receptor complexes in a site where the membrane begins to invaginate, followed by cell absorption and intracellular transport.

[3] GTPase Rab5 characterizes an early endosome. During endosome maturation, Rab5 is replaced with Rab7.

[4] This pathway is not only caveola- and clathrin-independent, but also dynamin- and Arf6-independent.

[5] This pathway is caveola-, clathrin-, dynamin-2-, and Arf6-independent, but cholesterol-, and tyrosine-kinase–dependent.

The bulging leads to the vesicle formation. Membrane budding is initiated by protrusion of plasmalemmal patches with carrier proteins of the outer membrane and their extracellular ligands. Membrane coat proteins, such as clathrins, assemble and capture the ligand-bound receptors. An initial inward protrusion of the plasmalemma generates a coated invagination.

Actin polymerization regulators as well as actin assembly and stabilization promoters associated with the coat stimulate actin filament formation. These actin filaments drive slow inward movement of the coat. Scission molecules separate the budded membrane from the adjoining plasmalemma to form a coated vesicle. The vesicle moves rapidly into the cell, being coupled to the cytoskeleton for efficient delivery to the target organelle, and the coat components are removed for another cycle of vesicle formation. When it reaches its target organelle, the vesicle is tethered to the target membrane before fusing with it. In summary, vesicle history can be divided into several successive steps: initiation, assembling, budding, detachment, uncoating, displacement, docking, and fusion.

5.1.1.5 Transport Factors

The cytoskeleton is involved in the spatial distribution, motility, and morphology of endocytic compartments. The actin cytoskeleton intervenes during early endocytosis, and acts in endosome dynamics and distribution. The microtubules are required for the motility of early endosomes.

Kinesins act as nanomotors. These ATPases translocate cargoes along microtubules. Certain kinesins (kinesin-8, and kinesin-13) also control microtubule polymerization dynamics, as they are microtubule depolymerases. Kinesin-1 drives the movement of rough endoplasmic reticulum, Golgi apparatus, mitochondria, and secretory and endocytic vesicles. Kinesin-1 contains two motor heavy chains (KHC) and two light chains (KLC). KLCs interact with multiple cargo molecules. Many different KLC1 splicing isoforms exist, but a given kinesin-1 has a single KLC isoform, and thus a given targeted cargo.

Cytoplasmic microtubule-activated ATPase dynein is the primary nanomotor for transport of vesicles, organelles, proteins, and RNA cargoes from the cell periphery toward the nucleus along the microtubule cytoskeleton.[6] Among microtubule-associated proteins, dynactin moves progressively along microtubules in the absence of molecular motors. Dynein requires dynactin for functioning. The skating domain of dynactin is used by motor dynein to maintain longer interactions with microtubules during each binding [506]. Tight binding prevents premature dissociation of the dynein–dynactin–cargo complex from

[6] Dynein is a minus-end-directed microtubule nanomotor, which can be controlled either by regulation of its subunits or regulation via accessory proteins, such as dynactin. Nuclear distribution genes encode dynein chains and dynactin components.

the microtubule. Moreover, dynactin maintains a bind with the microtubule but does not impede the dynein velocity along the microtubule.

Motor protein complexes, such as sets of kinesins and dyneins, operate with better efficiency than single molecules [507]. Multimotor transport depends on the architecture of the molecule assembly and its ability to bind multiple microtubule sites. Complexes formed by dynein, its activator dynactin, and actin-related protein ARP1, moves in the two directions (toward plus and minus microtubule ends) along microtubules [508]. The direction switch for backward excursions allows flexible navigation through a crowded cellular environment.

Transport myosins are made of two major domains: a motor domain that binds F-actin and a tail domain for cargo binding. Myosins-1, -5, -6, -7, -10 are involved in cellular transport. Processive myosins, such as myosin-5, can remain attached throughout the sequential steps of their activity along actin filament. Myosin-5 is a two-headed (dimeric) motor protein responsible for intracellular transport on actin filaments and positioning of certain vesicles (secretory vesicles, lysosomes, peroxisomes) and organelles.[7] Distant cargo-binding domains regulate cargo type (cargo-specific receptors), attachment/detachment and destination [509]. Myosin-5 has functional similarities with kinesin and differs from myosin-2 activity. Myosin-5 functioning is mediated by Rab GTPases, which bind to myosin either directly or via adapters. The light chain domain serves as a mechanical lever arm, due to tilting of the myosin neck. The two heads maintain association with actin during displacement. At each step, the trailing head becomes the leading head. Another orientational change occurs without producing significant motion along actin. The lever arms probe their local environment prior to executing a full step [512].

Cell organelles are carried along microtubules by kinesin, then locally along actin by myosin-5a. Myosin-5a can travel according to a spiral path along actin. At intersecting filaments (either actin or microtubules), the nanomotor adapts to transport its cargo to the cell periphery [513]. Myosin-5a not only easily maneuvers at actin filament intersections, but also when encountering a microtubule, steps onto the microtubule and begins a one-dimensional diffusive search for a kinesin.

[7] At nanomolar calcium concentrations, myosin remains folded, whereas at micromolar calcium levels the ATPase is activated and myosin unfolds [510]. Myosin-5 hydrolyzes ATP to move toward the plus end of actin filaments. Strain between the two heads of a moving myosin-5 can modulate the ADP release rate. ADP release is rate limiting in myosin kinetics. In addition, the forward motion rate of the nanomotor depends on ATP concentration, whereas load-dependent backstepping can occur in the absence of ATP, as ATP binding is inhibited by molecular loading. This mechanical asymmetry can be explained by the strength of actin binding of a nanomotor head that is modulated by the lever arm conformation, myosin-5 functioning as a ratchet [511].

Myosin-6, the single nanomotor that moves along actin filaments toward the minus end, participates in endocytosis. It is recruited to clathrin-coated pits and uncoated endocytic vesicles, as well as liposomes in the presence of calcium. Myosin-6 links clathrin-coated pits via the adapter protein Dab2. Adaptor synectin,[8], used in internalization of plasmalemmal receptors, is involved in the association of myosin-6 with uncoated endocytic vesicles [514].

Annexin-2 is a ubiquitous Ca^{++}-binding protein for actin-dependent vesicle transport.[9] Annexin-2 is present both as a cytosolic monomer and a heterotetrameric complex at the outer cytoskeleton linked to the plasmalemma, interacting with receptors and ion channels. Annexin 2 is located in cell regions undergoing actin remodeling. It indeed reduces the polymerization rate of actin monomers in a dose-dependent manner [515]. Annexin-2 inhibits filament elongation at the barbed ends.

Substrate transport within the cell requires a cluster of subtances: assembling and docking proteins, coat components, adaptors, effectors, and regulators, which target, tether, and fuse membranes. Vesicle movement between the cell compartments involves containers that are composed of coat protein complex (CoP1, and CoP2; Fig. 5.1), clathrin, and caveolin, involved in the different steps of endocytosis and exocytosis. These coats can also be linked to adaptors to ensure efficient substrate selection and coordination with vesicle formation.

Rab proteins constitute the largest set of Ras GTPases, reflecting the complexity of intracellular transport.[10] Rab cascades regulate the four steps in vesicular transport to achieve specificity and directionality in intracellular transport: budding,[11] delivery,[12] tethering,[13] and fusion[14] with the membrane of the target compartment (Fig. 5.2). Rab proteins undergo a membrane insertion and extraction cycle, which is partially coupled to the switch activity between GTP- and GDP-bound states. GDP dissociation inhibitor (GDI) binds to and maintains Rab protein in the cytosol. GDI displacement factor (GDF)[15]

[8] Synectin is also called the GAIP interacting protein-C terminus (GIPC).

[9] Annexin-2 is also required for the formation of tight junctions.

[10] The greater the plasmalemma complexity, the larger the Rab protein number.

[11] During the carrying vesicle formation, the suitable transport and fusion molecular complex must be incorporated into the vesicle before scission from the donor membrane, involving Rab proteins.

[12] Vesicles are transported through the cytoplasm toward target membranes by using either actin-dependent motors, such as myosins, or microtubule-dependent motors, such as kinesins or dyneins. These nanomotors or motor adapters are Rab effectors. Rabs often interact with nanomotors via intermediary proteins.

[13] Vesicle tethering brings the vesicle and target membrane into close proximity for merging.

[14] Rabs affect vesicle fusion via soluble N-ethylmaleimide-sensitive factor attachment protein receptors.

[15] GDFs are integral membrane proteins that displace GDI from the Rab proteins for activation.

allows membrane attachment of Rab proteins. Membrane-bound Rabs are active and can be stimulated by guanine nucleotide-exchange factors and bind to their specific effectors. In the absence of efficient intrinsic guanine nucleotide exchange, Rabs indeed interact with effectors via guanine nucleotide exchange factors and GTPase-activating proteins, which promote the cyclic assembly and disassembly of Rab-containing complexes. Rab effectors couple membranes to the cytoskeleton, recruiting kinesin and myosin, initiate vesicle docking, and mediate membrane fusion. Active Rabs are incorporated into specific membrane domains by effector- and GEF-mediated positive feedback loops [516]. Active Rabs can then recruit additional effectors into these domains for cell transport. Rab–GEF effector complexes stabilize activated Rabs on membranes. After inactivation by specific GTPase-activating proteins, the GDP-bound Rabs can be extracted from the membrane by GDI and recycled back to the cytosol. Rab proteins regulate the dynamic assembly and disassembly of proteic scaffolds implicated in vesicle motions. Rab-regulated hubs are building blocks for vesicle formation based on Rab GTPases, which function as coding systems regulating the dynamics of specialized plasmalemmal patches, the so-called *membrome* [517].

Rab5 regulates endocytic membrane dynamics from the vesicle formation to the endosome fusion. Clathrin-coated vesicles possess Rab5 exchange-factor activity. RabGAP5 affects endosomal Rab5 effector EEA1 and blocks endocytosis via endosomes [518].

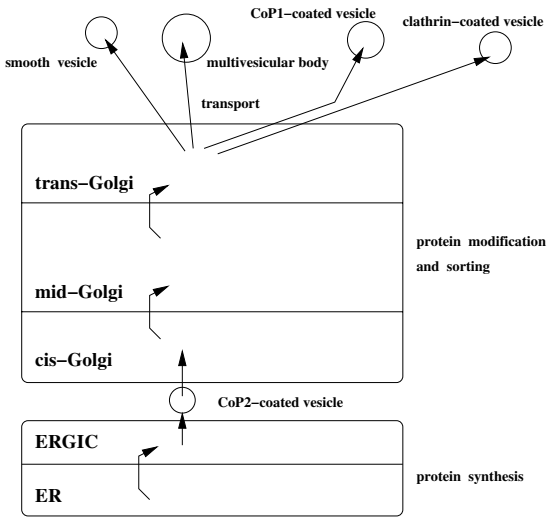

Figure 5.1. The Golgi apparatus receives proteins via transition vesicles from the endoplasmic–reticululm–Golgi intermediate compartments (ERGIC), a smooth region of the rough endoplasmic reticulum. The Golgi apparatus modifies proteins within its three compartments. The proteins are sorted for destination sites using different types of vesicles.

Rab GTPases and soluble N-ethyl-maleimide-sensitive factor attachment protein receptors, docking/fusion plasmalemmal proteins,[16] direct the transport of vesicles involved in endocytosis and exocytosis to their single destinations, SNAREs being required for membrane recognition and fusion. SNARE Proteins are divided into Q- and R-SNARE subgroups. Docking and fusion mediated by SNARE complexes are regulated by specific effectors that either promote or prevent SNARE assembly. There are several types of SNARE-binding proteins, such as *synaptotagmins*, which could regulate SNARE-mediated fusion, and *complexins*. These accessory proteins affect SNARE sorting and location. SNARE proteins are also regulated by protein kinases and phosphatases, and by other signaling proteins.

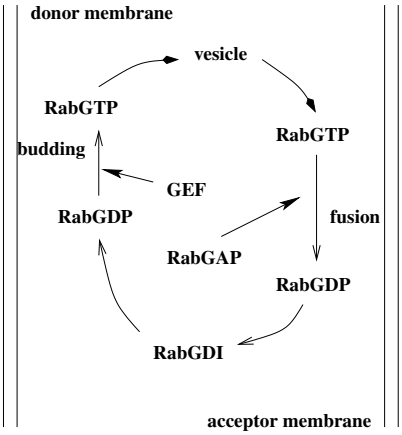

Figure 5.2. Regulation of vesicle transport by Rab GTPases (Source: [518]).

[16] Soluble N-ethylmaleimide-sensitive factor attachment protein receptors are important components of protein complexes that act as lipid mergers for membrane fusion between trafficking vesicles and target compartments. In particular, SNAREs form nanoclusters heterogeneously distributed in the plasmalemma, allowing docking of secretory vesicles. The stability of these nanodomains depends on steroid lipids, such as cholesterol. SNARE proteins form a family of 36 members in humans [519]. Many SNAREs reside predominantly or selectively in given cell compartments. Syntaxins-1, -2, -4, SNAP23, and SNAP25 are located at the plasmalemma, syntaxin-5, vesicle-associated membrane protein (or synaptobrevin) VAMP4 in the membrane of the Golgi apparatus. SNARE proteins involved in transport between two cell compartments are found in both compartment membranes, as well as in the associated vesicles. SNARE proteins clamp the membranes together and initiate fusion. Merged opposing membranes form an aqueous pore that connects the distal leaflets of the membranes in contact. After membrane fusion, SNARE complexes are disassembled and SNARE proteins are recycled owing to Aaa (ATPases associated with various cellular activities) proteins, N-ethylmaleimide-sensitive factor (NSF), and soluble NSF attachment proteins (SNAP cofactors).

Ras GTPases exist with their partners in the Golgi complex. Here, they are regulated with modes of activation and deactivation that differ from those at the plasmalemma.

Arf6 GTPase and its exchange factor, the ADP-ribosylation factor nucleotide site opener (ARNO), are involved in vesicle-coat formation and in actin cytoskeletal remodeling. Endosomes and lysosomes have an acidic luminal content induced by a proton-pumping vacuolar ATPase (V-ATPase). Arf6 and ARNO are recruited from the cytosol to endosomes owing to intra-endosomal acidification [520].[17] Hence, endosomal acidification promotes transport between early and late endosomes.

GEP100 (p100), a guanine nucleotide-exchange protein activator of the ADP-ribosylation factor, is partially colocalized with Arf6 in the cell periphery. It accelerates guanosine triphosphate binding by Arf6, which participates in protein transport near the plasmalemma, including receptor recycling.[18]

The cAMP–PKA pathway acts on many trafficking steps [521]. The cAMP–PKA pathway includes protein kinase-A, adenylyl cyclase, which synthesizes cAMP, adenylyl cyclase activators, such as Gα protein, phosphodiesterases which hydrolyze cAMP, and PKA-anchoring scaffold proteins. Protein kinase-A is actived when the catalytic and regulatory subunits dissociate in the presence of cAMP. The entire set of components is located on the plasmalemma. PKA recruits Arf1 to the Golgi complex. PKA phosphorylates certain SNAREs.

Protein kinase-C is recruited into cellular membranes for transport regulation. PKC is required in the transport of glucosaminoglycans from the Golgi apparatus to the plasmalemma. PKC is involved in carrier membrane fission from the trans-Golgi network.

Gα proteins are located in cellular membranes, particularly thoses of the Golgi complex. G proteins are involved in the regulation of the CoP1 transport and transport between the trans-Golgi network and plasmalemma.

Transport vesicles can be classified according to the components of the protein coat and transported molecules. Anterograde and retrograde transport is mediated by distinct sets of cytosolic coat proteins,[19] CoP1, and CoP2. Hence, three major kinds of coating proteins are involved in the transport between intracellular membranes and the plasmalemma: (1) the clathrin–adaptin complexes for endocytosis and vesicular trafficking between the Golgi, lysosomes, and endosomes; (2) the CoP1 complex for intra-Golgi and Golgi-to-endoplasmic reticulum displacement; and (3) the CoP2 complex[20] for vesicular motion from the endoplasmic reticulum to the Golgi stack.

[17] Arf1 is not recruited by an acidification-dependent mechanism.

[18] It is also involved in cell adhesion and cell migration, interacting with α-catenin, a regulator of adherens junctions and actin cytoskeleton remodeling.

[19] Coat proteins are heptameric complexes with β, γ, δ, and ζ subunits.

[20] Coat protein complex CoP2 is composed of: (1) Sar1 GTPase; (2) Sec23 and Sec24 (Sec23 is a Sar1-specific GTPase-activating protein; Sec24 selects transported substances); and (3) Sec13 and Sec31 (Sec13/31 has a structural role).

Sar1 GTPase, a member of the Ras family of small GTPases, recruits: (1) CoP2 vesicle to the membranes of the endoplasmic reticulum;[21] (2) Arf1, CoP1, and clathrin vesicles to the Golgi complex; and (3) plasmalemmal Arf6 phosphatidylinositol(4,5)bisphosphate kinase, Arl1, and ARFRP1 to the trans-Golgi network. Arf1 GTPase, involved in CoP1 coat assembly and recycling from Golgi stack to the endoplasmic reticulum, links with Rab1B, which modulates CoP1 recruitment to compartments of Golgi apparatus.[22]

Phosphorylated phosphoinositides are strongly implicated in endocytosis. Phosphatidylinositol(3)phosphate is found on the surface of endosomes, where it can recruit proteins. Among such proteins, certain ones coordinate the activity of the small GTPases Rab, some are involved in ubiquitination for subsequent lysosomal degradation, and others are required for multivesicular body formation. Phosphatidylinositol(4,5)bisphosphate is required in cell transport, especially in clathrin-dependent endocytosis of plasmalemmal proteins, such as nutrients and growth factor receptors. It is synthesized by phosphatidylinositol(4)phosphate 5-kinase in association with clathrin adaptor protein AP2. It also serves as a substrate for the synthesis of phosphatidylinositol(3,4,5)trisphosphate. Phosphatidylinositol 3-phosphate (PtdIns3P) or its metabolite PtdIns(3,5)P2 in endosomes is required for maturation of the transport of certain receptors assigned to recycling or degradation, at the microtubule-associated tubularization stage [523].

The *ubiquitin*[23] (Ub) is involved in signal transduction and endocytosis in particular [524, 525]. It acts on protein–protein interactions implicated in membrane fusion. Monoubiquitination causes the internalization of plasmalemmal receptors by clathrin-dependent or -independent mechanisms, according to the stimulation intensity and the ubiquitination status.[24] It is also required for the cellular transport associated with endosomal sorting.

Cargo partitioning into intralumenal vesicles of multivesicular endosomes usually involves protein ubiquitination, hepatocyte growth factor–regulated

[21] Transport from the endoplasmic reticulum to the Golgi complex requires assembly of the CoP2 complex at endoplasmic reticulum outlets. Cytosolic small CoP2-coated vesicles contain SNARE Sec22b necessary for downstream fusion [522].

[22] Arf1 also associates with Rab11A, dynamin-2, clathrin light chain-A, clathrin-binding protein HRS/HG, HRS/HG-interacting protein TSG101, and TSG101-interacting protein EAP30. Arf1 can form clusters with Rab1B/C, GEF TRAPPC4, Rab1 tether Grasp-55, TIP47, and CoP2 components Sec13R and Sec24C.

[23] The ubiquitin, activated by an ATP-dependent enzyme E1, is attached to target proteins by ubiquitin-conjugating enzyme E2 in association with a ubiquitin-protein ligase E3 to induce their degradation by a 26S proteasome. Ubiquitin-like proteins also control the activities of proteins. Ubiquitin-like proteins are activated by specific E1 and are transferred to their substrates by E2.

[24] Slight stimulations lead to clathrin-dependent endocytosis. Strong stimulations increase the level of receptor ubiquitination and favor membrane raft/caveola endocytosis.

tyrosine-kinase substrate, and endosomal sorting complexes required for transport. However, intralumenal vesicle sorting in fibril formation does not require hepatocyte growth factor–regulated tyrosine–kinase substrate [526].

Integrins[25] control the translocation of GTP-bound Rac to the plasma membrane, before Rac binding to effectors. Integrin–regulated Rac binding sites are within cholesterol-enriched membrane nanodomains. Integrins prevent the internalization of these plasmalemmal nanodomains, and hence control Rac signaling. This internalization is mediated by dynamin-2 and caveolin-1 [527].

Proteoglycans close to the external face of the plasmalemma participates to cell transport. The fibroblast growth factor FGF2 is secreted to transmit angiogenic signals via a ternary complex with FGF receptors and extracellular heparan sulfate proteoglycans [528].

5.1.1.6 Transport Sorting

Once membrane-bound vesicles enter the endosomal system, they are sorted for delivery to their specific destinations. Incorporation into the intraluminal vesicles of multivesicular bodies involves different mechanisms. Transport sorting is regulated by ubiquitination and by hepatocyte growth factor–regulated tyrosine-kinase substrate endosomal and endosomal sorting complexes required for transport ESCRT1, ESCRT2 and ESCRT3 [529].[26] Endosomal sorting complexes are necessary for transport select proteins to be included in vesicles. Another mechanism, which does not require direct ubiquitination exist for certain substances. The lipid-driven process of multivesicular body formation involves lipid lysobisphosphatidic acid [526]. Furthermore, protein processing can require a passage by intraluminal vesicles of multivesicular bodies.

The intracellular transport of substances deals not only with the flux of molecules from the extracellular spaces, but also with the transfer of compounds manufactured by the cell toward the targeted (intra- or extracellular) medium (Fig. 5.3). In both cases, the cell membrane must be crossed. The cell secretion from a donor organelle requires the budding of transport vesicles, their displacement, and the fusion of the vesicles with an acceptor membrane, once the vesicles loaded with a specific compounds have recognized the appropriate target. Large GTPase dynamin is recruited to coated pits before vesicle budding [530]. Dynamin binds to inositol lipids, constraining the membrane into tubular shapes. They form necks of vesicles and promote their scission from the membrane, via GTP hydrolysis.

[25] Integrins, involved in cell anchorage, regulate Rho GTPases. They also increase the membrane affinity for Rac, leading to RhoGDI dissociation and effector coupling (Sect. 2.2).

[26] Ubiquitin, used for degradation, is also a sorting tag on endocytic cargoes and a regulatory switch on endocytic adaptor proteins.

The vesicle-target membrane recognition requires the interaction of vesicle membrane and target membrane proteins. SNARE Proteins of the vesicle membrane (vSNARE) interact with target membrane ones (tSNARE). SNARE Proteins belong to the vesicle-associated membrane protein (VAMP) and syntaxin families.[27] Assembly of vSNARE–tSNARE complexes is modulated by Rab GTPases [531].

5.1.1.7 Receptor Endocytosis

Most receptors and ligands follow one of two distinct routes from the early endosome [105]. The intake path leads to late endosomes and lysosomes. It is required for nutrient uptake and receptor downregulation. The recycling route transfers unloaded receptors from the early endosome back to the plasma membrane. An additional retrograde transport connects the early endosome to the trans-Golgi network, Golgi apparatus, and endoplasmic reticulum.

The activity and recycling of membrane receptors need co-factors and regulators (Fig. 5.4). The low-density lipoprotein receptor–related protein (LRP) is a multiligand receptor. Sorting nexin-17 (Snx17) regulates the cell membrane level of LRP. Snx17 interacts with phosphatidylinositol(3)phosphate and binds to LRP, promoting LRP recycling in the early endosomes [532]. After ligand binding and phosphorylation of β-adrenergic receptors, recruitment of β-arrestin, and adaptor protein AP2[28] to the activated receptor allow the link of the receptor to clathrin-coated pits for internalization. Phosphoinositides(4,5)P2 in the plasmalemma is necessary for the budding of clathrin-coated vesicles. Phosphoinositide 3-kinase, which phosphorylates both proteins and lipids, is required in the endocytosis associated with the

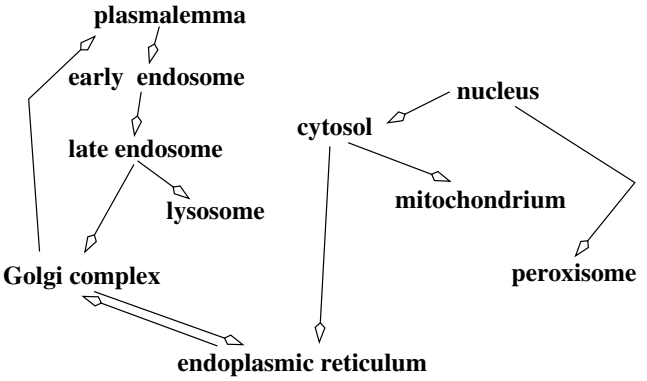

Figure 5.3. Transport between the main cell constituents.

[27] Neurotransmitter exocytosis involves syntaxins, SNAPs, and SNAREs.

[28] The adaptor complex AP2 is always associated with clathrin on the plasmalemma.

β-adrenergic receptor. Both protein and lipid kinase activities are involved in receptor endocytosis. Tropomyosin, a PI3K substrate, acts in the internalization of β-adrenergic receptors [533].

Signaling can be triggered at the plasmalemma or after receptor internalization from endocytic compartments [534]. Multiple plasmalemma receptors are ubiquitinated when they are stimulated by extracellular ligands. Ubiquitination also affects intracellular transport of receptors. Ubiquitination regulate receptor signaling by modulating the magnitude and duration of the signal. Ubiquitinated proteins are able to interact with other proteins, and, thus drive the different steps of the signaling pathway. Reciprocally, signaling regulates ubiquitination. Monoubiquitination can be required for receptor activation, receptor endocytosis, and lysosomal degradation.

Ligand binding to receptor tyrosine kinases activates the receptor to initiate the signaling cascade. The signaling lifetime is regulated, activated RTKs triggering negative feedback loops leading to receptor endocytosis and lysosomal degradation. This event requires the formation of large protein complexes that remove activated receptors from the plasmalemma. Certain ubiquitin ligases Cbl[29] (c-Cbl, and Cbl-b) especially downregulate plasmalemmal receptors by multiple monoubiquitination, and then act as endocytic adapters for subsequent lysosomal degradation. Cbl action is modulated by interactions

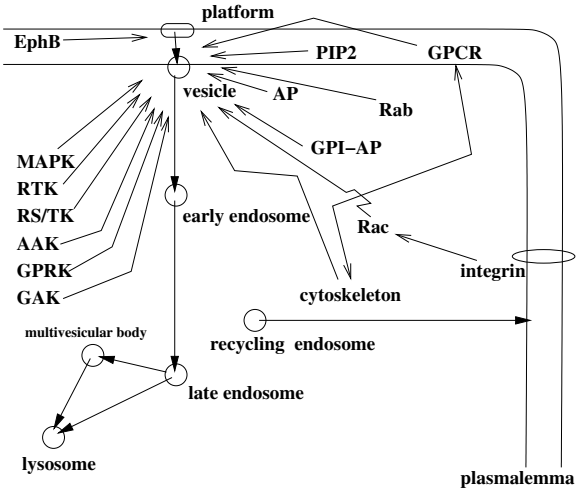

Figure 5.4. Examples of receptors, kinases, and pathways involved in endocytosis.

[29] Cbl forms with other proteins an interactome inside the cell that controls physiological processes, particularly cell proliferation, survival, and motion. Cbl is located in focal adhesions with actin linkers and small GTPases.

with regulators (CIN85,[30] and βPIX[31]), which promote or inhibit receptor endocytosis [535].

Kinases associated with the G-protein-coupled–receptor signaling pathways control clathrin-mediated endocytosis and regulate actin and tubulin cytoskeleton [536]. Kinases acting on the endocytosis are involved in cell adhesion. Each set of kinases exerts opposite effects on the two endocytic mechanisms (via caveola and membrane raft).

Other membrane-raft trafficking can be used for internalization of molecules [537]. The cells use various pathways to control plasmalemmal receptors, i.e., cell signaling features and receptor turnover. *Dynamin* is involved in cell transport, especially in apical transport to the plasma membrane and in receptor-mediated endocytosis. Carboxy-terminal binding protein 3/brefeldin A-ribosylated substrate (CtBP3/BARS) controls transport mechanisms distinct from the dynamin-required endocytosis, such as the basolateral transport from the Golgi apparatus to the plasma membrane [538].

5.1.1.8 Enzymatic Control

Phosphorylation–dephosphorylation cycles control the coated-vesicle transport (Fig. 5.4). A high number of kinases are involved clathrin- and raft-mediated transport with endosomes and caveosomes or membrane rafts, respectively [536]. Each endocytic route is regulated by a specific kinase group. Within this group, some kinases act directly, whereas other kinases modulate the endocytic path. Certain kinases exert opposite effects on the two main endocytic kinds for coordination between endocytic routes (Ca^{++} channels with kinase domains, Ca^{++}–calmodulin-activated kinases, effectors of Cdc42 and Rac1, Wnt signaling components, receptor Tyr kinases, and receptor Ser/Thr kinases (RSTK, MAPK).[32]

5.1.2 Clathrin-Mediated Transport

Clathrin-coated vesicles are required for intracellular trafficking between various compartments of both exocytosis and endocytosis. The clathrin-dependent

[30] The adaptor protein CIN85 binds to different molecules involved in the regulation of receptor endocytosis.

[31] The adaptor protein βPIX binds to p21-activated kinase.

[32] Metabolic kinases, the TOR signaling pathway, G-protein-coupled receptor–linked kinases (GRK), regulators of the cytoskeleton are involved in clathrin-mediated endocytosis. Regulators of the integrin-dependent focal adhesion, collagen receptor tyrosine kinase MRC2, and members of cell adhesion–dependent signaling regulate raft trafficking via focal adhesions. Factors of cortical actin depolymerization and polymerization, associated with Cdc42- and PIP2-dependent signaling act on the membrane raft/caveola endocytosis. Members of the Ark/Prk family of kinases, such as cyclin-G–associated kinase (GAK) and adaptin-associated kinase-1 (AAK1) are required in endocytosis, especially as they participate in clathrin-coated vesicles [539].

route is responsible for the internalization of nutrients, hormones, growth factors, receptors, low-density lipoproteins, and ferritin, as well as antigens and pathogens. Clathrin forms a scaffold around the corresponding vesicles, linked to the membrane by clathrin adaptors.

Concentrated receptors linked by coat proteins forms *clathrin-coated pits*. The relocation of activated receptors, such as G-protein-coupled β2-adrenergic receptor, epidermal growth factor receptor, low-density lipoprotein receptor, etc., after plasmalemma removal, allows: (1) interactions of receptors with other proteins in the endosome, (2) recycling of receptors back to the plasmalemma, and (3) degradation, with subsequent signaling shutdown, by directing receptors to lysosomes.

5.1.2.1 Clathrin

Clathrin is a membrane constituent. Clathrin can form structures of variable curvature. However, clathrin coats show an invariant local pattern of contacts that appear to stabilize the lattice and then control assembly and uncoating [540]. Clathrin-coated vesicles have three layers: (1) an outer clathrin layer, clathrin forming a scaffold on the vesicle surface, (2) a middle layer with various clathrin-adaptor molecules and assembling-regulation proteins, and (3) an inner layer with embedded transported substances.

Clathrin causes membrane deformation for budding, and can contribute to the activity of membrane-bending accessory proteins, serving as a flexible scaffold for the adaptors and accessory proteins [541].

5.1.2.2 Transport and Recycling

Clathrin-mediated endocytosis follows a set of transient and localized events. The first step is the binding of extracellular cargo molecules to specific plasmalemmal receptors. The receptors and other membrane proteins destined for endocytosis are then sequestered by intracellular adaptors. The formation of clathrin-coated vesicles at the plasmalemma begins with the recruitment of adapter complex-2-coated vesicle adapters, accessory proteins,[33] and clathrin to phosphatidylinositol(4,5)bisphosphate-enriched plasmalemmal nanodomains. Once the endocytic coat formed at the plasmalemma, clathrin drives coated pit invagination. Invagination and scission of clathrin-coated pits are tightly coupled. The motion of clathrin-coated pits away from the plasma membrane begins before membrane scission [542]. Cortactin recruitment occurs in the site of clathrin-coated pits before scission with a peak recruitment coincident with scission.

Membrane dynamics with changes in membrane curvature is required in cell transport. Membrane bending to the planed curvature is generated during

[33] Accessory proteins, such as clathrin assembly lymphoid myeloid leukemia protein, epsin, and eps15/eps15R coordinate the coat assembly.

vesicle budding, owing to amphiphysin, endophilin, epsin, and GTPase Sar1. Cytoplasmic *endophilin* (A1–A3; B1–B2)[34] bind to GTPase dynamin, a membrane scissor, and to phosphoinositide phosphatase synaptojanin, a clathrin uncoater. They contain a BAR domain that senses and generates membrane curvature. Epsin binds to PtdIns(4,5)P2, promotes membrane curvature and stimulates clathrin polymerization, thus stabilizing the generated curvature. The coated membrane invaginates and subsequently pinches off to form a vesicle.

Actin polymerization is tightly coupled to the vesicle budding, from the plasmalemmal invagination to form a coated pit to the vesicle scission. A transient burst of actin polymerization accompanies endocytic internalization. Actin polymerization at the neck of constricted coated vesicles pushes coated pits and nascent coated vesicles away from the plasmalemma into the cytosol.[35] Plasmalemmal myosins are recruited at the onset of actin polymerization. The Arp2/3 complex activators, N-WASP, and cortactin participate to actin organization and endocytosis.[36] The Arp2/3 complex activators are themselves regulated by many interacting proteins. Dynamin coordinates actin filament assembly at endocytic sites.

Once inside the cytosol, the clathrin coat of newly formed endocytic vesicles disassembles and clathrin is rapidly released for subsequent use. The naked vesicle fuses with endosomal membranes of other uncoated vesicles and forms early endosomes, to which their cargoes are delivered. Some ligands are separated from their receptors. Cargoes can be recycled back to the plasmalemma or transported to late endosomes and lysosomes for degradation.

Such a mechanism involves the microtubules (Sect 1.5). In certain cases, actin can associate with motile endosomes. Early endosomes can lead to tubules and even to vacuoles, characterized by a large fluid content. Sorted vesicles and other particles move along the cytoskeleton to fuse with acceptor membrane. Membrane proteins can be either recycled to the plasmalemma or retained in multivesicular endosome before being delivered to lysosomes.

[34] Endophilin-A1 is also called SH3P4, SH3GL2, and EEN-B1; endophilin-A2, SH3P8 and SH3GL1; endophilin-A3, SH3P13 and SH3GL3; endophilin-B1, SH3GLB1, and endophilin-B2, Bif1, SH3GLB2, and EEN.

[35] Actin polymerization at the plasmalemma either drives the protrusion of lamellipodia and filopodia at the leading edge of migrating cells or intracellular invaginations for endocytosis.

[36] Pan1 regulates the Arp2/3 complex involved in actin organization at endocytic sites. Pan1 contains a Wiskott-Aldrich syndrome protein (WASP) homology-2 (WH2)-like motif necessary for Arp2/3 activation. The Pan1 WH2-like motif binds to F-actin. F-actin binding to Pan1 is required prior to Arp2/3 activation [543].

5.1.2.3 Involved Molecules in Clathrin-Mediated Transport

Clathrin-coated vesicle formation and uncoating (duration of 30–90 s) requires interactions of numerous proteins. Various adapters allow sorting of cargoes into assembling clathrin-coated pits on a membrane. First, the membrane invaginates and then elongates. Then, a vesicle forms. To deform the membrane and form a tubular bud, and then a vesicle, the membrane elastic resistance forces must be balanced by forces that can be generated by actin polymerization and/or myosin-1 [544]. These active forces are also important in scission.[37] Membrane constriction leads to coated vesicle budding. The released vesicle quickly loses its clathrin coat. Various proteins regulate coat formation and disassembly. The uncoating implicates cytosolic ATPase Hsc70, which is recruited to clathrin-coated vesicles by its cofactor auxilin.[38] The timing of auxilin recruitment determines the onset of uncoating. A low level of auxilin is transiently incorporated during the growth of coated pits. However, immediately after vesicle budding, accumulation of a specific phosphoinositide recruit a sufficient amount of auxilin to trigger uncoating (second and major burst of auxilin recruitment) [545].

Clathrin adapters include amphiphysins-1 and -2, adaptor protein complexes, AP1, AP2, AP3 and AP4, which recognize different types of molecules, β-arrestins-1 and 2, AP180/clathrin assembly lymphoid myeloid leukemia protein (CALM),[39] autosomal recessive hypercholesterolemia protein (ARH),[40] disabled-2 (Dab2), epsins-1, -2, -3, and -R,[41] Huntington interacting proteins HIP1[42] and HIP1R, sorting nexin 9 (Snx9), Golgi-localized γ-ear-containing Arf-binding proteins GGA1, GGA2, and GGA3,[43] and Hrs[44] (Tables 5.1 and 5.2). These peripheral membrane proteins are recruited either via Arf/Arl/Sar GTPases and Rab proteins, or via phosphatidylinositol phospholipids, as these two mechanisms are interlinked.

α-*adaptin-associated kinase*-1 (AAK1), located in clathrin-coated pits, promotes AP2 conformational change to bind the compound to be transported [548]. Conversely, *synaptojanin*, which degrades AP2-recruiting phosphatidylinositol(4,5)bisphosphate, promotes AP2 dissociation from the plasmalemma and hinders the formation of clathrin-coated vesicles. Cyclin-G-associated kinase[45] (GAK) is involved in AP1 binding.

[37] GTPase dynamin does not always intervene.

[38] There are two auxilin variants, the brain-specific auxilin-1 and the ubiquitous cyclin-G-associated kinase, also called auxilin-2.

[39] CALM/AP180 binds PI(4,5)P2.

[40] ARH and Dab2 proteins are involved in the uptake of LDL receptors.

[41] Epsin-R is involved in TGN–endosome transport. The epsin family includes epsin-1, AP180/CALM, and HIP1/Hip1R, characterized by a PI(4,5)P2-binding domain.

[42] HIP1 can promote with AP2 clathrin recruitment and assembly.

[43] GGAs transport substances between the trans-Golgi network and endosomes.

[44] Hrs incorporates targeted molecules into multivesicular bodies from vesicles.

[45] Cyclin-G-associated kinase is also called auxilin-2.

Epsins and *amphiphysins* are implicated in membrane deformation. Clathrin-associated sorting proteins and arrestins recruit targeted molecules. Clathrin coats bind to *auxilin* and Hsc70, creating a distortion of the clathrin coat, destabilization, and uncoating [549].

Adaptin binds clathrin of clathrin-coated vesicles to the membrane. Adaptor protein complexes bind to clathrin to stimulate the formation of clathrin coat. The phospholipid bilayer via the phosphatidylinositol bisphosphate (PIP2) acts in the clathrin coat formation. After endocytosis, the membrane components in the endosome are either recycled back to the plasmalemma after dissociation from the ligand, or are degraded in the endosome fused with the lysosome.

EphrinB-EphB regulates clathrin-mediated endocytosis, acting on protein interactions and phosphoinositide turnover via phosphorylation of synaptojanin-1,[46] a phosphatidylinositol-phosphatase, which is required for clathrin-

Table 5.1. Clathrin adapters and membrane constituents (CHC: clathrin heavy chain; CLC: clathrin light chain; Sources: [546, 547]).

Clathrin adaptor	Membrane constituent	Binding partners
AP1	PI(4)P	CHC
AP2	PI(4,5)P2	CHC, epsin, auxilin,
	sSynaptojanin, amphilysin	
AP180	PI(4,5)P2	Clathrin, AP2, PLD
Epsin	PI(4,5)P2	Clathrin, AP2, intersectin
Arrestin	PI(4,5)P2	
Amphilysin	PI(4,5)P2	Clathrin, AP2, endophilin, PLD

Table 5.2. Clathrin-coated vesicle components and its interactions (Source: [546]).

Protein	Interacting partners
β-arrestin	Clathrin, APs, GPCRs
Dynamin	Amphilysin, endophilin, intersectin
	PIs, G-proteins, cortactin, profilin
	Grb2, Src, PI3K, PLCγ
Auxilin	Clathrin, APs, Hsc70
Synergin	APs, GGAs
Endophilin	Synaptojanin, amphilysin, dynamin
Ankyrin	Clathrin, spectrin, vimentin, tubulin (Ank1)
	Na^+ channel (Ank2)
	K^+-ATPase, IP3R, RC, PKC (Ank3)
GGA	Arf, GGAs

[46] More precisely, EphB2-mediated phosphorylation of synaptojanin-1 inhibits the interaction of synaptojanin 1 with the endophilin and lowers the phosphatase activity of synaptojanin-1.

mediated endocytosis [550]. EphB2 receptor activation and subsequent synaptojanin-1 tyrosine phosphorylation has two distinct effects on endocytosis. The inhibition of the interaction of synaptojanin-1 with *endophilin* promotes the early phase of clathrin-mediated endocytosis, whereas it hinders the late phase of endocytosis. The vesicle uncoating requires interactions with endophilin.

5.1.3 Caveolin-Mediated Transport

Caveolin-mediated transport represente another kind of transport used by the cell. Nanoindentations in the plasmalemma of many cell types, especially endothelial cells, can trap molecules. These membrane-lined recesses can extend from the inner to outer membrane layer. They are involved not only in cellular transport but also in signal transduction, as well as in cell displacement.

5.1.3.1 Caveolin and Caveola

The protein *caveolin*, a cholesterol-binding protein, forms caveolae [551]. Caveolae are invaginations (size 50–100 nm) of the plasmalemma, enriched in cholesterol, glycosphingolipids, and caveolins. Caveolin indeed forms oligomers that are able to connect to cholesterol and sphingolipids. Caveolin-1 also interacts with integrin and focal adhesions. Caveolae are abundant in endothelial cells, smooth-muscle cells, fibroblasts, and adipocytes. Aminopeptidase-P is abundant in the caveolae of endothelial cells. Caveolae form stable nanodomains but also function as carriers.

Caveolae are involved in endocytosis, transcytosis, and exocytosis, the transport being as rapid as with clathrin-coated vesicles. However, they are not involved in clathrin-independent intracellular transport under basal conditions [552].

Three types of caveolae might exist: caveolae, glycosphingolipid-enriched membranes[47] (GEM), and polyphosphoinositol-rich (PIP2-enriched) rafts. Caveolae function as signaling platforms at the plasmalemma. They can sense membrane physical and chemical changes. Caveolae can also be involved in lipid regulation in adipocytes and other cell types. Caveolae are particularly involved in cholesterol homeostasis.

The caveolin family has three members: caveolin-1 (Cav1), caveolin-2 (Cav2), and caveolin-3 (Cav3). Caveolin-1 is found in endothelia in particular. Caveolin-2 is co-localized and co-expressed with Cav1. Rab13 forms clusters with Cav1 and Cav2. Caveolin-3 is expressed in myocytes, including smooth muscle cells and cardiomyocytes.

[47] GEMs are caveolae without caveolin-1, an integral membrane protein that binds cholesterol.

5.1.3.2 Involved Molecules in Caveolin-Mediated Transport

Caveolae-mediated endocytosis involves SNAREs. Caveolar endocytosis is regulated by syntaxin-6, a tSNARE [553]. Syntaxin-6 is also required for the delivery of GM1 ganglioside and GPI/GFP-anchored protein from the Golgi complex to the plasmalemma.

Caveola-mediated trafficking uses caveolin-positive vesicles (cavicles, and caveosomes). The caveosome does not contain classical markers of the early endosome. The motility of caveosomes at the cell surface depends on cortical actin filaments. The cytoplasmic motion of caveosomes depends on the microtubule network. The caveosome route is regulated by non-receptor tyrosine kinase activity and depends on protein kinase-C activity [554]. Src kinase induces phosphorylation of Cav1 and dynamin, which is involved in fission of caveolae from the plasmalemma.

GTPase dynamin acts not only in clathrin-coated vesicle budding, but also in internalization of caveolae by membrane fission, hydrolyzing GTP. Phospholipase-D2 functions as a GTPase activating protein for dynamin. GTP hydrolysis leads to tubule twisting which causes longitudinal tension. The latter is released by tubule breakage [555].

Caveolae might have a role in mechanosensation in certain cell types. The high calveola density in endothelial cells, which are sensitive to changes in normal and tangential stresses, is associated with increased phosphorylation of Cav1 [556]. Moreover, caveolin is connected to integrins and Gq proteins. Caveolae can also sense membrane tension in smooth muscle cells.

5.2 Cell Motility

The spatial organization and motions of cells subjected to chemical and physical signals from neighboring cells, the surrounding fluid, and the extracellular matrix determine the formation and maintenance of tissues. These processes are regulated by the physical properties of the cell environment, especially its rigidity. In cell cultures, the support rigidity influences cell adhesion and cytoskeleton organization. The stability of focal adhesions depends on the support stiffness. In the presence of anisotropic stiffness, the growth and migration (haptotaxis) of epithelial cells occur according to the direction of greatest support rigidity [557].

5.2.1 Cell Remodeling during Displacement

Various protein pathways partition the migrating cell into functional front and rear compartments, including a leading lamellipodium, a cell body, and a trailing tail. The cell front is characterized by the regulation of actin and

focal adhesion dynamics associated with integrin signaling[48] [558]. The cell rear deals with maintenance and nuclear functions (nucleic acid metabolism, and cell cycle regulation).

The cytoskeleton reorganization and assembly and disassembly of focal adhesions and junctions between adjacent cells and the cell and the extracellular matrix are required for cell motility and tissue formation and remodeling. Cells can collectively migrate. Chemoattractant cues are translated into directed cell movement. Cell imaging measures cell shape changes and movements using labeled cytoskeletal components as well as other cellular elements without disturbing their function [559].

Cytoskeleton remodeling is regulated in space and time in response to external and internal signals to ensure cell adaptation to its environment as well as cell motion. The cell, indeed, displays a set of internal motions (cytoplasmic streaming, mitosis using mitotic spindle, vesicle-associated transport, etc.). Moreover, the cell is able to creep about and actively change its shape, to migrate with frequent direction changes. Cell motility is necessary for clotting, wound healing, and immune responses (Sect. 9.4 and Chap. 10). Cell motion is triggered by membrane receptors stimulated by an event cascade that ensures signal transmission down to the cortex deformation.

Cell locomotion requires molecular motor-driven motions, and assembly and disassembly of actin filament and cell adhesion sites. ENDO180–Rho–RoK–MLC2 signaling generates contractile forces via phosphorylation of myosin light chain-2, thereby promoting focal adhesion disassembly at the rear of the cell [560]. Migratory transmembrane receptor ENDO180,[49] highly expressed in motile cells, is recruited into clathrin-coated pits, then into endosomes. The activation of the Rho–RoK pathway requires ENDO180-containing endosomes. ENDO180 reduces the phosphorylation of three RoK substrates, LIM kinase-1 and -2, and myosin phosphatase-1.

Activation of the ERK pathway at the cell periphery is required for adhesion disassembly before cell spreading and migration. Moreover, ERK effectors, such as calcium-dependent calpain-2 and MLCK, which cleaves focal adhesion kinases and other components of focal adhesions and phosphorylates myosin light chain, respectively, are involved in focal adhesion turnover and disassembly. Adapter CAP, which binds to cytoskeletal proteins paxillin, vinculin, and actin, is located in adhesion sites between the cell and the extracellular matrix. CAP inhibits cell spreading via the PAK–ERK pathway and focal adhesion turnover [561].

Cell motility is associated with changes in cell configuration (filopodia, and lamellipodia) and mechanical state (sol-gel transition). When the membrane

[48] Integrin receptors sense changes in gradients of chemokines and physical features of the extracellular matrix. Ras/ERK pathway has peculiar substrates (MLCK) and scaffolds (MP1) in the lamellipodium. ERK activity can also be hindered by EphA2.

[49] ENDO180 is also called CD280.

is subjected to a local stimulus, the cortex gel undergoes a pressure from the incompressible sol which generates a bulge. The protuberance content gels to form a stable lamellipodium. *Gelsolin*, activated by Ca^{++}, dissociates actin gel for actin–myosin interactions. Cell motility results from actin polymerization into filaments and depolymerization.

Cell motion results from cytoplasmic extensions, the lamellipodia, and contraction of peripheral (cortex) cytoplasmic sheets, attracted by chemotactic molecules or growth factors. Phosphatidylinositols influences cell motility. Phosphatidylinositol 3-kinases are recruited at the plasmalemma of the leading edge during chemotaxis, generating accumulation of phosphatidylinositol(3,4,5)trisphosphate, whereas phosphatases PTEN and SHIP1 are recruited to the back and sides of cells where they can dephosphorylates PIP3. Chemotaxis amplification can be done by adenosine triphosphate release from the leading edge of neutrophils via P2Y2 receptors [562]. Neutrophils rapidly hydrolyze released ATP to adenosine, and A3-adenosine receptors are recruited to the leading edge. Adenosine via A3-receptors thus promotes cell migration.

Lamellipodia (length 2–10 μm, thickness 0.1 μm) ruffles back and forth, forming transient pointwise contacts (attachment sites) with the substrate, slowly stretching and pulling the remaining part. When the elongated cell begins to move, the actin filaments organized into linear bundles,[50] which span the whole cell in different directions with convergence to focal points, and then rapidly disassembles to form a diffuse mesh leading to thin filopodia and lamellipodia.

Growth and destruction of actin filaments in filopodia control formation and retraction of cell bulges during displacement. Filopodia are dynamic, long, narrow (finger-like) cell protrusions. Filopodia contain a core filled with parallel bundles of actin filaments with barbed ends located at the filopodial tip. Filopodial growth requires actin polymerization at these barbed ends. Myosin-10, also at the tips of filopodia, is a nanomotor required in filopodia formation downstream from Cdc42 GTPases [563].[51] Filopodia are involved in cell migration (wound healing, and angiogenesis), as well as in between-cell signaling. Filopodia have a sensory function.

Caveolin-1 has a polarized location in migrating endothelial cells, with caveolin-1 accumulation in the front of migrating cells or in the rear, whether endothelial cells cross a pore or move on a plane. The locomotion mode and the polarization of caveolin-1 depends on the substrate topology but not on chemoattractants [564].

[50] Filament bundles observed in immotile cells provides tensile strength and structural support.

[51] Several molecules are involved in the formation of filopodia, such as formins and the vasodilator-stimulated phosphoprotein (VASP). GTPase Cdc42 regulates filopodia formation. It can interact with N-Wiskott-Aldrich syndrome proteins to activate actin nucleators, such as Arp2/3 and formins. VASP proteins at the tips of filopodia can stimulate filopodia formation. Fascin is an actin-bundling protein in filopodia.

During cell travel, the cell membrane continuously moves from the progression front to the tapered cell back as tracked vehicles, but by the endocytosis-front-exocytosis cycle [565]. Cells involved in wound healing have a displacement speed from 0.1 to 10^{-3} mm/h. The velocity of clotting and immune cells is greater. 10^{11} neutrophils produced per day look after possible microorganisms throughout the body at a speed up to 2 mm/h. Macrophages migrate from the bloodstream to engulf possible invading bodies. Platelets deform and quickly spread out to plug vessel wall breaches.

Cell migration has long time scale directional persistence,[52] whereas lamellipodial dynamics has short time scales. The directional persistence of the cell path and lamellipodial dynamics has been studied using epidermal growth factor stimulation on fibronectin-coated substrata [566]. The short time scale kinetics of adhesion complex formation may modulate the directional persistence.

5.2.2 Molecules Involved in Cell Motion

5.2.2.1 Chemokines

Chemokines, small proteins involved in immune and inflammatory reactions, induces chemotactic migration of leukocytes as well as other cells, such as endothelial cells in angiogenesis. Chemokines do not target specific cells. A given cell responds to various kinds of chemokines. Many chemokine types are identified: C, CC, CXC and CX3C (Table 5.3). Chemokines interact with G-protein-coupled receptors, CCR (CCR1–CCR8), CXCR (CXCR1–CXCR5), and CX3CR (Table 5.4). A given receptor can bind different types of chemokines. Conversely, a given chemokine can link to several receptors. The receptor expression determines the action spectrum of chemokines, and subsequently the response of leukocytes to chemokines. Cells produce several chemokines with an overlapping action spectrum to yield a robust response.

5.2.2.2 Arp Complexes, and WASP and WAVE proteins

The actin assembly is required for cell shape determination, intracellular transport, and locomotion. Multiple actin nucleation factors collaborate to construct cytoskeletal structures, such as the *Arp2/3 complex* and the *formin* family of proteins. *N-Wiskott-Aldrich syndrome proteins* (N-WASP), which contain WASP homology 2 (WH2) domains, generate actin elongation for vesicular transport, but inhibit spontaneous actin assembly. Abi1, a scaffold protein for the assembly of molecular complexes, regulates both WAVE and N-WASP actin-dependent functions [568].

The *protein Spire* contains also a cluster of four WH2 domains, each of which binds an actin monomer. Spire induces rapid formation of filamentous actin structures [569].

[52] Migrating cells sustain a nearly constant direction of locomotion over time scales ranging from minutes to hours.

Table 5.3. Examples of receptors of constitutive chemokines: CCL13 (BCA1: B-cell attracting chemokine); CCL19 (ELC: EBV induced gene-1 ligand chemokine); CCL20 (LARC: liver- and activation-regulated chemokine); CCL22 (MDC: monocyte chemotactic protein); CXCL12 (SDF1: stroma cell-derived factor-1); as well as inducible chemokines: CXCL6 (GCP2: granulocyte chemoattractant protein 2); IL8 (interleukin 8); CCL10 (IP10: interferon γ-inducible protein 10); CX3CL1 (fractalkine); CCL2/8/7/13 (MCP1/2/3/4: monocyte [Mo] chemotactic protein; CCL5 (RANTES: regulated on activated normal T cell expressed and secreted); CXC (NAP2: neutrophil [Nφ] activating peptide 2); CCL3/4 (MIPα/β: macrophage inflammatory protein α/β) Source: [567].

Receptor	Ligand	Cell
CCR	CCL2/3/4/5/7/8	Bφ, Eφ, Nφ
	CCL13/17/19	Mo, dendritic cell
	CCL20/22	T and NK cells
CXCR	CXCL6	Nφ
	CCL10/13	B and NK cells
CX3CR	CX3CL1	Mo, T, and NK cells

Table 5.4. Chemokine receptors, their main targeted G-proteins, and principal ligands (Source: [241]).

Type	Main transducer	Preferential ligands
CCR1	Gi/o	CCL15, CCL23
CCR2	Gi/o	CCL2
CCR3	Gi/o	CCL11, CCL24, CCL26
CCR4	Gi/o	CCL22, CCL17
CCR5	Gi/o	CCL4
CCR6	Gi/o	CCL20
CCR7	Gi/o	CCL19, CCL21
CCR8	Gi/o	CCL1
CCR9	Gi/o	CCL25
CCR10	Gi/o	CCL27, CCL28
CXCR1	Gi/o	CXCL6, CXCL8
CXCR2	Gi/o	CXCL1, CXCL2, CXCL3, CXCL5, CXCL7, CXCL8
CXCR3	Gi/o	CXCL9, CXCL10, CXCL11
CXCR4	Gi/o	CXCL12α/β
CXCR5	Gi/o	CXCL13
CXCR6	Gi/o	CXCL16
CX3CR1	Gi/o	CX3CL1 (fractalkine)
XCR1	Gi/o	XCL1α/β(lymphotactins)

5.2.2.3 Small GTPases

Chemotaxis triggers the cell reorganization with a cell front and back in the direction of chemical gradients. Chemotactic pathways link the chemoattractant receptor to the pseudopod formation and cell motion. During the initial stage of chemotaxis (directional sensing stage), Ras GTPases localize rapidly (<3 s) to the plasmalemma, first uniformly, then at the cell front. Cell reorganization achieves a quick reversible asymmetry (chemotactic switch). Phosphatidylinositol 3-kinase is located at the front, and phosphoinositide-3 phosphatase PTEN at the back. Phosphatidylinositol(3,4,5)trisphosphate is produced at the cell fronts, ahead of the actin pseudopods that drive the cell migration. It is not an essential component of motility, but it accelerates the motion.

The *Rho* family of small GTPases, Rho, Rac, and Cdc42 regulates the actin cytoskeletal dynamics, especially cell motility and cell shape changes [570] (Table 5.5; Fig. 5.5). Mutual antagonism between Rac and RhoA GTPases contributes to their cell polarizing effects. Activation of RhoA GTPase and, subsequently, of its effector Rho kinase promotes retraction, suppressing lamella formation. RhoA promotes the formation of contractile stress fibers in the cell body and at the rear. Activation of Rac GTPase leads to actin polymerization and the protrusion of leading lamellae. At the leading edge, Rac1 and Cdc42 promote cell motility through the formation of lamellipodia and filopodia, respectively. Rac inhibits RhoA and conversely. Temporal and spatial control of these competing signalings allows the coordination of elongation and shortening of actin filaments, thereby the cycling between cellular protrusions and retractions for an effective cell displacement.

Signal transduction pathways involved in actin polymerization require small Rac GTPases and its Rac-associated phosphatidylinositol 5 kinase. Numerous effectors have been implicated in Rho GTPase-mediated signaling. The *p21-activated kinase* (PAK) is implicated in both Cdc42- and Rac-mediated organization of the actin cytoskeleton [571]. Rac activation can occur via *p21-interacting exchange factor* (PIX), a guanine nucleotide exchange factor. PAK-binding to PIX coordinates the activation of Cdc42 and Rac associated with phosphatidylinositol 3-kinase. PIX can associate with G-protein-coupled receptor kinase-interacting protein GIT1, a GTPase-activating protein. GIT1 makes a link between the PAK/PIX complex and focal contacts. Synaptopodin, an actin-associated protein, regulates RhoA signaling [572]. Synaptopodin competes with Smurf1 for RhoA binding, and thus prevents Smurf1-mediated ubiquitination of RhoA for proteasomal degradation.

FilGAP, a GTPase-activating protein specifically targeting Rac GTPase, binds filamin A [573], which manages the coordination of these antagonist reactions, residing at both the leading and trailing edges of polarized cells. Rac activation leads to actin-filament elongation. Rac is antagonized by filamin-A in membrane protrusions. FilGAP phosphorylation by Rho kinase stimulates its RacGAP activity, which requires filamin-A. FilGAP suppresses leading edge protrusions.

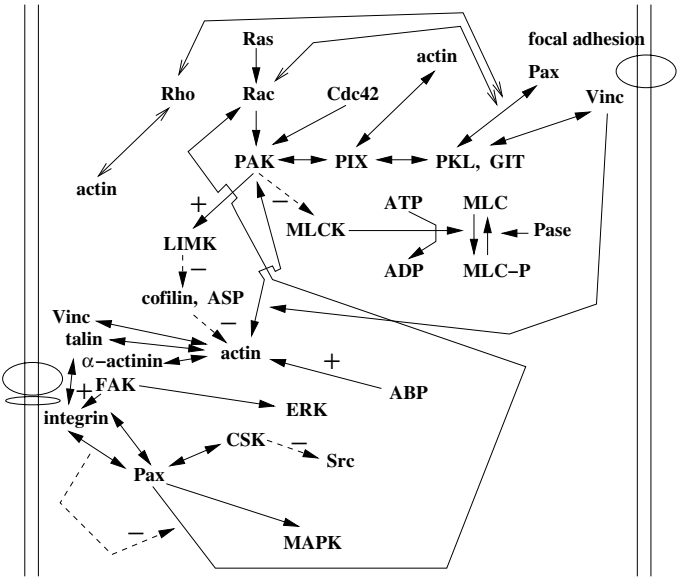

Figure 5.5. Rho GTPases, focal adhesions, and the cytoskeleton.

ArhGAP10, a Rho GTPase-activating protein (RhoGAP) for Cdc42 regulates the Arp2/3 complex and F-actin polymerization on Golgi membranes in coordination with Arf1 [574]. ArhGAP10 bridges Arf1 and Cdc42 signaling pathways, which require actin dynamics.

Cell migration is regulated by Ras proteins, which also regulate cell growth and differentiation. Ras proteins activate C-Raf and the extracellular signal-regulated kinase cascade. C-Raf activation requires prohibitin [575]. Ras proteins activate the Rac pathway. GTP-bound Rac activity depends on the mode of membrane anchoring.

Rnd proteins,[53] regulating the organization of the actin cytoskeleton, modulate contractility in smooth muscle cells, which involves RhoA (reciprocal inhibition). RhoE GTPase regulates the activity of actin filaments by bind-

Table 5.5. Rho GTPases and cell activity (cell motility, smooth muscle contraction/relaxation, endothelium permeability, platelet activation, and leukocyte migration).

GTPase	Function
Cdc42	Filopodium, focal complex
Rac	Lamellipodium, focal complex
Rho	Stress fiber, focal adhesion

[53] Rnd3 (RhoE) is also involved in the regulation of cell cycle.

ing and inhibiting RoK1,[54] which induces actin–myosin contractility. RoK1-phosphorylated and unphosphorylated RhoE are located in the cytosol and plasma membrane, respectively. RoK, stimulated by PDGF, phosphorylates RhoE, as the phosphorylation requires protein kinase-C pathway [576]. RhoE phosphorylation induces stress fiber disruption and, thus controls the RhoE stability. Rnd proteins interact with RhoGAP [577]. In addition, Rnd1 acts on adapter Grb7 involved in cell migration. Rnd1 inhibits calcium sensitization in smooth muscle cells, particularly in varicose veins with subsequent decreased contractility.

5.2.2.4 Integrins

Integrin-mediated cell motility can be decomposed into three stages: (1) formation of an integrin-dependent protrusion and new adhesion sites at the leading edge, (2) contraction of the actin/myosin cytoskeleton, and (3) detachment of the trailing edge after degradation of integrins at the cell back. The released integrins are recycled to the cell front, using actin binding motor protein myosin-10 [578]. Protein phosphorylation by protein kinase-C and other serine/threonine kinases regulate integrin transport. Protein kinase-B acts on the endosomal transport and recycling of integrins, by phosphorylating (inactivating) glycogen synthase kinase GSK3 [579]. Intermediate filaments also play a role in cell motility, especially vimentin. Vimentin phosphorylation affects both its assembly into polymers [580], as well as (like keratin [581]) the connections with intermediate filament–associated proteins. Protein kinase-C[55] phosphorylates vimentin. PKC hence controls the endocytosis of integrins to the plasmalemma via the association of vesicles with intermediate filaments, regulating cell motility [582].

5.2.2.5 Paxillin and its associated molecules

Paxillin (Pax) is a cytoskeletal and focal adhesion docking protein that regulates cell adhesion and migration. Pax acts downstream of focal adhesion integrins and other plasmalemmal receptors [583]. Pax is implicated in the regulation of integrin and growth factor signaling [584]. Pax indeed has binding sites for signaling molecules and structural proteins, such as vinculin (Vinc), *paxillin kinase linker*[56] (PKL), and focal adhesion kinase (FAK). Pax interacts with Rho GTPases,[57] with its downstream target LIMK, as well as with

[54] Conversely, Rho effector RoK1 (but not RoK2, also activated by RhoA) phosphorylates Rnd3.

[55] PKC activity is correlated with increased haptotaxis. PKC is associated with trafficking vesicles.

[56] PKL is a member of the GIT1 family.

[57] Rho GTPases regulate integrin-mediated adhesion via their effects on both the actin and microtubule.

the Rac pathway. Phosphorylated paxillin binds to its effectors and transduces external signals into cellular responses via Crk adapters (chicken tumor virus regulator of kinase) in association with Crk-associated substrate (CAS) and mitogen-activated protein kinase cascades [585]. In particular, Pax is phosphorylated following integrin stimulation by FAK and becomes a docking site for Crk [586] (Sect. 2.3). Moreover, negative regulators of these pathways, such as C-terminal Src kinase (CSK), an inhibitor of Src activity, bind directly to Pax. Vinculin and actopaxin bind actin directly to regulators of the actin cytoskeletal dynamics, such as paxillin kinase linker, PAK-interacting exchange factor, and p21-activated kinase. These proteins serve as effectors of the Arf and Rho GTPase families. Pax associates with the PKL–PIX–PAK complex, which regulates Rac and Rho. Rac activation at the leading edge of migrating cells is required for stable lamellipodia. The binding of Pax to α_4 integrin prevents Rac activation and thus inhibits lamellipodium formation [587]. Pax recruits an ADP-ribosylation factor GTPase-activating protein (ArfGAP), which decreases Arf activity, thereby inhibiting Rac.

5.2.2.6 Mitogen-Activated Protein Kinases

The cell adaptation to environmental osmolarity changes requires the activation of the p38 pathways. The protein SAP97/hDlg is phosphorylated by the SAPK3/p38 and dissociated from the guanylate kinase-associated protein (GKAP) and is therefore released from the cytoskeleton [588]. This process might regulate the integrity of intercellular junctional complexes and cell shape in response to osmotic stress.

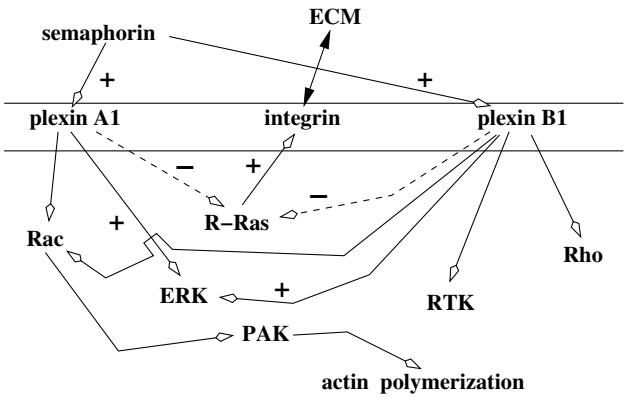

Figure 5.6. Semaphorins and their plexin receptors. Effects on cell-extracellular matrix adhesion and cellular cytoskeleton activity (adapted from [589]).

5.2.2.7 Semaphorins and Plexins

Semaphorins,[58] transmembrane or secreted glycoproteins, regulate cell motility and attachment in vascular growth, tumor progression, etc.. Plexins regulate Rho GTPases, activating Rho kinase and enhancing actomyosin interactions and formation of stress fibers. Plexins act on R-Ras, which in turn regulate integrins (Fig. 5.6) [589]. Plexins also interact with Rac, which activates p21-activated kinase initiating actin polymerization. GTPase Rnd1 binds to plexins. Semaphorin-bound plexins can play the role of GAP for R-Ras. Although other Ras-family members stimulate ERK, R-Ras slightly affects ERK, but regulates integrin activity. Active R-Ras increases integrin-based cell adhesion to the extracellular matrix. This effect is decreased by semaphorin binding to plexin.

5.2.2.8 Caspases

The pathway including caspase-11 and actin interacting protein-1 (Aip1), which cooperates with cofilin to optimize actin depolymerization, modulates the migration of inflammatory cells [590].

5.2.2.9 Growth Factors

The platelet-derived growth factor-B (PDGF, Sect. 10.1) stimulates cytoskeletal remodeling and chemotaxis via Nck adapters [591]. Nck adapters[59] transduce the signal from ligand-stimulated PDGFβ receptors using the scaffold protein p130Cas. Platelet-derived growth factor uses a pathway that implicates Src, the transcription/translation regulator hnRNP-K and the mRNA-encoding myosin regulatory light chain–interacting protein. The latter, an E3-ubiquitin ligase, leads to degradation of the myosin regulatory light chain required for reorganization of the actin cytoskeleton to prepare fibroblast locomotion [592].[60]

[58] Among the eight semaphorin classes, classes 3 to 7 are vertebrate semaphorins.
[59] There are two types of Ncks, Nckα or Nck1, and Nckβ or Nck2.
[60] Cell motility needs: (1) reduction in stress fibers and focal adhesions, and then (2) formation of lamellipodia.

6

Blood

The blood performs three major functions: (1) transport through the body, (2) regulation of bulk equilibria, and (3) body immune defense against foreign bodies. It supplies oxygen, and hence energy, and conveys nutrients (vitamins, mineral ions, glucose, amino acids, fatty acids among other glucids, protids, and lipids) to the tissues and removes carbon dioxide and waste products of cell metabolisms toward lungs and purification organs. The kidneys filter the blood. Toxins are not only removed in urine but also by sweating. The blood transmits metabolism factors and messengers, such as hormones, to the target organs. Blood volume and electrolyte concentration are regulated. Blood maintains the body temperature (36.4–37.1 °C) and acid–base equilibrium, controlling blood pH, which remains in the range 7.35 to 7.45.[1] The blood is involved in the body defense against infection, transporting immune cells and antibodies, and in repair processes after injury. It limits blood losses by clotting (Sect. 9.4). The blood contains living cells and plasma (Table 6.1). Eight to twelve hours after a meal, 100 ml of blood contains 19 to 23 g of solids and 77 to 81 g of water.

6.1 Plasma

Plasma is one of the body fluid compartments. Body fluids can indeed be broken down into several components. The two major compartments include intracellular and extracellular fluids. The extracellular fluids comprise: (1) interstitial fluids[2], (2) the plasma, and (3) minor components, such as lymph,[3]

[1] The major blood buffer is composed of bicarbonate ions, from which carbonic acid and water can be obtained. Carbonic acid is unstable and decomposes into carbon dioxide and water.

[2] The interstitial fluid, located between cells, corresponds to the interstice or interstitium.

[3] Because of the difference in tissue and blood pressures, blood plasma continuously leaks from the entrance segment of capillaries. Close-ended lymphatic

cerebrospinal fluid, digestive secretions, aqueous humor, and synovial fluids for joint lubrication. About two-thirds of body water is found in the intracellular space, about 25% in the intertitium, about 8% in the plasma, and about 3% in the minor compartments (Table 6.2).

Table 6.1. Blood composition and main characteristics in healthy adult man. The blood cells include erythrocytes (red blood cells), leukocytes (white blood cells) and platelets. Leukocytes are divided into five classes based on morphological and tinctorial characteristics. Neutrophils, eosinophils, and basophils are known as granulocytes due to granules in the cytoplasm. Monocytes and lymphocytes are involved in the body scavenging and defense. The blood plasma consists of water (90%), the remainder being electrolytes (sodium (Na^+), 142 mmol per liter, chloride (Cl^-), 102 mmol/l, and potassium (K^+), 5 mmol/l), carbohydrates, lipids, and amino acids, etc.

Erythrocytes	$4.5–5.2 \times 10^6/mm^3$
Hematocrit	41–47%
Leukocytes	$4–10 \times 10^3/mm^3$
Neutrophils	40–70%
Eosinophils	1–2%
Basophils	0.5–1%
Lymphocytes	20–40%
Monocytes	2–10%
Platelets	$2–4 \times 10^5/mm^3$
ions	295–310 mEq/l
Protids	70–80 g/l
Lipids	5–7 g/l
Glucids	0.8–1.1 g/l
Osmotic pressure	280–300 mosm
pH	7.39–7.41

Table 6.2. Approximative water content of body fluid compartments (l).

Total body water	41–47
Intracellular space	27–30
Extracellular space	14–17
Interstitial fluid	11–13
Plasma	3–4

capillaries return protein-rich lymph via lymphatic vessels and lymphatico–venous junctions back to the venous compartment of the blood systemic circulation.

6.1.1 Plasma Constituents

Plasma represents approximately 55% of the blood volume. The remaining is *hematocrit* (Ht), i.e., percent of packed cells[4] (Ht 38–46% in women, 42–53% in men). Plasma is mainly composed of water, a suspending fluid (or solvent) for various solutes (Table 6.3). Plasma contains 92% water, 8% proteins ($\sim 7\,\mathrm{g/dl}$), and other substances.

6.1.1.1 Electrolytes

Electrolytes, or ions, contribute to the osmotic pressure (Π), which is mainly regulated by the kidneys. Major electrolytes are cations Na^+, K^+, Ca^{++}, Mg^{++}, and anions HCO_3^-, Cl^-, HPO_4^{2-}, and SO_4^{2-}.

Cations and anions are unevenly distributed in body fluid compartments (Table 6.4). Sodium ion (Na^+) is the major cation and chloride (Cl^-) the major anion outside the cell. Inside the cell, potassium ion (K^+) is the major cation and phosphate (HPO_4^{2-}) the major anion. At physiological pH, proteins are negatively charged.

Ion transports through the cell membrane between the body fluid compartments require specialized plasmalemmal proteins (Fig. 6.1). The ion displacements in the different compartments affect the blood volume.

6.1.1.2 Glucids

Glucids are composed of: (1) oligosaccharids (glucose, fructose, and galactose), (2) disaccharids (saccharose, lactose, and maltose), and (3) polysaccharids (glycogen). Glucose and fructose can be used by cells. Other glucids require degradation into glucose. Glucids are important nutrients because they are energy sources.

Table 6.3. Plasma approximate composition (%). Plasma, the suspending fluid for peripheral blood cells, is composed of water, electrolytes, proteins (albumins 60–80%, globulins 16–36%, and fibrinogen 4%), amino acids, lipoproteins, other lipids, and glucids. Conveyed hormones, vitamins, and enzymes belong to these substances. The normal plasma volume is 40–90 ml/kg of body weight.

Water	92
Proteins	7
Electrolytes	0.9
Lipids	0.6
Glucids	0.1

[4] Because erythrocytes (RBC) represent 97% of blood cell volume (Table 6.7), Ht estimates RBC concentration. The normal sex-dependent range has different limits according to the authors: $\mathrm{Ht} = 0.47 \pm 0.07$ and $\mathrm{Ht} = 0.42 \pm 0.05$ in male and female subjects, respectively.

Glycemia ($\sim 1\,\mathrm{g/l}$) is the blood glucose concentration, which depends on the exogenous supply and degradation of hepatic glycogen. Glycemia is stabilized by two pancreatic hormones, insulin and glucagon. The insulin decreases glucose level by cell use and storage, especially in the liver and the muscles. The glucagon increases glucose concentration.

6.1.1.3 Plasma Proteins

Serum is plasma without *fibrinogen* (195–365 mg/dl) and other clotting factors. Fibrinogen acts on erythrocyte aggregation,[5] hence in blood rheology

Table 6.4. Distribution of ions and proteins (mEq/l) in intra-, and extracellular spaces, and plasma.

	Cell	Intertitium	Plasma
Sodium (Na^+)	10–15	130–150	135–145
Calcium (Ca^{++})	10^{-4}	2–5	5–10
Potassium $(K)^+$	135–150	4–5	3–5
Magnesium (Mg^{++})	30–35	1–3	1–3
Cations	180	152	155
Chloride (Cl^-)	3–9	108–125	100–108
Bicarbonate (HCO_3^-)	10–12	27–30	23–28
Phosphate (HPO_4^{2-})	40–80	2–3	1–3
Sulfate (SO_4^{2-})	20	1	1
Proteins	35–55	~ 0	14–16
Organic acid	20	5	5–6
Anions	180	152	155

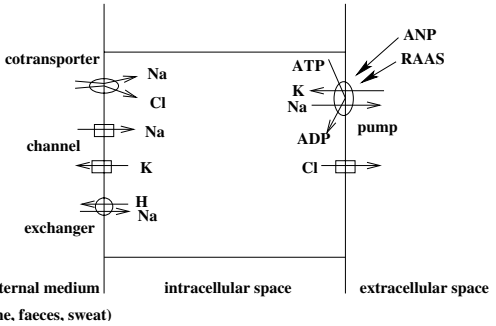

Figure 6.1. Types of ion motions in the three main fluid compartments, extracellular (particularly the blood), intracellular, and external media.

[5] Fibrinogen bridges erythrocytes to form rouleaux. RBC aggregation depends on the concentration of fibrinogen and β-globulins. The *fractal dimension* of 2D finite RBC aggregates is equal to 1.59 ± 0.03 [593], which is similar to the result

(part II), and in coagulation (Sect. 9.4). Serum proteins are composed of *albumin* and *globulins* (Table 6.5). Albumin is the main plasma protein (3.3–4.5 g/dl) synthetized in the liver. It binds many small molecules for transport through the blood and participates in blood colloidal osmotic pressure (Π), which keeps fluids within the vascular system. Like fibrinogen, globulins induce reversible RBC aggregation in stagnant blood regions.[6] Several kinds of globulins exist: α globulins, which transport chemical species such as thyroxine and retinol (vitamin-A), β-globulins, such as transferrin, and γ-globulins, most of the antibodies (0.1–0.4 g/dl of α1 globulins, 0.5–1 g/dl of α2 globulins, 0.7–1.2 g/dl of β globulins, and 0.5–1.6 g/dl of γ globulins). Main non-protein nitrogens (NPN) are urea, uric acid, creatine, creatinine, ammonium salts, and amino acids.

6.1.1.4 Plasma Lipids and Lipoproteins

The lipoprotein structure shields water-insoluble lipids (cholesteryl esters and triglycerides) from water by encapsulating them with polar lipids and proteins. However, core lipids can move between lipoproteins. The four main types of circulating lipoproteins, which differ in size, density, and content, include *chylomicrons*, *very low density lipoproteins* (VLDL), *low density lipoproteins* (LDL), and *high density lipoproteins* (HDL) (Table 6.6, Fig. 6.2). The lipoproteins convey cholesterol esters and triglycerides in blood.[7] Triglycerides (TG) are delivered to muscles and adipose tissues for energy production and storage[8] (blood TG concentration [TG] < 1.2 g/l at 20 years old, [TG] < 1.6 g/l at 60 years old). Excess lipid and glucid intakes lead to conversion into triacylglycerols in the liver, which are packaged into VLDLs and released into the circulation. VLDLs (size 0.03–0.08 μm) contain also *cholesteryl esters* (CsE)

Table 6.5. Plasma protein composition (%).

Albumin	∼0.60
α1-Globulin	∼0.04
α2-Globulin	∼0.08
β-Globulin	∼0.12
γ-Globulin	∼0.16
Fibrinogen	∼0.03

(1.55) of a chemically-limited aggregation model [594], which give in 3D a fractal dimension of 3.

[6] Hemodynamic stresses avoid aggregation, limit aggregation growth, and progressively break existing RBC rouleaux and possible rouleau networks.

[7] Analysis of serum lipids commonly yields the concentration of LDLs, HDLs, and TGs.

[8] The lipoprotein lipase (LPase) removes TGs from chylomicrons and VLDLs for fat storage or energy source in adipose tissues and muscles, respectively.

and apolipoproteins (ApoB100, ApoC1, ApoC2, ApoC3, and ApoE). *Intermediate density lipoproteins* (IDL) are formed from VLDLs by the lipoprotein lipase after triacylglycerol removal.[9] IDLs become LDLs (size 0.02 µm) after losing the whole TG content. The plasma LDL contains cholesterol esters in its core and a hydrophilic coat composed of cholesterol, phospholipids, and ApoB100.

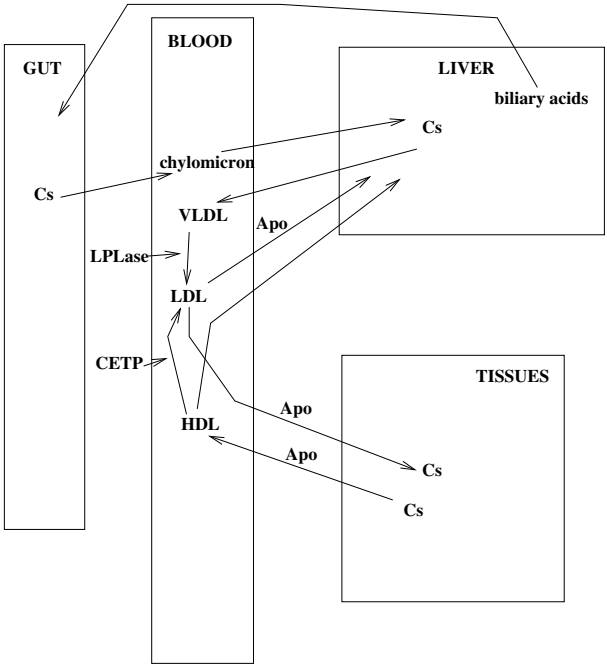

Figure 6.2. Cholesterol turnover.

Table 6.6. Size and lipid content (%) of lipoproteins.

	Chylomicron	VLDL	LDL	HDL
Size (nm)	80–500	∼50	∼20	∼10
Cholesterol	∼0.02	∼0.07	∼0.08	∼0.04
Cholesterol esters	∼0.03	∼0.12	∼0.42	∼0.15
Phospholipids	∼0.07	∼0.18	∼0.22	∼0.30
Proteins	∼0.02	∼0.08	∼0.22	∼0.47
Triiglycerides	∼0.86	∼0.55	∼0.06	∼0.04

[9] IDLs retain cholesteryl esters. Some IDLs are quickly taken up by the liver, whereas others remain in the blood and are converted to LDLs.

Cholesterol (Cs, Chap. 2), from extracellular sources as well as de novo synthesis[10] is transported in blood mainly as cholesteryl esters associated with lipoproteins (blood Cs concentration [Cs] $< 2.2\,g/l$ at 20 years old, [Cs]$<2.6\,g/l$ at 60 years old). Dietary CsEs are carried from the small intestine to the liver by chylomicrons.[11] A part is excreted in the biliary ducts as free cholesterol or bile acids (partial cyclic travel) and another part is conveyed in blood within LDLs[12] or VLDLs.[13] LDLs link to specific plasmalemmal receptors. They are incorporated in vesicles, which fuse with lysosomes for degradation.[14] LDL receptor-mediated endocytosis requires ApoB100. ApoE, synthesized in various organs and found in high concentration in interstitial fluid, is a ligand for LDL receptors that participate in Cs redistribution[15] [596].

HDLs (5–17 nm, 1.06–1.21 g/ml) are synthesized in the liver[16] and small intestine.[17] They extract cholesterol from cell membranes with ApoA1 and

[10] Endogenous cholesterol is synthesized in the liver and carried to tissues by VLDLs.

[11] Cs esters and triglycerides, packed into chylomicrons in gut, are carried in lymph and then in blood. The chylomicron is removed from blood by the liver.

[12] Endogenous and exogenous Cs, which exceeds hepatic needs, is carried in the blood circulation by LDLs.

[13] The liver synthesizes VLDLs. LDLs are plasma carriers of cholesterol after conversion of VLDLs to LDLs by lipoprotein lipases, in particular in endothelial cells.

[14] The sequential steps of the cell LDL receptor destiny include: (1) LDL receptor binding, (2) coated pit formation, (3) endocytosis (coated vesicle and afterward endosome), (4) lysosomal hydrolysis, and (5) metabolism via a passage into the endoplasmic reticulum and the Golgi complex to come back to the membrane [595].

[15] ApoE mutant, which is inappropriate to bind LDL receptors is associated with familial type III hyperlipoproteinemia, a genetic disorder with elevated plasma cholesterol levels and coronary artery disease.

[16] Synthesis of new high-density lipoproteins begins with the secretion of apolipoprotein A1 from the liver. Apolipoprotein A1 can bind to the ATP-binding cassette transporter A1 (ABC-A1) of plasmalemma of macrophage in the subendothelial space. ApoA1 promotes efflux of cholesterol and phospholipids from macrophage, which form nascent HDL [114]. These particles can then be modified by the lecithin-cholesterol acyltransferase into larger, less dense HDL2 and smaller, denser HDL3. HDL2 and HDL3 can receive additional cholesterol from macrophages before entering into blood circulation.

[17] The intestine is an important source of HDLs. The ATP-binding cassette class A, member 1 (ABCA1) gene in the intestine plays a key role in the production of high-density lipoproteins. HDL concentrations in mice with deleted ABCA1 gene specifically in the intestine are 30% lower than in normal mice [597]. When the ABCA1 gene is deleted in both the liver and intestine, HDL concentrations are reduced by over 90%.

ApoA2[18] [599]. HDLs are circulating stores of cholesterol, cholesteryl esters and Apos (ApoA, ApoC, ApoD, ApoE, and ApoJ[19]) deliverd to the liver, where they are endocytosed. HDL cholesterol could be esterified in the plasma by lecithin-cholesterol acyltransferase. Free and esterified cholesterols are removed from HDL in the liver, with the mediation of the scavenger receptor SR-B1 (class B, type 1), and afterward excreted into the bile. Several proteins, such as lecithin-cholesterol acyltransferase (LCAT), paraoxonase (Pon), platelet-activating factor acetylhydrolase (PAFAH), are associated with HDLs.

Several HDL types exist that differ in density, size, shape, and surface charge. Therefore, HDLs are heterogeneous in structure, physicochemical properties, metabolism, and activity. Small, lipid-poor, nascent HDLs are made of apolipoproteins embedded in a lipid monolayer of phospholipids and free cholesterol. They are mainly produced by the liver and intestine or can be released as fragments from triglyceride-rich lipoproteins. Large, mature HDLs contain a core of cholesteryl esters and triglycerides. Nascent HDLs acquire cholesterol and phospholipids at cellular membranes via efflux mediated by ATP-binding cassette transporter A1. Esterification of HDL cholesterol from pre-β HDLs by lecithin cholesterol acyltransferase generates: (1) small, dense, cholesterol-poor HDL3; and (2) large, light, cholesterol-rich HDL2, which, in turn, can be converted to HDL3 (Fig. 2.3). HDL metabolism involves several processes, such as exchange of cholesteryl esters and triglycerides between HDLs and apoB-containing lipoproteins, uptake of cholesteryl esters by hepatocytes mediated by scavenger receptor B1, and phospholipid and triglyceride hydrolysis by hepatic lipase. Cholesterol acquired by HDLs can also be transferred to VLDLs and LDLs by cholesterol ester transfer protein (CETP).[20]

[18] Membrane-associated cholesterol is esterified by HDL-associated lecithin cholesterol acyltransferase (LCAT). Cs efflux especially requires HDL containing ApoA1 [598].

[19] ApoA1, ApoA2, ApoA4, ApoB, ApoC3, ApoD, ApoE, ApoH, LCAT, and CETP are detected in the ApoA4-containing lipoprotein particles (A4LP) [600].

[20] The glycoprotein cholesteryl ester transfer protein (CETP) acts in cholesterol distribution among plasma lipoproteins. CETP transfers not only cholesteryl esters, but also triglycerides and phospholipids between lipoproteins, including VLDL, LDL, HDL, and chylomicrons. "Bad" cholesterol is mainly found in low-density lipoproteins, "good" cholesterol in high-density lipoproteins. CETP mediates the transfer of cholesteryl esters from HDL to ApoB-containing lipoproteins in exchange for triglycerides. ApoC1 abolish CETP activity [601]. Lipid transfer inhibitor protein (LTIP or ApoF) regulates CETP activity in LDLs.

HDLs are considered antiatherogenic[21] because they transport the cholesterol to the liver for excretion and degradation. Apolipoprotein M (apoM), a HDL component, is required for the formation of preβ-HDL and cholesterol efflux to HDL, thereby hampering atherosclerosis [602]. Cholesterol diffusion and the scavenger receptor B1 efflux pathway, which allows bidirectional cholesterol exchange between cells and HDL, might also be involved in macrophage cholesterol efflux. The cholesterol efflux from arterial wall cells corresponds to the initial stage of cholesterol transport to the liver (*reverse cholesterol transport*).

The inflammation modifies lipid and lipoprotein metabolism to hamper the toxicity of various agents and enhance tissue repair by nutrient redistribution to immune cells. The inflammatory cascade decreases the concentration in cholesterol-rich HDLs, which favors the synthesis and accumulation of phospholipid-rich VLDLs[22] aimed at binding bacterial products and other toxic substances [603]. Alterations in lipoproteins implicated in HDL metabolism, associated with depletion in several nuclear hormone receptors (peroxisome proliferator–activated receptor, liver X receptor, farnesoid X receptor, and retinoid X receptor), impair the reverse cholesterol transport and increase cholesterol delivery to immune cells [604]. ATP binding cassette A1-dependent lipid efflux is also disturbed. During severe infection, VLDL clearance decays due to decreased lipoprotein lipase and apolipoprotein E in VLDL. Enrichment of lipoproteins in ceramide, glucosylceramide, and sphingomyelin enhances uptake by macrophages. Changes in binding capacity of associated apolipoproteins and enzymes alter the HDL antioxidant capacity. HDLs become proinflammatory molecules. Oxidation of LDLs and VLDLs increases.

HDLs possess multiple antiatherogenic activities, which include reverse cholesterol transport from the arterial wall to the liver for excretion, and antioxidative,[23] anti-inflammatory,[24] antithrombotic, and vasodilatory actions.

[21] Atherosclerosis (Part II) is a chronic inflammation of the artery wall, which can be identified by increased serum levels of C-reactive protein. Wall inflammation associated with endothelial dysfunction is responsible for oxidative stresses, with possible excessive transport and intimal retention of LDL and with monocyte extravasation. Oxidized LDLs induce cholesterol accumulation in macrophages. An additional source of influx is provided by the vasa vasorum.

[22] The inflammation acute phase, mediated by cytokines, is characterized by high plasma levels of triglycerides with VLDL secretion caused by adipose tissue lipolysis, increased hepatic fatty acid synthesis, and suppression of fatty acid oxidation.

[23] LDLs could be the major target of HDL antioxidative effects. The antioxidative action depends on apolipoproteins, such as ApoAI, by the removal of oxidized phospholipids from LDL, and enzymes that hydrolyze LDL-derived oxidized phospholipids, such as platelet-activating factor acetylhydrolase (also known as lipoprotein-associated phospholipase-A2) and lecithin cholesterol acyltransferase.

[24] HDL anti-inflammatory activity can be characterized by inhibited expression of adhesion molecules, reduced neutrophil infiltration within the arterial wall and

Lipid-free apolipoproteins, primarily apoAI, cause specific efflux of cellular cholesterol and phospholipids via ATP-binding cassette transporter-A1, whereas ATP-binding cassette transporters-G1 and -G4 mediate cholesterol efflux to mature HDL particles. Mature HDLs induce a cholesterol efflux mediated by the scavenger receptor-B1, an HDL receptor. Small, lipid-poor HDL3 thus represents more efficient cholesterol acceptors than HDL2.

Small, dense HDLs3 have antioxidative activity, transferring oxidized lipids from the tissues and blood circulation to the liver. HDL3 could hence be the HDL subset mostly responsible for anti-inflammatory activity under normal conditions. Alterations in HDL composition, such as: (1) a decrease in cholesteryl esters, apoAI, and enzymes bound to HDLs; and (2) an increase in triglyceride content and serum amyloid-A occur during inflammation. HDLs undergo structural and functional changes especially in *atherogenic dyslipidemia* [115].[25] Replacement of cholesteryl esters by triglycerides alters the conformation of apoAI, and hence the lipid binding capacity. The selective uptake of cholesteryl esters from high-density lipoprotein by the scavenger receptors-B1, is strongly affected by dysfunctional triglyceride-enriched HDLs [605]. HDL enzymes (paraoxonase-1, platelet-activating factor acetylhydrolase, and lecithin cholesterol acyltransferase) can become dysfunctional and depleted. Serum amyloid-A (SAA) can replace apoAI and other HDL apolipoproteins [606]. HDL3 enrichment in serum amyloid-A[26] reduces cholesteryl ester uptake by hepatocytes, but can increase HDL binding to macrophages [607]. Sustained redirection of cholesterol from the liver to macrophages corresponds to a maladaptive response in the long term.

Bile acids[27] interacts with triglycerides. Bile acids are not only required in dietary lipid absorption in the digestive tract and cholesterol homeostasis, but also also have endocrine functions [608]. Bile acids bind G-protein-coupled receptor TGR5, activate mitogen-activated protein kinase pathways and nu-

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decreased generation of reactive oxygen species. HDL enzymes can hydrolyze oxidized lipids.

[25] Atherogenic dyslipidemia, a major risk factor for cardiovascular diseases, is defined by higher circulating levels of proatherogenic, cholesterol-rich, apolipoprotein-B-containing lipoproteins, such as LDLs, and lower concentrations of antiatherogenic apolipoprotein-AI-containing HDLs.

[26] Lipid-free SAA, a ligand for SR-B1, inhibits HDL binding on SR-B1. HDL-associated SAA has little effect on HDL binding to SR-B1, but decreases selective cholesteryl ester uptake of HDL.

[27] Bile acids are synthesized in the liver from cholesterol. Bile acids secreted from the hepatocytes into the bile canaliculi are stored in the gallbladder and transported down the duodenum. They are absorbed again in the terminal ileum, and convected back to the liver via the portal vein. Bile acids decrease their synthesis using a negative feedback. The main bile acids include: (1) primary bile acids, cholic acid, and chenodeoxycholic acid, as well as their glycine and taurine conjugates; and (2) the secondary bile acids, deoxycholic acid, and lithocholic acid.

clear hormone receptors such as farnesoid X receptor α (FXR).[28] Farnesoid X receptor-α induces apolipoprotein-C2 expression. ApoC2 is a co-activator of lipoprotein lipase, which lowers serum triglyceride and VLDL levels.

6.1.2 Blood gas

One of the main tasks of the blood circulation is to transport dissolved gas, oxygen and carbon dioxide. Oxygen dissolves less and needs to bind to a suitable transporter in erythrocytes, the hemoglobin. Oxygen-carrying capacity is related to the quantity of oxygen transported by hemoglobin. Another factor is the condition and rate of association and dissociation of oxygen. Hemoglobin loading and unloading, indeed, happen under different conditions. The higher the oxygen availability, the greater the quantity of oxygen taken up by hemoglobin. The lower the local oxygen concentration, the higher the release of oxygen by hemoglobin. The dissociation curve levels off toward the saturation line (100% of oxygenated hemoglobin). Almost full loading is ensured at usual oxygen concentration (97% of oxygenated hemoglobin). The dissociation curve exhibits two main features, a sigmoid shape and a left-right position with respect to the oxygen concentration. A shift toward the left depicts an increase in oxygen availability. The higher the carbon dioxide concentration, the greater the right shift. Consequently, the higher the CO_2 concentration, the greater the oxygen dissociation and delivery.

6.2 Blood Cells

There are three main kinds of flowing cells: erythrocytes, leukocytes and platelets (Table 6.7). Blood cells need to communicate between them and with other body cells. They secrete cytokines, which have a broad activity spectrum. Cytokines allow communications among immune system cells and between immune system cells and other body cells. They thus target manifold cell types. Four action modes are used: *autocrine* (on the producing cell; αυτο: inside) or *intracrine* (intra: within); *juxtacrine* (on neighboring cells; juxta: immediately adjacent, adjoining); *paracrine* (on relatively closely related cells; παρα: close to); and *endocrine* (on remote cells; ενδδοκρινω: release inside) processes, via suitable receptors. Chemokines are inducible chemotactic cytokines, which mobilize nearby responsive cells according to a chemotactic direction (Chap. 10).

[28] In the liver and intestine, activated farnesoid X receptor-α protects against toxic accumulation of bile acids by transport and excretion of bile acids. Bile acids are also involved in liver regeneration by activation of farnesoid X receptors, as well as, but at a lower extent and transiently, the xenobiotic receptors CAR [609].

Table 6.7. Blood cell approximated geometry and relative concentration.

Blood cell	Quantity ($/mm^3$)	Relative proportion	Size (μm)	Cell volume percentage
Erythrocyte	5×10^6	1	~ 8	97%
Leukocyte	5×10^3	10^{-3}	~ 15	2%
Thrombocyte	3×10^5	6×10^{-2}	~ 3	1%

6.2.1 Blood Cell-Targeted Growth Factors

Blood cell genesis, growth, proliferation in their bone marrow niches, and their mobilization in the bloodstream and in the body tissues are regulated by numerous substances (Chap. 10).

6.2.1.1 Cytokines

Cytokines are growth factors implicated in immunity and hematopoiesis (Table 6.8, Sect. 6.3 and 10.1). Cytokines exhibit both pleiotropy and redundancy. Pleiotropic actions of cytokins can be explained by the presence of receptors for a given cytokine on multiple cell lineages and the activation of multiple signaling pathways contributing to different functions. Overlapping actions of different cytokines can be explained by similar cellular distributions of specific receptors for different cytokines, as well as by share of signaling pathways. Moreover, cytokines can signal via several types of receptors and several cytokins can bind to given receptors. Also, cytokin receptors have common structural components.[29] Cytokines are downregulated by cytokine antagonists. Soluble cytokine receptor fragments in the blood and the extracellular fluid, after enzymatic cleavage of the extracellular domain of cytokine receptors, can bind cytokines, thereby neutralizing their activity.

Most cytokine receptors, as well as growth hormone[30] and prolactin receptors, of the immune and hematopoietic systems belong to the family of class 1 cytokine receptors. Most class 1 cytokine receptor are dimers, with a

[29] Interleukins IL3 and IL5, as well as granulocyte–monocyte colony-stimulating factor (GM-CSF) share a common cytokine receptor βc-chain. Receptors of IL6, IL11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), novel neurotrophin-1/B cell-stimulating factor-3/cardiotrophin-like cytokine (NNT1/BSF3/CLC), and cardiotrophin-1 (CT1) share the gp130 signal-transducing molecule. IL2, IL4, IL7, IL9, IL15, and IL21 share the common cytokine receptor γc-chain, IL12 and IL23 IL12Rβ1, IL4, and IL13 the type 2 IL4 receptor, and IL7 and TSLP the receptor IL7Rα [620].

[30] The growth hormone is a type 1 cytokine. It acts via the JaK–STAT pathway, which mediates activation of STAT transcription factors and the Ras–MAPK pathway for phosphorylation of the transcription factor Elk1.

cytokine-specific subunit and a signal-transducing subunit, which is usually not specific for the cytokine. Certain receptors are trimers. Class 1 cytokine receptors are subdivided into subsets composed of receptors with identical signal-transducing subunits: (1) granulocyte–monocyte colony-stimulating factor (GM-CSF) subset includes the receptors for IL3, IL5, and GM-CSF; (2) IL6 subset the receptors for IL6, IL11, and IL12; and (3) IL2 subset the receptors for IL2, IL4, IL7, IL9, and IL15. The ligands for class 2 cytokine receptors are interferons Ifnα, Ifnβ, and Ifnγ. The TNF receptor family includes TNF receptor-1 (TNFR1), TNF receptor-2 (TNFR2), CD40, and Fas. Certain cytokine receptors belong to the immunoglobulin superfamily, such as receptors for IL1 and macrophage colony-stimulating factor (M-CSF).

Signal transduction via class 1 and 2 cytokine receptors begins with cytokine-induced dimerization of receptor subunits. These cytokines use the JaK–STAT pathways. Plasmalemmal JaK phosphorylates tyrosine residues of STAT. STAT then activates genes via transcription factors. The JaK–STAT pathway is also used by other growth factors and hormones.

6.2.1.2 Chemokines

Chemokines form a family of inflammatory inducible cytokines. Chemokines have potent chemotactic activity. Most of them belong to two main subsets according to their architecture: CXC-chemokines (α-chemokines, encoded by human chromosome 4q12-21), and CC-chemokines (β-chemokines, which map to human chromosome 17q11-32). Most CXC-chemokines are chemoattractants for neutrophils, whereas CC-chemokines generally attract monocytes, lymphocytes, basophils, and eosinophils. Two additional subsets are composed of C-chemokines, more or less lymphocyte specific, and CX3C-chemokines, which target leukocytes. The chemokine receptors are composed of seven transmembrane proteins. They transduce signals via G-proteins. Chemokine receptors are classified into specific, shared, promiscuous, and viral groups.

Table 6.8. Cytokines in immunity and hematopoiesis. Erythropoietin, thrombopoietin, growth hormone, and prolactin have similar structures and signaling mechanisms to interleukins and colony-stimulating factors, referred to as type I cytokines. Interferons are referred to as type II cytokines. Many cytokines are pleiotropic. Different cytokines have overlapping activities (TGFβ: transforming growth factor-β; TNFα: tumor necrosis factor-α).

Immunity	Hematopoiesis
Interleukins	Interleukins
TGFβ	Colony stimulating factors
Interferons	
TNFα	
Chemokines	

6.2.2 Erythrocytes

The erythrocyte or red blood cell (RBC) is a hemoglobin (Hb) solution[31] bounded by a thin flexible membrane (non-nucleated cell). In its undeformed state, it has a biconcave disc shape with a greater thickness in its outer ring (diameter $7.7 \pm 0.7\,\mu m$, central and peripheral thicknesses $1.4 \pm 0.5\,\mu m$ and $2.8 \pm 0.5\,\mu m$, aspect ratio ~ 0.4, surface $\sim 140\,\mu m^2$, volume $\sim 90\,\mu m^3$, membrane thickness $\sim 0.02\,\mu m$). It is then susceptible to deform[32] (negligable bending resistance), in particular with a parachute shape, when moving through tiny capillaries. Its mass density is equal to about $1100\,kg/m^3$. It lives an average of 120 days. The RBC number is age- and sex-dependent (4.2–$5.5\times10^6/mm^3$ in women, and 4.5–$6.2\times10^6/mm^3$ in men).

The membrane is constituted by a phospholipid bilayer with protein inclusions, ion channels for chloride Cl^- and bicarbonate HCO_3^- in particular, and sialoglycopeptides (blood groups),[33] with glucids ($< 10\%$), and with cytoskeleton meshing and mooring proteins. A peripheral skeleton, a mesh made of spectrin, actin and other proteins (tubulin,[34] etc.), resides at the inner surface of the plasmalemma. It binds to RBC membrane (ankyrin–spectrin bonds)[35] [611]. It gives RBC shape.

The erythrocyte experiences large reversible deformations during its 120-day life span. Its plasmalemma and the cell cortex, with the spectrin network tethered to the phospholipid membrane, are the main load-bearing components. Spectrin tetramers in the unstressed state form dimers under shear and the cell periphery undergoes fluidization beyond a shear threshold [613]. Spectrins remodel into tetramers upon unloading and the stiffness of the erythrocyte wall raises. RBC stretching by optical tweezers gives estimated shear modulus of 4–10 $\mu N/m$ [614].

The RBC shape represents an equilibrium configuration that minimizes the curvature energy of a closed surface (a vesicle) for given surface area and volume with a geometrical asymmetry.[36] RBCs respond to Π changes

[31] The kinematic viscosity (ν) of the Hb solution is greater than that of water: $\nu \sim 6\,mPa\,s$ [610].

[32] RBC deformability can be estimated by transit time during filtration.

[33] RBCs have surface antigens associated with blood groups (glycosphingolipids ABO, Rh, MN, Duffy, Lewis, and Kell).

[34] Microtubules, involved in marginal bundles of RBCs, as well as in mitotic spindles and centrioles, are composed of tubulins (Sect. 1.5).

[35] Ankyrins (ANK) link spectrin–actin cytoskeleton to the membrane. Ankyrin deficiency is also known as hereditary spherocytosis. ANKs bridge between the spectrin–actin cytoskeleton and proteins involved in ion transport (Cl^-/HCO_3^- anion exchanger, Na^+/Ca^{++} exchanger, Na^+/K^+ ATPase, IP3 receptor, ryanodine receptor, and voltage-gated Na^+ channels), cell adhesion, and membrane trafficking [612].

[36] The phospholipid outer layer of the RBC membrane has slightly more molecules and a greater surface area than the inner layer.

by swelling[37] and shrinking (crenated RBC)[38] in hypotonic and hypertonic fluids, respectively. RBC membrane has thermal agitation. These spontaneous undulations are explained by the high membrane deformability. Furthermore, the membrane is excited by permanent impacts from water molecules.

Red blood cells defend against osmotic changes by controlling their intracellular ion content, especially keeping low $[Na^+]_i$ and high $[K^+]_i$ by the activities of the ATP-driven Na^+K^+ pumps and ion exchangers. Hereditary stomatocytosis and spherocytosis are characterized by an uncontrolled excessive membrane permeability to Na^+ and K^+ associated with amino-acid substitutions in the intramembrane domain of the anion exchanger AE1 [616].

Each RBC contains about 280×10^6 Hb molecules [617]. Hb consists of four globin chains α and β. It contains four iron atom Fe^{++}, in the center of hemes. Hb carries oxygen (O_2) from lungs to tissues and help to transport carbon dioxide (CO_2) from tissues to lungs.[39] Hb is also involved in pH regulation. *Reticulocytes* are slightly immature cells, characterized by a filament network and by granules. Reticulocytes amount to 0.5–2% of the total RBC count (more in anemia).

Erythrocytes express the gap junction protein pannexin-1, which forms a mechanosensitive and ATP-permeable channel in the plasmalemma [618]. Erythrocytes release ATP in response to hypoxemia or shear stress.

In adults, definitive erythropoiesis occurs in the bone marrow. Erythroblasts are associated with macrophages in anatomical units (*blood islands*). Nuclei are disconnected from reticulocytes, expelled and then engulfed by the macrophages. Phosphatidylserine, often used as an apoptosis signal, is also used for the nucleus engulfment [619].

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[37] In low-osmolarity solutions, RBCs becomes spherical after swelling (spherocyte). With further swelling (hemoglobin solution leakage), RBCs are reduced to the membrane (erythrocyte ghost). Hyperosmotic shock triggers the release of prostaglandin E2 (PGE2) [615]. PGE2 activates Ca^{++} channels in erythrocyte membranes. Subsequent Ca^{++} influx induces ankyrin-R degradation and erythrocyte shrinkage.

[38] Two types of spiculed cells exist: echinocyte and acanthocyte, whether spicules are uniformly distributed or not.

[39] One O_2 molecule can reversibly bind to each heme to form oxyhemoglobin. The reaction is reversible because O_2 is taken when $[O_2]$ is high and released when $[O_2]$ is low. O_2 combination to and release from Fe^{++} is accelerated by already existing O_2–Fe^{++} interactions. Under conditions of lower temperature, higher pH, and increased O_2 partial pressure (p_{O_2}), O_2 fixes to Hb. When temperature is higher, pH and O_2 partial pressure lower, the reverse reaction is promoted. With alveolar values of $p_{A_{O_2}}$ =13.7 kPa and $p_{A_{CO_2}}$ =5.3 kPa, the arterial and mixed venous blood values are p_{aO_2} =12.7 kPa, p_{aCO_2} =5.1–5.3 kPa, p_{vO_2} =4.9 kPa, and p_{vCO_2} =5.9–6.1 kPa. 95% of the CO_2 generated in the tissues is carried in RBCs and about 5% dissolves in the plasma. A part of CO_2 is bound to hemoglobin (carbaminohemoglobin). The rest is converted by carbonic anhydrase contained in RBCs into bicarbonate ions HCO_3^- and hydrogen ions H^+, which bind to the hemoglobin and do not affect pH ($CO_2 + H_2O \Leftrightarrow H^+ + HCO3^-$).

The ABO and Rhesus blood groups are the main blood groups considered in transfusion. The ABO blood group is related to the presence on the erythrocyte plasmalemma (as well as on the surface of endothelial and most epithelial cells) of two monosaccharides at the termini of oligosaccharide chains on glycoproteins and glycolipids responsible for A- and B-antigen activity.[40] Group A has anti-B antibodies targeting B-antigen; group B has anti-A antibodies; and group O has both (group O red blood cells lack both A- and B-antigens. Group O cells have at the termini of their oligosaccharide chains fucose (H-antigen). The five main Rhesus antigens are transmembrane proteins C, D, E, c and e (genotype cde/cde (d means absence of D-antigen) is RhD-negative, whereas the others are RhD-positive).

6.2.3 Leukocytes

The leukocyte, or white blood cell (WBC) plays a role in immune defense. Five kinds of leukocytes exist, three types of *granulocytes*, which have about the same size, neutrophils, eosinophils and basophils, and two kinds of agranular leukocytes, lymphocytes and monocytes. Granulocytes are innate immune cells that contain granules involved in immune defense. WBC life span in blood is several days.

The *neutrophil* (Nφ; size 8–15 μm) has small plasma granules and frequently multilobed nucleus. It is able to phagocytize foreign cells, toxins, and viruses. Normally, neutrophils account for 50–70% of all leukocytes. Scouting neutrophils look after possible invading agents.

The *eosinophil* (Eφ; size $\sim$ 15 μm) has large granules and a nucleus which often has two lobes. The granules contain digestive enzymes which are effective against parasitic larves. These cells also phagocytize antigen–antibody complexes. These cells account for less than 5% of all leukocytes.

The *basophil* (Bφ; size 12–15 μm) has numerous large basophilic granules. Committed basophils release two mediator kinds: (1) preformed granule-associated mediators, such as histamine (which causes vasodilation), serotonin, bradykinin, heparin (which is an anticoagulant), and cytokines; and (2) newly generated mediators, such as prostaglandins (PG) and leukotrienes (Lkt, Sect. 8.4). They represent less than 1% of all leukocytes. Basophils are a distinct cell type from mast cells. Normally, mast cells are not found in circulation.

Mast cells, which are leukocytes, contain granules storing inflammatory mediators, including histamine, serotonin, proteolytic enzymes cleaving complement components, heparin, chondroitin sulfate, and chemotactic factors, in particular for eosinophils and neutrophils. Mast cells are tissue-resident sentinels. They recruit other inflammatory cells.

The *lymphocyte* (Lφ; size 8–15 μm) has a very large nucleus. It is much smaller than the three granulocytes. It accounts for 25–35% of the WBCs. It

[40] The monosaccharides of A- and B-antigens are N-acetylgalactosamine and galactose, respectively.

plays an important role in immune response. There are *T lymphocyte* subsets: (1) inflammatory T cell which recruits macrophages and neutrophils to the site of tissue damage (CD4+ T cell); (2) cytotoxic T lymphocyte (CTL or CD8+ T cell)[41] which kills infected cells (by virus, bacterium, protozoan), allograft and tumor cells; and (3) helper T cells which enhance the production of antibodies by B cells. The *B lymphocyte* produces antibodies. B cells can be activated independently from T cells by bacterial polysaccharides and microorganism-derived Toll-like receptor ligands, generating short-lived, low-affinity extrafollicular plasma cells. However, interactions between T and B cells in secondary lymphoid tissues[42] are required for B-cell memory and antibody responses, specific for foreign antigens, producing high-affinity memory B cells and long-lived plasma cells. Such a process particularly involves a follicular population of T cells, *the follicular B helper T cells* (FBHT), which expresses CXC-chemokine receptor 5 (CXCR5),[43] like primed CD4+ T cells in the tonsils and memory CD4+ T cells in the blood[44] [622]. The lymphocytes that become T cells migrate from the bone marrow to the thymus where they mature. The CD4+ cells, or helper T cells, bind to antigen presented

[41] It has cytoplasmic granules that contain perforin and granzymes [621].

[42] After binding antigen, B cells interact with primed T cells in the T-cell zones of secondary lymphoid tissues. B cells upregulate expression of CC-chemokine receptor 7 and are attracted by a gradient of CC-chemokine ligand 21 to the outer T-cell zone of secondary lymphoid tissues, where B cells interact with T cells. B cells then differentiate using two pathways: (1) a follicular pathway, which induces germinal centers, and (2) an extrafollicular pathway. In the extrafollicular pathway of both T cell-dependent and -independent responses, B cell genitors migrate to the splenic bridging channel that connects the T-cell zone with either the spleen red pulp or the medullary cords in the lymph nodes. They then differentiate into short-lived plasma cells, which produce low-affinity antibody, and form extrafollicular foci of such cells. In the follicular pathway, B-cell genitors of germinal centers give birth to high-affinity memory B cells and long-lived plasma cells. Germinal centers of follicules, at least in the tonsils, are composed of: (1) a dark zone with centroblasts (dividing germinal-center B cells which undergo mutation of their immunoglobulin genes); (2) a light zone with centrocytes (dark zone-exiting centroblasts), follicular dendritic cells, and follicular T cells (with a large proportion of CD4+CD57+ T cells); (3) an outer zone, with CD4+CD57− T cells; and (4) a mantle zone with IgD+ resting B cells and CD4+ T cells. The interaction with FBHT cells select high-affinity centrocytes, leads to the differentiation into long-lived plasma cells and memory B cells, stimulates centrocytes to recycle into centroblasts.

[43] CXCR5 is a receptor for CXC-chemokine ligand 13 (CXCL13 or B cell-attracting chemokine 1), a chemokine secreted by follicular stromal cells, FBHT cells, and myeloid and plasmacytoid dendritic cells.

[44] FBHT cells differ from T helper-1 (TH1) and -2 (TH2) cells. Various lineages of T helper cells indeed exist, which have distinct differentiation pathways, such as CD4+IL17+ cells implicated in autoimmunity.

by B cells and form clones of *plasma cells*[45] secreting antibodies against the antigenic material. The major production sites of T and B cells are the thymus and bone marrow, respectively. Both B cells and T cells reside in lymph nodes and in the spleen where they mature into fully functional cells. A small fraction (∼2%) of the circulating lymphocytes are *natural killer* (NK) cells.[46]

Lymphocytes provide antigen-specific acquired immunity (immunological memory). Immature T- and B-cell activity is restricted mainly to lymphoid tissues. Activated T cells migrate more efficiently than antigen-unexperienced T cells. Antigen-experienced T cells include: (1) central memory cells, which circulate through lymphoid tissues; and (2) effector memory cells, which do not move. Cell history can lead to tissue-specific cells according to the residential type of lymphoid organs; but such a property can be reverted. Effector cell behavior is influenced by cytokines involved in T helper TH1 and TH2 responses. T-cell exit from lymphoid tissues and peripheral tissues requires sphingosine 1-phosphate and CCR7, respectively.

The *monocyte* (Mo; size 15–25 μm) is the largest leukocyte. Its nucleus is most often U- or bean-shaped. Monocytes are originated in the bone marrow from a myeloid progenitor shared with neutrophils. It leaves the blood stream by diapedesis, where they circulate for several days. Circulating monocytes give rise to mature tissue-resident *macrophages* (Mφ). These phagocytic cells defend the body against viruses and bacteria. They account for 3–9% of all leukocytes. Monocytes can be identified by expression of large amounts of lipopolysaccharide receptor component CD14.[47] Both monocyte[48] and macrophage lineages are characterized by heterogeneity.

Monocytes differentiate to give birth not only to *macrophages*, but also to *dendritic cells*. Dendritic cells are antigen-presenting cells. They collect and process antigens and migrate to lymphoid tissues to activate T lymphocytes.[49] Several dendritic cell subsets exist with distinct immunological activities, tis-

[45] Plasma cells (size ∼13 μm) are located in the bone marrow and lymphoid organs. They derived from B lymphocytes.

[46] NK cells, distinct from T and B cells, kill virus-infected and mutant cells by antibody-dependent cellular cytotoxicity. Killing is done by exocytosis of granules that contain perforin and granzymes. NK cells secrete cytokines such as Ifnγ and TNFα.

[47] Classic monocytes are CD14hiCD16-, which express CC-chemokine receptor CCR2. CD14+CD16+ monocytes express higher amounts of MHC class II molecules, CD32, and CC-chemokine receptor CCR5 [623]. A third monocyte subset express CD14, CD16, and CD64.

[48] Circulating monocytes are variable in size, granularity, and nuclear morphology.

[49] Dendritic cells are indeed required for priming CD4+ and CD8+ T cells against exogenous antigens. The dendritic cells in inflammatory sites upregulate homing receptors CCR7 and then can enter in draining lymph vessels, which express the CCR7 ligands CCL19 and CCL21. In lymph nodes, these antigen-loaded dendritic cells activate immature T cells, which migrate to the inflammation site.

sue distribution, and migratory properties.[50] Myeloid and plasmacytoid dendritic cells are the main producers of interferon-α. Dendritic cells can differentiate from tissue-resident progenitors, such as bone marrow-derived myeloid precursors, or blood monocytes.

Macrophages can be classified as either *M1 cells*,[51] or *M2 cells*.[52] *Tumor-associated macrophages* release growth and angiogenic factors.[53]

6.2.4 Thrombocytes

Thrombocytes (TC; size 2–4 μm) or platelets, are cell fragments involved in coagulation.[54] The average life time is 10 days.[55] Platelet activation is affected by hemodynamic forces. The usual concentration is equal to 250–$500\times10^3/\mathrm{mm}^3$.

The cytoplasmic organization is involved in shape change, secretion, and clot contraction. The shape rapidly changes on contact with foreign surfaces. Thrombostenin is a contractile protein of platelets organized in microfilaments. The cortex contains actin filaments and a circumferential bundle of microtubules. An intracellular tubular system is open to the cell surface via

[50] Different sets of dendritic cells are defined according to cellular origin, location, function, and life span in peripheral tissues, particularly in the spleen (where they have a relatively short life) and lymph nodes. Conventional dendritic cells originate from bone marrow-derived myeloid precursors for monocytes and dendritic cells [624]. Inflammatory dendritic cells, born from monocytes during inflammation, migrate to lymphoid organs. Plasmacytoid dendritic cells, which produce large amounts of interferon-1, might derive at least partially from myeloid progenitors. Antigen-presenting Langerhans cells, located in the skin, might be formed from skin-resident progenitors.

[51] M1 cells produce interleukin-12, granulocyte–macrophage colony-stimulating factor, interferon-γ, and tumor-necrosis factor.

[52] M2 cells secrete interleukin-10, transforming growth factor-β, vascular endothelial growth factor, and inducible nitric-oxide synthase (iNOS or NOS2) [625].

[53] Tumor-associated macrophages release tumor-necrosis factor, interleukin-1, IL6, CXC-chemokine ligand 8, vascular endothelial growth factor and colony-stimulating factor-1 (M-CSF). Production of reactive nitrogen species after iNOS stimulation can drive additional DNA mutations. Immunity cell contribution to tumorigenesis might depend on the balance between tumor-promoting cytokines (interleukin-6), and tumor-curtailing cytokines (interleukin-10, and transforming growth factor-β).

[54] Platelet aggregation and fibrin formation both require thrombin, Ca^{++}, and other clotting factors.

[55] The life duration of platelets is determined by the interplay between pro-survival BclXl of the Bcl2 family and the pro-apoptotic Bak, a target of BclXl in the apoptotic pathway [626]. Thrombocytes can survive for days. When BclXL degrades, aged platelets are destined to die. The balance between BclXl and Bak thus constitutes a molecular clock for thrombocyte life, BclXl maintaining platelet survival by restraining Bak.

invaginations of the plasma membrane. Dense tubules contain Ca^{++} stores and enzymes involved in arachidonic acid metabolism, which are required for platelet secretion and aggregation. The granular central part is embedded in a matrix.

Platelets are involved in various processes of vascular biology by the expression of multifold molecules (caspases, osteonectin, ubiquitin, vimentin, tissue inhibitors of matrix metalloproteinases, etc.). The two major secretory granule types include numerous α-granules and large dense granules (Table 6.9). Platelet dense granule membranes contain both granulophysin and P-selectin. α-Granule membrane contains growth factors,[56] clotting factors,[57] adhesion molecules,[58] and chemotactic compounds. Granules fuse by exocytosis with the plasmalemma for content release or with the membrane of the tubular system. Vessel wall collagen and various molecules (platelet-activating factor, serotonin,[59] and products of arachidonic acid metabolism), secreted by platelets and other cells, activate platelets. Activated platelets become spherical (contraction of actin filaments and microtubules), form pseudopodia for adhesion, secrete substances, and aggregate.

Circulating platelets are kept in an inactive state particularly by prostacyclin and nitric oxide released by the endothelial cells (Sect. 9.4). Endothelial cells also express plasmalemmal CD39, which inhibits platelet activation by adenosine diphosphate, ADP being converted into AMP. At injury sites, the platelets adhere to the exposed subendothelium and interact with collagen, von Willebrand factor, and fibronectin via their receptors, integrins, and glycoprotein Ib-IX. Both thrombin and ADP activate platelets, which then secrete ADP, platelet-derived growth factor, and fibrinogen from storage granules, as well as thromboxane-A2 by immediate synthesis. Platelet integrins, the glycoproteins IIb and IIIa, bind to fibrinogen, leading to platelet aggregation. With the simultaneous formation of a fibrin mesh, this process lead to the formation of a platelet thrombus.

[56] Platelet growth factors target fibroblasts and smooth muscle cells for wound healing (Chap. 10).

[57] Fibrinogen and von Willebrand factor (vWF) can be used as α-granule markers.

[58] CD9 (p24) and PECAM1 (CD31) are glycoproteins (GP) of the platelet membrane implicated in platelet adhesion and aggregation. CD9 and PECAM1 also line the membrane of α-granule, thus appear to have an intracellular distribution identical to GP IIb–IIIa, a major aggregation platelet receptor [627].

[59] Dense granules contain serotonin (5-hydroxytryptamine) after transport from blood. Serotonin causes vasoconstriction in blood vessels with damaged endothelium and activates platelets.

P2 receptors[60] are implicated in platelet activation. The activation of phosphatidylinositol 3-kinases by G-proteins coupled to the ADP receptors triggers platelet aggregation.

The platelet can also be considered as an inflammatory cell, synthesizing proteins involved in inflammatory pathways (Sect. 10.5). The α-granules of platelets indeed contain inflammatory modulators, such as P-selectin,[61] CD40 ligand,[62] thrombospondin-1, platelet factor-4, transforming growth factor-β, and IL1 precursors.

6.3 Hematopoiesis

The turnover of hematopoietic (αιματοσ: blood, ποιεο: to do) cells is estimated to be about 10^{12} cells per day [630]. All blood cell types are produced in the bone marrow and arise from a *pluripotent stem cell* (PSC). PSCs give birth

Table 6.9. Platelet released substances (Sources: [628, 629]).

Location	Substance	Target
α granule	PDGF, IGF, TGF	ECM
	PF4, β-thromboglobulin	GF activation
	Growth factors	
	FN, Fng, Tsp	TC aggregation
	Albumin	
	Protease nexin-2	protease inhibition, growth factor
Dense granule	ADP, ATP, Ca	TC aggregation
	5HT, catecholamines	Vasoconstriction
Cytosol	vWF	TC adhesion
	FV	Coagulation
	PAF	TC activation
	TXA2, 12-HETE	Vasoconstriction

[60] There are at least three subtypes of P2 receptors, P2X1, P2Y1, and P2Y12 (Sect. 3.2.1.1). The P2X1 ion channel, activated by ATP during collagen-induced platelet activation, generates a rapid influx of calcium, which leads to a change in platelet shape and platelet aggregation. The P2Y1 receptor, a G-protein-coupled receptor (Gq), also induces modifications in platelet shape and aggregation via calcium influx. Adenosine diphosphate binds to the P2Y1 receptor. The P2Y12 receptor, another G-protein-coupled receptor (Gi) for ADP, inhibits adenylyl cyclase.

[61] P-selectin leads to aggregates of activated platelets and leukocytes.

[62] CD40 ligand allows platelets to bind to macrophages, T cells, and endothelial cells, as well as to other platelets via glycoprotein IIb/IIIa receptors. CD40 ligand also activates an inflammation cascade involving E-selectin, VCAM, and ICAM1, thus attracting leukocytes.

by unipotential stem cells (USC) to the different lineages via proerythroblasts and erythroblasts, megakaryoblasts, myeloblasts and promyelocytes, and lymphoblasts. These stem cells express CD34 and produce by mitosis either stem cells of the same type (self-renewal) or progenitors leading to blood cells. In steady state, most of stem cells are dormant whereas few ones are active [632].

Hematopoietic stem cells either reside in bone marrow in a dormant state or enter the cell formation. In quiescent cells, protein kinase B remains inactivated, and its downstream target FOXO3a stays in the nucleus. When used as progenitors, membrane raft clustering leads to PKB phosphorylation and FOXO3a relocates in the cytoplasm [633].

6.3.1 Stem Cell Niches

In adults, hematopoietic stem cells (HSC), the progenitors of blood cells, have their own bone marrow microenvironment,[63] close to the endosteal[64] surface of bone marrow cavities in trabecular regions of long bones, whereas more differentiated hematopoietic progenitors are mainly located in the central bone marrow region [634]. The endosteal niche is characterized by a sinusoidal endothelium. Changes in the HSC niche does not allow appropriate HSC maintenance in vivo. Osteoblast cell lines secrete multiple cytokines which promote the proliferation of hematopoietic cells. Specialized spindle-shaped N-cadherin-expressing osteoblasts of the endosteum have direct contacts via N-cadherin of hematopoietic cells.[65] Many stromal cell lines of endosteal bone surfaces, which are involved in bone modeling, are required in HSC maintenance. The endosteal niche can contain quiescent and self-renewing HSCs. *Osteopontin*, inhibiting HSC proliferation, might maintain HSC quiescence (Fig. 6.3). *Angiopoietin-1* at the osteoblast surface interacts with Tie2 on stem cells to maintain stem cell quiescence in the niche.

Molecular cross-talks between HSCs and other cellular constituents of endosteal niches regulate the balance between HSC self-renewal and differentiation. Hematopoietic stem cells belong to a bone marrow cell population that expresses high levels of lineage stem cell antigen-1 (LinSCA1) and stem cell membrane-bound Kit,[66] but not plasmalemmal markers of lineage-committed

[63] Extramedullary transient hematopoiesis, in the liver or spleen, occurs after bone marrow stress; but after reparation, bone marrow hematopoiesis must follow.

[64] The endosteum is a bone remodeling site, characterized by high concentrations of calcium ions. Extracellular Ca^{++} concentration is assessed by calcium-sensing receptors.

[65] Receptor tyrosine kinases Tie1 and Tie2 are required in HSC maintainance in HSC microenvironment. Tie2, activated by angiopoietin-1 secreted by osteoblasts, upregulates N-cadherin expression in hematopoietic stem cells and maintains HSC quiescence via cyclin-dependent kinase inhibitor p21.

[66] Kit ligand is the stem-cell factor. Membrane-bound stem-cell factor, after proteolytic cleavage, binds and activates Kit. Membrane-bound stem cell factor

hematopoietic cells.[67] This cell subset is hence defined as LinSCA1+ Kit+ cells (LSK cells). Many plasmalemmal receptors control HSC location, among which is a calcium-sensing receptor [637].[68] Calcium-sensing receptors are particularly expressed by LSK cells.

Certain situations, such as inflammation, trigger the activity of osteoclasts along the stem cell niche, the secretion of enzymes (cathepsin G, elastase, MMP9, etc.) and cytokines (interleukin IL8), and the mobilization of progenitors from the bone marrow to the circulation [638]. Osteoclasts require RANKL signals from osteoblasts for proliferation and bone resorption.[69] Activating osteoclasts via either RANKL or other stimuli leads to emigration of hematopoietic stem cells into the circulation. In contrast, increased osteoblast activity via parathyroid hormone receptor or inactivation of the bone morphogenic protein receptor-1A causes a proliferation of hematopoietic stem cells.

The vascular bone marrow HSC niche is a second specialized HSC microenvironment in the bone marrow, with a large quantity of CD150+ HSCs attached to the fenestrated endothelium of bone marrow sinusoids. Bone marrow vascular and endosteal niches cooperate to control HSC quiescence and self-renewal. A small number of hematopoietic stem cells is constantly released into the blood circulation. Endothelial cells of the bone marrow sinusoids differ from endothelial cells of the microvasculature of any other organ.

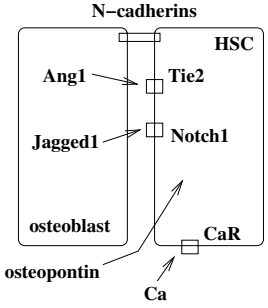

Figure 6.3. Endosteal niche of hematopoietic stem cells. Interactions between hematopoietic stem cells and osteoblasts (Source: [634, 635]).

stimulates adhesion of HSCs and hematopoietic progenitors to stromal cells, because it activates integrins.

[67] Hematopoietic stem cells can be precisely distinguished from other hematopoietic progenitors by the plasmalemmal expression of signaling lymphocytic activation molecules (SLAM), such as CD48, CD150, and CD244 [636].

[68] The endosteal niche for HSCs is characterized by a high Ca^{++} concentration (more than twenty-fold the plasmatic one, due to active bone remodeling in the endosteum by osteoclasts.

[69] RANKL is a member of the TNF family required for osteoclastogenesis.

These endothelial cells express cytokines, such as CXC-chemokine ligand 12[70] (CXCL12), like osteoblasts, and adhesion molecules for HSC mobilization, homing,[71] and lodging in endosteal bone marrow HSC niches [634]. CD150+ HSCs close to sinusoids could monitor the concentration of blood elements.

Hematopoietic stem cells without the calcium-sensing receptor can engraft in extramedullary spaces, as in the spleen or the liver, and produce extramedullary hematopoiesis.

6.3.2 Regulation Molecules

Notch- and Wnt-signaling pathways interact and regulate the self-renewal of the hematopoietic stem cells (HSC). The regulation of HSC self-renewal, via inhibition of differentiation and induction of proliferation, can be uncoupled. Notch signaling inhibits differentiation, thus maintaining HSCs in an undifferentiated state [639]. Notch signaling is required for Wnt-mediated maintenance of undifferentiated HSCs but not for entry into the cell cycle in vitro. Inhibition of the Notch-signaling pathway accelerates HSC differentiation in vitro. Notch signaling is specifically active in vivo in the HSC population of the bone marrow and in immature thymocytes. It is reduced or absent in more mature differentiated cells. Decrease in Notch signaling, with subsequent HSC differentiation, remains to be determined.

Nuclear transcription factor-Y (NF-Y) induces the expression of many genes encoding proteins that regulate HSC self-renewal and differentiation [640]. NF-Ya, the regulatory and DNA-binding subunit of NF-Y, thus acts in several pathways implicated in HSC self-renewal (Notch1, LEF1, telomerase RNA, etc.), regulating HSC self-renewal. Also, Bag1 is necessary for survival of hematopoietic stem cells [641].

Self-renewal, differentiation, which leads to given lineage types, and selection of lineages by the multipotential progenitors, are intrinsic properties of the progenitors and are stochastic. Survival and proliferation are regulated by cytokines and hormones [630] (App. A and Sect. 10.1). Both growth factors and hormones bind to plasmalemmal receptors (Table 6.10). Many kinds of hematopoeitins ensure a dynamic balance between cell differentiation and proliferation.

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[70] CXCL12 can induce motility, chemotaxis and adhesion of cells expressing its receptor, CXC-chemokine receptor-4, as well as secretion of matrix metalloproteinases and angiogenic factors, such as the vascular endothelial growth factor. CXCL12-CXCR4 association is required for retention and maintenance of adult hematopoietic stem cells.

[71] Homing corresponds to recruitment of circulating hematopoietic stem cells to the bone marrow vasculature and subsequent extravasation, which requires adhesion molecules, such as selectins and integrins. Rac1 and Rac2 GTPases are also implicated in homing and retention of hematopoietic stem cells in the endosteal bone marrow HSC niches.

Erythropoietin (Epo), a glycoprotein hormone synthesized mainly by the kidney cortex stimulated by hypoxemia in the renal arteries and by the liver, activates the production of erythrocytes.[72] Erythropoietin acts in the bone marrow by a specific erythropoietin receptor located mainly on erythroid progenitors. It stimulates proliferation of erythroid progenitors and maintains their viability. More precisely, Epo induces proliferation of burst forming unit-erythroid (BFU-E, early erythroid progenitor) and differentiation of colony forming unit-erythoid (CFU-E, later erythroid precursor) [631]. Epo also promotes megakaryocyte differentiation, B-cell proliferation, and endothelial cell chemotaxis. Furthermore, erythropoietin prevents the destruction of viable tissue surrounding injuries, such as infarction.

Thrombopoietin (Tpo) participates in hematopoiesis in general, and thrombopoiesis in particular. Tpo, assisted by interleukin IL11, stimulates the production of megakaryocytes via myeloid progenitor and, afterward of platelets (megakaryocyte fragmentation produces platelets). Tpo is manufactured by the liver, kidneys, smooth muscle, and marrow in particular. Tpo has the longest half-life (30 hours) in the circulation among hematopoietic growth factors.

Colony stimulating factors (CSF) are cytokines that stimulate the proliferation of stem cells of the bone marrow in adults. *Granulocyte–monocyte colony stimulating factor* (GM-CSF) induces the commitment of granulocyte–monocyte progenitors. GM-CSF stimulates proliferation of multi-lineage progenitors and the growth of BFU-E, granulocyte, macrophage, and eosinophil colonies [631]. GM-CSF also enhances phagocyte (neutrophil, macrophage, eosinophil) functioning. GM-CSF is secreted by lymphocytes after stimulation by antobodies on the one hand and fibroblasts and endothelial cells when they are activated by IL1 and tumor necrosis factor (TNF) produced by macrophages and T cells on the other hand. GM-CSF can also be synthesized

Table 6.10. Examples of hematopoietic growth factor receptors (Source: [631]).

Receptor	ligand
Type 1 cytokine	IL1–IL9, IL13, IL18, GM-CSF, G-CSF, Epo, Tpo
Type 2 cytokine	IL10, INF
Type 3 cytokine	M-CSF, SCF
Chemokine (G-protein-associated))	IL8
TNF	TNF

[72] Erythropoietin (Epo) and its receptor (EpoR) are also synthesized by neoplastic cells and tumor endothelial cells.

by macrophages and mast cells. *Granulocyte colony stimulating factor* (G-CSF), produced by the endothelium, macrophages, and immune cells, stimulates proliferation and maturation of monopotent neutrophil progenitors that differentiate into neutrophils. G-CSF thus has a relatively specific activity. Further stimulated by IL5, the granulocyte–monocyte progenitors develop into eosinophils. G-CSF is produced by fibroblasts and monocytes/macrophages stimulated by GM-CSF and IL3. IL3 participates in the differentiation of basophils. G-CSF enhances neutrophil functioning (chemotaxis, and phagocytosis) and is involved in neutrophil removal from the blood circulation. Stimulated by *macrophage colony stimulating factor* (M-CSF) the granulocyte–macrophage progenitor cells differentiate into monocytes. M-CSF also enhances cytokine production (G-CSF, IL6, and IL8) and other functions in monocytes and macrophages.

Stem cell factor (SCF) is produced by marrow endothelial cells, fibroblasts, and other sources. SCFs act in synergy with Epo, Tpo, IL3, GM-CSF, and G-CSF on BFU-E, CFU-GM, and CFU-granulocyte–erythroid–macrophage–megakaryocyte, and possibly preCFU-C, as well as on megakaryocytic progenitors [631]. SCF enhances IL7-induced proliferation of preB cells and stimulates T lymphocyte development and maturation. With IL2, SCF promotes differentiation of CD34+ hematopoietic cells into NK cells.

Other cytokines affect the hematopoietic development. *Tumor necrosis factor* α (TNFα) stimulates granulopoiesis and inhibits erythropoiesis. *Insulin like growth factor*-1 (IGF1) induces erythroid colony formation.

Interleukins (IL) are also involved in hematopoeisis (Table 6.11). IL1[73] modulates the expression of genes[74] and surface receptors for IL2, IL3, IL5, and GM-CSF in particular. IL1 increases the production of CSFs and SCFs (Table 6.11). IL1 enhances the activation of T cells in response to antigen. This activation leads to increased T-cell production of IL2, which raises T-cell activation (*autocrine loop*). IL1 also induces expression of interferon-γ (Ifnγ) by T cells. This effect of T-cell activation by IL1 is mimicked by tumor necrosis factor-α secreted by activated macrophages. IL1 is secreted by macrophages, neutrophils, ECs, SMCs, B and T cells, and fibroblasts in particular. IL2 is responsible for T-cell proliferation. It also acts on macrophages, B cells, NK cells, and lymphokine-activated killer cells [631]. NK cells secrete TNFα, Ifnγ, and GM-CSF in response to IL2, which activates macrophages. IL2 is secreted by activated CD4+ T helper cells. Its expression is induced by IL1.

Synthesized by activated T cells and NK cells, IL3 acts on hematopoietic progenitors, in combination with other cytokines. IL3 induces proliferation of

[73] IL1 α and IL1 β are agonists, whereas and IL1 receptor antagonist (IL1RA) is a specific antagonist.

[74] IL1 increases the expression of itself and other cytokines, including IL1RA, as well as of mediators, like cyclooxygenase and nitric oxide synthase, of growth factors (fibroblast growth factor, keratinocyte growth factor, hepatocyte growth factor, nerve growth factor, and insulin-like growth factor), of clotting factors, neuropeptides, extracellular matrix molecules, etc. [631].

several lineages and enhances the effects of GM-CSF. Activated CD4+CD8+ T cells, basophils, and mast cells produce IL4 and IL13. IL4 is a T-cell GF and promotes the differentiation of Th0 precursors toward the Th2 lineage [631]. IL13 elicits many actions of IL4. IL5, manufactured by activated CD4+CD8+ T cells and mast cells, influences eosinophil and basophil production and functioning. IL5 enhances IL2-dependent differentiation and proliferation of T cells.

Fibroblasts, endothelial cells, activated B cells, monocytes, and T helper cells produce IL6, which promotes development and functioning of both B and T lymphocytes and megakaryocyte maturation [642]. IL6 acts in synergy with IL1 and TNF. IL6 enhances the production of immunoglobulin and glucocorticoid synthesis. IL7 acts on the lymphocyte production. IL7 stimulates stem cells to form lymphoid progenitors, which lead to B and T cells. In particular, IL7R play a role on the common lymphoid progenitor, the source of all lymphoid lineages [643].

Stimulated monocytes, neutrophils, T cells, NK cells, fibroblasts, and endothelial cells secrete IL8. IL8 is chemoattractant for fibroblasts, neutrophils, basophils, T lymphocytes, and endothelial cells[75] [644]. IL8 also activates integrins (Sect. 2.2) in monocytes and eosinophils for adhesion to endothelial

Table 6.11. Interleukins associated with vascular and blood cells.

Interleukin	Sources	Targets and functions
IL1	Mφ, Nφ, B Lφ	Granulocyte, EC, Mo, FB Hematopoiesis, inflammation Cn synthesis, chemotaxis, Proliferation
IL2	TH1 and NK Lφ	B, T, and NK Lφ
IL3	T Lφ	Hematopoietic progenitor cells
IL4	TH2 and mast cells	B Lφ, Eφ, mast cells
IL5	TH2 and mast cells	Eφ
IL6	TH2 and B Lφ, Mφ	B and T Lφ, thrombopoiesis
IL7	Thymic and marrow cells	B and T lymphopoiesis
IL8	Mφ, Nφ	Nφ, T Lφ
IL9	T Lφ	Hematopoiesis, thymopoiesis
IL10	TH2, CD8+ T and B Lφ, Mφ	B and mast cells Inhibition of cytokine production Anti-inflammatory
IL11	Stromal cells	Hematopoiesis, thrombopoiesis
IL12	B Lφ, Mφ	NK Lφ
IL13	TH2-Lφ	B Lφ, Eφ, mast cells

[75] During extravasation, the leukocytes migrate stimulated by IL8 concentration gradient and accumulate at locations of high concentration.

cells. IL9 acts on erythroid progenitors, in the presence of Epo, and T and B cells [645].

IL10 is expressed by CD4+ T cells (Th0, Th1, and Th2 clones), CD8+ T cells, monocytes, and macrophages [631]. IL10 inhibits the synthesis of Th1-derived (IL2, Ifnγ, and GM-CSF) and monocyte-derived cytokines (IL1α and β, IL6, IL8, TNFα, GM-CSF, and G-CSF), but induces IL1RA production by macrophages. In the presence of monocytes and/or macrophages, IL10 inhibits proliferation of resting T cells, the reduced proliferation being only partially due to decreased IL2 production. IL10 can also enhance the cytotoxic activity of CD8+ T cells. IL10 stimulates B cells and mast cells [646].

IL11 is expressed by fibroblasts, and endothelial cells among others. In synergy with IL3, IL4, IL7, IL12, IL13, SCF, and GM-CSF, IL11 stimulates the proliferation of primitive stem cells as well as commitment and differentiation of multi-lineage progenitors [647]. IL11 acts synergistically to stimulate: (1) megakaryocytopoiesis and the thrombopoiesis with IL3, Tpo or SCF; (2) erythropoiesis with IL3, SCF or Epo; and (3) myeloid colony formation with SCF.

IL12 is produced by macrophages, activated monocytes, and neutrophils. A positive feedback exists between IL12 and Ifnγ, this loop being controlled by IL10, TGFβ, IL4, and IL13, which downregulate IL12 production and the ability of T and NK cells to respond to IL12 [631]. IL12 activates NK cells and enhances via Ifn$\gamma$the phagocytic activity. IL12 favors TH1 cell differentiation and functioning and inhibits TH2 cell differentiation. IL12 synergizes with other hematopoietic factors to promote proliferation of early multi-potent hematopoietic progenitors and lineage-committed precursors [648].

IL15 is expressed in heart, skeletal muscle, lungs, liver, and kidneys, as well as in activated monocytes, macrophages, endothelial cells, fibroblasts, etc. IL15 triggers the proliferation of activated B lymphocytes and their immunoglobulin production, as well as the proliferation of NK cells and activated CD4+CD8+ T cells. IL16 synthesized by CD4+CD8+ T cells, eosinophils, and mast cells is a chemokine for CD4+ cells, monocytes, and eosinophils.

IL17 is secreted by CD4+CD45+RO+ activated memory T cells [649]. IL17 stimulates T cells, endothelial cells, fibroblasts, and macrophages to express various cell-specific cytokines [650]. IL17 also exhibits indirect hematopoietic activity by enhancing the capacity of fibroblasts to sustain the proliferation of CD34+ hematopoietic progenitors and their differentiation into neutrophils [651]. T lymphocytes, NK cells, and macrophages are targeted by IL18, which promotes the production of Ifnγ and TNF [652].

Proliferation and maturation of committed progenitors is controlled by late-acting factors such as Epo, M-CSF, G-CSF, and IL5, most of them being lineage-specific [630]. Progenitors at earlier stages of development are controlled by a group of intermediate-acting lineage-non-specific cytokines: IL3, GM-CSF, and IL4. Primitive progenitors are committed by early-acting cytokines, which include IL6, IL11, IL12, and G-CSF. Certain cytokines are either stimulatory or inhitory. However, others exhibit both effects like IL4.

Among inhibitors, lineage-non-specific interferons act at various development stages, whereas TGFβ affects early phases of hematopoiesis [653]. In addition, the synthesis of cytokines by the vascular endothelium is stress dependent (Sect. 9.5). Steady laminar shear stress increases the production of GM-CSF by ECs [654].

6.4 Blood Therapies

6.4.1 Blood Cell Disorders

Anemia is due to acute or chronic factors. Anemias are classified according to the hemoglobin level (Table 6.12). Injuries are common examples of acute anemias by hemorrhage. Another usual cause is cancer. Tumor anemia is due to tumor bleeding, hemolysis, and deficiency in folic acid and vitamin B12. Release of cytokines, especially interferon-γ, interleukin-1, and tumor-necrosis factor-α, impairs erythropoietin synthesis in the kidney. Activation of macrophages can lead to a shorter erythrocyte half-life and a decrease in iron use. Chemotherapy and radiotherapy further aggravate anemia. Recombinant human erythropoietins (epoetins) avoid transfusions, but thromboembolic complications have been observed [655].

Platelet and leukocyte deficiencies can also occur. Thrombocytopenia and leukopenia can result from damage to hematopoeitic stem cells or precursors. Lymphocytopenia (or lymphopenia) can be also due to lack of zinc, long-term heavy alcohol consumption, and certain infections.

6.4.2 Blood Substitutes

Blood substitutes have been studied for universal compatibilility, availabililty and storability. They are based either on hemoglobin, encapsulated or cross-linked to avoid toxicity, or perfluorocarbons, which carry O_2 and CO_2. Nowadays, the production of cultured human RBCs using culture support that mimics bone marrow environment (cytokine supply, coculture, etc.) provides an alternative to blood transfusion. Cultured RBCs can also be used: (1) as

Table 6.12. Anemia grading.

Grade	Degree	Hemoglobin
0	Normal	12–16 g/dl (women) 14–18 g/ dl (men)
1	Mild	10–12/14 g/dl
2	Moderate	8–10 g/dl
3	Severe	6.5–8 g/dl
4	Life threatening	<6.5 g/dl

model of human RBC-targeted infections, and (2) for gene therapy. In vitro production of mature and functional human RBCs from hematopoietic stem cells has been demonstrated [656].

6.4.3 Plasmapheresis

Plasmapheresis separates plasma and blood cells. The filtration flux per unit of plasma filter area is approximately proportional to the wall shear rate at least up to 7500/s and depends on the effective membrane length $L^{-1/3}$ [657]. Pulsatile flow with the time-mean flow rate equal to the steady flow enhances the filtration flux, with optimal modulation frequency 1 Hz and volume amplitude 1.5 ml [658]. Mathematical and numerical analysis of filtration across a fibrous medium has been performed [659], using an homogeneization technique [660–666].

7

Heart Wall

The heart is made of two synchronized pumps with a common wall, the septum between the left and right atria at its upper part, and the left and right ventricles at its lower part. These twin pumps are composed of two chambers, upstream atrium and downstream ventricle, to adapt to the pressure difference between the upstream venous compartment and downstream arterial bed. The two pumps function in parallel. The left heart propels blood through the systemic circulation (arterial oxygenated blood), and the right heart through the pulmonary circulation (arterial deoxygenated blood; the artery corresponds to a vessel leaving the cardiac pump, without relation with the blood gas content).

7.1 Cardiogenesis

The cardiogenesis is a regulated process, disturbances of which lead to cardiac malformations. Body organ morphogenesis, indeed, involves the perfect orchestration of cell differentiation, proliferation, and migration (Fig. 7.1). Mesodermal cells, which differentiate into cardiac cells during heart morphogenesis, segregate into two distinct populations of cardiac progenitors: the primary heart field and anterior heart field, which contribute to the left ventricle and right ventricle and outflow tract, respectively. Both heart field cells are tagged by the transcription factor NKX2.5, whereas the transcription factor islet1 is only involved in the differentiation of second heart field cells. Second heart field cells persist in the postnatal heart. These progenitors can differentiate into several types of cardiac cells.[1] Fibroblast growth factor-8

[1] Damaged heart, valves, vessels, conduction tissue and myocardium must be regenerated. The mobilization of endogenous progenitors and the differentiation of exogenous stem cells participate to tissue repair. Adult cells are genetically equivalent to embryonic cells. Differential gene expression results from reversible epigenetic (phenotypic) changes gradually imposed on the genome during

(FGF8), which promotes cardiogenesis, is found in the anterior heart field and in a subset of the primary heart field cells [667, 668]. Autocrine FGF8 signaling is required in the anterior heart field to promote cell proliferation and survival. Diversin controls fusion of heart precursors independently of β-catenin [669]. Diversin binds dishevelled, to activate Rac and Rho GTPases, then c-Jun N-terminal kinase.

Morphogenesis of endothelium-lined tubes leads to the development of a functional circulatory system. The formation of endothelial vacuoles followed by intracellular and intercellular fusion drives vascular lumen genesis [670]. These endothelial pinocytic vacuoles quickly occur, fuse together and enlarge to create the tube lumen. Endothelial cells form extensive contacts between themselves and merge their intracellular vacuoles without cytoplasmic mixing. The vacuolar compartments in adjacent endothelial cells associate and form a continuous lumen in developing blood vessels.

During early embryogenesis, the heart is a single, relatively straight tube that bends and twists. Different myocardial progenitors form the various regions of the heart. The embryonic heart begins to pump blood before the development of valved chambers. The pumping action results from suction into the heart tube [671]. The bloodstream in the developing heart favors directed fluid motion in a stressed gel-like medium. The embryonic heart,

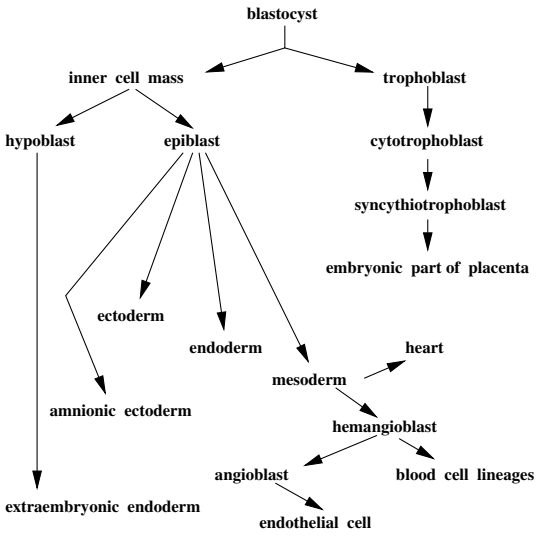

Figure 7.1. The cardiovascular organs develop from the mesoderm. The blood vessels form independently from the heart; yet the heart is the first functional body organ.

development. However, adult cells have a reprogramming capacity toward an undifferentiated embryonic state, as differentiation is reversible.

whereas pulsating, is transformed from a simple tube into a four-chambered pump via multiple transluminal septation.

Relationships among the epicardium, coronary beds, and conduction paths are observed during heart development. Cells of the proepicardial organ (PEO), a more or less diffuse cluster of extracardiac mesothelial cells, are precursors for epicardial epithelial cells, myocardial connective tissue cells, coronary smooth muscle cells, and endothelial cells. After proliferation, PEO cells travel over the myocardial surface and differentiate into a epithelium to form the primitive epicardium. A set of epithelial cells migrate into the subepicardium and undergo an epithelial-to-mesenchymal transformation.[2] Epithelial-to-mesenchymal transformation is implicated in manifold steps of cardiovascular morphogenesis, such as heart valve development, coronary artery formation, inflow and outflow tract septation [672]. Epithelial-to-mesenchymal transformation is the first step of morphogenic events that transform the mesenchyme into functional structures, such as septa and valves, required for unidirectional pumping activity. A set of epithelial cells in the cardiac wall give rise to cardiac fibroblasts and participate in coronary vasculogenesis. Another set interacts with the myocardioblasts for myocyte differentiation into Purkinje cells. Certain epicardium-derived cells migrate in the core of the endocardial cushions to produce atrioventricular valve leaflets.

Epithelial-to-mesenchymal transformation occurs also in adult life, especially in cardiovascular diseases such as atherosclerosis, intimal hyperplasia, and aneurisms. Ubiquitous periostin is involved in epithelial-to-mesenchymal transformation and subsequent mesenchymal maturation, with differentiated mesenchyme and condensed matrix in embryonic heart valves. Periostin is also highly expressed in the myocardium in patients with heart failure. Periostin inhibits cardiomyocyte spreading and adhesion of cardiac fibroblasts [673].

During normal heart development, epicardial-derived cells (EPDC) undergo an epithelial-to-mesenchymal transition in response to BMP, FGF, and TGFβ released from the myocardium. Secreted myocardial thymosin-β4 then induces EPDC migration into the myocardium, where they respond to angio- (VEGF/FGF2) or arteriogenic (PDGF/TGFβ) growth factors and differentiate into either endothelial or smooth muscle cells, respectively, leading to a capillary plexus and stabilizing coronary vessels.

Two myosin heavy chain genes are expressed in the mouse heart: β-MHC in the embryo, and α-MHC postnataly. Cardiac stresses favor β-MHC with negative repercussions on the cardiac function.

Developing cardiac valves, like other avascular tissues, express transcription factors, such as Sox9, NFATc8, Cbfa1, and MSX2, and growth factors,

[2] During epithelial-to-mesenchymal transformation, epicardial and endocardial (endothelial) cells transform into mesenchymal cells, which generate different tissues. Epithelial-to-mesenchymal transformation involves fibroblast growth factor, epidermal growth factor, vascular endothelial growth factor, transforming growth factor-β (Sect. 10.1), and transcription factor WT1.

such as BMP2 and TGFs zlig2. Sox9, in conjunction with Sox5 and Sox6, targets the gene Chm1[3] in avascular tissue, coding for chondromodulin-1. In the interstitial space, chondromodulin-1 inhibits endothelial cell proliferation and angiogenesis [674]. Chondromodulin-1 expression persists in normal cardiac valves throughout life. It is downregulated in degenerative cardiac valves. Loss in chondromodulin-1, indeed, leads to vascularization, calcification, lipid deposition, and inflammation of cardiac valves.

With its structural and functional heterogeneity, the heart achieves a coordinated contraction of its myofiber population in the two in-parallel pumps with its in-series twinned chambers to eject blood in both circulations during each cycle. This composite material is optimally organized. The activation phase of the myofibers matches their mechanical heterogeneity for a suitable electromechanical coupling.

7.2 MicroRNAs

MicroRNAs affect heart development and function because they regulate the expression of proteins generated from messenger RNA transcript of genes involved in the regulation of cardiac morphogenesis, in the propagation of electrochemical waves, in the myocardium hypertrophy and contractility (Table 7.1). MicroRNAs commonly bind to mRNAs, thereby inhibiting mRNA translation with or without mRNA cleavage. The miR-1 level increases during cardiac infarctions specifically in the ischemic regions [675]. Overexpression of miR-1 increases the occurrence of postinfarct arrhythmias and promotes arrhythmias in healthy hearts. miR-1 Overexpression slows conduction and depolarizes the plasmalemma by repressing transcripts of KCNJ2 gene encoding the Kir2.1 subunit of the potassium channel, which sets the resting membrane potential, and of GJA1, which encodes connexin 43 of gap junctions. The functions of miR-1 are dose sensitive [676]. Moreover, miR-1-2 regulates not only cardiac morphogenesis, but also the cardiomyocyte cycle. In mouse embryos, homozygous deletion of muscle-specific miRNA miR-1-2 induces ventricular septal defects, possibly due to an increased level of transcription factor Hand2, a regulator of cardiac morphogenesis. Surviving mice exhibit cardiac arrhythmias due to defects in potassium channels associated with increased abundance of the transcription factor Irx5, a repressor of the potassium channel gene Kcnd2. Moreover, they have an increased rate of cardiomyocyte mitosis. The muscle-specific miRNAs miR-1 and miR-133, which are implicated in skeletal myoblast proliferation and differentiation, respectively, hinder hypertrophic growth of the myocardium. MicroRNA miR-133 targets transcripts for RhoA and Cdc42, associated with cytoskeletal and myofibrillar rearrangements during hypertrophy, as well as NelfA/Whsc2, a nuclear factor involved in cardiogenesis [677]. Conversely, in cardiac hypertrophy, miR-133 and miR-1 are downregulated. Cardiac contractility depends on

[3] Chm1 is also called Lect1.

the expression of two MHC genes. α-myosin heavy chain and β-MHC, indeed, are contractile proteins of the heart, which are regulated in a antithetical manner by various signals.[4] Cardiac-specific microRNA miR-208, encoded by an intron of α-MHC gene, controls the expression of β-MHC gene, which is elevated by physical stress and hypothyroidism [678]. MicroRNA miR-208 targets thyroid hormone receptor-associated protein THRAP1,[5] a regulator of the thyroid hormone receptor, which hampers β-MHC expression.

7.3 Cardiac Progenitor Cells and Precursors

The adult heart contains c-Kit+ stem cells for its regeneration [679]. Multipotent progenitor cells generate the four major cell types of the heart: cardiomyocytes, nodal cells, smooth muscle cells, and endothelial cells. Both embryonic and postnatal cardiovascular progenitor cells, characterized by the expression of the transcription factor islet1, can differentiate into cardiac smooth muscle cells or endothelial cells. Cardiomyocytes come from the primary and secondary heart fields in the embryo [680]. Tripotent progenitor cells from the secondary heart fields express transcription factor islet1, Nkx2.5, and VEGFR2 [681]. Certain cardiac tripotent progenitor cells express the marker

Table 7.1. MicroRNAs and heart structure and activity in mice (Sources: [675–678]. MicroRNAs regulate cardiac structure and activity, more precisely, heart morphogenesis and growth, conduction of the electrochemical signal, and myocardium contraction. Cardiac miR-1 targets a gap-junction protein and cardiac transcription factor Irx5, which regulates the nodal tissue. miR-1 Overexpression is associated with cardiac arrhythmia. Loss in miR-1-2 leads to ventricular septum defects (septal holes), cardiomyocyte hyperplasia, and abnormal conduction in the ventricular nodal tissue. MicroRNA miR-133 prevents cardiac hypertrophy in response to mechanical and chemical stimuli. Heart-specific miR-208 is transcribed from a sequence of the intron of the gene of the heavy chain of α-myosin. MicroRNA miR-208 is required for the expression of β-myosin heavy chain during stress or hypothyroidism.

MicroRNAs	Cardiac effect
miR-1	Conduction
miR-1-2	Morphogenesis, CMC number, conduction
miR-133	Growth
miR-208	Growth

[4] The slower adenosine triphosphatase β-MHC is the dominant isoform expressed in the embryonic heart, whereas faster ATPase α-MHC is upregulated postnataly. The β-MHC level rises during cardiac diseases when α-MHC is down-regulated. Thyroid hormone T3 stimulates α-MHC and inhibits β-MHC transcription after birth.

[5] THRAP1 is also known as TRAP240.

brachyury at a specific stage of embryonic development [682]. c-Kit+ Nkx2.5+ differentiated embryonic stem cells, which serve as cardiac progenitor cells, give rise to cardiomyocytes and smooth muscle cells but not to endothelial cells [683].

Regulators of coronary vasculogenesis are also involved in repair of ischemic heart damage resulting from vascular insufficiency. Coronary vasculogenesis from epicardium involves friends-of-GATA2 (FOG2) and VEGF [684].[6] Thymosin-β4, a G-actin monomer-binding protein, promotes vascularization from the embryonic epicardium. Thymosin-β4 stimulates Tie2+ progenitor cells derived from quiescent adult epicardial cells and triggers their migration and differentiation into fibroblasts, smooth muscle cells when stimulated by platelet-derived growth factor and transforming growth factor-β, and endothelial cells, in response to a combination of vascular endothelial growth factor and fibroblast growth factor-2 [685]. The thymosin-β4 product by peptidase cleavage, the pro-angiogenic N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), stimulates differentiation from adult epicardial cells into endothelial cells.

Growth factors of cardiac myogenesis include members of the bone morphogenetic protein and Wnt families. Both canonical and non-canonical Wnt pathway can promote cardiomyocyte formation. Transcription factor Sox17 acts on transcription factors of cardiac specification in primitive mesoderm Mesp1 and Mesp2 [686].

Wnt–beta-catenin signaling exhibits developmental stage-specific effects on cardiomyogenesis, hematopoiesis, and vasculogenesis [687]. In the early phase during embryoid body formation, it enhances differentiation of embryonic stem cells into cardiomyocytes and suppresses differentiation into hematopoietic and vascular cell lineages. In the late phase after embryoid body formation, it prevents bone morphogenetic protein signaling, thus inhibiting differentiation into cardiomyocytes and enhancing differentiation into hematopoietic and vascular cell lineages.

Smooth muscle α-actin exists in progenitor cells of cardiomyocytes. During cardiomyocyte differentiation, α-actin gene activity is downregulated as soon as rhythmic contractility starts. The level of LIM-only protein CRP2 is correlated with smooth muscle gene activity [688]. CRP2 is a transcription co-adaptor that recruits serum response factor and remodels silent chromatin in adult cardiomyocytes toward smooth muscle gene activity.

7.4 Wall Structure

The heart wall is composed of three layers: (1) the internal thin *endocardium*, (2) the thick muscular *myocardium*, and (3) the external thin *epicardium*. The

[6] FOG2(−/−) hearts are characterized by a thin ventricular myocardium, common atrioventricular canal, tetralogy of Fallot, and absence of coronary vasculature. Transgenic re-expression of FOG2 in cardiomyocytes leads to coronary vessel development.

double-layered *pericardium* is composed of the outer parietal pericardium and the inner visceral pericardium, or epicardium, which is thus attached to the myocardium. The pericardial cavity separates the two pericardium layers. It contains a lubrificating fluid ($V \sim 25$–35 ml). The parietal pericardium consists of an outer layer of thick, fibrous connective tissue and an inner serous layer with a *mesothelium* and connective tissue. The anchoring parietal pericardium is attached to the diaphragm and fuses with the outer wall of blood vessels entering and leaving the heart. The visceral pericardium has an external layer of flat mesothelial cells lying on a support tissue that contains elastic fibers. The coronary arteries reside in the epicardium and send branches into the myocardium.

The heart wall is composed of various cell types that are embedded in an extracellular matrix, especially with fibrillar collagen. The cell set includes not only cardiomyocytes (at least one-third of all cells) and nodal cells, but also endothelial cells, vascular smooth muscle cells, and fibroblasts. Reciprocal regulation of the collagen turnover exists between stimulators (e.g., angiotensin-2,[7] endothelin-1, catecholamines, aldosterone, fibroblast growth factor FGF2, insulin-like growth factor, etc.) and inhibitors (prostaglandins, nitric oxide, natriuretic peptides, etc.) [689].

The epicardial adipose tissue, a source of free fatty acid, generates various substances, such as *adiponectin* (App. A.2.1), *resistin* (App. A.2.3), and *adipokines* (inflammatory cytokines). Adiponectin[8] (Apn), produced by adipocytes has cardioprotective effects, via its anti-inflammatory and anti-atherogenic properties. Adiponectin binds to adiponectin receptors AdipoR1 and AdipoR2, which also link adapters. Apn also increases sensitivity to insulin in muscle and liver (insulin-sensitizing hormone). Resistin is associated with insulin resistance. Epicardial adipose tissue may serve as a cardiac risk marker and be implicated in the development of cardiac pathology [690].

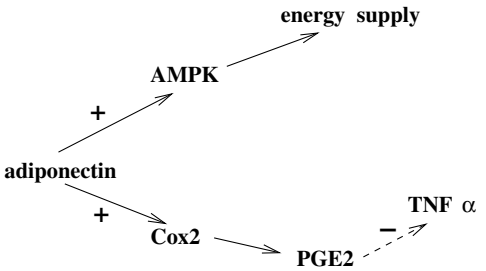

Figure 7.2. Adiponectin activities. Adiponectin stimulates cyclooxygenase-2 (Cox2) and the synthesis of prostaglandin-E2 (PGE2), and thereby inhibits TNFα. It also acts via AMP-activated protein kinase (AMPK).

[7] Angiotensin-2 favors the production of collagen-1 and -3.

[8] Adiponectin is also called ACRP30, AdipoQ, or gelatin-binding protein-28. It is involved in the regulation of lipid and glucose metabolism.

The adiponectin receptors, AdipoR1 (also observed in skeletal muscles) and AdipoR2 (also found in the liver), are expressed in the heart. Stimulated AdipoRs increases AMP-activated protein kinase, mediates peroxisome proliferator-activated receptor-α ligand activities, glucose uptake and fatty-acid oxidation [691]. Low levels of circulating adiponectin, as well as AdipoR1/R2, are measured in obesity, which lead to insulin resistance.

Adiponectin acts either via AMP-activated protein kinase or via cyclooxygenase Cox2, involved in prostanoid synthesis (AMPK-independent pathway; Fig. 7.2). AMPK, which is also activated by an increased ratio of AMP to ATP, helps fatty-acid catabolism and glucose uptake and inhibits glucose synthesis. AMPK also protects cardiomyocytes from ischemic injury, particularly hypoxia–reoxygenation-induced apoptosis. Suppression of cardiac AMPK activity indeed leads to increased injury when myocardium perfusion is reduced. Phosphorylation (activation) of AMPK after cardiac ischemia-reperfusion injury is attenuated in hearts of adiponectin-deficient mice [692]. Apn prevents TNFα production by upregulating Cox2, and subsequently prostaglandin PGE2. It thus reduces inflammation. Intravenous Apn injection, either shortly before or after the induction of ischemia in mice reduces ischemic injury [692].

The myocardium is mainly composed of *cardiomyocytes* (CMC). In both ventricles, papillary muscles and chordae tendinae tether the valve. The endocardium is composed of three layers. The outer layer is made of collagen fibers, which merge with collagen surrounding adjacent cardiomyocytes. This layer contain the Purkinje fibers of the *nodal tissue*. The thickest middle endocardial layer contents more regularly arranged collagen fibers and in parallel arranged elastic fibers. The inner layer is constituted of endothelial cells (EC), which are continuous with the vessel endothelium. The endocardium is thicker in the atria than in the ventricles.

The heart has a *fibrous skeleton* with its central fibrous body, which prevents early propagation of action potential. The central fibrous body provide extensions: (1) the valve rings, to which are inserted the cardiac valves, and (2) the membranous interventricular septum,[9] which can bulge into the right ventricle to form aneurism.

7.5 Heart Valves

The cardiac valves are sheets of connective tissue, which begin at the *annulus fibrosus*. The valve cusps are passive soft tissues attached to the wall at the insertion line. The aortic and pulmonary valves have smooth ventricular and wavy arterial faces. The free edge is indented. The middle part of the free edge is thicker with the *Arantius nodule*, characterized by a high concentration of collagen fibers. These geometry features affect both valve motion during the

[9] The pars membranacea septi correspond to the upper part of the interventricular septum.

cardiac cycle,[10] as well as flow in the aortic sinus when it is open, and thus the transvalvar pressure. Effective boundary conditions or wall laws for laminar pattern can be introduced. Roughness elements are supposed to be periodic with a length scale greater than the flowing cell size [693].

The valves, covered by the endothelium and containing smooth muscle cells, are reinforced by many internal bundles of collagen and elastic fibers (Table 7.2). Sparsely distributed valvular interstitial cells have extensive contact with proteoglycans of the extracellular matrix, as well as with elastin and collagen fibers. Collagen-1 forms a dense content beneath the endothelium. Collagen fibers of the outer layer of the cusp are mainly oriented in the azimuthal direction. Collagen fibers are more randomly oriented in the cusp central part. Elastin fibers are more abundant near the fibrous ring. In the fibrous ring, collagen fibers form massive wavy twisted bundles and elastin fibers have different directions. Also in the commissures, collagen fibers form massive twisted bundles. Chordae tendinae mingle with the valvar connective tissue. The myocardium penetrates into the valve leaflets. The valve components and their estimated elastic moduli are given in Part II. Whatever the transvalvar pressure at which the porcine aortic valves are fixed ($< 12\,\mathrm{kPa}$), the fibers mainly run along the circumferential direction [694]. Moreover, strong collagen fiber bundles that travel through the valve cusps and attach to the arterial walls behave like suspension cables for force transmission.

The cusp is a multilayer structure. The three layers are the *fibrosa*, the *spongiosa* (which is absent in the coaptation region), and the *ventricularis* (Fig. 7.3). The fibrosa, located toward the arterial wall, contains a large number of circumferential[11] wide collagen bundles. These circumferential bundles are arranged in a corrugated manner for radial expansion. A matrix with elastin surrounds the collagen bundles. The ventricularis contains collagen and elastin fibers, which are less organized than in the fibrosa. However, elastin sheets provide recoil, which retains the folded shape of the fibrosa. The loosely organized spongiosa, between the fibrosa and ventricularis, contains glycosaminoglycans, collagen, and elastin mostly in the radial direction. The spongiosa could act as load buffer for the fibrosa and the ventricularis.

The heart structure provides the three properties of contractibility, automatism, and conduction due to two kinds of cardiac muscular cells: cardiomyocytes and nodal myocytes.

Mathematical Histology

Structure–function features of the heart have been mathematically investigated. Two fiber networks have been particularly studied: (1) the network of

[10] In addition to the valve cross curvature of the open leaflet, the Arantian nodule induces axial curvature due to stiffer medial thickening.

[11] Collagen bundles run from commissure to commissure, spreading out near the cusp belly and combining again toward the opposite commissure.

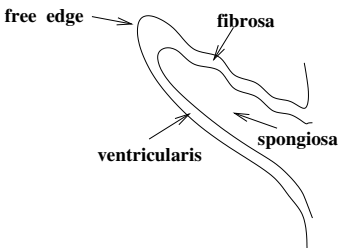

Figure 7.3. Ventriculo-arterial valve layers, according to the internal collagen network of the valve leaflets: fibrosa, spongiosa and ventricularis from the wavy arterial face to the smooth ventricular face. Collagen fibers provide strength, elastin fibers elasticity and proteoglycans swelling. Collagen architecture is mediated by mechanical loading.

Table 7.2. Structural elements of heart valves, with its three layers: (1) the ventricularis (rich in elastin); (2) the central spongiosa; and (3) the fibrosa (with packed collagen). Forces applied on the valve leaflets (pressure, shear, tension, and bending) are borne by structural rearrangement (fiber reorientation, folding, uncrimping, compaction). Collagen and elastin fibers cooperate. Alignment of packed collagen bundles transfers forces between the cusps and the wall. Corrugations of the cusp face in front of the wall (fibrosa) help size and shape changes during the cardiac cycle. Microscopic collagen foldings (crimps) allows lengthening from low stresses. The anisotropic behavior is responsible for required differences in radial and circumferential extensibility. Cusp coaptation avoids valve prolapse. The trileaflet semilunar ventriculo–arterial valves are inserted and supported by the aortic and pulmonary roots. The atrioventricular valves are inserted on an annulus. Chordae tendineae and papillary muscles maintain the atrioventricular valve closed during blood ejection from the ventricles into the corresponding arteries (Source: [695]).

Component	Function
Cells	Interstitial cells synthesize and remodel ECM (fibroblasts, smooth muscle cells, myofibroblasts)
	Valvar endothelial cells regulate the behavior of flowing and mural cells
Matrix	Elastin fibers extend in closed valve and shrink in open valve
	Collagen fibers maintain coaptation in closed valve
	Proteoglycans associate with matrix fibers for stress accomodation
Blood vessels	Valve cusps are avascular
	Nutrient/waste transport from heart cavity and myocardial vessels
Nerves	Regulation of coordinated activity of heart components

Table 7.3. Autoexcitation frequency (Hz) in nodal tissue. SAN frequency is decreased by the vagal influence (X).

SAN	$2.0–2.3 \xrightarrow{X} 1.2–1.3$
AVN	0.5–1.0
His bundle	0.3–0.6
Purkinje fibers	0.2–0.5

collagen fibers of the aortic valve cusp, and (2) the myofibers of the left ventricle wall, using a simple model of mechanically loaded fibers. The structure of the aortic leaflet has been derived from its function, which is assumed to consist of supporting a uniform pressure load undergone by a single family of fibers under tension [696]. The equation of equilibrium for the fiber structure is solved to determine its architecture. The computed fiber architecture resembles the real one. Assuming a constant myofiber cross-sectional area, symmetry with respect to the ventricle axis, small wall thickness with respect to the other dimensions, and a stress tensor resulting from hydrostatic pressure and myofiber stress, the bundles of myofibers have been shown to be located on approximate geodesics on a nested set of toroidal surfaces centered on a degenerate torus in the equatorial plane of the cylindrical part of the left ventricle [697].

7.6 Conduction System

Nodal cells are small ($80–100 \times 10–20\,\mu m$) muscular cells with few myofibrils, which create or quickly spread depolarization in the myocardium. The conducting cells are wider than the cardiomyocytes. They are not branched. Nodal tissue myocytes are sparse with dispersed gap junctions that have connexin-40 and -45. The cell of the Purkinje fibers have abundant gap junctions with high-conductance connexin-40 and low-conductance connexin-45 [698].[12] There is an automatism hierarchy between different regions of nodal tissue, according to the emission frequency of the action potential (Table 7.3). The propagation speed of the electrochemical signal also varies with the heart region (Table 7.4).

The electrochemical signal starts with spontaneous depolarization (automatic self-excitation) of the nodal cells of the *sinoatrial node* (SAN; length $\sim 8\,mm$, width $\sim 2\,mm$), the "natural pacemaker,"[13] located at the top of the

[12] Many types of connexins aggregate to form gap junctions between adjacent cells. They form homomeric or heteromeric hemichannels and homotypic, heterotypic, or heteromeric channels with different conductance, permeability, and gating properties. Connexin type expression varies according to the heart site. Cells that express human connexin Cx31.9 exhibit much faster transport than cells expressing other connexin types [699].

[13] SAN has the highest emission frequency of action potential (Table 7.3).

right atrium, between the orifice of the superior vena cava and the auricle. The sinoatrial node is composed of different cell types, stellate and elongated spindle cells. Stellate myocytes, the primary pacemaker cells exhibit a faster functioning rate and longer action potential duration.

Since SAN fibers fuse with the surrounding atrial cardiomyocytes, the action potential spreads through the atria at a rate at least of ∼0.3 m/s and produces atrial contraction. However, several nodal bundles conduct action potentials with a greater speed of ∼1 m/s. Three conduction preferred paths have been observed in the right atrium wall: the anterior Thorel, the mid Bachman, and the posterior Wenckebach bundles.

The action potential reaches the *atrioventricular node* (AVN; ∼5 mm long), which is located in the right atrium near the lower part of the interatrial septum. AVN is adjacent to the coronary sinus. AVN is subdivided into three regions: atrio-nodal, nodal, and nodal-His, according histological and electrophysiological criteria. Ovoid cells are observed in nodal and nodal-His regions, rod-shaped cells in the atrio-nodal region. The conduction speed is slower in the nodal region. AVN imposes a delay at most of ∼100 ms in impulse transmission to the ventricles, when atria have completed their contraction (Table 7.5). Hence, it travels at a speed of 1.5–4 m/s.

The action potential runs in the *His bundle*, beneath the endocardium in the interventricular septum, which divides after a short distance into two branches, the right and left bundle branches, which descend along respective sides of the interventricular septum.

His bundle gives birth to the *Purkinje fibers*, which penetrate into the myocardium to end on cardiomyocytes. The Purkinje cells allow a very rapid and almost simultaneous impulse distribution to activate the bulk of the ventricular myocardium.

The action potential propagates through the ventricular myocardium at 0.3–0.5 m/s. Ventricular depolarization duration is ∼80 ms (Table 7.6). Excitation begins in the subendocardial layer in the interventricular septum.

Tight junctions (Sect. 2.3.3) facilitate action-potential propagation. Gap junctions (Sect. 2.3.4) also transmit electrochemical signals in the excitable myocardium, in which response speed and tissue activity synchronization are crucial, by ion diffusion.

Table 7.4. Estimated conduction speeds (m/s) in nodal tissue.

SAN	0.05
Atrium	0.3–1
AVN	0.02–0.10
His bundle	1–2
Purkinje network	3–5
Ventricle (axial)	0.6–1
Ventricle (transverse)	0.2–0.5

Both the sinoatrial and the atrioventricular nodes are supplied with sympathetic and parasympathetic fibers. When stimulated, the sympathetic and parasympathetic fibers release *noradrenaline* (NAd) and *acetylcholine* (ACh), respectively.

7.7 Cardiomyocyte

Cardiomyocytes (length 70–150 μm, width 20–35 μm) are striated, nucleated cells that are electrically excited to rhythmically contract and relax. In the rat ventricular myocardium, there is about one capillary per cardiomyocyte couple for optimized irrigation and oxygenation [701].

Cardiomyocytes have a richer supply of mitochondria and greater dependence on ATP than skeletal muscle. PGC1α and PGC1β are highly expressed in the heart. The expression of peroxisome proliferator-activated receptor PPARγ coactivator PGC1α rises when ATP production must increase, i.e., when the cardiac activity augments. PGC1α coactivates PPARα, estrogen-related receptor ERRα, and other nuclear factors that control the transcription of genes involved in cardiac fatty acid oxidation and mitochondrial functional capacity [25]. Moreover, PGC1α stimulates the mitochondrial genesis in cardiomyocytes, although it is not essential for mitochondrial biogenesis. In the absence of PGC1α, mitochondrial density remains normal, but expression of the genes of oxidative phosphorylation decays [702]. ATP and phosphocreatine homeostasis are disturbed. The β-adrenergic–cAMP pathway, calcineurin-A,

Table 7.5. Approximate depolarization instants in the heart wall (ms). The myocardium depolarization takes ∼ 50–100 ms after reaching the junction between the Purkinje fibers and the subendocardial layer of the myocardium.

Atria	$5 \rightarrow 85$
AVN	50 (impulse arrival)
	125 (activation departure)
His bundle	130
His branches	145
Purkinje fibers	150
Endocardial myofibers	$\rightarrow \sim 190$
Epicardial myofibers	$\rightarrow \sim 250$

Table 7.6. Left ventricle activation timing (ms; Source: [700]).

Mid left part of ventricular septum	0–10
Inner part of LV wall (septum, apex)	10–20
Ventricular septum & RV, LV inner apex	20–30
Heart apex & external RV, LV lateral walls	30–45
Bases of RV & LV	45–80

calcium–calmodulin-dependent protein kinase, AMP-activated protein kinase, p38, and NO activates PGC1α expression.

Cardiomyocytes are joined by regions of interdigitating sarcolemmas, the so-called *intercalated discs*, which contain clusters of gap junctions to allow electrochemical impulses, or action potentials (APl), to spread rapidly and orderly so the cell contraction is almost synchronized. Cardiomyocytes then act as a syncytium. Stimulation of an individual cell results in the contraction of the whole myocardium. Intercalated discs contain not only gap junctions for chemical communications, but also mechanical junctions, composed of fascia adherens (adherens junctions, with N-cadherin, catenins, and vinculin) and desmosomes (containing desmin, desmoplakin, desmocollin, and desmoglein) [698]. The fascia adherens anchors myofibrils and desmin cytoskeleton on cadherin membrane sites. The fascia adherens occupies between-cell interdigitating regions, transversally with respect to the sarcomere, whereas desmosomes and gap junctions are located in membrane parts in the direction of the sarcomere axis.

Cardiomyocytes are surrounded by a trellis of collagen and elastin, which supports the cells and limits dilation (Fig. 7.4). The helical mesh of collagen avoid excessive CMC stretching. Moreover, cardiomyocytes are related to each other by collagen–elastin cross struts. The two sets of fibers develop recoil forces and store energy during myocardium contraction, which can be restituted during diastole [703].

The collagen network of the left ventricle wall includes three major components: (1) a collagen weave network, which surrounds groups of cardiomyocytes; (2) an array of myocyte-to-myocyte collagen struts (bore 120–150 nm), which extend from the basal lamina of a cardiomyocyte to the basal laminae of contiguous cardiomyocytes; and (3) an array of myocyte to capillary col-

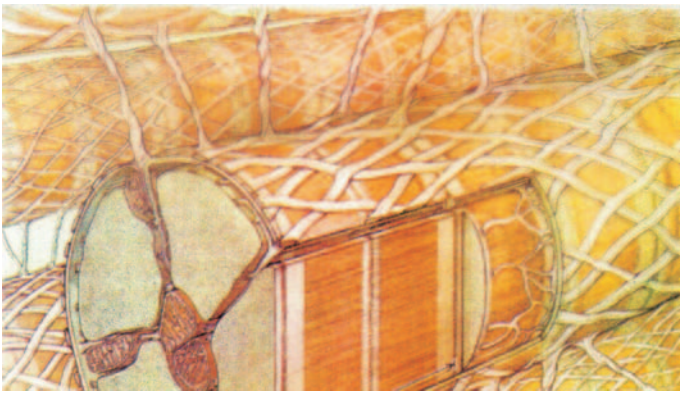

Figure 7.4. Cardiomyocyte, its collagen envelope and struts; the sarcolemma and its T-tubules; inside, the sarcomere, mitochondria and sarcoplasmic reticulum (from [703]).

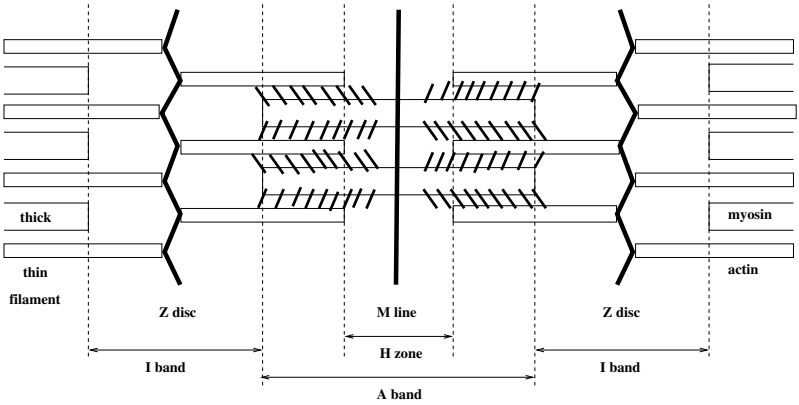

Figure 7.5. The sarcomere, its microscopic structure and the myofilament arrangement. The M line and the Z discs are proteic scaffolds that orientate myosin and actin filaments, respectively. The H zone (bisected by M line) contains only thick myosin filaments, the I band (bisected by Z disc) only thin actin filaments, and the region of the A band close to the Z disc both filament types.

lagen struts (of similar caliber) from the basal lamina of capillaries to the basal laminae of contiguous cardiomyocytes [704]. Collagen connections between cardiomyocytes and between these cells and the perfusing capillaries allow bulk motions during the cardiac cycle.

Four types of connective tissues can then be distinguished: (1) an endomysium-like connective tissue made of beams of collagen fibers that connects cardiomyocytes; (2) a connective tissue that links cardiomyocytes and capillaries; (3) a perimysium-like connective tissue that forms sheath of spiral collagen fibers around cardiomyocytes; and (4) an epimysium-like connective tissue, layers of elastin and collagen fibers that limit the endocardic and epicardic surfaces.

Myocytes have special components inside the sarcoplasma, an array of myofibrils and a sarcoplasmic reticulum (Fig. 7.4).

7.7.1 Sarcomere

Myofibrils, formed by packed contractile proteins (actin, myosin, and associated proteins), fill most of the cytosol. The striated appearance of the muscle fiber is created by a transverse pattern of alternating dark A bands, bisected by the H zone with a mid M line, and light I bands, divided by the Z line (Fig. 7.5). The sarcomere (length $\sim 2\,\mu m$), the array of thick and thin filaments between the Z lines, is the anatomical unit of muscular contraction, and the hemisarcomere is the functional unit. Repeating sarcomere units are precisely aligned in the cardiomyocyte.

The sarcomere length is a predictor of muscle function. Non-invasive measurements have been developed to assess the sarcomere structure. Tissue

scattering is related to the refractive index distributions associated with the morphology and the composition of the explored tissue. Sarcomere structural changes can be monitored by optical reflectance on muscle, a strong correlation existing between muscle optical scattering properties and sarcomere length used as a predictor of muscle function [705].

The sarcomere includes four filament complexes: (1) the thin filament, which is predominantly made of F-actin,[14] (2) the thick filament, mainly consisting of myosin[15] (3) titin, and (4) nebulin. The lateral boundaries of the sarcomeric units, Z discs, relate contractile apparatus to cytoskeleton (Sect. 7.7.4).

Sarcomere Z discs, which anchor myofilaments and stabilize their assembly, are densely packed cellular structures that include actin, titin, and nebulin, as well as smaller proteins, such as α-actinin and telethonin. Two giant *titins* are assembled into a complex by *telethonin*, a Z-disc ligand that has a symmetric structure [707].

B-crystallin, a molecular chaperone (Hsp) abundant within the myocardium, could act in the assembly and remodeling of sarcomeres. B-crystallin and Hsp47 chaperones might play complementary roles in cross-bridging actin filaments in the Z disc of the sarcomere.

7.7.2 T-system and Sarcoplasmic Reticulum

Excitation-contraction coupling requires Ca^{++} mainly provided by the sarcoplasmic reticulum, which store calcium ions from diastolic uptake for systolic release.[16] The sarcolemma repeatedly invaginates to form transverse tubules, the so-called *T-tubules*, which plunge into the sarcoplasma (T-system; Fig. 7.4). The T-tubules are close to the sarcoplasmic reticulum. The action potential sweeps quickly along the sarcolemma and along the T-tubules to trigger Ca^{++} release from the sarcoplasmic reticulum. Ca^{++} then diffuses to bind to troponin.[17] This bond leads to the interaction between actin and myosin and sarcomere contraction. Owing to its speed, the action potential arrives almost simultaneously at all tubules of the T system, ensuring that all sarcomeres have a coordinated contraction. When the process ends, Ca^{++} is pumped back into the sarcoplasmic reticulum (Sect. 7.9).

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[14] Striated muscles express two actin isoforms, cardiac actin and skeletal actin. In adult myocardium, cardiac actin is the major isoform (80%) [706].

[15] There are three isoforms of non-muscle myosin, myosin-2A, myosin-2B, and myosin-2C. Myosin-2 is also observed in muscles. Myosin-2B is the single isoform found in adult cardiomyocytes.

[16] In cytoplasma around myofibrils, free calcium concentration $[Ca] \sim 10^{-6}$ mole/kg and $[Ca^{++}] \sim 10^{-9}$ mole/kg. These amounts are insufficient for contraction. Ca influx $\sim 10^{-12}$ mole/cm^2 cannot explain contraction. Most of Ca^{++} comes from SR (SR $[Ca^{++}] \sim 10^3$ sarcolemma $[Ca^{++}]$) [700].

[17] When $[Ca^{++}]$ is high, Ca^{++} binds to troponin, which then acts on tropomyosin, initiating conformal changes.

The sarcoplasmic reticulum located throughout the myoplasm can be divided into three main components, junctional (JSR), corbular (CSR), and network (NSR). The sarcoplasmic reticulum indeed broaden out at multiple sites to form junctional SR cisternae tightly coupled to the sarcolemma and T-tubules. The junctional compartment is positioned within 12 to 15 nm to T-tubules. Together with the T-tubule, JSR defines a restricted subspace in which Ca^{++} can reach high concentration. JSR contains the majority of the ryanodine receptors.[18] Ca^{++} release from the sarcoplasmic reticulum storage occurs as discrete loci in the JSR–T-tubule subspace, the Ca^{++} sparks, although other secondary Ca^{++} transient currents are initiated by other ion carriers. The corbular region is located near the T-tubules at a distance of several μm from the sarcolemma. CSR releases Ca^{++} without interaction with sarcolemmal L-type Ca^{++} channels. Sarcoplasmic reticulum Ca^{++}-ATPases are predominantly located in NSR.

The sarcomere is bounded at both ends by the deep cylindrical invaginations of the sarcolemmal T-tubular system. The density of T-tubules affects the Ca^{++} influx. The contraction is caused by the opening of sarcolemmal voltage-gated calcium channels, which are mostly located in T-tubules. These channels trigger the calcium influx and local accumulation, which activates calcium release from its sarcoplasmic reticulum stores, especially the close junctional regions of this myocyte organelle. A decreased number of T-tubules, particularly in atrial myocytes and Purkinje cells, is correlated to local slow rate of Ca^{++} ingress [708].

7.7.3 Myofibrils

Each myofibril is made of arrays of parallel filaments. The thick filaments (diameter ~ 15 nm) are composed of *myosin*[19] (Fig. 7.7). Myosin filaments produce the A bands. The thin filaments (diameter of ~ 5 nm) are composed of *actin*, with small amounts of two regulatory proteins, *troponin* (TN) and *tropomyosin* (TMy, τροποσ: belt).[20] Actin filaments extend in direction normal to the Z lines from each side. They create the I band where they do not overlap the thick filaments. In the H zone, the thick and thin filaments never overlap (Fig. 7.6).

The myosin structure has been defined in two regions, light (LMM) and heavy (HMM) meromyosin. LMM represents part of the tail and is responsible for the assembling of myosin (Fig. 7.7). HMM contains the two heads and the

[18] The ryanodine receptor is a sarcoplasmic reticulum Ca^{++} channel, which is able to bind ryanodine.

[19] The myosin molecule (length 1.55 μm, diameter 12–15 nm) can be divided into two heavy chains and four light chains [709].

[20] G-actins polymerize in the presence of Ca^{++}, Mg^{++}, and ATP to form F-actin in a double helix chain with a pitch length of 35–36 nm. One tropomyosin runs in the groove of the F-actin chain. One troponin exists for each tropomyosin.

remaining part of the tail. HMM is further divided into globular fragment-1 (S1) and rod fragment-2 (S2). S2 contains binding sites for essential and regulatory light chains (MLC1 and MLC2). S1 has ATP-hydrolysis and actin-binding domains. The thick filament also contains M-protein at the M-line, myomesin, C-protein, and titin. Titin is involved in the connections between the ends of myosin filaments and Z disks, and can span the entire sarcomere length. Myomesin acts for lateral registration. S1 has helical arrangements around the filament axis.

Cardiac myosin isoform-5.1 has higher ATPase and contractile activities than isoform-5.3 [710]. Myosin isoforms might have a heterogeneous regional distribution.

Heterodimer troponin is composed of three subunits: TN-C, TN-I, and TN-T. Troponin-C has a binding site for Ca^{++} (calcium receptor/handling

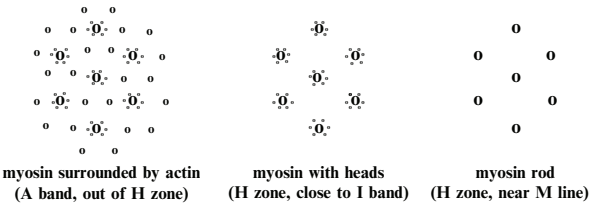

Figure 7.6. The sarcomere cross section and the myofilament arrangement. The inner region of the H zone (in the M line and its vincinity) contains the rode part of the myosin filament (bare zone). Myosin heads are located in the outer regions of the H zone and A band. Myosin filaments are surrounded by actin filaments in the outer region of the A band. The I band (not represented) is composed only by actin filaments.

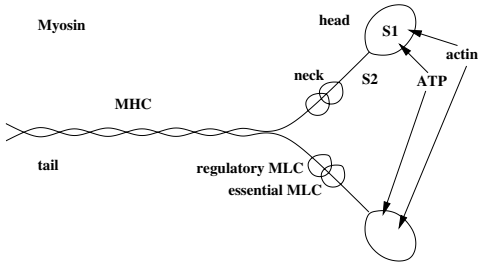

Figure 7.7. Myosin filaments (MHC: myosin heavy chain, MLC: myosin light chain). The neck has binding sites for light chains, the head for ATP and actin. For example, myosin-2 consists of two head segment S1, composed of heavy chains, connected to a long dimeric rod by a flexible coiled-coil segment S2 (S2δ in β-myosin-2). The coiled-coil light meromyosin rod tails of myosin-2 are packed side by side, whereas both heads point away from the myosin filament surface (or light meromyosin dimer axis). S1 subfragments act as ATP-driven motors, conformation and actin affinity of which change according to the status of ATPase sites. Actomyosin cross-bridges use chemical energy liberated by the hydrolysis of cardiac actomyosin Ca^{++}-stimulated MgATPase.

protein). Troponin-I[21] inhibits actin–myosin binding in the absence of Ca^{++}. Troponin-I is regulated by TN-C, which relieves the inhibition once TN-C binds Ca^{++}. Troponin-T is a tropomyosin binding protein. Troponin-C–Ca^{++} complex deforms TN-C–TN-I element and weakens TN-I–TN-T, exposing the myosin binding site on actin.

Troponin-I and C-protein are substrates for protein kinase-A. Phosphorylating troponin-I, PKA reduces the Ca^{++} sensitivity but not the cross-bridge cycling rate [712]. Protein kinase-C targets troponin-I, troponin-T, myosin light chain-2, and C-protein. Protein kinase-C might reduce the cross-bridge cycling rate.

Two actin polymers placed side by side form a double helix, which behaves like a moving rack (Fig. 7.8). The screw-shaped myosin has a twisted tail and a head that emerges to form grooves in which the actin filaments slide like a wrench jaw. Contraction is induced by the four-time nanomotor composed of interdigited myosin and actin filaments (Fig. 7.9). (1) The myosin head detaches from actin and fixes ATP. (2) ATP is hydrolized and the myosin head binds to actin. (3) The myosin head releases the phosphate and undergoes

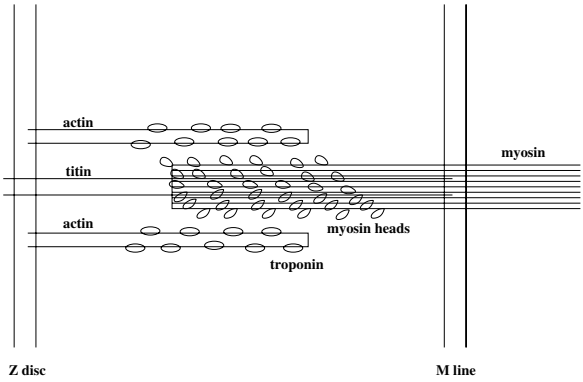

Figure 7.8. Interacting myosin and actin in the sarcomere. The thick filaments are composed of myosin and its partners (myosin binding proteins-C, -H, and -X. The thin filaments contain cardiac actin, α-tropomyosin, and troponins-T, -I, and -C. Myocardium contraction is generated by cyclic interactions between actin and myosin. Conformational changes in the actin–myosin binding interface are related to ADP release and ATP binding. Nanoscale conformational changes are converted into macroscale myocardium displacements. The dissociation rate of actomyosin bonds was postulated to depend on strain in the Huxley model. The lifetime of the actomyosin bond indeed depends on the instantaneous load and loading history.

[21] Troponin-I has a key role in ischemia-induced contractile dysfunction. Ischemia leads to intracellular acidosis. Troponin-I modifies the Ca^{++} sensitivity of actin. The fetal cardiac isoform of troponin-I protects more from acidosis than the adult cardiac isoform, enhancing Ca^{++} activation of the sarcomere activity. The composition of troponin-I thus affects the cardiac performance [711].

a conformational change [713]. Actin displacement is generated by a bascule motion of the myosin head, which pulls on the actin filament. The myosin head rotates with angle of about 45 degrees. This rotation leads to an actin displacement of about 10 nm. (4) The myosin releases ADP and remains anchored to actin. The interaction force is equal to about 1 pN [714]. Interdigitated actin and myosin filaments slide over each other to shorten the sarcomere during contraction (sliding-filament model). As a sarcomere contracts, Z lines are closer, the widths of the I bands and H zone decrease, whereas the width of the A band remains constant. Conversely, as a myofibril is stretched, the width of the I bands and H zones increases (constant width of the A band). Sarcomere shortening generates myofibril and muscle shortening. Each molecule of myosin contains a *myosin head*, which is a binding site for actin and ATP (Fig. 7.10). Troponin and tropomyosin allow actin to interact with myosin heads in the presence of Ca^{++}. Activation of the muscle fiber causes the myosin heads to bind to actin. Actin is drawn a short distance (~ 10 nm) past myosin. When linkages break, bonds are reform farther along actin to repeat the process.[22] The sarcomere length is maximum near the endocardium [715].

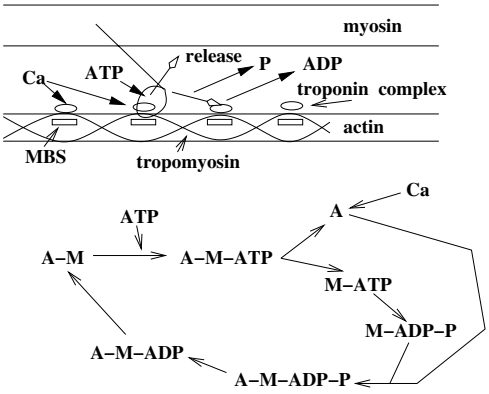

Figure 7.9. Sliding-filament model of actin–myosin interactions: actin and myosin fixation, sliding and detachment cycle (nanomotor cycle). In the absence of Ca^{++}, tropomyosin locks the myosin binding sites of actin. When Ca^{++} binds to the troponin complex, troponin and tropomyosin free the mysosin binding site (MBS) of actin. The myosin head then binds to actin, forming a cross-bridge. Myosin detachment from actin is caused by ATP binding. ATP hydrolysis induces a rotation and a weak then strong rebinding of myosin to adjoining myosin binding site of actin, associated with release of phosphate (P) and then ADP (phosphate release induces a rotation in myosin head). ADP unbinding leads to a new cycle if Ca^{++} and ATP are available.

[22] The filaments are pulled past each other in a ratchet-like action.

Myofibrils are held in position by scaffolds of *desmin* filaments, anchored by *costameres*, the cortical parts of the cytoskeleton, enriched in *vinculin*[23] along the sarcolemma (Fig. 7.11). The costameres maintain the spatial

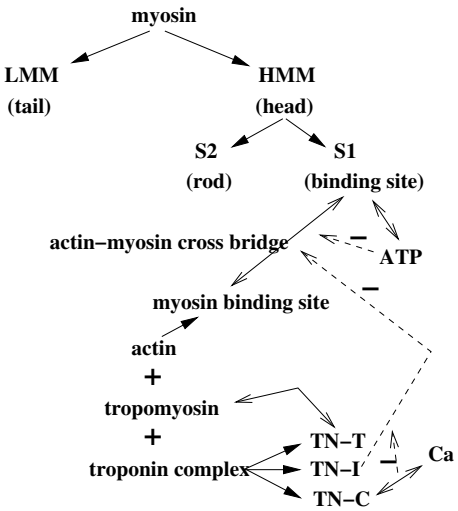

Figure 7.10. Actin and myosin filaments. The thick filament is composed of many myosin units lined up in a bidirectional arrangement, each unit being made of two myosin chains wrapped around each other like a twisted golf club. The combination of calcium ions to troponin-C, which liberates the myosin binding site on actin, followed by the inactivation of the tropomyosin–troponin complex, allows myosin head displacement and actin sliding. The heterotrimeric troponin complex is formed by regulatory troponin-C, inhibitory troponin-I, and tropomyosin-binding troponin-T. It is positioned along the actin filament at every seventh actin monomer. Troponin-C and calmodulin are the main targets of calcium ions. Both TN-C and Cam contain four Ca^{++}-binding domains. Calcium binding to the regulatory site of the cardiac troponin-C (cTN-C) affects both its interaction with TN-I and the interaction of TN-I with TN-T and tropomyosin. In the absence of Ca^{++}, TN-I inhibits the interaction of myosin-2 with actin. Ca^{++} binding to cTN-C relieves the TN-I inhibition, ensuring contraction. Ca^{++}-induced conformational changes of troponin-C are imparted to all actin monomers via TN-I and TN-T acting via two tropomyosin threads on both sides along the entire actin filament. cTN-I phosphorylation by either PKA or PKC lowers the TN-C affinity for calcium ions (positive lusitropy). Two main mechanisms change contractibility: (1) modifications in the amplitude and/or duration of Ca^{++} fluxes transient, and (2) modifications in the sensitivity of the contractile filaments to calcium by phosphorylation of the myosin light chain and/or troponin-I. Moreover, sarcomere compression due to stretching associated with rising diastolic filling brings the contractile filaments closer together thus facilitates actin–myosin interaction and increases the TN-C affinity for calcium.

[23] Vinculin is also observed in intercalated discs, T-tubule membrane and adherens junctions, between the myofibrils and sarcolemma.

structure of sarcomeres and couple the cardiomyocytes to the extracellular matrix. The membrane skeleton is made from *spectrin* and *dystrophin*, adapting the sarcolemma to CMC functioning and contributing to the force transmission. The costameres, the membrane skeleton and the cytoskeleton are linked to the extracellular matrix by integral membrane proteins such as integrins, dystrophin–glycoprotein complexes and β dystroglycan-laminin bounds. Dystrophin and β-*dystroglycan* are membrane proteins that are distributed in the sarcolemma and T-tubules.

The cardiac isoform of myosin binding protein-C (cMBP-C), a member of the immunoglobulin superfamily, regulates the sarcomere structure. The cMBP-C phosphorylations at different sites by Ca^{++}–calmodulin-activated kinase and protein kinases PKA and PKC maintain the thick filament spacing [716]. cMBP-C binds titin and actin, and thus participates in the organization of the sarcomere.

β-myosin-2 flexible coiled-coil segment S2δhas a charge distribution with three prominent rings of negative potential [717]. Charge interactions can regulate cardiomyocyte activity via myosin-binding protein-C bound to S2, a modulator of sarcomere contractility [718]. Adrenergic stimulation of the myocardium leads to quick phosphorylation of the cardiac isoform of myosin-binding protein-C by protein kinase-A, as well as to phosphorylation of troponin-I and phospholamban. These phosphorylations contribute to the positive inotropy effect of adrenergic substances. cMyBP-C phosphorylation decreases during ischemia-reperfusion injury and pathological hypertrophy (Part II), reducing the binding quality between actin and myosin.

7.7.4 Z Disc, a Stretch-Sensing Structure

The Z discs not only serve as cross-linkers of thin filaments and transmitters of force generated by the myofilaments, but also as stretch receptors and signal transducers [719]. Various signaling molecules interact with Z-disc proteins. Several interacting substances shuttle between the Z disc and other cellular compartments.

The Z discs are mainly composed of α-actinins assembled in successive layers (width $\sim 20\,\text{nm}$), which provide a skeleton for the insertion of actin filaments, titin and nebulin (Fig. 7.12). Z discs of neighboring sarcomeres are aligned in parallel and connected via desmin. The sarcomere is coupled to the sarcolemma by costameres, which comprise peripheral Z-disc molecules and subsarcolemmal proteins. Z disc and costameric proteins are involved in mechanotransduction.

α-*Actinin*-2 is the main component of the Z disc. α-Actinin-2 forms homodimers aligned in an antiparallel fashion. It contains three domains, the actin-binding domain, central rod domain, and domain with two calcium-binding motifs. α-Actinin-2 interacts with multiple molecules, such as the

Rho effector serine/threonine protein kinase PKN, phospholipase-D2,[24] and

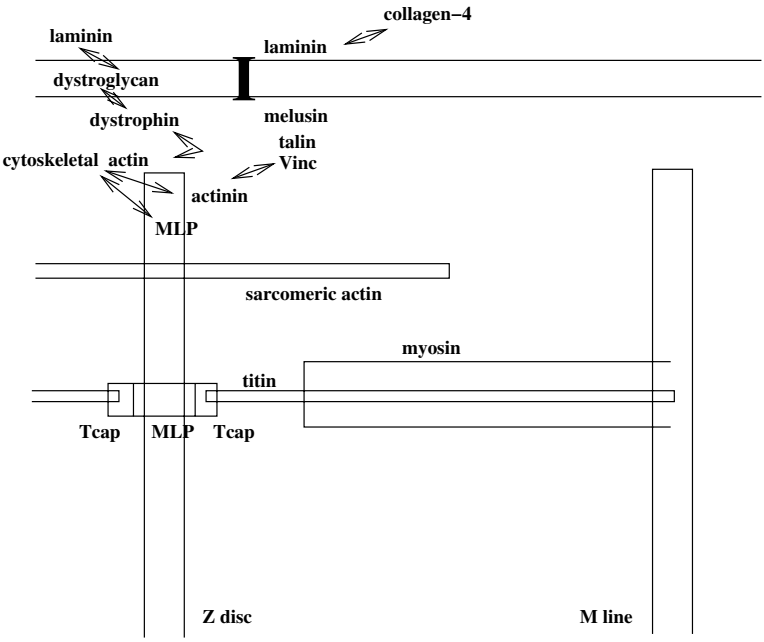

Figure 7.11. Costamere is the junctional structure connecting the sarcomere to the sarcolemma and the extracellular space. Costameres, indeed, contain three main proteic complexes: (1) focal adhesions, (2) spectrin-based complex, and (3) dystrophin-associated complex. Costameric complexes, like the proteic complex Z disc, contain α-actinin and muscle LIM protein (MLP). α-Actinin binds to or is indirectly associated with vinculin (Vinc), talin, and melusin (a possible stretch sensor), among other proteins. α-Actinin is thus connected to the sarcoplasmic domain of β1D-integrin (I; melusin links the costamere to integrin). The integrin extracellular domain links to laminin. The costamere hence coordinates the deformation of the sarcomere, sarcolemma, and extracellular medium (T-cap (telethonin): titin-cap). In addition, dystrophin binds actin on the one hand, and interacts with the dystrophin-associated proteins of the sarcolemma and sarcoplasma (dystroglycans, sarcoglycans, syntrophins, dystrobrevin, and sarcospan) on the other hand. Integral membrane proteins interact with components of the extracellular matrix via α/β-dystroglycan–α2-laminin complexes. Therefore, dystrophin also connects the sarcomere to the sarcolemma and extracellular matrix. Focal adhesions are composed of sarcoplasmic proteins (i.e., vinculin, talin, tensin, paxillin, zyxin), which connect with cytoskeletal actin filaments and transmembrane proteins (α- and β-dystroglycan, α-, β, γ, and δ-sarcoglycans, dystrobrevin, and syntrophin).

[24] Phospholipase-D2, mainly located at the sarcolemma, regulates Ca^{++} fluxes. PLD2 is inhibited by α-actinin and Arf1 [720].

G-protein-coupled receptor kinases.[25] CapZ interacts with α-actinin. CapZ is a dimer composed of α and β-subunits.

Titin[26], the largest human protein, spans the sarcomere from the M line to the Z line, where it is anchored. Titin acts as a spring for sarcomere retraction of stretched cardiomyocytes. Titin near the Z-disk segment binds to actin, near the M-line segment to myosin and myosin binding protein-C. In the relaxed state, the titin I-band segment is highly folded [722]. When the sarcomere is stretched, the extensible I-band segment elongates and develops a tension. During contraction below slack length, myosin filaments moves into the near Z-disk segment of titin, which then generates restoring force, useful

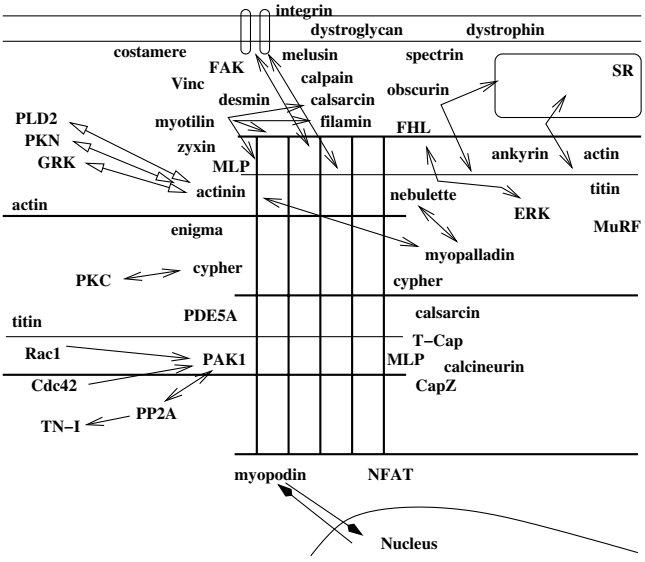

Figure 7.12. Z-disc proteins and partners, and their interactions (Source: [719]). Z disc is a lattice of interdigitating proteins, such as nebulette, myopalladin, myotilin, filamin, capZ, Cypher/ZASP, FATZ, etc.). This proteic complex particularly contains α-actinin, titin-cap (T-cap; or telethonin), muscle LIM protein (MLP), which binds actin and titin. Titin extends over half a sarcomere. (Titin crosses the Z disc.) Titin then associates the Z disc to the M line, both serving as sarcomere scaffolds (supporting and organizing actin and myosin filaments in parallel arrays by cross-linking antiparallel actin and titin). Desmin filaments surround the Z disc, connecting it to to cortical (subsarcolemmal) costameres. The sarcomere is also linked to costameres via sarcomeric α-actinin and non-sarcomeric actin (i.e., γ-actin).

[25] Phospholipase-D2 modulates GRK activities. In the presence of calmodulin and α-actinin, GRK5 phosphorylates soluble but not plasmalemmal substrates. In the presence of phosphatidylinositol(4,5)bisphosphate and α-actinin, GRK5 phosphorylates plasmalemmal but not soluble substrates [721].

[26] Titin is also named connectin.

for diastolic filling. The I-band segment is composed of many subsegments of different rheological properties. Among these segments, N2A and N2B in the cardiomyocyte sarcomere determine titin isoforms (N2B/N2BA).[27] The A-band and the near Z-disk segments are inextensible.

N2B interacts with LIM-family protein members, which binds creatine kinase and adenylyl cyclase for appropriate compartmentation. N2B is a substrate for protein kinase-A. Ventricular stiffness could then be modulated by adrenergic signaling via titin phosphorylation. Calcium binding to titin, especially to N2BA, also affects the titin rheology. Calcium also alters interactions between titin and actin. Ca^{++} binding protein S100A1, which exists in high concentration in the myocardium, inhibits the interaction between titin and actin.

Titin crosses the Z disc up to its binding partner *T-Cap (telethonin)* at the periphery of the adjacent Z disc. Titin function serves not only as a structural component but also as a sensor. Titin binds T-cap, which links titin to signaling and structural molecules, such as K^+ channel in the T-tubule and small ankyrin-1. Titin also binds obscurin, which associates to ankyrin. The interactions between the Z-disk segment and various compounds could act in the positioning of the sarcoplasmic reticulum and T-tubule close to the I band and ensure structure mobility according to sarcomere activity. T-cap also interacts with muscle LIM protein (MLP), a nuclear regulator of myogenic differentiation and possibly a stretch sensor mediating natriuretic peptide release.

Titin is connected to the sarcoplasmic reticulum via obscurin and ankyrin. *Obscurin* and *ankyrin* regulates ryanodine channel distribution. Obscurin binds to calmodulin in a calcium-independent fashion. Titin kinase and zinc finger protein Nbr1 form a signaling complex at the M band, which can recruit the ubiquitin ligase muscle-specific ring finger MuRF2. MuRF2 binds to the serum response factor, an inductor of the expression of many muscle proteins. T-Cap not only binds to titin, but also the delayed rectifier potassium channel. It also binds via titin to ankyrin-1, which couples T-tubules to the sarcoplasmic reticulum.

Nebulin is an actin-binding protein. *Nebulette* is the cardiac-specific small isoform of nebulin. *γ-filamin*[28] is the filamin isoform specific to striated muscle. γ-filamin binds to several partners, such as sarcoglycan, myotilin, calsarcin, and β1-integrin. *Myotilin* binds to α-actinin-2, γ-filamin, and actin. It also interacts with calsarcins-1 and -2. *Myopalladin* interacts with manifold proteins, such as nebulette, α-actinin, and cardiac ankyrin repeat protein. *Myopodin*[29] moves from Z discs to the nucleus.

[27] The N2BA distribution depends on the cardiac wall region (atria express more N2BA than ventricles) and transmural layer within the ventricular myocardium. N2BA titins are less extensible than N2B titins.

[28] γ-filamin is also called filamin-C, ABP 280, or filamin-2.

[29] Myopodin is also called synaptopodin-2.

The PDZ/LIM protein family[30] contains several members. The actinin-associated LIM protein is expressed at lower levels in the heart than in skeletal muscles. Z-band alternatively spliced protein (ZASP) interacts with α-actinin-2. Its isoform cypher-2 binds to various but not all PKC types. Enigma is able to bind actin, β-tropomyosin and protein kinases. CLIM1 and enigma homologue protein (EnH) also interact with α-actinin-2.

The four and a half LIM-only protein (FHL) family include FHL1,[31] FHL2,[32] and FHL3. FHLs acts in extracellular signal-regulated kinase signaling. *Zyxin* (ubiquitous member of LIM-only protein family) is involved in the cytoskeletal organization. It is mainly located in focal adhesion complexes close to the Z disc. It binds to α-actinin and muscle LIM protein.

The Z disc of cardiomyocytes contains a stress sensor, made of muscle-specific LIM protein,[33] titin-cap, and titin. Muscle LIM protein (MLP) binds to α-actinin, telethonin/T-Cap, nebulin-related actinin-binding protein N-Rap, PP3, and β-spectrin. Cultured cardiomyocytes deficient for MLP do not express natriuretic peptides ANP and BNP in response to stretch.

Calsarcins are calcium–calmodulin-dependent phosphatases, the calcineurin-associated sarcomeric proteins, which are involved in cardiac hypertrophy. Calsarcin-1 is exclusively expressed by the adult myocardium and slow-twitch skeletal muscle, calsarcin-2,[34] and calsarcin-3 by fast-twitch skeletal muscle fibres. Calsarcins interact with α-actinin, telethonin, γ-filamin, cypher, and myotilin.

Calcineurin (protein phosphatase PP2B or PP3), a calcium–calmodulin-bound heterodimer, interacts with calsarcin-1 at the Z disc. It induces myocardial hypertrophy (Sect. 7.7.7). It dephosphorylates transcription factors of the NFAT family for translocation into the nucleus. Cytoplasmic calcineurin-dependent NFATs can be detected at the Z disc.

Protein kinase-Cϵ, located at the Z disc, regulates cardiomyocyte contractility and hypertrophy. Activated by GPCRs, PKCϵ binds anchoring proteins, the receptors for activated C-kinases (RACKs). PKCϵ induces normal growth (with preservation of the cardiomyocyte contractility and without fibrosis), rather than maladaptive hypertrophy. cGMP-specific phosphodiesterase-5A localizes to the Z disc. It dissociates from the Z disc during heart failure. Arg-binding protein-2, an Abelson-family non-receptor tyrosine kinase, forms a complex with ubiquitin ligase Cbl, a regulator of the actin cytoskeleton

[30] PDZ and LIM are acronyms of the first three identified PDZ proteins (PSD-95/SAP90, septate junction protein Discs-large, and ZO1) and the first three described LIM domain-containing proteins (LIN-11, Isl1, and MEC-3), respectively.

[31] FHL1 is also known as SLIM1.

[32] FHL2 is also termed down-regulated in rhabdomyosarcoma (DRAL).

[33] Muscle-specific LIM protein, a member of the C-reactive protein family that belongs to the cardiomyocyte cytoskeleton, is also known as cysteine-rich protein-3 or cysteine- and glycine-rich protein-3.

[34] Calsarcin-2 is also designated as myozenin or FATZ.

and apoptosis. Arg-binding protein-2 binds to α-actinin at the Z disc. p21-Activated kinase-1, a Ser/Thr protein kinase activated by GTPases Rac1 and Cdc42, is located at the Z disc, sarcolemma, intercalated discs, and nuclear membrane. PAK1 interacts with protein phosphatase-2A, decreasing the phosphorylation of cardiac troponin-I, and enhancing calcium sensitivity.

Calcium-dependent cysteine proteases, the *calpains*, target sarcomeric proteins and focal adhesions during cell migration. Calpain-3, the isoform specific of striated muscle, is located at the Z discs, costameres with desmin and ezrin, the M line, and the I band. Calpain-3 activates NFκB.

Desmin, a muscle-specific intermediate filament protein, links the costameres to the Z disc. It is a component of the intercalated disc in cardiomyocytes. Desmin interacts with nebulin, spectrin, ankyrins, calpain-3, and the intermediate filament proteins synemin and syncoilin. *Integrins* couple the cytoskeleton, especially the costameres, to the extracellular matrix. Sarcolemmal α1 and α2-integrins, expressed in the embryonic heart, can be re-expressed in the adult heart bearing unusual mechanical loading. β1D-integrins are specific to cardiomyocytes. Integrins associate with focal adhesion kinase, integrin-linked kinase and its partner affixin, calreticulin, and melusin. *Melusin* at the costamere in the vicinity to Z disc phosphorylates (inactivates) glycogen synthase kinase, thereby preventing transcription factor activity.

7.7.5 Endocrine Function

Atrial cardiomyocytes are long, slender, and poor in T-tubules. Atrial cardiomyocytes contain small granules, especially in the right atrium. These granules secrete *atrial natriuretic peptide* (ANP) immediately when the atrial cardiomyocytes are stretched excessively. ANP increases the excretion of water, sodium, and potassium ions by the kidney distal convoluted tubule (Part II). It also decreases blood pressure by inhibiting *renin* secretion by the kidneys and *aldosterone* secretion by the adrenals. Ventricular cardiomyocytes synthesize *B-type natriuretic peptide* (BNP). BNP levels reflect long-term overload [723]. Three receptors have been described for the natriuretic peptides. ANP and BNP are guanylyl cyclases, that induce the production of cyclic guanosine monophosphate. cGMP targets cGMP-dependent protein kinases, cGMP-gated ion channels and cGMP-regulated phosphodiesterases.

7.7.6 Expression of Regulators

Hemojuvelin is mainly expressed in skeletal and cardiac muscles and, to a lesser extent, the liver. It modulates the expression of HAMP, the gene encoding hepcidin, via the bone morphogenetic protein pathway [724]. BMP belongs to TGFβ family [725]. Hepcidin is a liver-derived iron regulatory peptide acting on cells to limit iron flux toward the plasma. Hepcidin deficiency induces iron overload, whereas hepcidin excess induces anemia.

7.7.7 Cardiomyocyte Adaptive Growth

The heart is capable of acute and chronic hypertrophy.[35] Developmental growth deals with normal growth of the heart after birth. Long-term regular exercise and pregnancy induce physiological adaptive growth. Both developmental growth and physiological hypertrophy are characterized by coordinated myocardium growth matched with heart chamber size, normal organization of sarcomeres, and enhanced cardiac function. The increase in cardiomyocyte length is larger than the rise in width. Maladaptative hypertrophy is induced by diseases such as systemic arterial hyperpressure and aortic valve stenosis. Pathological cardiac hypertrophy is associated with alterations in the extracellular matrix, possible fibrosis, and increased cardiomyocyte apoptosis. Remodeling leads to a greater increase in cardiomyocyte width than length. Maladaptative hypertrophy is defined by myocardial dysfunction.

The heart undergoes hypertrophy to accommodate loading alterations. Hypertrophy is initiated by mechanical causes and/or biological factors (hormones, cytokines, chemokines, and other growth factors). Animal models have shown that cardiac hypertrophy involves the mitogen-activated protein kinase, the calcineurin–nuclear factor of activated T-cells pathway, the insulin-like growth factor-1–phosphatidylinositol(3)kinase–protein kinase-B–mammalian target of rapamycin cascade, the cyclin-dependent kinases CDK7 and CDK9, and class 2 histone deacetylases [726].

Signaling pathways involved in the development of cardiac hypertrophy, via the PP3–NFAT (Sect. 3.1.1.2), PI3K–PKB–GSK3, and MEF2–HDAC cascades, and via NFκB activated by G-protein-coupled receptors of endothelin-1 and angiotensin-2, or by cytokine receptors of TNFα and IL1, operate with cross-talk at various levels. This integrated signaling network regulates gene expression via many transcription factors, such as NFκB, MEF2, NFAT, and GATA4, as well as histone deacetylases. Long-term inhibition of cGMP phosphodiesterase PDE5A deactivates signaling pathways of cardiomyocyte hypertrophy.

Catecholamines, angiotensin-2, and endothelin-1 bind to specific G-protein-coupled receptors (α1-adrenergic receptor, angiotensin-2 receptor, and endothelin receptor) of the cardiomyocyte plasmalemma (Fig. 7.13) [726]. The corresponding Gαq/α11 are coupled to phospholipase-Cβ for production of di-

[35] Hypertrophy is defined by an increase in size of cardiomyocytes. Myocardial hypertrophy can be either adaptive or maladaptive according to cardiomyocyte contractility, the former being associated with normal or even improved function, and the latter with impaired contractile function. Increased cardiomyocyte size relative to the normal cell results from myocardial adaptation. The increase in the size of cardiomyocytes subjected to a hypertrophic stimulus is associated with augmented sarcomerogenesis and elevated expression of natriuretic peptides. Increase in the number of cardiomyocytes means hyperplasia. Hyperplasia is characterized by increased myocardial dry mass, normal cardiomyocyte size, and the absence of an abnormal myocardial component.

acylglycerol, which binds to and activates protein kinase-C, and subsequently inositol(1,4,5)trisphosphate. The latter binds to intracellular IP3 receptor, leading to the release of internal Ca^{++} stores. Diacylglycerol also augments the concentration of cytosolic free calcium in cardiomyocytes. Calcium ions then stimulate the PP3–NFAT pathway and inactivate the calmodulin-dependent kinase–histone deacetylases pathway. Activated $G\alpha q/\alpha 11$ stimulate MAPK

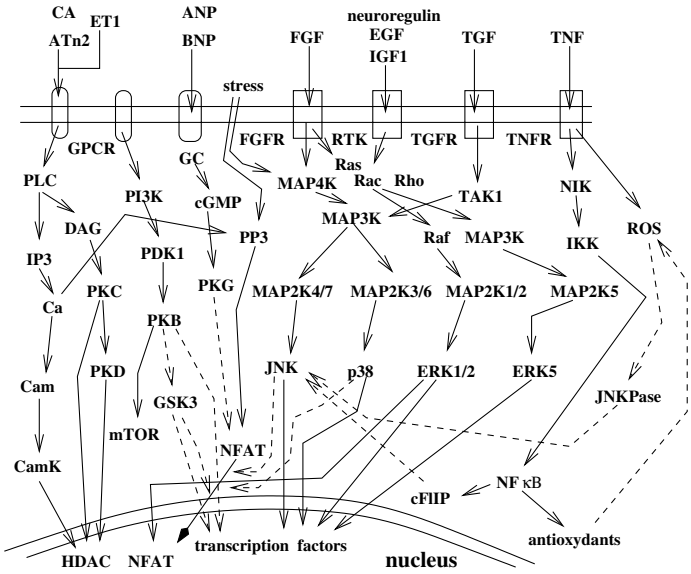

Figure 7.13. Signaling pathways that initiate the cardiac hypertrophy (Source: [726]). Signaling starts at the sarcolemma by ligands (natriuretic peptides ANP and BNP, angiotensin-2 [ATn2], endothelin-1 [ET1], fibroblast growth factor [FGF], epidermal growth factor [EGF], insulin-like growth factor-1 [IGF1], transforming growth factor-β [TGF], and tumor necrosis factor-α [TNF]), which bind to receptors (G-protein-coupled receptors [GPCR] and receptor tyrosine kinases [RTK]), activating cytosolic effectors (guanylyl cyclase-A [GC]; protein kinases [PKB, PKC, PKD, and PKG]; phospholipases [PLA2 and PLC]; mitogen-activated protein kinase pathways [MAP4K, MAP3K, Raf, MAP2K, ERK, JNK, and p38]; small GTPases [Ras, Rac, and Rho]; diacylglycerol [DAG] and inositol trisphosphate [IP3]; phosphatidylinositol 3-kinase [PI3K]; phosphoinositide-dependent kinase [PDK]; glycogen synthase kinase-3β [GSK3]; calmodulin-dependent kinase [CamK]; mammalian target of rapamycin [mTOR]; nuclear factor of activated T-cells [NFAT]; NFκB-inducing kinase [NIK]; inhibitor of NFκB kinase [IKK]; and cyclin-dependent kinase), then transcription factors (nuclear factor-κB [NFκB], and nuclear factor of activated T-cells [NFAT]), and nuclear components (histone deacetylases [HDAC]). NFκB impedes prolonged JNK signaling induced by TNFα via antioxydants and cellular flice-inhibitory protein (cFlIP), thus subsequent apoptosis. Transient MAPK stimulation by TNFα favors cell growth.

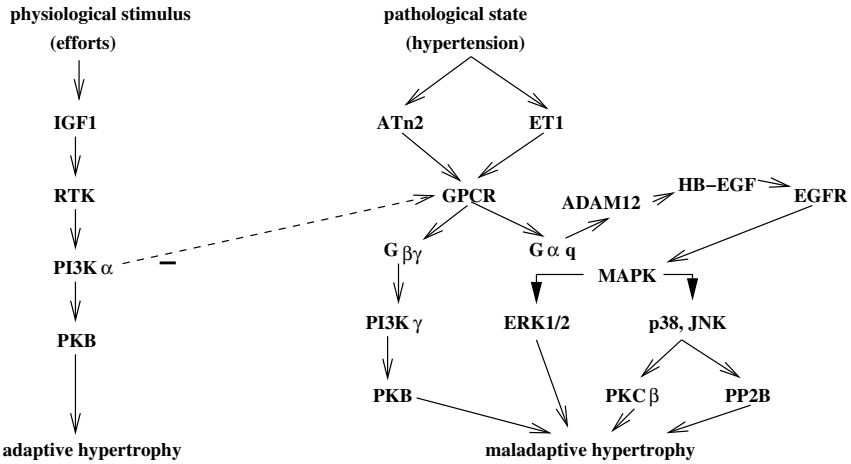

Figure 7.14. Signaling cascades implicated in adaptative and maladaptive hypertrophy. Different pathways involve different isoforms of phosphoinositide 3-kinase with different effects in the cardiomyocyte (Source: [727]).

signaling. These biochemical cascades mediate cardiomyocyte growth and possible hypertrophy.[36]

The p110α isoform of phosphoinositide 3-kinase is strongly implicated in exercise-induced hypertrophy (Fig. 7.14) [727]. Moreover, elevated PI3Kα activity improves cardiac function and limits fibrosis; hence, it attenuates maladaptative cardiac hypertrophy in mouse model of dilated cardiomyopathy subjected to pressure overload caused by ascending aorta constriction.

Exercise activates the IGF1–PI3Kp110α–PKB pathway. PKB activated by RTK (IGF1R) and PI3K(p110α) promotes growth and survival of cardiomyocytes. On the other hand, PKB stimulated by GPCR (ET1R) and PI3K(p110γ) induces maladaptive hypertrophy. Besides, myostatin, a transforming growth factor-β family member, is a negative regulator of striated myocyte hypertrophy, inhibiting p38 and PKB phosphorylation, but does not suppress PKB phosphorylation induced by IGF1 [728]. Furthermore, PI3K(p110α) negatively regulates the signaling pathway, including G-protein-coupled receptor, PI3K(p110γ), and PKB in isolated cardiomyocytes (Fig. 7.14). PI3K(p110α) inhibits ERK1/2 activation, acting upstream from ERK1/2.

A-kinase anchoring protein AKAP-Lbc is a Rho-specific guanine nucleotide exchange factor. It activates RhoA involved in certain pathways of cardiomyocyte hypertrophy, transducing hypertrophic signals from α1-adrenergic receptors coupled to Gα12 [729]. Inactivation occurs via AKAP-Lbc phospho-

[36] Physiological cardiac hypertrophy over a limited time preserves the ventricular function. Pathological hypertrophy responds to sustained long-term pressure overload.

rylation by anchored PKA and subsequent recruitment of regulator 14-3-3. α1-adrenergic receptors coupled to Gαq also play a role in cardiac hypertrophy.[37]

Angiotensin-2 causes cardiomyocyte hypertrophy via the nuclear factor of activated T-cells, after activating phospholipase-C, which produces inositol-trisphosphate and diacylglycerol (Fig. 7.13). The former generates calcium influx via IP3R, emptying the intracellular calcium stores; the latter enhances the frequency of calcium oscillations triggered by membrane depolarization via the activation of store-operated diacylglycerol-sensitive TRPC channels (Sect. 3.1.1.1), especially TRPC3 and TRPC6 channels [730]. The resulting Na^+ and Ca^{++} influxes change sarcolemmal potential and subsequently stimulate L-type voltage-gated calcium channels.

The receptor tyrosine kinase ErbB2 links to ErbB3 or ErbB4 and binds neuregulin, negatively regulating cardiomyocyte growth (Fig. 7.15). The receptor tyrosine kinases of insulin-like growth factor-1[38] and fibroblast growth factor, the receptor Ser/Thr kinases of transforming growth factor-β, and cardiotrophin-1, as G-protein-coupled receptors and stresses, stimulate the MAPK cascades.

EGFR2/HER2 receptor tyrosine kinase is involved in cardiogenesis and acts as a survival factor in adult myocardium. HER2 inhibition causes mitochondrial dysfunction in cardiomyocytes. Certain HER2 inhibitors (herceptin) favor cardiomyopathy. Other HER2 inhibitors (GW2974) activate AMP-activated protein kinase and protect the cardiomyocyte against TNFα-induced death [731]. HER2 inhibition blocks HER3 transactivation by HER2, increasing intracellular calcium level. Calcium-activated calmodulin-dependent kinase kinase-β phosphorylates (activates) AMPK. AMP-activated protein kinase switches the cardiomyocyte from ATP consumption (fatty acid, cholesterol, and protein synthesis) to ATP production from fatty acid and glucose oxidation.[39]

Cardiotrophin-1, a member of the IL6 cytokine family and one of the most potent cardiac myocyte survival factors, acts via gp130 cytokine receptor. Cardiotrophin-1 stimulates the JaK2–STAT3 pathway and induces cardiac myocyte hypertrophy. Cardiotrophin-1 phosphorylates STAT3. Activated

[37] Gαq activates phospholipase-C, leading to calcium release from intracellular stores, and regulates calcineurin, which controls transcription factor NFAT. Gαq also stimulates the mitogen-activated protein kinase cascades and protein kinase-C–protein kinase-D pathway.

[38] IGF1 binding to its receptor activates PI3Kα, which converts plasmalemmal phosphatidylinositol(4,5)bisphosphate to phosphatidylinositol(3,4,5)trisphosphate, hence activating signaling.

[39] AMPK inhibits acetyl-CoA carboxylase and 3-hydroxy 3-methylglutaryl-CoA reductase. AMPK also inhibits mTOR and eEF2. AMPK activates mitochondrial ERRα and PGC1α. Fatty acid oxidation is indeed regulated by PPARs and their coactivators PGC1α. Estrogen-related receptor ERRα, ERRβ, and ERRγ regulate cardiac energy metabolism. ERRα interacts with PGC1α.

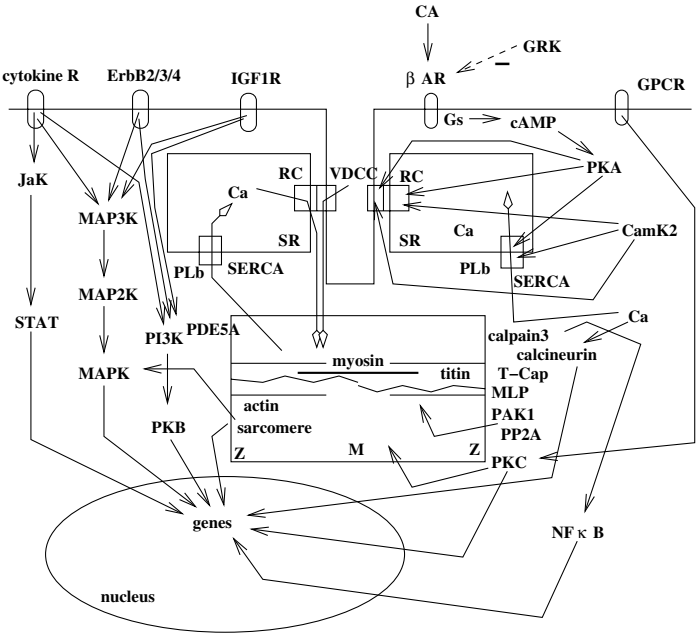

Figure 7.15. Cytokine receptors, ErbBs, and insulin-like growth factor-1 receptor (IGF1R) activate the Janus kinase–signal transducer and activator of transcription (JaK–STAT) pathway, mitogen-activated protein kinase (MAPK) pathway, and phosphatidylinositol 3-kinase–protein kinase-B (PI3K–PKB) pathway, to regulate gene expression (Source: [794]). Calcium-activated calcineurin dephosphorylates NFAT transcription factors involved in cardiac remodeling. Mechanical stresses stimulate Z-disc sensors. The titin–T-Cap–muscle-specific LIM protein (MLP) sensor binds calcineurin for mechanotransduction. Excitation-contraction coupling requires calcium ions. Calcium fluxes govern myofilament contraction and relaxation, particularly via the L-type calcium channel (VDCC), ryanodine channel (RC), and sarcoplasmic reticulum calcium ATPase (SERCA) associated with its inhibitor phospholamban (PLb). The activity of these three calcium channels is regulated by protein kinase-A (PKA) and calcium–calmodulin-dependent kinase-2 (CamK2). PKA is stimulated by G-protein-coupled receptors, such as the β-adrenergic receptor (βAR). Protein kinase PKCϵ (PKC), located at the Z disc, regulates cardiomyocyte contractility and hypertrophy. p21-Activated kinase-1 (PAK1) interacts with protein phosphatase-2A (PP2A), targeting troponin-I and enhancing calcium sensitivity. Calpain-3 activates NFκB. Phosphodiesterase-5A (PDE5A) dissociates from the Z disc during heart failure. In heart failure, GRK2 desensitizes G-protein activation of ACase.

STAT3 increases angiotensinogen expression in cardiomyocytes (autocrine regulation) [732]. Cardiotrophin-1 binds to IL6R/gp130 and triggers the signaling cascade, which also involves MAPK.

Activated p38, JNK, and ERK phosphorylate multiple intracellular substances such as transcription factors.[40] ERK1/2 signaling causes hypertrophy in vivo. ERK5 signaling is also implicated in cardiac growth. In contrast, p38 and JNK induce cardiomyopathy and hinder cardiomyocyte hypertrophy. Cardiomyopathy results from the overexpression of Ras GTPases.

Activated PI3K leads to the sarcolemmal recruitment of protein kinase-B and phosphoinositide-dependent kinase-1, which phosphorylates (activates) protein kinase-B. Glycogen synthase kinase-3β (GSK3β), targeted by protein kinase-B, negatively regulates hypertrophic transcription effectors. However, protein kinase-B also activates the Ser/Thr protein kinase mammalian target of rapamycin (mTOR), thereby favoring cardiac hypertrophy.

Protein kinase-C, protein kinase-D, and calmodulin-dependent kinase phosphorylate histone deacetylases for translocation from the nucleus to the cytoplasm. In the nucleus, HDAC2s are recruited to genes involved in the regulation of cardiomyocyte growth. HDAC2s negatively regulate hypertrophy, inhibiting myocyte enhancer factor, whereas HDAC1s favor cardiac hypertrophy.

Atrial and B-type natriuretic peptides, and nitric oxide produced by the heart activate guanylyl cyclases, which generate cGMP and activate PKG1. The latter hampers cardiac hypertrophy.

Epoxyeicosatrienoic acids inhibit NFκB activation, and thus have beneficial effects on cardiac hypertrophy. Soluble epoxide hydrolase catalyzes the conversion of epoxyeicosatrienoic acids into dihydroxyeicosatrienoic acids. Inhibitors of soluble epoxide hydrolase, which indirectly block NFκB activation, can prevent development of cardiac hypertrophy, as well as cardiac arrhythmias associated with cardiac hypertrophy, using murine model with aortic banding [733].

Ubiquitous focal adhesion kinases are activated by integrins and neurohumoral factors, which lead to extracellular signal-regulated kinase activation and cFos expression. Focal adhesion kinases are implicated in compensatory hypertrophic remodeling [734].

Kruppel-like factor 15 (KlF15), a member of the subset of the zinc-finger family of transcriptional regulators expressed in cardiomyocytes, hinders cardiac hypertrophy [735]. KlF15 acts, at least partially, by inhibiting nuclear factor GATA binding protein GATA4 and myocyte enhancer factor MEF2.

Cardiomyocytes detect deformation possibly via plasmalemmal integrins, such as melusin, and via the muscle LIM protein in the sarcomere Z disc. Melusin acts in the phosphorylation (inactivation) of glycogen synthase kinase-3β. Muscle LIM protein might trigger the calcineurin–nuclear factor of activated T-cell pathway. Both molecules negatively control cardiomyocyte growth. Cardiomyocyte stretch acts on the angiotensin-2 type 1 receptor, which links to Janus kinase-2, hence translocating G-proteins into the cytosol,

[40] MAP2K1 and MAP2K2 activate ERK1 and ERK2, MAP2K6 and MAP2K3 p38 kinases, and MAP2K4 and MAP2K7 JNK kinases.

activating the extracellular signal-regulated kinase and leading to hypertrophy.

Re-expression of fetal β-myosin heavy chain (β-MHC) gene in cardiomyocyte subsets are supposed to yield a marker of normal aging, as well as pathological cardiac hypertrophy [736]. β-MHC-expressing cells in normal and old hearts, as well as in hypertrophic hearts are predominantly located in subendocardial clusters within and surrounding fibrosis sites. β-Myosin heavy chain, thereby, is a marker of fibrosis rather than hypertrophy.

7.7.8 Cardiomyocyte Orientation

Dog cardiomyocytes form *muscular fibers* with a given orientation, which varies from the endocardium to the epicardium, with an angle from $+70$ to -70 degrees, with respect to the horizontal (equatorial) plane, the major axis of the truncated ellipsoid-like left ventricle being vertical [737]. The wall circumferential sheet thickness is given by the cross size of four to six cardiomyocytes. Subepicardial and subendocardial myofibers follow helical paths, whereas midmyocardial myofibers are roughly circumferential. The myofiber orientation can be quantified by helix[41] and transverse[42] angles. Cardiac myofiber postmortem orientation has been measured in five healthy goat hearts using diffusion tensor imaging DTI (Part II) [738]. The helix angle is evaluated at several sectors[43] in the equatorial LV slice.[44] From the endocardium to the epicardium, the helix angle varies from nearly 90 to -60 degrees in the anterior sector, from nearly 90 to -30 degrees in the posterior sector, and from nearly 50 to -80 degrees in the septal sector. The transverse angle was determined in the midmyocardial free wall for all slices from apex to base and averaged over the circumference. The mean transverse angle varied from 12 ± 4 degrees near the apex to 9 ± 4 degrees near the heart base.

In fetal and neonatal human heart, the direction of muscular fibers has been provided for each studied slices parallel to the equatorial plane by elevation and azimuthal angles with respect to a given horizontal axis [739]. The technique is based on polarized light transmission and resulting image processing. In slices near the heart basis, myocardial fibers cross the myocardium from the endocardium to the epicardium with a counterclockwise manner, whereas

[41] The helix angle is the angle between the myofiber direction and the plane perpendicular to the local major axis.

[42] The transverse angle is the angle between the local circumferential direction and the projection of the fiber direction on the plane normal to the local major-axis direction.

[43] The helix angle is determined in anterior, interpapillary muscle, posterior, and septal angular wall sectors of 20 degrees.

[44] The equatorial slice is defined as the slice positioned at one-third of the major-axis length from the heart base, the base corresponding to the slice nearest to the outflow tract, but outside from it, the apex the first slice showing the cardiac wall from the chest wall.

the reverse is observed in slices near the apex. Near the equatorial plane, the elevation angle continuously ranges from −50 degrees near the epicardium to +70 degrees near the endocardium. Near the heart basis and near the apex, a discontinuity in elevation angle variation is observed at mid-thickness, the angle varying from +40 to +70 degrees near the endocardium for both basis and apex, and from −40 to −50 or to −65 degrees near the epicardium in apex and basis slices, respectively. Myocardial fibers are geodesic curves running on surfaces as demonstrated by quantitative polarized light microscopy in the whole left ventricle[45] [740]. The Clairaut number indeed remains invariant on the geodesic of the revolution surface. Sheets of geodesic fibers between two left ventricle slices have been reconstructed. An anatomical model of toroidal surfaces, fitted into each other inside the ventricular wall, over which run the myocardial fibers with a spiral pattern has also been proposed [741]. Spatial distribution of ventricular wall stresses is sensitive to the orientation of the muscular fibers [742]. The structure of the left ventricle is designed for maximum homogeneity of fiber strain during ejection [743]. CMC orientation might result from an optimization process (minimization of muscular tension for a given pressure).

Transmural differences in myofiber orientation is equal to about 20 degrees per mm in human heart [744]. Therefore, transmural discretization of wall computational models must be small enough to compute strains and stresses.

7.7.9 Ion Carriers

The main involved Na^+, K^+, and Ca^{++} proteic channels span the sarcolemma and the membranes of the cell organelles, especially of the sarcoplasmic reticulum [745] (Fig. 7.16). Their permeability depends on the protein conformation. The sarcolemma Na^+–K^+ *adenosine triphosphatase pump* (Na–K ATPase) slightly contributes to the resting membrane potential by admitting two K^+ into the cell and expelling three Na^+ from it to the extracellular fluid for every ATP consumed. To avoid local acidosis, H^+ is expelled from the cell by Na^+–H^+ *exchanger*.

Cardiomyocytes exhibit two classes of *voltage-dependent* Ca^{++} *channels* (VDCC): L- and T-type. L-type Ca^{++} channels[46] (CaV1), are located primarily at junctions between the sarcolemma and sarcoplasmic reticulum close to the ryanodine channels (ryanodine receptors). Voltage-gated calcium channels

[45] Each cardiac slice is discretized into 130×130 µm squares over which two square-averaged angles are measured: (1) an elevation angle γ_e between the fiber and the slice plane; and (2) an azimuth angle γ_a between the fiber projection in the slice and the east-west direction. The fiber direction $\mathbf{f}$ is then given by: $f_x = \cos\gamma_e \cos\gamma_a$, $f_y = \cos\gamma_e \sin\gamma_a$, and $f_z = \sin\gamma_e$. Angles close to zero degree and greater than 75 degrees cannot be accurately measured. The measurement error is estimated to be equal to 1 degree.

[46] L-type Ca^{++} channels are also named dihydropyridine receptors

are composed of three main subunits, $\alpha 1$,[47] β and $\alpha 2\delta$, and of channel-resident calmodulin [746]. A subgroup of L-type Ca^{++} channels is located in caveolae made of caveolin-3 in ventricular myocytes that form a signaling complex with $\beta 2$-adrenergic receptors, in association with Gα-protein, adenylyl cyclase, protein kinase-A, and protein phosphatase-2A [747].

The amount of Ca^{++} influx during depolarization is limited by Ca^{++}-dependent inactivation of the cytosolic side of the channels due to the calmodulin bound to the channels [748]. SR Ca^{++} release also contributes to the Ca^{++}-dependent inactivation of the calcium current (double negative feedback) [749]. The feedback processes require interaction with Ca^{++}–calmodulin and a channel motif IQ of $\alpha 1$ subunit [746].

Voltage-gated L-type Ca^{++} channels (CaV1.2) are composed of a pore-forming $\alpha 1$ subunit and auxiliary $\alpha 2\delta$ ($\alpha 2\delta 1$–$\alpha 2\delta 4$), β, and γ subunits. Major myocardial $\alpha 2\delta 1$ and $\beta 2$ subunits[48] regulate excitation-contraction coupling in the cardiomyocyte [750]. Loss in these subunit leads to prolonged action

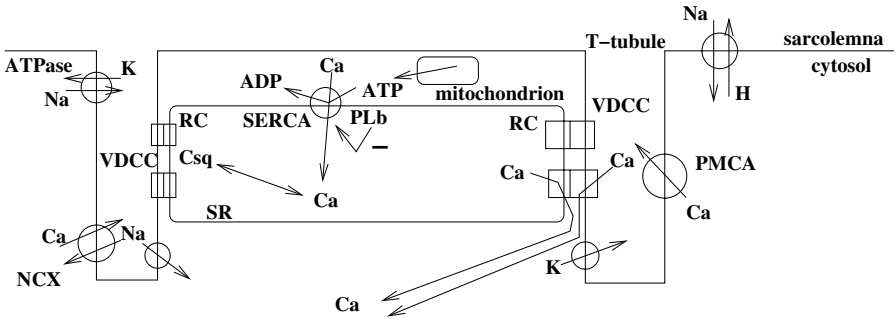

Figure 7.16. The cardiomyocyte and its main ion carriers. Voltage-dependent L-type Ca^{++} channels (VDCC) of the sarcolemma trigger the ryanodine calcium channels (RC) of the sarcoplasmatic reticulum (SR), the main cellular storage source of Ca^{++}. The cytosolic concentration of calcium determines the number of actomyosin cross-bridges per time unit, hence the maximal velocity of sarcomere shortening. At the end of systole, calcium dissociates from troponin-C and is transported into the sarcoplasmatic reticulum by the sarco(endo)plasmatic reticulum Ca^{++} ATPase pump (SERCA). Calcium efflux from the myocyte to the extracellular space also balance the calcium amount that has entered due to the action potential. During relaxation, Ca^{++} is exchanged for Na^{+} by the Na^{+}/Ca^{++} exchanger (NCX) and sarcolemmal calcium ATPase pump (PMCA). The frequency-dependent control of Ca^{++} flux is due to faster systolic calcium release from SR and faster diastolic calcium reuptake by SR (positive lusitropy). Voltage-gated Ca^{++} channels are short-term regulators of intracellular Ca^{++} level. An accumulation of cytosolic calcium increases the force of contraction.

[47] Subfamilies of voltage-gated calcium channels can be defined by $\alpha 1$ subunit: 1.x for L-type, and 3.x for T-type.

[48] The $\alpha 2\delta 1$ subunit is also expressed at high levels in vascular smooth muscle cells.

potential and increased Ca^{++} transients. (CaV1.2 determines the plateau duration of the action potential and control Ca^{++}-induced Ca^{++} release from the sarcoplasmic reticulum.) $\alpha 2\delta 1$ Subunit is not necessary for plasmalemmal expression level of the calcium channel but boosts activation and inactivation kinetics of CaV1.2.

T-type Ca^{++} channel (CaV3) density is large in Purkinje cells (involved in action potential transmission rather than contraction) and atrial myocytes (atrial contraction do not play a major role in atrium emptying), but small in most ventricular myocytes. T-type Ca^{++} channels inactivate very rapidly. The contractions initiated by Ca^{++} entry in Purkinje cells via T-type Ca^{++} channels are characterized by a longer delay to the onset of sarcomere shortening and slower rates of shortening and relaxation [751].

Although T- and L-type Ca^{++} channels have similar unitary Ca^{++} conductances, L-type Ca^{++} channels more efficiently trigger cardiomyocyte contraction. Ca^{++} influx via L-type Ca^{++} channels produces greater amplitude and faster contractions. Many of the voltage-dependent L-type calcium channels are localized within T tubules. L-type Ca^{++} channels release a relatively small and localized amount of calcium from the sarcolemma. However, L-type Ca^{++} currents control the main calcium flux by release from the sarcoplasmic reticulum via interaction of closely apposed L-type Ca^{++} and ryanodine channels.

Another mechanism, voltage-activated Ca^{++} release, activated directly by the membrane depolarization without Ca^{++} entry, can cause Ca^{++} transient currents [752]. This additional mechanism, which is depressed in heart failure, depends on internal [cAMP] [753]. Phosphorylations can then occur via adenylyl cyclase–protein kinase-A and Ca^{++}–calmodulin-dependent kinase pathways, to regulate the cardiac strength.

Three types of *ryanodine* (calcium) *channels* (RC) exist, two being cardiac isoforms [754]. They are arranged on the sarcoplasmic reticular membrane in arrays with contacts between the cytoplasmic domains. These arrays are located at the junctions between the sarcoplasmatic reticulum and sarcolemma on the CMC surface and in T-tubules beneath L-type VDCCs [755]. These coupled channels exhibit simultaneous gating due to FK506 binding protein [756]. The ryanodine channel forms a junctional proteic complex. This complex includes calmodulin (which exerts Ca^{++}-dependent modulation of RC function), FK binding protein, PKA, phosphatases-1 and 2A and sorcin, as well as other proteins at the luminal SR surface, triadin, junctin and calsequestrin. The latter proteins participate in Ca^{++} buffering and modulation of Ca^{++} release.

The cardiac ryanodine channel complex RC2 consists of four monomers and various other components, including FKBP12.6[49] and phosphodiesterase 4D. In the normal heart, the opening is regulated by FKBP12.6 and phosphodiesterase-4D. Loss of phosphodiesterase-4D from RC2 is associated with

[49] FKBP is also known as calstabin-2.

increased local levels of cAMP. Thereby, PKA activity rises, phosphorylating RC2 monomers with loss of FKBP12.6 from the complex. Consequently calcium leaks, leading to heart failure and arrhythmia [757].

Na^+–Ca^{++} *exchangers* (NCX; Sect. 3.1.1.2) yield inward or outward Ca^{++} motions (outward or inward Na^+ movements, respectively, with the ratio of three Na^+ for one Ca^{++}) whether the intracellular concentration $[Na^+]_i$ or $[Ca^{++}]_i$ is high. Na^+–Ca^{++} exchangers induce slow contractions. The unitary conductance for Ca^{++} of Na^+–Ca^{++} exchanger is smaller than the one of L-type Ca^{++} channel. However, Na^+–Ca^{++} exchangers can induce a Ca^{++} influx able to trigger reticulum sarcomplasmic release of Ca^{++} [758]. Furthermore, Na^+–Ca^{++} exchangers have a limited activity, because the intracellular Na^+ concentration decays during the depolarization.

The cytoplasmic Ca^{++} concentration is controlled by an ATP-dependent proteic pump in the membrane of the calcium storing sarcoplasmic reticulum that temporarily sequesters Ca^{++}. *Sarco(endo)plasmic reticulum* Ca^{++}-*ATPase pumps* (SERCA; Sect. 3.1.1.2) indeed transport the calcium ions from the sarcomere and the cytosol into the sarcoplasmic reticulum.[50] *Plasma membrane* Ca^{++}-*ATPase pumps* (PMCA; Sect. 3.1.1.2), Na^+–Ca^{++} *exchangers*[51]

Table 7.7. Main CMC ion carriers of the sarcolemma and membrane of the sarcoplasmic reticulum and ion fluxes between the cytosol and either the extracellular space or the sarcoplasmic reticulum.

Carrier	Ion flux (with respect to cytosol)
Sarcolemma	
Na^+/K^+ ATPase pump	Na^+ efflux K^+ influx
Ca^{++} ATPase pump	Ca^{++} efflux
K^+ channels	K^+ efflux
Na^+/Ca^{++} exchanger	Ca^{++} efflux (forward mode)/influx (reverse mode) Na^+ influx (forward mode)/efflux (reverse mode)
voltage-gated Na^+ channels	Na^+ influx
voltage-gated Ca channels	Ca^+ influx
Sarcoplasmic reticulum	
ryanodine receptor	Ca^{++} influx
Ca^{++} ATPase pump	Ca^{++} efflux

[50] Three SERCA types exist: type 1 in fast-twitch skeletal muscles; type 2a in the myocardium and in slow-twitch skeletal muscles; 2b in smooth muscle cells; and type 3 in endothelial cells.

[51] NCX competes with PMCA for Ca^{++} efflux, but it might induce less than one third of Ca^{++} efflux.

in their forward mode, and *mitochondrial* Ca^{++} *uniporters* participate to Ca^{++} removal from the CMC cytosol (Table 7.8).

Voltage-gated potassium channels are characterized by a functional diversity relevant to action potential repolarization in the myocardium. Multiple voltage-gated K^+ currents contribute to the shape variations of action potentials in different regions of the myocardium (Sect. 7.8). Voltage-gated potassium channels are made of several subunit sets, Kv1 (Kv1.1–Kv1.8), Kv2 (Kv2.1 and Kv2.2), Kv3 (Kv3.1–Kv3.4), Kv4 (Kv4.1–Kv4.3), Kv5, and Kv6 [760]. Additional Kv1 subunits, ERG and KvLQT are found in the myocardium. Functional voltage-gated K^+ channels comprise four subunits. Members of this channel family combine various kinds of subunits with different time and voltage-dependent properties.

Three major potassium channels, with a varying concentration in the myofiber sarcolemma according to the transmural location, contribute to ventricular myofiber repolarization: the *inward rectifier* (Kir, IK1 current), which closes during depolarization, *rapid* (IKr current) and *slow* (IKs current), *delayed rectifiers*, and *transient outward* K^+ *channels* (Ito current).

Transient outward K^+ channels set the rate of the early phase of repolarization. They are quickly inactivated (Table 7.9). Two types can be identified, fast and slow, according to the rates of inactivation and recovery from inactivation. Fast types are associated with Kv4a subunits, whereas Kv1.4 underlies slow types. Hence, the transient outward heterotetrameric K^+ channels are made of pore-forming α subunits (Kv4.2, predominant Kv4.3) and accessory β subunits (KChIP2, frequenin/NCS-1) [760].

Delayed K^+ rectifiers activate later and inactivate slower during the repolarization than transient outward K^+ channels. Rapid delayed K^+ rectifiers have faster activation and deactivation kinetics and more negative threshold potential than slow delayed K^+ rectifiers. A third type exists, the ultrarapid

Table 7.8. Calcium efflux in ventricular myocytes during relaxation. Relative participation of the involved carriers. The Ca^{++} amount entering the mitochondria is unsignificant with respect to excitation-contraction coupling, but cumulative changes in intra-mitochondrial [Ca^{++}] can stimulate dehydrogenases that produce nicotinamide adenine dinucleotide (NADH) and ATP to match increased energetic demands [759]. The relative activity (%) of the carriers depends on their concentrations (species dependency), here given for rabbit and rat. Ca^{++} efflux in ferret, dog, cat, guinea-pig and human ventricle is quantitatively similar to the rabbit. (rat ventricles do not mimic the human heart with respect to Ca^{++} efflux.)

Carrier	Rabbit	Rat
SR Ca^{++}-ATPase	$\sim$ 70	$\sim$ 92
Sarcolemmal Na^+/Ca^{++} exchanger	$\sim$ 28	$\sim$ 7
Sarcolemmal Ca^{++}-ATPase and Mitochondrial Ca^{++} uniporter	$\sim$ 1	$\sim$ 1

delayed K^+ rectifiers. These three delayed K^+ rectifiers underlie repolarization phase 3. ERG1 and KvLQT1 subunits are related to the rapid and slow delayed K^+ rectifiers, respectively (Table 7.10). hERG (ERG1, and KCNH2) potassium channels are responsible for the rapid delayed rectifier K^+ current during the third phase of repolarization. hERG Potassium channels exist not only in cardiomyocytes but also in smooth muscle cells and other cells. At negative membrane potentials, hERG channels are in a closed state. Membrane depolarization slowly opens the channels, which are then rapidly inactivated. Kv1.5 underlies the ultrarapid component of outward delayed K^+ rectifiers in the atrium.

G-protein–dependent *inward rectifier* K^+ (KG), activated by $G\beta\gamma$ subunit[52], has negative chronotropic effect and explains acetylcholine-induced bradycardia. KG channels of the sinoatrial node indeed decelerate pacemaker activity. K^+ flux depends on the electrochemical gradient for K^+ across the sarcolemma. KG channels are heterotetramers of inward rectifier K^+ (Kir) channel subunits Kir3, combined in a cell-specific manner.[53] Each subunit has one $G\beta\gamma$ binding site. The subcellular localization of KG channels involves PDZ domain-containing anchoring proteins. Cell membrane hyperpolarization and depolarization lead to fast[54] and slow ("relaxation")[55] increase and decrease in channel activity, respectively [763]. During sarcolemma repolarization, the GTPase-accelerating protein action of the regulators of G-protein signaling is inhibited by phosphatidylinositol(3,4,5)trisphosphate, which binds to the regulators of G-protein signaling RGS4 [764]. The voltage-dependent formation of Ca^{++}–calmodulin leads to its binding to regulators

Table 7.9. Activation and inactivation rates of voltage-gated potassium channels (from [761]). There are more transient outward K^+ channels in epicardium than in endocardium, and in right than in left venticle epicardium.

K^+ channel	Activation	Inactivation
Fast transient outward	Fast	Fast
Slow transient outward	Fast	Slow
Ultrarapid delayed rectifier	Fast	No
Rapid delayed rectifier	Moderate	Fast
Slow delayed rectifier	Very slow	No

[52] The availability of each channel subunit is dictated by $G\beta\gamma$ concentration, the available status being characterized by a higher affinity for $G\beta\gamma$.

[53] The KG channel of the atrial cardiomyocyte is composed of Kir3.1 and Kir3.4.

[54] The fast channel flux is due to the blockade of outward K^+ motion through the channel by intracellular Mg^{++} and polyamines. This feature is common to almost all Kir channels.

[55] The slow channel flux is specific of KG channel. It is due to the voltage-dependent action of regulators of G-protein signaling, which accelerate the intrinsic GTP hydrolysis of the $G\alpha$ subunit.

of G-protein signaling. Thereby, Ca^{++}–calmodulin relieves the inhibition by phosphatidylinositol-trisphosphate and decreases free $G\beta\gamma$ level due to the restoration of the activity of regulators of G-protein signaling. Consequently, the number of active KG channels decays during systole.

Cardiac sarcolemmal ATP-sensitive K^+ channels, composed of Kir6.2 and SUR2A subunits, as well as glyceraldehyde 3-phosphate dehydrogenase,[56] are regulated by intracellular ATP [765]. They couple glycolysis to membrane excitability. ATP-sensitive K^+ channels are responsible for ECG ST segment elevation during ischemia [766].

The transcription factor Irx5 from the Iroquois homeobox genes controls the action potential. Irx5 represses expression of gene Kcnd2 encoding Kv4.2 subunit of voltage-gated potassium channel via the recruitment of the transcription repressor mBop [767].

Table 7.10. Major voltage-gated potassium channels in the heart. Inward-rectifier (Kir), voltage-dependent (Kv), and calcium-activated (Kca) K^+ channels also regulate the caliber of arteries and arterioles, especially in the cerebral territory (Source: [762]).

Type	current	subunits
Ultrarapid delayed rectifier (phase 1, 2)	IKur	Kv1.5 (KCNA5)
Rapid delayed rectifier (plateau end, phase 3)	IKr	hERG (ERG1, KCNH2) minK, MiRP1 (KCNE1, KCNE2)
Slow delayed rectifier (plateau end, phase 3)	IKs	KvLQT1 (KCNQ1) minK (KCNE1)
Rapid transient outward (phase 1)	Ito(r)	Kv4.3 (KCND3) KCNIP2
Slow transient outward (phase 1)	Ito(s)	Kv1.4 (KCNA4) MiRP1 (KCNE2)
Inward rectifier (phase 3, rest)	IK1	Kir (KG) Kir2.1 (KCNJ2) Kir2.2 (KCNJ12)

[56] GAPDH produces (1,3)bisphosphoglycerate, an opener of ATP-sensitive K^+ channels. Hence, they regulate the channel activity.

Ca^{++}-activated Cl^- current[57] occurs in the same action potential period than transient outward K^+ current and contribute to ventricular repolarization. Main features of ion carriers are summarized in Tables 7.11 and 7.12.

7.7.10 Main Ion Currents

Activated ion channels generates ionic influxes (Table 7.13) and effluxes (e.g. Table 7.10) in and out of the nodal cells and cardiomyocytes. Ionic currents

Table 7.11. Main features of CMC sodium and potassium carriers.

Potassium carriers	
Inward rectifier	Small efflux at positive potential
	Open at negative potential
	Repolarization down to −80 mV
Delayed rectifier	Phase 3 repolarization
	Slow activation beyond −40 mV
	Slow inactivation
Transient outward	Activation by depolarization
	Phase 1 repolarization
Muscarinic (ACh)	Inward motion
	Hyperpolarization
	Background current in SAN
ATP	Opening by low [ATP],
	by increase in [ADP],
	and by adenosine, H^+
Sodium carriers	
Fast activating	Influx
	Voltage- and time-dependent opening
	Scarse in SAN
Hyperpolarization activated	Slow opening at $[-60, -40\,\text{mV}]$
	Depolarization of pacemaker
	Stimulation by catecholamines-cAMP
	Inhibition by ACh
Background	Inward flux
	Attenuation of resting membrane potential
NCX	Activated by increase in cytosolic $[Ca^{++}]$
	3 Na^+ vs. 1 Ca^{++}
	Early Ca^{++} influx
	Na^+ influx in late plateau
NaK pump	3 Na^+ vs. 2 K^+
	Minor contribution to resting potential

[57] Ca^{++}-activated Cl^- temperature-dependent currents have been observed in atrial and ventricular myocytes, as well as Purkinje cells, at least in the rabbit heart.

and action potentials vary between mammal species, and within an animal specy, between cell types. Mathematical models of ion fluxes across the plasmalemma and organelle membranes of nodal cells or cardiomyocytes as well as ions motions and sequestration within the cytosol and certain organelles are aimed at restituting the conduction velocity and the action potential duration of the explored region of the heart wall.

Pacemaker Cells

Major currents in the sinoatrial nodal cells, as well as atrial cells, are provided by fluxes of sodium, potassium, and calcium ions [768–770]. (Table 7.14; Fig. 7.17). Hyperpolarization-activated current, an inward depolaring current, is due to Na^+ and other ions. This current is Cl^--sensitive and Ca^{++}-

Table 7.12. Main features of CMC calcium and chloride carriers.

Calcium carriers	
Voltage-gated	T type activated at more negative potential than L type channel
	Early plateau current (L type)
	Ca-Induced Ca Release (L type)
	SAN and AVN action potential (L type)
	Pacemaker depolarization (T type)
	Fast inactivation (T type)
Ca ATPase	Efflux
chloride carriers	
cAMP-dependent	Stimulation by cAMP, β1 agonists
	Phase 1 repolarization after β1 activation
Ca-dependent	Stimulation by cytosolic Ca^{++}
	Phase 1 repolarization
	Pacemaker potential
Stretch-activated	Opening by osmotic swelling

Table 7.13. Main inward anion currents and corresponding channels in the heart. If: non-selective cation current (Source: [762]).

Current	Channel	Genes
INa	NaV1.5	SCNA5
		SCN1B, SCN2B
ICa,L	CaV1.2	CACNA1C, CACNL1A1
		CACNB1, CACNB2, CACNA2D1
ICa,T	CaV3.1	CACNA1H
If		HCN1, HCN2, HCN4
		KCNE2

dependent. The hyperpolarization-activated cyclic nucleotide-gated cation channel HCN4 is involved in If current of cardiac pacemaker activity.[58]

Sodium fluxes lead to several currents, a small fast inward Na^+, a Na^+–Ca^{++} exchange , a Na^+–K^+ pump, and a Na^+ background current. Among the voltage-dependent K^+ currents, the outward K^+ delayed rectifier current plays a major role in pacemaker activity. Only a small fraction of nodal cells of the sinoatrial node yields a transient outward K^+ current. Furthermore, inward rectifier K^+ current, although observed in the atrium and part of the atrioventricular node, is not found in the sinoatrial node, whereas it is the dominant background current in ventricular myocytes. Additional K^+ currents are provided by ligand-gated channels (ATP, adenosine, and acetylcholine-sensitive[59] channels). Transient and long-lasting Ca^{++} currents have been recorded in the sinoatrial node, associated with T and L-type Ca^{++} channels. A stretch-activated Cl^- channel can provide an inward backgroung current. The decay in outward K^+ current and inward backgroung current lead to initial cell depolarization to the threshold. The hyperpolarization-activated current modulates pacemaker depolarization. Ca^{++} current is a main factor during late depolarization. ATP-sensitive K^+ channels are activated by ATP depletion. Na^+–K^+ pumps and Na^+–Ca^{++} exchangers influence the pacemaker activity. Intracellular ion concentrations and regulators affect the functioning of pacemaker ion carriers (Table 7.14).

Cholinergic signals activate M2 cholinergic receptors, which decrease cAMP levels, thus limiting the activation of protein kinase-A, and slowing pacemaker rate. In contrast, adrenergic cues activate β-adrenergic receptors in pacemaker cells, thus increasing cAMP concentration, activating PKA, and increasing pacemaker frequency. PKA regulates numerous proteins in nodal cells, especially ion channels. A-kinase-anchoring proteins control PKA activity. AKAP10 variants in human are associated with increased cardiac frequency and decreased heart rate variability (decaying sensitivity to external stimuli). AKAP10 modulates the sensitivity of cardiomyocytes to vagus nerve stimuli [771].

Ventricular Cells

Mathematical models of the ventricular action potential are aimed at reproducing the different action potential shapes corresponding to the three transmyocardial regions (endo-, epi-, and midmyocardia) of the ventricles and their different rate dependencies [770, 778]. Basal models of ventricular myocyte

[58] During development, HCN4 location becomes restricted to the dorsal wall of the right atria, then to the junction of the right atrial appendage and the superior vena cava, where the sinoatrial node appears [772]. HCN4 channels are highly expressed in the adult sinoatrial node.

[59] The activity of both adenosine and acetylcholine-sensitive K^+ channels involves GTP-binding proteins. ATP and Mg^{++} are co-factors of acetylcholine-sensitive K^+ channels.

Table 7.14. Regulation of pacemaker currents (Source: [768]).

Effector	Target	Effect
Ca^{++}	Na^+–Ca^{++} exchanger	Activation
	Delayed rectifier K^+ channel	Activation
H^+	Inward rectifier K^+ channel	Inhibition
	Ca^{++} channel	Inhibition
	Na^+–HCO_3^- cocarrier	
β-Adrenergic agonist	Gs/ACase/cAMP/PKA	
	L-type Ca^{++} channel	Activation
	Delayed rectifier K^+ channel	Activation
α1-Adrenergic agonist	G/PLC/DAG/PKC	
	Delayed rectifier K^+ channel	Activation
Muscarinic agonist	Gi/ACase/cAMP/PKA	Inhibition
	Ach-activated K^+ channel	Activation
Adenosine	ACase	
	Adenosine-sensitive K^+ channel	Activation
ATP	ATP-sensitive K^+ channel	Inhibition
	Ca^{++} pump	Activation
	Na^+–K^+ pump	Activation

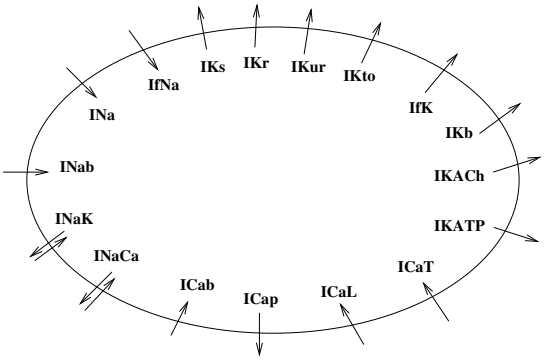

Figure 7.17. Main ionic currents across the sarcolemma of sinoatrial node and atrial cells, associated with the action potential (Sources: [769, 770]). IcaL: L-type Ca^{++} current; IcaT: T-type Ca^{++} current; ICap: sarcolemmal Ca^{++} pump current; ICab: background Ca^{++} current; INaCa: Na^+/Ca^{++} exchanger current; INaK: Na^+/K^+ pump current; INa: inward Na^+ current; INab: background Na^+ current; IfNa: hyperpolarization-activated Na^+ current; IfK: hyperpolarization-activated K^+ current; IKur, IKr, IKs: ultrarapid, rapid, and slow K^+ delayed rectifier current; IKb: background K^+ current.

functioning comprise three major components: (1) membrane ion currents (fast sodium, L-type calcium, transient outward potassium, rapid and slow delayed rectifier potassium, inward rectifier potassium, sodium/calcium exchanger, sodium/potassium pump, calcium and potassium plateau, and background currents [778]; Fig. 7.18); (2) intracellular calcium compartments (subspace between the T-tubule and junctional sarcoplasmic reticulum,[60] junctional and network sarcoplasmic reticulum, sarcomere, and bulk myoplasm); and (3) calcium buffers (mainly the sarcoplasmic Ca^{++} buffer calmodulin, sarcomeric Ca^{++} buffer troponin, and sarcoplasmic reticulum Ca^{++} buffer calsequestrin).

Very small inward currents of sodium and calcium ions maintain a long plateau of depolarization. The action potential plateau is also maintained by the activity of the sodium–calcium exchanger, driven by transmembrane voltage and Na^+ and Ca^{++} concentration gradients. During the plateau phase of the action potential, the Na^+–Ca^{++} exchanger initially works in reverse mode, importing Ca^{++} and exporting Na^+, afterward it switches to forward mode, extruding Ca^{++}.

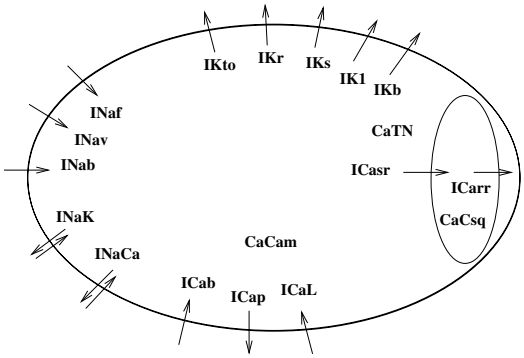

Figure 7.18. Main ionic currents associated with the action potential and calcium buffers in the ventricular cardiomyocyte (Sources: [773–778]). IcaL: L-type Ca^{++} current; ICap: sarcolemmal Ca^{++} pump current; ICab: background Ca^{++} current; Icarr: Ca^{++} ryanodine release current; ICars: sarcoplasmic reticulum Ca^{++} pump (uptake) current; INaCa: Na^+/Ca^{++} exchanger current; INaK: Na^+/K^+ pump current; INaf: fast Na^+ current; INab: background Na^+ current; IKto: transient outward K^+ current; IKr, IKs: rapid and slow K^+ delayed rectifier current; IK1: inward K^+ rectifier current. The three main transient buffers are the complexes of Ca^{++} with calsequestrin (CaCsq) in the sarcomplasmic reticulum, troponin (CaTN) in the sarcomere, and calmodulin (CaCam) in the sarcoplasma. The ventricular cell model can also include accumulation and depletion of calcium ions in diadic spaces, between the sarcolemma and sarcoplasmic reticulum.

[60] Sarcoplasmic reticulum calcium channel respond to increase in calcium ion concentration in the subspace caused by the local sarcolemmal influx.

The excitation-contraction process is triggered by calcium and involves especially the complexes (couplons or dyads) formed by sarcolemmal L-type calcium channels and the ryanodine channels of the junctional region of the sarcoplasmic reticulum. Reverse-mode Na^{+}–Ca^{++} exchangers and T-type Ca^{++} channels also trigger the Ca^{++} release, but with less efficiency [779].

7.8 Action Potential

The ionic composition, in particular sodium, potassium and calcium contents, of the CMC sarcoplasma is controlled by ion pumps and exchangers, which maintain steep ion concentration and electrical gradients across the thin ($\sim 6\,\mathrm{nm}$) sarcolemma that surrounds myofibrils (Table 6.4). The sarcolemma has a high and low conductance for K^{+} and Na^{+}, respectively. The resting membrane potential is about equal to $-88\,\mathrm{mV}$ (inside negative).

The heterogeneity of the action potential shape according to the nodal cell type is illustrated in Fig. 7.19. Purkinje and ventricular action potentials exhibit a plateau (phase 2). Atrial action potentials have a blunt triangular shape (absence of phases 1 and 2). The upstroke is slow because the fluxes elicited by Ca^{++} channels are smaller and these channels are activated more slowly than Na^{+} channels (triangle-shaped action potential). Activated non-selective cation channels induce a slow increase in transmembrane voltage after the end of the action potential, up to the triggering threshold for action potential.

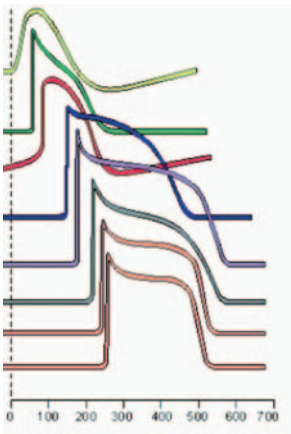

Figure 7.19. Varying shapes (t in ms) of the action potential during its propagation in the nodal tissue, starting from the sinoatrial node (top), travelling through the atrial myocardium, reaching the atrioventricular node, afterward running through the His bundle and its branches, then Purkinje fibers, to stimulate the venticular myocardium (bottom; from [780]).

Table 7.15. Nodal tissue and ionic currents. According to the types of involved ion channels, the initial depolarization ramp (upstroke; V/s) is more or less steep, action potential duration (APD) more or less long (ms), and firing frequency more or less high (beats/mn). APD is longer in the crista terminalis than the pectinate muscles in the right atrium, and longer in the right atrium than the left auricle. The short action potential in atria is partially explained by IKur. ICa,L and ICa,T are responsible for phase 0 in SAN, ICa,L in AVN. If in AVN is responsible for a secondary pacemaker activity. (A: atrium; V: ventricle; SAN: sinoatrial node; AVN: atrioventricular node; H: His bundle; P: Purkinje fibers; mid: midmyocardial; endo: subendocardial; epi: subepicardial layers of the myocardium; If: non-selective cation current; (Source: [762]).

Current	SAN	A	AVN	H	P	V
IKur	0	+	0	0	0	0
IKr	+	+	+	+	+	+
IKs	+	+	<SAN	+	+	+
Ito		+	+	+	+	Mid, epi > endo
IK1	∼0	Low	Low	<V	<V	+
IK,ACh	+	+	+			+
IK,ATP		+		+	+	+
If	+	+	+	+	+	+
INa	0	+	+	+	+	+
ICa,L	+	+	0	<V	<V	+
ICa,T	+	+	+	+	+	+
Frequency	70–80	50	30–60	20–40	15–30	15–30
Upstroke	2–5	150–250	10–20	400-600	500-600	
APD	150	150	150	400	400	epi < endo < mid

The features of the action potential (initial depolerization rate, duration, etc.) depend on the activities of involved ion channels of the nodal cells in the different compartments of the conduction network and cardiomyocytes in the wall of the atrium and ventricle, and within the ventricle, according to the myocardium layer (midmyocardial, subendocardial, and subepicardial; Table 7.15). The duration of the action potential depends on the nodal tissue territory with the following sequence of increasing order: (1) distal Purkinje fibers (∼ 400 ms, with upstroke of 500–600 V/s), (2) proximal Purkinje fibers and His bundle, (3) midmyocardial layer, (4) subendocardial layer, (5) subepicardial layer, and (6) atria, SAN and AVN (∼ 150 ms, with upstroke of 2–10 V/s) [762].

Non-pacemaker cells have a resting membrane potential (electrical diastole, phase 4), whereas pacemaker cells generate regular, spontaneous action potentials (Fig. 7.20).

7.8.1 Sinoatrial Node Action Potential

Cardiac pacemaker cells exhibit automaticity associated with membrane depolarization and repolarization. Atrial pacemaker potential is characterized by two distinct phases: an initial gradual depolarization (D1) followed by a steep slope (D2). The sinoatrial node yields spontaneous action potentials. In comparison with the standard action potential exhibited by ventricular myocytes, only three phases can be defined. Spontaneous depolarization (equivalent to phase 4 of standard action potentials; Fig. 7.20) is due to a K^+ outflux associated with Na^+ influx and a small Ca^{++} influx. Once the depolarization reaches a threshold of about −40 mV, a new action potential is triggered. A quick depolarization (corresponding to phase 0) is mainly caused by an augmented Ca^{++} influx. Repolarization occurs (corresponding to phase 3) when Ca^{++} influx decreases and K^+ outflux increases.

Involved mechanisms include the modulating hyperpolarization-activated inward Na^+ current,[61] the inward T- and L-type Ca^{++} currents, time-dependent decay of K^+ conductance, Na^+–Ca^{++} exchange, and low background K^+ conductance. Voltage-activated Ca^{++} release (Ca^{++} sparks) significantly contributes with the activity of multiple sarcolemmal ion channels

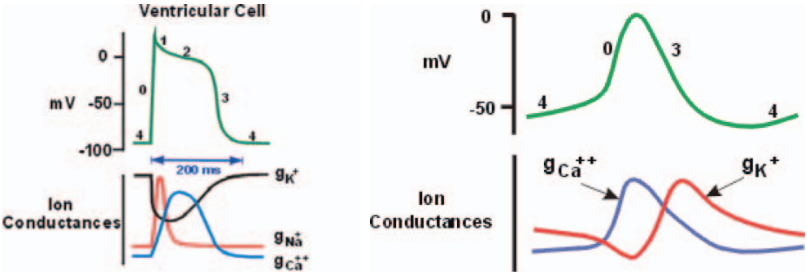

Figure 7.20. (**Left**) action potential of the ventricular myocyte (classical model) with its five phases 0–4, phase 4 being the resting membrane potential. Phase 0, the rapid depolarization phase, is due to opening (influx) of fast Na^+ channels (with quick rise in membrane conductance g_{Na^+}, current INa) and closure of K^+ channels. Phase 1 is due to closure of the fast Na^+ channels and transient outward fluxes of K^+ and Cl^-. Phase 2 (plateau) is caused by a balance between Ca^{++} influx using long-lasting channels and K^+ outflux. Phase 3, repolarization down to the resting membrane potential, is induced by a decrease in Ca^{++} influx and K^+ outflux. During phases 0, 1, 2, and part of phase 3, the cell is refractory to the initiation of a new action potential. (**Right**) action potential spontaneously generated (automaticity) in pacemaker cells of the sinoatrial node. A continuous K^+ outflux causes slow depolarization. Phase 0, fast depolarization due to Ca^{++} influx. Phase 3, repolarization, is due to inactivation of voltage-gated Ca^{++} channels, decaying Na^+ flux, and rising K^+ flux (from [831] (web site) with author permission).

[61] Pacemaker cells lacking hyperpolarization-activated inward Na^+ current have a lower frequency.

to heart electrical excitations originating from the sinoatrial node as well as latent atrial pacemaker. This voltage-gated intracellular Ca^{++} release is triggered by the activation of T-type Ca^{++} channels [781]. Atrial pacemaker cells do not contain T-tubules; therefore, the subsarcolemmal SR cisternae are located along the cell periphery with a subspace (size $\sim 25\,\text{nm}$) rich in T-type Ca^{++} channels.

7.8.2 Ventricular Action Potential

The action potential is initiated by depolarization of the sarcolemma, disturbing the ionic gradient across the sarcolemma. Separate voltage sensitive channels[62] for Na^+, K^+, and Ca^{++} exist in the sarcolemma which transiently open and increase the passage of these ions.

The Na^+ channels open first and then rapidly inactivate (`Phase 0`; Table 7.16). The depolarization, indeed, inactivates these Na^+ channels. The quick cellward Na^+ motion increases transmembrane potential up to about $+30\,\text{mV}$. The conductance of the inward K^+ rectifiers strongly decreases from the onset of depolarization.

`Phase 1` occurs after action potential peak. It corresponds to the first stage of rapid repolarization associated with a transient outward motion of K^+. Sarcolemmal repolarization, especially the phase 1 rate, which affects the number of activated L-type Ca^{++} channels and the ion flux rate through them, modulates the recruitment of the ryanodine channels via L-type Ca^{++} channels; hence, the Ca^{++} flux to the sarcomere. A second transient flux for repolarization is induced by Ca^{++}-activated Cl^- channels.

`Phase 2` is associated with a slow inward Ca^{++} current (i_{Ca}) for several hundred milliseconds (plateau). L-type Ca^{++} channels are indeed secondarily activated, with two opening modes: mode 1 by short opening bursts, and mode 2 by long-duration opening. Ca^{++} channels inactivate more slowly than Na^+ channels. Mode 2 Ca^{++} channels are involved during phase 2 of action potential with Ca^{++} flux from extracellular space to sarcoplasma. They elicit

Table 7.16. Phases of action potential and main ionic motions.

Phase	Ion motion	Path
0	Na^+ influx	Na^+ channels (quick and short opening)
1	K^+ efflux	Transient outward K^+ channels
2	Ca^{++} influx	VDCC (mode 1 and 2 L-types), RC
3	K^+ efflux	Delayed rectifier K^+ channels
4	K^+ efflux	Inward rectifying K^+ channels

[62] Channel protein conformations are influenced by the membrane potential, so that voltage-gated ion channels are only permeable over a narrow range of membrane potentials. Ligand-gated ion channels require specific chemical activators.

additional Ca^{++} release from the sarcoplasmic reticulum for contraction with slow inactivation.

The plateau of ventricular action potentials is prolonged because the K^+ currents are slow to occur and corresponding channels have a reduced conductance at positive transmembrane potentials. K^+ effluxes from rapid and slow delayed K^+ rectifiers balance the Ca^{++} import. The kinetics and conductance of L-type Ca^{++} channels and delayed K^+ rectifiers determine plateau duration. A long action potential ensures adequate time for entry of extracellular Ca^{++} into the cardiomyocyte for optimum excitation-contraction coupling. Delayed repolarization also yields cardiomyocyte refractory to premature excitation.

Other ionic fluxes arise from Na^+-Ca^{++} exchangers, with its transport reversal during repolarization, and from Na^+-K^+ pumps. The transition between phases 2 and 3 is characterized by an increase in repolarization rate due to the beginning in activity of inward K^+ rectifiers, expelling K^+ ions.

During `phase 3`, delayed rectifier K^+ channels induce rapid repolarization by K^+ current, which counters depolarization induced by the inflow of Na^+ and Ca^{++}. These membrane channels remains closed in the presence of ATP, but as soon as ATP levels fall, they open for K^+ efflux, which leads the cardiomyocyte to a hyperpolarized inactive state.

Throughout `phase 4`, the resting membrane potential is regulated by a background K^+ current. A Na^+ efflux can balance the K^+ influx generated by the inward K^+ rectifiers to ensure a rest cell potential. This process can be modulated by voltage-sensitive Cl^- channels activated by catecholamines via cAMP. The action potential is shortened by Cl^- influx and lengthened by adrenergic effect on Ca^{++} channels.

7.8.3 Electrophysiological Myocyte Types

Transmural differences in electrochemical properties of the ventricular myocytes are attributed to changes in both quantity and quality of sarcolemma (K^+ transient outward, rapid, and slow delayed K^+ rectifiers [782], inward K^+ rectifier, Na^+–Ca^{++} exchanger with its reverse/forward modes, and late Na^+ channel), as well as sarcoplasmic reticulum carriers (sarcoplasmic reticulum Ca^{++}-ATPase).[63] Such variations lead to electromechanical coupling differences, which can also be due to variations in intracellular Ca^{++} buffering, as well as in contractile protein isoform expression.[64]. These transmural differences might be required for coordinated contraction/relaxation of the

[63] The expression of sarcoplasmic reticulum Ca^{++}-ATPase is greater in epicardial than midmyocardial and endocardial myocytes [783]. Consequently, epicardial myocytes exhibit a quicker decay of intracellular Ca^{++} content than endocardial myocytes. Transmural expression of Na^+/Ca^{++} exchanger do not significantly vary [783]. Regional differences in ryanodine channel expression and/or its binding protein might exist.

[64] Myosin isoforms do not have similar ATPase and contractile activities [784].

ventricular myocyte population across the ventricular wall stimulated at different instants by the propagating electrochemical, with a delay of the order of few tens of milliseconds.

The ventricular myocardium contains at least three electrophysiologically distinct myocyte types: epicardial, midmyocardial, and endocardial myocytes. The epicardial action potential exhibits a prominent early notch (spike-and-dome morphology). The action potential notch is due to a transient outward K^+ current [785], which decays in amplitude from the epicardial to endocardial surfaces (the notch is smaller in midmyocardial myocytes and absent in endocardial myocytes). The transient outward K^+ current is also larger in right than left ventricular epicardial myocytes. Higher transient outward K^+ currents lead to faster rate of repolarization, hence causes larger L-type Ca^{++} current and greater increase in $[Ca^{++}]_i$ than slower currents [786]. Epicardial and, to a lesser extent, midmyocardial myocytes indeed exhibit faster sarcomere shortening kinetics than endocardial myocytes. The number of inward K^+ rectifiers and the characteristics of delayed K^+ rectifiers differ between endocardial and epicardial cells. Because the number of sarcoplasmic reticulum Ca^{++}-ATPase is greater in epicardial myocytes, Ca^{++} reuptake is quicker in ventricular epicardium than endocardium.

There is no transmural difference in rapid-activating delayed rectifier K^+ currents and inward rectifier K^+ currents. But, apicobasal differences can exist.

Midmyocardial or M myocytes,[65] which can extend to the deep subendocardium, exhibit longer action potential duration due to smaller slow components of the delayed K^+ rectifier density and/or unitary conductance [782], as well as a larger density in Na^+–Ca^{++} exchanger [789], and higher late Na^+ current [790]. In the midmyocardium, higher density of sodium channels, larger channel conductance, and greater probability of reopening yield stronger late Na^+ currents. Prolonged action potential augments myocyte contraction by increased $[Ca^{++}]_i$. M myocytes have a greater rate of depolarization (higher conduction velocity than in other cardiomyocytes, but lower than in nodal cells). M myocytes are more sensitive to frequency changes.

Following depolarization and ventricular contraction, repolarization initiates cardiac relaxation. Ventricular repolarization takes a longer time in the heart apex than base, and in endocardium than epicardium. Endocardial cardiomyocytes have smaller rates of repolarization than epicardial cardiomyocytes, because of a lower density of voltage-gated potassium channels (transient outward K^+ flux) in endocardial and septal myocardia than in epi-

[65] M cardiomyocytes have been observed in the human heart [787]. Midmyocardial myocytes are located between the deep subendocardium and midmyocardium in the anterior wall, between the subepicardium and midmyocardium in the lateral wall, throughout the wall of the right ventricle outflow tracts, as well as in papillary muscles, trabeculae and interventricular septum [788].

cardial myocardium.[66] A space gradient in voltage-gated potassium channels is required for coordinated cardiac repolarization.

Differences in between-myocyte electrophysiological characteristics are associated with variations in shortening kinetics (Fig. 7.21) [791]. Behavior features of epicardial, endocardial, and midmyocardial myocytes are summarized in Table 7.17.

7.9 Excitation-Contraction Coupling

Cardiomyocyte contraction and relaxation are effected by cyclic association and dissociation of actin and myosin. This cycle is regulated by Ca^{++}, the troponin complex, and tropomyosin. Troponin-C binds Ca^{++}, troponin-I links to actin and blocks interaction with myosin when $[Ca^{++}]$ is low, and troponin-T connects to tropomyosin. When the cytoplasmic $[Ca^{++}]$ rises (mainly after Ca^{++} release from the sarcoplasmic reticulum because the opening of voltage-dependent Ca^{++} channels is insufficient to cause contraction), Ca^{++} binds to TN-C. The Ca^{++}–TN-C complex moves TN-I away from myosin binding sites on actin, alters the interaction between TN-T and tropomyosin, and frees the binding sites between myosin and actin to initiate a new cross-bridge cycle at many points along the filament, which promotes contraction. ATP provides the energy for contraction. ATP hydrolysis stops as soon as Ca^{++} is removed from cytosol.

Calcium ions are a major factor with ATP in cardiac excitation-contraction coupling. Ca^{++} influx from the extracellular medium and mainly cellular stores to the sarcomere, together with ATP from cytolosic sources and mitochondria, and outflux from the sarcomere to the sarcoplasmic reticulum,

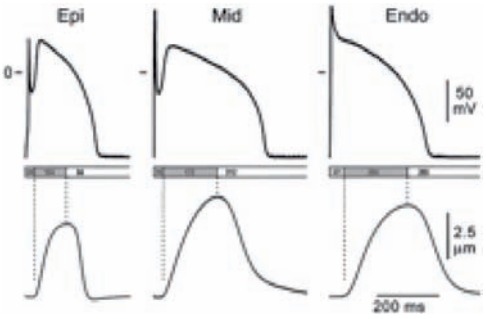

Figure 7.21. (**Top**) action potentials in epicardial (Epi), midmyocardial (Mid), and endocardial (Endo) myocytes. (**Bottom**) corresponding cell shortening (from [791] used with permission).

[66] Loss in repolarization heterogeneity can lead to polymorphic ventricular tachycardia.

mitochondria, and extracellular fluids are strongly regulated. Moreover, the effects influence the causes. The membrane potential modulates Ca^{++} transport, which in turn modifies the membrane potential.

The magnitude and the duration with respect to the cardiac cycle of CMC contraction is regulated by $[Ca^{++}]_i$, which depends on protein carriers on cell and SR membranes (Fig. 7.16, Tables 7.7 and 7.18). There are four main Ca^{++} compartments in the cardiac nanomotor: (1) the extracellular space, (2) the sarcoplasmic reticulum for capture and release, (3) troponin-C transiently,

Table 7.17. Main features of action potentials and cell shortening in unloaded epicardial, endocardial, and midmyocardial myocytes (Sources: [783, 791]).

	Epicardial myocyte	Midcardial myocyte	Endomyocardial myocyte
Action potential			
Spike-and-dome configuration	+	+	No
Duration	Shortest	Longest	
Peak time			Longest
Ca^{++} carriers			
Ca^{++} influx onset		Similar time	
$[Ca^{++}]$ decay rate	++	+	+
SR Ca^{++} content	Largest		Smallest
L-type Ca^{++} channel		Similar density	
Sarcoplasmic reticulum Ca^{++} ATPase	++	+	+
Na^+–Ca^{++} exchanger		Similar density	
Cell shortening			
Contraction onset	Shortest		Greatest
Peak time	Shortest		Longest
Relaxation	Fastest		

Table 7.18. Main calcium-handling proteins in CMCs. The intracellular calcium concentration in CMC is regulated by ion carriers and Ca^{++} binding by intracellular proteins.

RC	Release from SR	Contraction (inotropy)
L-type VDCC	Influx across sarcolemma	Contraction
Troponin-C	Ca^{++} binding	Contraction
Calmodulin	Ca^{++} binding	Influx limitation
NCX	Influx across sarcolemma	Contraction (high $[Na^+]_i$)
	Efflux across sarcolemma	Relaxation (high $[Ca^{++}]_i$)
SERCA	SR sequestration	Relaxation (lusitropy)
Calsequestrin	storage	Relaxation

and (4) calmodulin (Cam). Oxygen is the combustible molecule. Cytoplasmic [Ca^{++}], and hence CMC contraction/relaxation are controlled.

During the systole, two mechanisms, Ca^{++}-induced Ca^{++} release and a voltage-sensitive release mechanism induce Ca^{++} flux from the sarcoplasmic reticulum via ryanodine channels, or SR Ca^{++} release channels [792]. Ca^{++}-induced Ca^{++} release deals with Ca^{++} flux through L-type Ca^{++} channels eliciting Ca^{++} release from ryanodine channels. The voltage-sensitive release mechanism, once activated by potential of about $-60\,\mathrm{mV}$, generates Ca^{++} release. Influx of Ca^{++} triggers Ca^{++} release from the sarcoplasmic reticulum into the cytoplasm, which sufficiently increases the cytosolic [Ca^{++}] to induce contraction.[67] Na^{+}–Ca^{++} *porter* which exchanges three Na^{+} for one Ca^{++} operates during phase 2. It stabilizes intracellular [Ca^{++}] by a repeated entry of Ca^{++}.

Relaxation requires Ca^{++} removal from the cytosol. During the diastole, cytosolic [Ca^{++}] decreases by Ca^{++} uptake: (1) into the sarcoplasmic reticulum via sarco(endo)plasmic reticulum Ca^{++}-ATPases and Na^{+}–Ca^{++} exchangers (NCX); (2) into mitochondria by mitochondrial Ca^{++} uniporters; and (3) by extrusion for a small amount via sarcolemmal Ca^{++}-ATPases (PMCA) and NCXs. Because the Na^{+}–Ca^{++} exchanger in its forward mode, the sarcolemmal and the sarcoplasmic reticulum Ca^{++}-ATPases restore cytosolic Ca^{++} concentration to resting levels, they have a lusitropic effect.

Most Ca^{++} returns to the sarcoplasmic reticulum, where Ca^{++} is stored by bonds with *calsequestrin* (Csq).[68] ATP is not only associated with pumping of Ca^{++} back into SR and removing Ca^{++} surplus, but is also required for relaxation of the actin–myosin cross-bridges. The stronger the contraction, the greater the Ca^{++} reuptake.

Phospholamban (PLb), which is associated with SERCA, inhibits this pump. Its phosphorylation[69] accelerates Ca^{++} uptake by the SERCA pump; it is enhanced by β-adrenergic stimulation, which thus has a positive lusitropic effect. Phosphorylation by protein kinase-A of *L-type* Ca^{++} channels increases the number of functional channels, increases the opening duration, and decreases the channel activation threshold and deactivation rate. PKA phosphorylation of delayed rectifier K^{+} channels increases the activity and shortens action potential duration. PKAs are stimulated by activation of Gs protein. Catecholamines increase inotropy in particular by phosphorylating sarcolemmal Ca^{++} channels and increasing Ca^{++} influx into the cytosol during the plateau phase 2. Cellular acidosis decreases the sensitivity of the contractile

[67] About 10% of the calcium needed for cardiac contraction enters from the extracellular space. This trigger calcium induces release of the remainder from the sarcoplasmic reticulum.

[68] There are two isoforms, one in the skeletal muscle, the second in the myocardium [754].

[69] Phosphorylation of phospholamban by cAMP-dependent (PKA) or calmodulin-dependent protein kinases (CamK2) relieves the inhibition of SERCA by phospholamban.

elements to Ca^{++} (Table 7.19). Inorganic phosphate at a concentration achieved during ischemia reduces maximum developed force and sensitivity to Ca^{++}.

Heart failure is characterized by a decrease in calcium removal into the sarcoplasmic reticulum associated with SERCA2 activity and dysregulated calcium release from the sarcoplasmic reticulum via ryanodine channels (Fig. 7.15). Many types of heart failure are associated with downregulation of SERCA2 expression, impaired SERCA2/phospholamban ratio, and decreased phosphorylated phospholamban. Heart failure can, therefore, be stopped and even reversed by correcting sarcoplasmic reticulum calcium release and storage (SR Ca^{++} cycling). Inhibition of phospholamban enhances SR Ca^{++} uptake and rescues heart failure in several animal models of dilated cardiomyopathy and post-myocardial infarction chronic heart failure [794].

*S*100A1[70] is expressed in the myocardium, where it interacts with contractile filaments and proteins of the sarcoplasmic reticulum (titin, SERCA, phospholamban, and the ryanodine channel). The Ca^{++} binding protein S100A1 increases Ca^{++} release from the sarcoplasmic reticulum by interacting with ryanodine channels. It also interacts with SR proteins, regulating Ca^{++}-induced Ca^{++}-release. Cardiomyopathies are associated with altered S100 protein levels. S100A1 is upregulated in right ventricular hypertrophy, which can be an adaptive response of myocardial Ca^{++} homeostasis to a higher load [797], and downregulated in end-stage heart failure [798]. S100A1 gene transfer restores contractile function of the failing myocardium[71] [799].

Table 7.19. Myofilament Ca^{++} sensitivity. CMC stretching enhances the actin–myosin interaction partly by transverse compression of the filament lattice (Source: [759]).

Increased	Decreased
Stretching	Acidosis (ischemia)
Caffeine	Elevated phosphate concentration (ischemia)
Certain inotropic drugs	Elevated Mg^{++} concentration (ischemia)
	β-Adrenergic activation

[70] S100 multifunctional proteins are diversified according to the structure, ion-binding property (Ca^{++}, Zn^{++}, or Cu^{++}), spatial distribution in the intra- or extracellular space, and ability to form homo- and heterodimers [795]. Several S100 proteins (S100B, S100A4, S100A8, S100A9, S100A12, and S100A13) are secreted and have cytokine-like functions through interactions with the receptor for advanced glycation end (RAGE) products. S100A4 has angiogenic effects [796].

[71] Adenovirus-mediated S100A1 gene delivery normalizes S100A1 protein expression in a post-infarction rat heart failure model and reverses contractile dysfunction of failing myocardium. Moreover, S100A1 gene transfer decreases elevated $[Na^{+}]_i$ to levels detected in non-failing cardiomyocytes and restores energy supply in failing cardiomyocytes.

Nervous Influences

Adrenergic signaling in the myocardium contributes to the positive control of the heart rate (chronotropy +), contraction strength (inotropy +), and relaxation rate (lusitropy +) by changes in levels of intracellular calcium ions or modifications in sensitivity of regulatory proteins to calcium ions. Inotropic effects are due to increased Ca^{++} current and greater availability of stored Ca^{++}.

Adrenaline and noradrenaline released by the sympathetic system increase calcium currents conducted by heteromeric L-type CaV1.2 channels of cardiomyocytes.[72] β-Adrenergic signaling causes a three- to fourfold rise in calcium current due to the activation of adenylyl cyclase and inhibition of phosphodiesterases. Both activated β1- and β2-adrenergic receptors increase the channel activity via phosphorylation by protein kinase-A in association with A-kinase anchoring protein AKAP15. However, the calcium current predominantly results from activation of β1-adrenergic receptors.[73]

The sympathetic system stimulates the heart through β-adrenergic receptors. About 75% of cardiac β-adrenergic receptors are β1-receptors. They are distributed throughout the sarcolemma and coupled to Gs-protein. Less abundant β2-adrenergic receptors reside predominantly in caveolae. Several other ion carriers exist in caveolae of cardiomyocytes: voltage-dependent Na^+ channel, voltage-dependent K^+ channel Kv1.5, Na^+/Ca^{++} exchanger, HCN4 pacemaker channel, and voltage-gated L-type Ca^{++} channels.[74] β2-Adrenergic receptors signal via the set of cardiac voltage-gated L-type Ca^{++} channels of caveolae in ventricular myocyte [747]. L-type Ca^{++} channels are co-located with caveolin-3, a major structural protein of caveolae in striated muscle.[75]

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[72] Cav1.2 channels are responsible for the major calcium current in cardiomyocytes which triggers release of stored calcium from the sarcoplasmic reticulum for contraction.

[73] β2-Adrenergic receptor-mediated phosphorylation depends on intracellular calcium concentration, which must be increased. β2-Adrenergic receptors do not significantly participate to the calcium flux at basal calcium levels [800]. β2-Adrenergic receptors associated with adenylyl cyclases, AKAP15, and calcium channels in the cardiomyocyte sarcolemma stimulate a small set of Cav1.2 calcium channels. Protein kinase-C and calcium–calmodulin-dependent protein kinase-2 can also phosphorylate voltage-gated calcium channels to increase the calcium influx.

[74] These cardiac voltage-gated L-type Ca^{++} channels outside of junctional complexes do not strongly intervene in excitation-contraction coupling but regulate cellular functions.

[75] Caveolae (Sect. 5.1.3) are tiny invaginations of the plasmalemma with cholesterol, sphingolipids and caveolin. Many proteins involved in cellular Ca^{++} activity are located in caveolae. They include IP3 receptor, Na^+/Ca^{++} exchanger, Ca^{++}-ATPase, members of the transient receptor potential family. Caveolin-3, Cav1.2 channel, β2-adrenergic receptors (but not β1-adrenergic

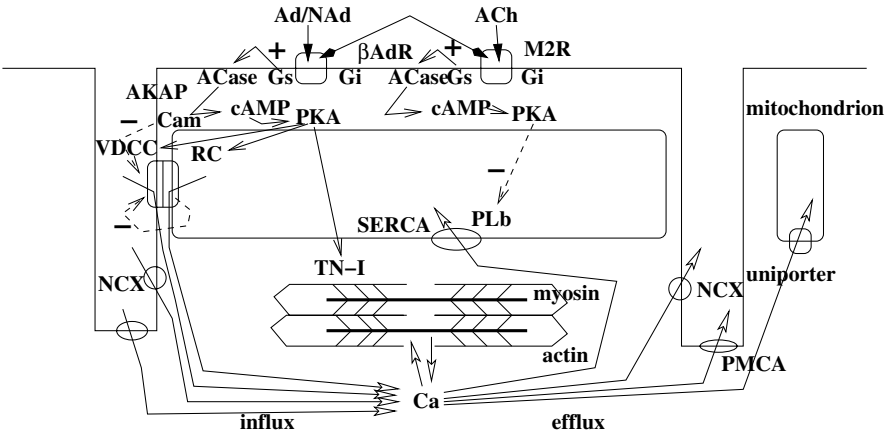

Figure 7.22. β-Adrenergic and M2 muscarinic interacting receptors and CMC functioning (Source: [759]).

β-Adrenergic receptor stimulates a Gs protein, which activates adenylyl cyclase, producing cAMP, which in turn activates protein kinase-A (Fig. 7.22). PKA phosphorylates phospholamban (lusitropic effect), L-type VDCC, ryanodine channel, troponin-I (speed up dissociation of Ca^{++} from the myofilaments), and myosin binding protein-C. The increased L-type Ca^{++} currents through Cav1.2 channels by activated β-adrenergic receptors requires phosphorylation by protein kinase-A anchored to the channel via an A-kinase anchoring protein AKAP15. AKAP15 and PKA form an inhibitory complex [801].

M2-Muscarinic receptor activation can either decrease or increase cAMP concentrations, whether these receptors interfere with β1- or β2-adrenergic receptors, respectively [793].

Both compartmentation and dynamics in cyclic adenosine monophosphate and protein kinase-A signaling in cardiomyocytes affect cardiac inotropy. cAMP buffering by protein kinase-A corresponds to a rate-limiting node of PKA pathway downstream from the β-adrenergic receptor.[76] Prostaglandin E1 stimulates higher PKA activity in the cytosol than at the sarcolemma, likely due to differences in cAMP diffusion [802]. Restricted diffusion, cAMP degradation by phosphodiesterases, and cAMP accumulation near protein kinase-A contribute to both spatial and temporal signaling variations.

receptors), Gαs protein, Gαi protein, adenylyl cyclase, protein kinase-A, and protein phosphatase-2A form signaling complexes in caveolae of cardiomyocytes.

[76] cAMP synthesis can greatly exceed requirements for PKA activation.

Cell Excitability Adjustment

The cardiomyocyte adapts its sensitivity to repeated stimulations. Moreover, the *electrochemical delay*, i.e., the duration between the membrane depolarization and contraction (cross-bridge formation) is small enough (< 50 ms) to limit asynchrony of the myocardium activity.

The *refractory period* is a protective mechanism to maintain efficient successive blood fillings and ejections. The CMC refractory period is longer than contraction and relaxation periods. Several periods, which overlap each other, starting from the beginning of phase 0, have been defined (Table 7.20, Fig. 7.23). From phase 0 to initial/mid phase 3, i.e., during the *absolute refractory period* (ARP), the CMC unexcitability is complete. During the *relative refractory period* (RRP), a strong stimulus causes an evoked excitation, but the amplitude response is too low for propagation. During the *effective refractory period* (ERP), the potential propagates when the stimulus is supraliminal. The *supernormal period* (SPN) is a short phase at the end of repolarization, during which the potential initiated by an infraliminal stimulus propagates. The excitability is maximum and decreases to its rest value at the end of the *total recovery period*. Refractory periods vary with excitation rate.

7.10 Nervous Inputs

The control of the function of the cardiovascular system involves both fast-acting responses and long-lasting adaptations acting on gene expression. The cardiovascular system is quickly regulated by a set of mechanisms, which mainly involve the central nervous system (fast neural command) and the release of hormones from endocrine organs (delayed humoral command, triggered particularly by catecholamines, vasopressin, angiotensin-2, and natriuretic peptides).

Table 7.20. CMC excitability and refractory periods. Cardiomyocytes and nodal cells are inexcitable during absolute refractory period (ARP). Even strong stimuli do not produce any excitation. The cell is refractory to the initiation of new action potentials. The absence of excitability appears because Na^+ channels remains inactivated until sufficient repolarization occurs in the initial part or middle of phase 3. At the end of ERP, a stimulus generates an action potential that can propagate. The recovery of Na^+ channels allows cell excitablity recovery. The recovery of excitability is delayed in the atrioventricular node because Ca^{++} channels rather than Na^+ channels elicit the upstroke.

ARP	No excitability
RRP	No conduction
ERP	Conditional propagation
SPN	Maximal excitability

The heart is innervated by both components of the autonomic nervous system, parasympathetic and sympathetic nerves, which send signals to the cardiac cells via plasmalemmal receptors, thus triggering signaling pathways (Tables 7.21 and 7.22). Signaling pathways involve sequential protein phosphorylation cascades that ultimately activate transcriptional factors driving gene expression. Protein phosphatases terminate nervous signaling. Within the cells, particularly in cardiomyocytes and vascular smooth muscle cells, calcium ions trigger contraction, metabolism, and growth. Cell activity, in particular cardiomyocyte contraction, ends when calcium ions are removed from regulatory proteins, such as calmodulin and troponin. Coronary vessels also receive both sympathetic and parasympathetic innervation. The cardiovascular centers of the brainstem get signals from various sensors distributed throughout the body. Efferent nerves connect to nodal tissue and myocardium, controlling primarily the heart frequency and contractibility. Efferent nerves to the smooth muscles in coronary vessel walls control arterial tone, arteriolar resistance, and blood volume in the large veins. Normally, parasympathetic innervation is the dominant neural influence on the heart. However, the adrenergic system, with its various specific G-protein-coupled receptors and their corresponding cell signaling cascades, yields the major regulatory system, whereas the parasympathetic system plays a minor role in responses to environmental stimuli.

Galanin (Gal), a neuropeptide that is cleaved from preprogalanin, inhibits neurotransmitters. Galanin increases mean arterial blood pressure and heart rate, inhibiting cholinergic activity in the heart in cooperation with neuropeptide Y (NPY). Activated Gal1 and NPY-Y2 receptors of parasympathetic terminals of vagal neurons in the heart reduce acetylcholine release, especially

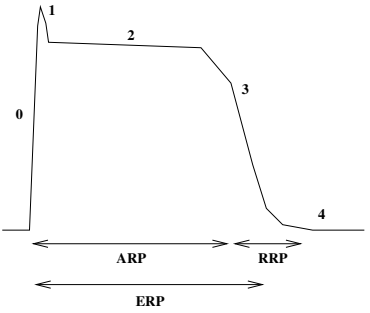

Figure 7.23. Refractory periods and recovery of excitability. No stimulus can generate another action potential during absolute refractory period (ARP). During the relative refractory period (RRP), an excitation stronger than the one for the electrical diastole is required to evoke an action potential, and a higher threshold potential is set up. Effective refractory period (ERP) extends from the upstroke beginning to the middle of RRP. ERP corresponds to the time interval of the action potential without conduction.

during prolonged attenuation of parasympathetic slowing following activation of the cardiac sympathetic nerve [803].

Adrenergic G-Protein-Coupled Receptors

The myocardial systolic and diastolic function are mainly governed by β1 and β2-adrenergic receptor activities. However, α-adrenergic receptors leads to positive and negative inotropy. The family of adrenergic receptors consists of nine subtypes (Table 7.23): α1A, α1B, α1D, α2A, α2B, α2C, β1 (∼70%),[77] β2 (∼10%), and β3 (∼1%). Dimerization can occur between adrenergic receptors, such as between α2 and β1 or β2-adrenergic receptors. Scaffold A-kinase-anchoring proteins interact with adrenergic receptors, especially β2-adrenoceptors. Stimulated adrenergic receptors activate pathways leading to

Table 7.21. Parasympathetic and sympathetic (α) neurotransmitters, receptors, and effectors. Gs Protein activates adenylyl cyclase (ACase), which triggers the PKA pathway, leading to positive inotropy (faster, stronger, shorter contraction) and lusitropy (faster relaxation). Gs Protein stimulates Rho GTPases and mitogen activated protein kinases (MAPK). Gi/Gβγ Protein inhibits ACase and stimulates PLC, NOS, and PI3K pathways, thus targeting PKC (cell conditioning), PKG (antiproliferative and negative intropy), and PKB (antiapoptotic), respectively. Gq/Gβγ Protein also activates PLC, NOS and PI3K pathways. PKC stimulates and PKA inhibits the activity of adenylyl cyclases (positive and negative feedback loops). Reduced arginine can form superoxide and reactive nitric oxide species (RNOS). Superoxide rapidly converts NO to RNOS (Source: [804]).

	Parasympathetic	Sympathetic (α)	
Neurotransmitter	Acetylcholine	Noradrenaline	
GPCR	M2	α1	α2
G Protein	Gi/Gβγ	Gi/Gβγ Gq/Gβγ	Gs Gi/Gβγ
First effectors	ACase (−) PLC NO/GCase PI3K	ACase (−) PLC NO/GCase PI3K	ACase (−) PLC NO/GCase PI3K
Second messengers	 IP3/Ca/DAG RNOS/cGMP PDK1	 IP3/Ca/DAG RNOS/cGMP PDK1	cAMP IP3/Ca/DAG RNOS/cGMP PDK1
Protein kinases	PKB/PKC/PKG	PKB/PKC/PKG	PKA/PKB/PKC/PKG

[77] β1-adrenergic receptors are downregulated in hypertrophic and ischemic cardiomyopathy.

non-receptor protein Ser/Thr kinases (PKA, PKB, PKC, and PKG).[78] Adrenergic G-protein-coupled receptors can also activate Ras GTPase and MAPK cascades. Phosphorylation by G-protein-coupled receptor kinases, with arrestin and adapter AP2, desensitizes the nervous signaling.[79]

Activated α1-adrenoceptors interact with Gαq, which signals via phospholipase-C leading to: (1) diacylglycerol, then activating protein kinase-C; and (2) inositol trisphosphate and calcium influx mainly from the sarcoplasmic reticulum. α2-Adrenoceptors coupled to Gαi inhibit plasmalemmal adenylyl

Table 7.22. Sympathetic (β) neurotransmitters, receptors and effectors (see caption of Table 7.21; Source: [804]).

	Sympathetic (β)		
Neurotransmitter		Noradrenaline	
GPCR	β1	β2	β3
G Protein	Gs	Gs Gi/G$\beta\gamma$ Gq/G$\beta\gamma$	Gs Gi/G$\beta\gamma$
First effectors	ACase(+/−)	ACase(+/−) PLC NO/GCase PI3K	ACase(+/−) PLC NO/GCase PI3K
Second messengers	cAMP	cAMP IP3/Ca/DAG RNOS/cGMP PDK1	cAMP IP3/Ca/DAG RNOS/cGMP PDK1
Protein kinases	PKA	PKA,PKB, PKC,PKG	PKA,PKB PKC,PKG
Effectors	Ras, MAPK		

[78] Inactive PKA binds to cAMP, then dissociates into cAMP-bound dimer and active PKAs. PKC family members are activated via phospholipase-C. PKCα, PKCβ1/2, and PKCγ require a phospholipid, Ca^{++}, and DAG for activation; PKCδ, PKCϵ, PKCη, and PKCθ needs DAG but not Ca^{++}; PKCζ and PKCλ do not have Ca^{++} and DAG binding sites.

[79] GRK2 and GRK5 are expressed in cardiomyocytes.

Table 7.23. Adrenergic receptors; corresponding signaling pathways; and functions in nervous, cardiac, and vascular tissues. Effectors include: adenylyl cyclase (ACase); cyclic adenosine monophosphate (cAMP); protein kinase-A (PKA); guanylyl cyclase (GCase); cyclic guanosine monophosphate (cGMP); protein kinase-G (PKG); nitric oxide synthase (NOS); phospholipase-C (PLC); diacylglycerol (DAG); inositol trisphosphate (IP3); calcium ions; protein kinase-C (PKC); phosphoinositide 3-kinase (PI3K); phosphoinositide-dependent kinase-1 (PDK1); protein kinase-B (PKB); Rho GTPases; and mitogen activated protein kinases (MAPK; Source: [804]).

Receptor	Effectors	Functions
α1A	Gq-PLC-IP3-Ca^{++} Rho-Rho kinase MAPK (ERK, JNK, p38)	Inotropy +, myocyte growth, vasoconstriction
α1B	Gq-PLC-IP3-Ca^{++} ERK, p38	Inotropy +, myocyte growth, vasoconstriction
α1D	Gq-PLC-IP3-Ca^{++} MAPK (weakly)	Vasoconstriction
α2A	Gi (presynaptically),	Sympathetic output reduction hypotension
α2B	Gi (postsynaptically),	α2A Counteraction peripheral vasoconstriction, hypertension
α2C	Gi at low frequency nerve activity),	Sympathetic output reduction (presynaptically, cAMP Antagonist (postsynaptically), Vasoconstriction (cold), Hypotension
β1	Gs-ACase-cAMP-PKA	Inotropy +, lusitropy +, metabolism, growth
β2	Gs-ACase-cAMP-PKA Gi/G$\beta\gamma$-PLC-DAG-PKC G$\beta\gamma$-PI3K-PDK1-PKB	Inotropy +, lusitropy +, metabolism, growth, CMC survival, Relaxation of bronchial and vascular SMC
β3	Gi/G$\beta\gamma$-PLC-DAG-PKC Gi/G$\beta\gamma$-NOS-NO-GCase-cGMP-PKG	Inotropy −, CMC survival

cyclase,[80] activating K^+-channels and inhibiting sarcolemmal L-type Ca^{++} channels.

[80] Among the nine known isoforms (ACase1–ACase9), adenylyl cyclase-5 is specifically expressed in cardiomyocytes; adenylyl cyclase-6 is expressed in other

β1-Adrenoceptors are coupled to Gαs, inducing positive inotropy and lusitropy via adenylyl cyclase and protein kinase-A. Both β2 and β3-adrenoceptors activate Gαs, Gαi, or Gαq according to the humoral context. Gαs- and Gαq-protein sets work with adenylyl cyclase and PLCβ pathways, respectively. The Gαi-protein set family can act via Gβγ. Gαq/Gβγ Protein activates phosphoinositol-3 kinase.

β2-adrenoceptors are preferentially coupled to Gαs, but they also bind Gαi- or Gαq-coupled receptors. When stimulated by noradrenaline, β2-adrenoceptors interact much faster with Gαs than with Gαi or Gαq. Besides, PKA-dependent phosphorylation of β2-adrenoceptors switches the G-coupling from Gαs to Gαi, thus, inhibiting ACase and activating the mitogen-activated protein kinase cascade.

β3-adrenoceptors lack phosphorylation sites. They thus are not desensitized by GRKs, PKA, or PKC. α1, α2, β1, β2, and β3-Adrenoceptors exist post-synaptically in cardiomyocytes, smooth muscle cells, and endothelial cells. β-Adrenoceptors are major regulators of the cardiac activity.

Positive inotropic and lusitropic effects of β1- and β2-adrenoceptors are mediated by PKA phosphorylation of phospholamban, troponin-I, L-type Ca^{++}-channels, and ryanodin channels-2. Cardiac phosphodiesterases degrade cAMP, thus limiting the magnitude of β-adrenoceptor stimulation. At least four phosphodiesterase types (PDE1–PDE4), mostly in the cell cortex, exist in cardiomyocytes.[81]

Positive chronotropy can be achieved by shifting the activation curve of the ion channels to more positive voltages. Such a process is used by cAMP and cGMP to increase HCN channel activity. Cyclic nucleotides binds to HCN channels. HCN channels are activated during membrane hyperpolarization after the termination of an action potential and yield Na^+ influx, which slowly depolarizes the sarcolemma. There are several types of voltage-gated cation channels HCN (HCN1-HCN4). HCN4 is the predominant type in the sinoatrial node. Sympathetic stimulation of the sinoatrial node raises cAMP concentrations, hence accelerating depolarization and the cardiac frequency. Stimulation of muscarinic receptors slows the heart rate by inverse action.

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cardiac cells. Other isoforms are expressed in the heart to a lesser extent. The fine tuning in ACase regulation controls the adrenergic signal transmission rate. Elevated intracellular calcium levels during sustained activity inhibit adenylyl cyclases ACase5 and ACase6.

[81] PDE1 activity depends on the calcium–calmodulin complex, the quantity of which augments concomitantly with elevation in cytoplasmic calcium level. PDE2 is stimulated by cAMP. PDE3 is inhibited by cAMP. PDE4 is insensitive to cAMP.

8

Vessel Wall

As blood pulses in an artery, its wall alternatively stretches and recoils. S. Hales described in 1733 blood systolic storage and diastolic restitution. O. Frank in 1899 modeled arterial capacitance by a windkessel element, i.e., a damping air chamber incorporated in a rigid pipe network. Blood vessels are pressure and volume buffers. Veins, which are the major blood storage compartments, accommodate volume changes by shrinking to possible collapse.

Wall expansion and relaxation depend on the rheological properties of the vessel wall, and thus to its composition and structure. In particular, distension and subsequent recoil are achieved by the architecture and interactions between coupled elastin and collagen fibers. In addition to passive caliber changes, the vessel wall undergoes active variations due to vasomotor tone. Vascular smooth muscle activity fits the blood flow to the tissue requirements.

8.1 Wall Structure

Structure components exist in every kind of blood vessel, except capillaries, although the element amount and structure vary between vessel types. Endothelial cells, of shear-dependent shape (50–100 × 10 × 0.5–2 μm), line the blood–wall interface of the whole vasculature (Chap. 9). Specific cells of connective tissues (Sect. 2.4) are *fibroblasts* (FB), which produce ground matrix and fibers in growing tissues, and *fibrocytes* (FC). The ground matrix fills the space between cells and fibers. It is composed of interstitial fluid, which contains electrolytes and plasma molecules, proteoglycans, and glycoproteins. *Elastin* forms low-stiffness elastic fibers, taut in the unstretched configuration, which can stretch up to 60% with elastic behavior. *Collagen* makes high-stiffness fibers that are tortuous in the unstretched configuration and are responsible for structural integrity. Thereby, elastin provides vessel distensibility and collagen tensile strength.[1] *Smooth muscle cells* are

[1] Wall distensibility increases when tension rises. Elastin is involved at low stresses and collagen is progressively recruited when tension increases.

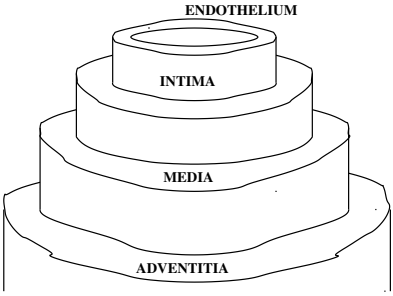

Figure 8.1. Wall tunicae. The media (intermediate layer) is bounded by the elasticae interna and externa from the tunicae intima (inner layer) and adventitia (outer layer).

responsible for vessel tone and, thus for lumen size (Sect. 8.2). They affect vessel impedance.

The wall structure is characterized by a tunica set (Fig. 8.1). Tunica number and structure vary according to vessel type and size.

8.1.1 Large Vessels

The wall of large blood vessels has a circumferentially layered structure. The internal thin *intima* is composed of the inner *endothelium* and a subendothelial connective tissue tunica. The endothelium is made of a monolayer of endothelial cells supported at its abluminal surface by a basal lamina. The endothelium modulates wall structure and functions. The *internal elastic lamina* (IEL) delimits the intima from the media. The fenestrated internal elastic lamina is characterized by a quasi-uniform distribution of pores. Water and solutes are transported through these fenestrae. Cells communicate and migrate via these pores. The number of fenestrations is greater at the branching point.

The middle muscular *media* is formed by layers of circumferential smooth muscle cells (SMC) and connective tissue with fibers. The *external elastic lamina* (EEL) is located between the media and adventitia. Action potentials trigger SMC contraction after a delay ranging from 80 to 100 ms that lasts from 10 to 15 s [805].

The external *adventitia* consists mainly of connective tissue with some smooth muscle cells. In the large vessels, the adventitia contains nerves, vessels (*vasa vasorum*) and possibly lymphatic vessels.

The media is the main site of histological specializations of artery walls (Table 8.1). Vessels proximal to the heart are *elastic arteries*. Wall distension during pressure wave propagation for efficient windkessel effect is facilitated by thin concentric fenestrated lamellae (lamella: diminutive of lamina, or lamna: thin plate, membrane, layer) of elastin in a thick media, between which are

smooth muscle cells and collagen fibers (Table 8.2). The lamella number depends on vessel size, between-lamella space being nearly constant. Inside the elastic lamella, elastic fibers have a helical orientation.[2] The external elastic

Table 8.1. Artery wall structure. Downstream from the heart, the arterial tree successively comprises elastic, transition, and muscular arteries, and arterioles. The different artery types act as conducting, distributing, and resistive arteries with increasing distance from the heart. During the systole, elastic arteries store a part of the blood volume ejected by the left ventricle for diastolic restitution owing to wall recoil. Peripheral muscular arteries and arterioles locally control the lumen size and the vessel resistance.

Layer	Elastic artery	Muscular artery	Arteriole artery	Cerebral artery
Caliber	Great	Mid	Small	Mid
Intima			Absence of subendothelium	
IEL	Thin elastic lamella	Important, scalloped	Thin Thin	Well developed
Media	Thick Numerous elastic lamellae	Thin Numerous layers of SMCs	1–5 layers of SMCs	Almost no EnFs no EnFs
EEL	Badly defined	Clearly defined	Badly defined	Absent
Adventitia	Thin	Thick		

Table 8.2. Wall structure.

Wall layer	Components
Endothelium	EC
Basement membrane	Ln, Cn4,
Subendothelium	Cn3,-4,-5, PoG, GP (Ln, FN, vWF, TSP), FB, ±SMC
IEL	En
Media	En, Cn, PoG, FB, SMC
EEL	En
Adventitia	FB, En, Cn
	Vasa vasorum, nerve endings, lymphatics

[2] The orientation of the elastin fibers in a given rabbit artery slice is parallel to the local artery axis or nearly perpendicular to it, whether the elastin fibers belong to the fenestrated internal elastic lamina or medial elastic bundles (P. Farand, personnal communication). Therefore, elastin fibers of the internal elastic lamina and media sustain longitudinal and circumferential loadings, respectively. Bridges exist between axially and circumferentially oriented elastin fibers.

lamina is not very well defined and the adventitia is thinner. The orientation and extension of the collagen fibers of the adventitia of human aortas that undergo uniaxial tension are correlated to the applied stress [806]. After straightening and suitable orientation, collagen fibers can bear increasing loads.

Muscular arteries have thinner intima. The media is characterized by numerous concentric layers of smooth muscle cells with elastic fibers and few collagen fibers. The external elastic lamina can be clearly observed. The wall content in elastin and collagen varies according to the artery type, elastic, transitional, or muscular (Table 8.3). The fibers have a helical orientation with a variable pitch angle from the inner to the outer vessel wall. Besides, cerebral arteries are thin walled without external elastic lamina.

Vein walls are thinner than artery walls, whereas the bore is larger (Table 8.4). The intima is very thin. Internal and external elastic laminae are either absent or very thin. The media is thinner than the adventitia. Medium-sized veins are characterized by the presence of *valves* to prevent transient blood return to upstream segments during muscular compression (Figs. 8.2 and 8.3). The largest veins of the abdomen and thorax have very thick adventitia, which contains bundles of longitudinal SMCs and vasa vasorum. Valves are absent. The main features of the wall are given for different kinds of veins, sorted by their bore, in Table 8.5.

Table 8.3. Wall composition in elastin and collagen (dog, % dry defatted tissue) in different segments of proximal arteries and elastic modulus (kPa) range given in biomechanics textbooks (Sources: [190, 807]).

	Collagen	Elastin
Ascending aorta	15–20	40–50
Thoracic aorta	15–30	20–40
Abdominal aorta	25–35	15–20
Femoral artery	35–45	15–25
Elastic modulus	10^2–10^6	10^2–10^3

Table 8.4. Comparison between a vein and its associated artery walls.

	Artery	Vein
Shape	Circular	Elliptical
Wall	Thick	Thin
Intima		Very thin
Media	Prominent	Weak
IEL		Quasi-absent

Table 8.5. Vein wall structure.

Vein type	Caliber	Wall
Microvenule	15–100 µm	Endothelium, thin layer with CnFs, ±FBs, SMCs if caliber > 50 µm
Minivenule	100–300 µm	Continuous layer of SMCs, ±postcapillary sphincter
Venule	300–500 µm	Media, thin adventitia (EnFs, CnFs, FBs)
Drainage vein	0.5–2 mm	Intima with subendothelium, ±EnF network, adventitia
Collecting vein	2–9 mm	Intima sometimes bounded by EnF network, media poorly developed, except in inferior limbs, thick adventita, valves in zones of greater caliber
Central vein	20–30 mm	Adventitia of vena cava with CMCs in the heart vicinity

Different gene expression occurs between arterial and venous walls, exposed to different pressures. Plakoglobin, galectin-7, sciellin, and SPRR3 are expressed in arteries but not veins [808].

Venous Valves

Perforating veins usually contain venous valves that prevent reflux of blood from the deep veins into the superficial veins (*ostial valve*). Although venous drainage in the human head and neck commonly does not counter gravity, venous valves are also found throughout veins of these body parts [809]. *Vieussens valve* is the ostial valve of the great cardiac vein located near the coronary sinus, which is provided at its ostium by the *Thebesian valve* [810].

Venous valves most often are bicuspid and located in stiffer expanded segments (Figs. 8.2 and 8.4). Venous valves are made of connective tissue with elastic fibers and few smooth muscle cells, covered by the endothelium. Veins have usually elliptical cross-sections. Valve leaflets are inserted on each face of the vein wall,[3] whereas vein edges receive perforating and tributary

[3] Valvular free borders of the superficial veins of inferior limbs are parallel to the skin surface.

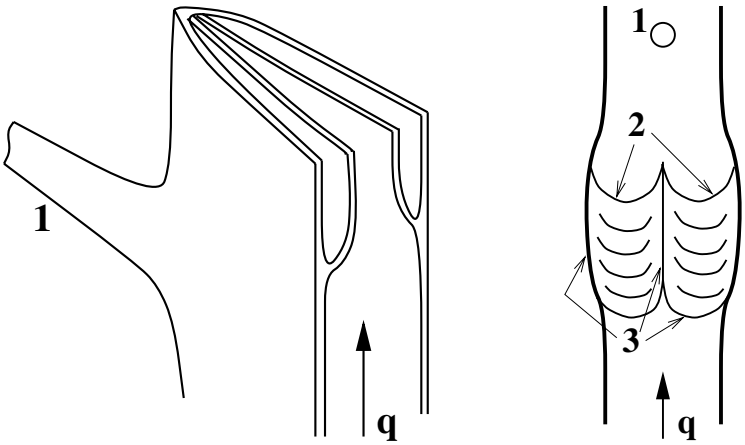

Figure 8.2. Bicuspid venous valves. (**Left**) cross and axial cut of a vein (1: orifice of communication vein at the vein edge; 2: cusp free border in front of the vein face; 3: valve insertion lines). (**Right**) open and spread vein after axial incision of a wall edge. The valvular sinuses are behind the cusps. The luminal face is smooth; the parietal face is rough.

veins [814]. Endothelial cell density is significantly higher on the surface of valve leaflets (about 10 cells per 1000 μm^2) than on other venous surfaces (about 7 cells/1000 μm^2) [815]. Endothelial cells on the medial surface and on the lateral surface of the valve leaflet are oriented parallel to the vein axis of the vessel and perpendicular to it, respectively.

8.1.2 Small Vessels

Arterioles are composed of an endothelium surrounded by one or few concentric layers of smooth muscle cells that regulate blood flow. Arterioles receive both sympathetic and parasympathetic innervation. Capillaries[4] are small exchange vessels composed of endothelium surrounded by a basement membrane with three structural types. *Continuous capillaries*, found in muscle, skin, lung, and central nervous system are defined by a continuous basement membrane and tight intercellular clefts and have the lowest permeability. *Fenestrated capillaries*, found in exocrine glands, renal glomeruli, and intestinal mucosa, are characterized by perforations in endothelium and, thus, by relatively high permeability. *Discontinuous capillaries*, found in liver, spleen, and bone marrow, are defined by large intercellular and basement membrane gaps

[4] M. Malphighi, in the second half of the seventh century, identified the capillaries that close the blood circulation loop between arteries and veins, demonstrated earlier by W. Harvey. About at the same time, C. Aselius discovered the lymphatic vessels.

and, consequently, very high permeability. *Venules* are composed of endothelium surrounded by basement membrane for the postcapillary venules and smooth muscle for the larger venules (Table 8.5).

8.1.3 Blood–Brain Barrier

The average distance from capillary to neuron ranges from 8 to 20 µm. The microvasculature of the central nervous system (CNS) is aimed, among usual goals, at maintaining the homeostasis of the internal medium. The blood–brain barrier (BBB) is the specialized endothelium of cerebral capillaries that avoids any fluctuation in substance concentration in the extracellular space (ECS), especially ions, whereas blood supplies the brain with the required nutrients (Fig. 8.5). Unlike peripheral capillaries, which allow substance filtration across and between endothelial cells, BBB strictly limits transport into the brain through tight junctions[5] (Sect. 2.3) and membrane carriers.

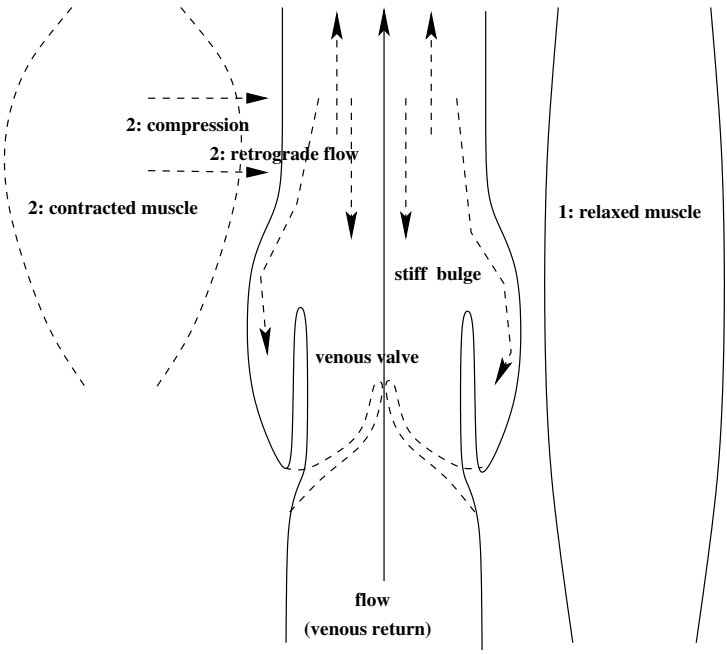

Figure 8.3. Venous valves are fully open when neighboring muscles are relaxed. Adjoining muscle contraction compresses much more avalvular segments of veins than valvar parts, which are slighly dilated and stiffer. Consequently, venous return is favored, but is associated with retrograde flow. The latter is impeded by valve closure due to blood stream between the vein wall and the valve.

[5] The intercellular cleft width is equal to about 20 nm. The zonula occludens (tight junction) are characterized by completely occluded areas between external

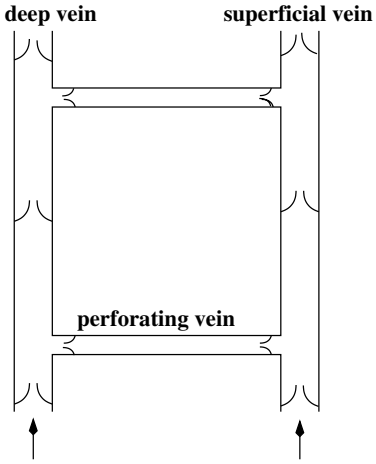

Figure 8.4. Limb valved vein network, with a superficial vein and a deep vein anastomosed by a communicating vein equipped with ostial valves for one-way circulation from superficial to deep vein.

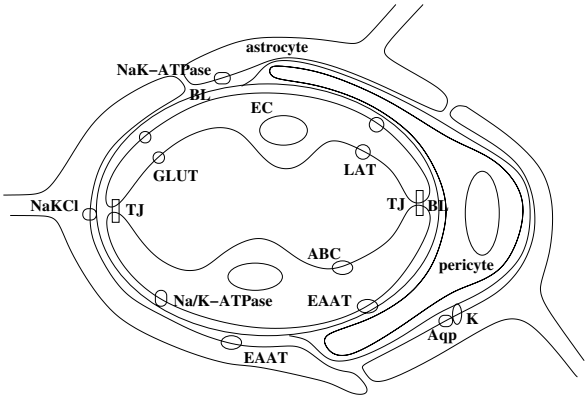

Figure 8.5. Schematic drawing of the blood–brain barrier, with endothelial cells (EC) of the cerebral capillary, surrounded by basal lamina (BL), perivascular extensions of astrocytes and pericytes. Endothelial cells are joined by tight junctions (TJ). The endothelium contains carriers for glucose (GLUT), amino acids (LAT), glutamate (EAAT), ions (Na/K-ATPase), and ABC transporters. Astrocyte extensions facing the capillary wall have patches with aquaporins Aqp4 and K^+ channels (K). They contain also Na/K-ATPase, Na-K-Cl cotransporters and EAATs (EAAT: excitatory amino acid transporters; GLUT: glucose transporter; LAT: L-system for large neutral amino acids; Source: [821]).

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membrane sheets of adjoining cells. External layer of adjacent cell surfaces fuse. Cerebral capillary endothelial cells contain tight junctions, that seal cell-to-cell contacts between adjacent endothelial cells. The tight junctions between cerebral endothelial cells lead to high endothelium electrical resistance.

Small gaseous molecules such as O_2 and CO_2 can diffuse freely through the lipid membranes, as well as small lipophilic agents. Specific carriers on the luminal and abluminal membranes regulate transcellular transport. The endothelium contains carriers for glucose, amino acids (such as bidirectional L-systems for large neutral amino acids LAT1), purine bases, nucleosides, choline, and other substances. Efflux carriers are energy dependent.

The brain endothelium has a much lower capacity of endocytosis/transcytosis than that of peripheral endothelia. Furthermore, intracellular and extracellular enzymes yield a metabolic barrier. Ecto-enzymes, such as peptidases and nucleotidases, are able to metabolize peptides and ATP, respectively. Intracellular enzymes such as monoamine oxidase and cytochrome P450 can inactivate many neuroactive and toxic compounds. Renin, angiotensin converting enzyme, and aminopeptidase-A acting on angiotensin belong to degrading enzymes of the BBB. Certain substances that are not carried by the endothelium, are metabolized in endothelial cells such that they cannot enter into the brain. The cerebral endothelium has enzymes that mainly target neuroactive substances, found in low concentration or absent in other capillaries.

The luminal and abluminal membranes of brain capillaries are biochemically and functionally different. Asymmetrical distribution of carriers is observed between luminal and abluminal membrane parts, due to either amount difference or absence (transport polarity). Whereas alkaline phosphatase activity is located in both the luminal and antiluminal cytoplasmic membranes of the brain capillary endothelial cells, the K^+-dependent phosphatase activity associated with Na^+/K^+-ATPase is only found in the abluminal membrane [816]. The main glucose carrier isoform GLUT1 at the BBB, which is bidirectional, has a 1:4 ratio of luminal/abluminal distribution [817]. Na^+-dependent glutamate[6] carriers such as excitatory amino acid transporters EAAT1–EAAT3, which allow glutamate efflux against concentration gradient, are mostly abluminal.

The cerebral capillary wall is thinner than that of the muscle capillary [818]. The wall thickness could modulate the restrictive permeability of the BBB with a shorter transendothelium transport time. There might be a greater mitochondrion number in BBB endothelial cells than in other endothelial cells, in relation to energy-dependent transcapillary transport [819].

Continuous capillaries, with transport systems for nutrients and wastes, are then caracterized by: (1) between-EC tight junctions, which occlude the intercellular clefts more effectively than in other endothelia;[7] (2) absence of

[6] Glutamate is a cerebral excitatory transmitter. Astrocytes recycle glutamate. Glutamate influx particularly via excitatory amino acid transporters-1 and -2 is followed by conversion into glutamine to be taken by adjoining neurons.

[7] The brain tight junctions restrict even the movement of small ions such as Na^+ and Cl^-.

interstice, except in the hypothalamic-pituitary axis;[8] and (3) astrocyte extensions[9] that surround the capillary endothelium. Therefore, transit across the BBB involves transport successively through the endothelium wetted membrane, cytoplasm, antiluminal membrane, basal lamina and astrocyte extension. Astrocytes provide the cellular link to the neurons.

Biochemical bridges exist between the endothelium and astrocytes. The basal laminae of cerebral capillaries contain *agrin*, a heparin sulfate. Agrin binds to *α-dystroglycan*, a member of the dystrophin–dystroglycan complex, which links to aquaporin-4 of the astrocyte extension via *α-syntrophin*, another member of the dystrophin–dystroglycan complex. α-Syntrophin also binds to the Kir4.1 K^+ channel of the astrocyte extension.

The endothelium forms functional neurovascular units with astrocytes and neurons characterized by strongly regulated blood–brain barrier functioning. The distance between any neuron and brain capillary usually is lower than 20 μm.[10] The brain endothelium supplies the brain with essential nutrients and mediates efflux of waste products. Specific ion carriers regulate ionic motions to produce appropriate brain interstitial fluid, avoiding damaging fluctuations in ionic composition, and then disturbances in axonal signaling.

Three main cell layers with tight junctions provide a barrier: (1) the brain endothelium (BBB), (2) the arachnoid epithelium, which forms the middle layer of the meninges, and (3) the choroid plexus epithelium. *Choroid plexi* form an active barrier between blood and *cerebrospinal fluid*[11](CSF). They secrete CSF and ensure CSF chemical stability. They are located at the surface of the lateral, third, and fourth ventricles. They are made of two cell layers, which are bound by tight junctions: a capillary endothelium and a choroid epithelium, which are separated by the brain interstitial fluid. Epithelial cells produce 90% of CSF (~ 0.4 ml/mn/g) [820]. CSF is composed of 99% of water,

[8] The hypothalamus, above the pituitary gland, or hypophysis, is located in the middle of the base of the brain, encapsulating the ventral portion of the third ventricle. The hypothalamus controls many body functions. The anterior pituitary (adenohypophysis) secretes protein hormones, under control by hypothalamic hormones. The posterior pituitary (neurohypophysis) is an extension of the hypothalamus. They release several hormones controlling endocrin gland function.

[9] Astrocyte extensions facing the capillary wall have peculiar features, such as high density of patches with aquaporin-4 and Kir4.1 K^+ channel, which are involved in ion and volume regulation.

[10] The distance between a neuron and cerebrospinal fluid is on order 1 millimeter or even centimeter.

[11] The cerebrospinal fluid is located within the head ventricles, spinal canal and subarachnoid spaces. CSF volume ranges from 140 to 150 ml, with 30 to 40 ml in the ventricles. Hence, CSF is mainly in the subarachnoid space, basal cisterns, and around the spinal cord. The production rate is equal to about 21 ml/hour and the turnover rate of total CSF is smaller than 7 hours. CSF cushions the brain, regulates brain extracellular fluid, distributes neuroactive substances, and collects brain wastes.

of vitamins, of ions, of glucose, of amino acids, etc. The choroid plexi also have a CSF purification role.

Claudins-3, -5, and possibly -12 contribute to the high transport resistance of the brain tight junctions. Junctional adhesion molecules JAM-A, JAM-B, and JAM-C maintain tight junctions. Adapters ZO-1, ZO-2, and ZO-3, Ca^{++}-dependent serine protein kinase, membrane-associated guanylate kinase MAGI1, MAGI2, and MAGI3, multi-PDZ-protein-1, partitioning defective proteins PAR3 and PAR6 participate in the cytoplasmic plaques associated with tight junctions. The cytoplasmic plaque is associated with another proteic complex of regulatory and signaling molecules, small GTPases, regulators of G-protein signaling RGS5, ZO-1-associated nucleic acid-binding protein, junction-associated coiled-coil proteins, etc.

Astrocytes, pericytes, perivascular macrophages, and neurons regulate BBB features, such as tight junction properties, endothelium polarity, and enzymatic activity. Astrocyte-derived factors, such as transforming growth factor-β, glial-derived neurotrophic factor, basic fibroblast growth factor, and angiopoetin-1 can act on endothelial receptors [821]. Conversely the endothelium acts on the growth and differentiation of perivascular cells, particularly via endothelium-derived leukemia inhibitory factor.

Cell interactions occur not only for development but also for functioning modulation. Astrocytes, after sensing neuronal cues, can send signals to the capillaries to supply nutrients, using inositol(1,4,5)trisphosphate, glutamate, and ATP through gap junctions or via receptor stimulation. Moreover, water and ions are redistributed between the components of the functional neurovascular units.

Active neurons release neurotransmitters and K^+ and take up Na^+. They generate water during glucose metabolism. Neurotransmitters and ions are mostly recycled. The perivascular space acts as a K^+ space buffer for recycling, as the astroglial plasmalemma has a high density of K^+ channels[12] on its perivascular extension and the cerebral endothelium has a low K^+ and water permeability. Excess water must be removed from the perivascular spaces, using lymph and CSF.

Blood flow is locally increased in active cerebral regions. Neural activity consumes energy (functional hyperemia). Neural activity cues are transmitted to the vasculature by astrocytes. During neuronal activity, glutamate released by the neuron activates glutamate receptors of the astrocyte plasmalemma and triggers a calcium wave. Calcium ions reach the vascular extension of the astrocyte, where it stimulates phospholipase-A2 for arachidonic acid production. Arachidonic acid is then metabolized by cyclooxygenase COx1[13] into a prostaglandin vasodilator PGE2 (Fig. 8.6) [822]. COx1,

[12] Astrocyte extensions particularly have inwardly rectifying Kir4.1 channels, Na^+-K^+-ATPase, and Na^+-K^+-Cl^- cotransporters.

[13] COx1 is constitutively expressed by astrocytes, whereas COx2 is expressed in response to inflammatory stimuli.

which is highly expressed in astrocyte vascular endfeet, is the main mediator of astrocyte-induced vasodilation. Moreover, Ca^{++} stimulates production of inositol trisphosphates, which cross gap junctions and trigger calcium motions and prostaglandin synthesis in neighboring astrocyte extensions, that ensheath the vessel wall.

The blood–brain barrier prevents the delivery of most therapeutic molecules to their targets within the brain parenchyma, such as neurons and glial cells. A solution for large molecules consists of triggering transcytosis via cell receptors of the blood–brain barrier (low-density lipoprotein receptor, transferrin receptor, insulin-like growth factor receptor) that bind ligands to facilitate their transport to the central nervous system by adding the receptor-binding domain of the targeted ligand (so-called molecular Trojan horses) [823]. A non-replicating lentivirus vector system is used to deliver a substance to neurons and astrocytes by fusing it to the binding domain of ApoB for low-density lipoprotein receptor and adding a secretory sequence to allow its release. Such a method provides a source (from either a central or peripheral organ, such as liver, spleen, or muscle, in the cells of which genes are introduced) for prolonged production and release in the blood flow of a BBB-crossing drug, but fusion proteins must be immunocompatible.

8.1.4 Lymphatic System

The circulatory system has a specialized compartment conveying the *lymph* in lymphatic vessels. The lymphatic system maintains the fluid balance and is involved in immunity. Lymph vessels collect excess tissue fluid and transport lymph into the veins, returning it to the blood. Unlike blood circulation,

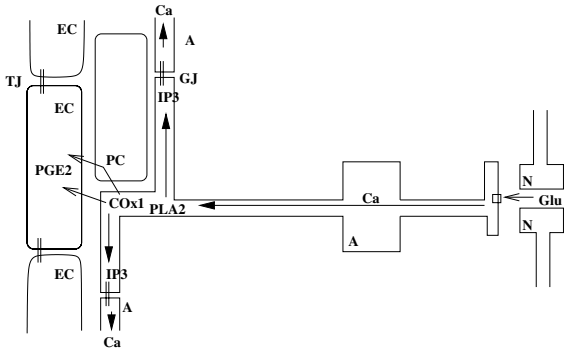

Figure 8.6. Intercations among neurons (N), astrocytes (A) and vascular cells, pericytes (PC) and endothelial cells (EC) for functional hyperemia (TJ: tight junction, GJ: gap junction; adapted from [822]). With their extensions to both the vasculature and the nerves, astrocytes are anatomical links between the circulatory and nervous systems. Induced vasodilation involves cyclooxygenase COx1, and inositol trisphosphate (IP3).

the collecting lymphatic system is an open circuit (lymphatic capillaries have a closed upstream end). The lymph circulation is affected by breathing and skeletal muscle activity. Lymphatics exist in all tissues except the bone, cartilage, central nervous system, and certain vessel walls. Lymph flow can cease during periods of complete physical inactivity.

8.1.4.1 Lymphatics

Lymphatic capillaries are thin walled, composed of endothelium[14] with intercellular gaps. The surrounding basement membrane is either small and permeable or absent. The endothelium of terminal lymphatics lacks a continuous basement membrane and tight junctions. Lymphatic capillaries are similar in size to venules, without a sheath of pericytes or smooth muscle cells. Larger collecting lymphatic vessels have smooth muscle cells and are similar to veins with thinner walls. The wall is surrounded by a basement membrane. Lymphatic vessels have numerous semilunar valves, every few millimeter, which prevent back and forth lymph motion. Lymph from the upper right quarter of the body drains into the right lymphatic duct, and subsequently into the right subclavian vein, otherwise into the thoracic duct, and then into the left subclavian vein. Additional lymphatico-venous anastomoses occur in renal, hepatic, and adrenal veins, as well as lymph nodes.

8.1.4.2 Lymphoid Tissues

Lymphatic vessels are associated with lymph nodes, oval-shaped filters enclosed by a fibrous capsule, generally organized into clusters. Lymph nodes not only filter the lymph and phagocytose toxins and microorganisms, but also are maturation sites for some lymphocytes and monocytes. Lymphatic vessels process the lymph in lymph nodes via dendritic cells.

There are several types of lymphoid tissues [824]. Genetically preprogrammed anatomically distinct lymphoid organs include lymph nodes and spleen. Prepatterned environment-dependent mucosal-associated lymphoid tissues are represented by Peyer patches, tonsils, adenoids, nasal-associated lymphoid tissue, and bronchial-associated lymphoid tissue. These two groups of secondary lymphoid organs trap and concentrate antigens at many loci throughout the body to initiate an adaptive immune response. The spleen detects blood-borne pathogens. T-cell maturation occurs in the thymus.

Lymphoid neo(organo)genesis is defined by cellular assemblies during chronic inflammation in any region of the human body. These tertiary lymphoid organs have structural similarity to lymph nodes. Hence they are able to carry out angiogenesis and lymphangiogenesis.

[14] Lymphatic vessel hyaluronan receptor-1 LYVE1 is a marker for lymphatic endothelial cells. Plasmalemmal podoplanin is also assigned to lymphatic endothelial cells.

Lymph nodes are surrounded by a fibrous capsule and an underlying subcapsular sinus. The inner region of lymph nodes can be decomposed into three parts: cortex, paracortex and medulla. The cortex contains primary follicles, clusters of B lymphocytes and follicular dendritic cells. After antigen stimulation, B cells undergo intense proliferation and give rise to secondary follicles, the so-called germinal centers. The paracortex is composed of T lymphocytes and dendritic cells. The medulla consists of medullary cords separated by lymph-filled medullary sinuses. Lymph nodes have lymphatic vessels and venules. Afferent lymphatics enter the lymph node at several sites to fill the subcapsular sinus. T and B cells circulate inside the lymph node via venules. They can cross the endothelium and enter the lymph node. Afterward, they exit via efferent lymphatics.

Follicular dendritic cells and dendritic cells are antigen presenting. Dendritic cells present antigens in the context of major histocompatibility complex classes I and II to stimulate T cells. Follicular dendritic cells target B cells with antigen–antibody complexes.

The recruitment of lymphocytes and dendritic cells in the lymph node is regulated by lymphoid chemokines. The lymphoid chemokine set is composed of four ligands, CCL19, CCL21,[15] CXCL12, and CXCL13[16], and two receptors, CCR7 and CXCR5.

8.1.4.3 Lymph

The lymph has a composition close to that of the plasma. The lymph-to-plasma protein concentration ratio is equal to about 0.75. The compositions in lipid (free cholesterol, cholesterol ester, triacylglycerol, and phospholipid) of plasma and lymph lipoproteins either are similar or differ according to lipoprotein types. The protein-rich lymph contains cells such as extravasated leukocytes and activated antigen-presenting cells. In the small intestine, lymphatic vessels of the intestinal villi absorb the dietary fat released by enterocytes, which form chylomicrons.

8.1.5 Perivascular Nerves

Dual regulation of vascular tone is done by perivascular nerves and endothelial cells. Endothelial cells can yield both vasodilatation and vasoconstriction. The endothelial cells indeed produce, store, and release vasoactive substances, either vasodilators, such as nitric oxide and acetylcholine, or vasoconstrictors, such as endothelin (Sect. 9.5). Perivascular nerves and endothelial cells interact.

[15] CCL19, CCL21, and CCR7 are expressed by stromal cells of T-cell region. CCL21 is expressed by the endothelium of venules of the lymph node, as well as of lymphatics of non-lymphoid tissues.

[16] CXCL13 is expressed by follicular stromal cells.

Perivascular nerves are not limited to adrenergic or cholinergic axons. Four types of perivascular nerves exist: sympathetic, parasympathetic, sensory-motor, and projection neurons from intrinsic ganglia of the heart and other organs [825]. Cotransmission usually occurs. Sympathetic nerves use the following transmitters: noradrenaline, adenosine triphosphate, and neuropeptide-Y (NPY). The parasympathetic signals via acetylcholine and vasoactive intestinal peptide (VIP). Messages of sensory-motor nerves[17] are coded by calcitonin gene-related peptide (CGRP), substance P, and ATP. Neurons from organ ganglia release many types of neurotransmitters, including peptides,[18] purines (purinergic nerves), nitric oxide (nitrergic transmission), and classical transmitters.

Artemin (Artn), which is expressed in vascular smooth muscle cells, serves as a guidance factor for the growth of sympathetic nerve fibers along blood vessels [826]. Artemin is a member of the glial cell line-derived neurotrophic factor (GDNF) family of ligands.[19] These ligands signal via a receptor complex composed of Ret tyrosine kinase and a co-receptor, which is a glycosylphosphatidyl-inositol-anchored protein of the GDNF family receptor-α.

8.2 Vascular Smooth Muscle Cell

Several subpopulations of smooth muscle cells (length 20–60 µm, center width ~ 4 µm) can be described in the arteries, such as spindle-shaped smooth muscle cells (sSMC) and rhomboid SMCs (rSMC). The SMC morphology can be modulated by growth factors and environmental stimuli [827]. A large proportion of rSMCs is observed after stent implantation, with high proliferative and migratory activities (Part II).

The vascular smooth muscle cells include phasic and tonic types. The tonic variety does not generate action potentials, whereas the phasic kind, in the portal system and lymphatics, induces contraction waves to propel the luminal fluid contents toward the heart.

Myocardin-related transcription factors, coactivators of the transcription factor serum response factor, are required in vascular smooth muscle as well as in cardiomyocyte differentiation. The serum response factor regulates genes involved in cell proliferation, migration, cytoskeletal dynamics, and myogenesis [828].

The vascular smooth muscle cell carries out slow and sustained contractions, whereas CMC contractions are rapid and of short duration ($\mathcal{O}$[100 ms]). Contractile proteins are involved in the vasomotor tone of blood vessels. The ratio between actin and myosin (15/1) is greater in smooth muscle cells than

[17] Certain afferent nerves have an efferent function and axonic reflex control of vascular tone.

[18] Peptidergic nerves contain substance P, CGRP, and VIP.

[19] The glial cell line-derived neurotrophic factor family ligands include the glial cell line-derived neurotrophic factor, neurturin, persephin, and artemin.

striated muscle cells (7/1).[20] α-Smooth muscle (αsm) actin, localized in microfilament bundles, is typical of smooth muscle cells. Myogenic activity has been demonstrated in arteries, arterioles, venules, veins, and lymphatics. Actin and myosin in smooth muscle cells are not arranged into distinct bands, but organized for maintainance of tonic contractions. Vascular smooth muscle contraction can be initiated by mechanical, electrical, and chemical[21] stimuli, in particular SMC stretching causes contraction (myogenic response). Manifold isoforms of actin [829] and myosin light and heavy chains [830] exist. The difference in isoforms of contractile proteins between small and large arteries may induce different characteristics. SMC contraction speed is very low, but it can achieve a much greater degree of shortening.

SMC activity is regulated by cytosolic $[Ca^{++}]_i$ due to: (1) membrane depolarization and Ca^{++} entry via L- and T-types of voltage-gated Ca^{++} channels (VDCC, Fig. 8.7); (2) to Ca^{++} release from endoplasmic reticulum[22] (Ca_i^{++} stores); and (3) to receptor-dependent Ca^{++} channels (Table 8.6). Calcium motions from the intra- to the extracellular space use pumps and exchangers. Smooth muscle cells often have a graded response to membrane depolarization. The activity of ion pumps and exchangers on the SMC membrane is modulated by several factors, such as vasoactive substances and growth factors (Fig. 8.8). Moreover, gap junctions between smooth muscle cells are required.

The *myosin* regulatory system is more important in smooth muscles than striated muscles. *Myosin light chain* (MLC) phosphorylation state, the biochemical determinant of contraction, is tightly controlled by the relative activities of the counter-regulatory enzymes myosin light chain kinase (MLCK) and myosin phosphatase (Fig. 8.7). *Myosin light chain kinase*, activated by calcium and calmodulin, increases myosin calcium sensitivity and regulates actin–myosin interactions. MLC phosphorylation leads to cross-bridge formation between the myosin heads and actin filaments, and hence to smooth muscle contraction. Dephosphorylation of myosin light chains by protein kinase-C leads to smooth muscle relaxation. The *cGMP-dependent protein kinase-1α* (cGK), the principal effector that mediates SMC relaxation in response to nitrovasodilators, binds directly to the myosin binding subunit (MBS) of the myosin phosphatase. The disruption of the cGK-MBS interaction prevents NO–cGMP from myosin phosphatase activation [833]. The *RhoA/Rho kinase* pathway inhibits the myosin phosphatase. GPCR stimulation generates smooth muscle cell contraction via Ca^{++}-calmodulin-dependent activation of MLCK and subsequent myosin phosphorylation. Rho kinase also regulates MLC phosphorylation. *Myosin phosphatase–Rho interacting*

[20] This ratio is very large in non-muscle cells.

[21] Noradrenaline, angiotensin-2, vasopressin, endothelin-1, and thromboxane-A2 cause SMC contraction.

[22] Like CMCs, smooth muscle cells have Ca^{++} influx from ECF and mainly from endoplasmic reticulum with its ryanodin channels and Ca^{++} resequestration from SERCA.

proteins are found with cGK and myosin phosphatase and colocalize with myofibrils in smooth muscle cells.

The SMC contraction is regulated not only by the phosphorylation of the myosin light chain by Ca^{++}–calmodulin-dependent MLCK, but also by caldesmon–calmodulin complex, PKC, and calponin. *Caldesmon* (Cdm)

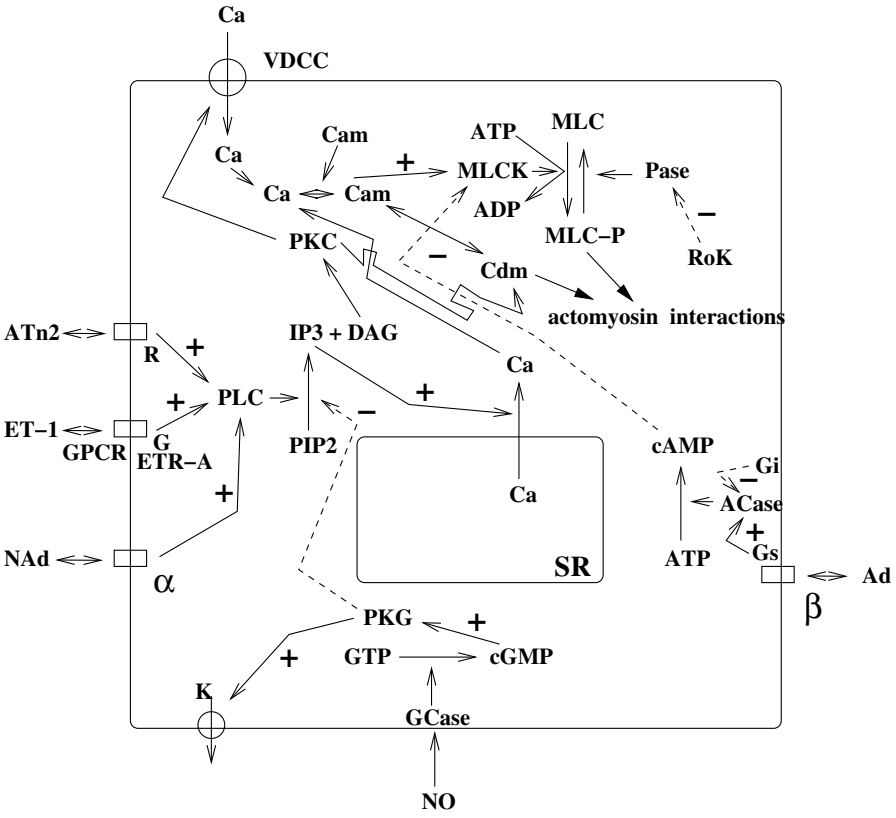

Figure 8.7. The smooth muscle cell function and its regulation (adapted from [831]). Two pathways are involved: (1) the phosphatidylinositol pathway, and (2) the Gs-protein pathway. A ligand activates a GPCR, which stimulates phospholipase-C (PLC), producing inositol trisphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol (PIP2). IP3 releases calcium from the sarcoplasmic reticulum (SR) and DAG activates protein kinase-C (PKC). PKC acts on the contractile proteins and ion channels. Calcium and calmodulin (Cam) stimulate a myosin light chain kinase (MLCK), leading to actin–myosin interactions. Noradrenaline (NAd), acting via α1-adrenoceptors, angiotensin-2 (ATn2) via specific receptors and endothelin-1 (ET1) via ETRA receptors, activate phospholipase-C (PLC). Gs-protein, activated by a β2-adrenoceptor agonist, stimulates adenylyl cyclase (ACase), which catalyzes the formation of cAMP. cAMP inhibits MLCK and induces vasodilation.

reversibly binds to F-actin and actin–tropomyosin filaments[23] and blocks the ATPase activity. Caldesmon also is a calmodulin-binding protein. Caldesmon may act on F-actin either by phosphorylation by PKC and Ca^{++} or by forming a caldesmon–calcium–calmodulin complex that is unable to bind to actin. Mitogen-activated protein kinase activity, which is regulated by PKC, in

Table 8.6. Calcium transport in muscle cells. Phosphorylation by cAMP-dependent protein kinase acts for channel opening. PKA also phosphorylates phospholamban to increase SERCA activity.

Transport path	Activity	Influence factor
	Plasmalemma/sarcolemma	
Voltage-dependent channel	Ca^{++} influx	PKC
L-type	Slow inactivation	PKA
T-type	Quick inactivation	
Strech-activated channel	Ca^{++} influx	
Receptor-operated channel	Ca^{++} influx	α_1-Adrenergic receptor
ATPase pump	Ca^{++} efflux	Cam
Na^{+}–Ca^{++} exchanger	Ca^{++} efflux	
	Endoplasmic/sarcoplasmic reticulum	
Ryanodine-sensitive	Ca^{++} influx	
Ca^{++}-sensitive	Ca^{++} influx	
IP3-sensitive channel	Ca^{++} influx	
SERCA	Ca^{++} resequestration	PLb
	Mitochondrion	
Ca^{++} uniporter	Ca^{++} uptake	

Table 8.7. Regulatory proteins of contraction. Caldesmon can be sequestred away from actin by calcium–calmodulin complexes; it is phosphorylated by PKC.

CMC	SMC
Troponin	Caldesmon

[23] Caldesmon is a protein implicated in the regulation of actomyosin interactions in smooth muscle and nonmuscle cells (Table 8.7). Cdm is also known to bind caltropin [834] and tubulin [835].

particular caldesmon phosphorylation, increases under SMC stretching [836]. Moreover, caldesmon enhances the binding of ATP-bound heavy meromyosin (HMM), as well as (but to a lesser extent) the subfragment S1-ATP (in smooth muscle cells, and HMM-ATP in skeletal muscle cells), to actin [837]. This effect is hindered by Ca^{++}–calmodulin. Caldesmon also interacts with myosin in a process regulated by phosphorylation by casein kinase-2 [838].

Calponin, a Ca^{++} and calmodulin-binding protein, hampers actin-activated Mg-ATPase activity of myosin in smooth muscle cells [839]. Calponin binds to tropomyosin, independently of Ca^{++}. Calponin also binds to calmodulin in the presence of Ca^{++}. Calponin phosphorylated by PKC and Ca^{++}-calmodulin-dependent protein kinase-2 is not able to inhibit the actomyosin ATPase.

Vasodilator and vasoconstrictor influences are exerted upon a basal vascular tone. A vasomotor tone is indeed spontaneously developed in most arterioles [840]. In isolated arterioles and arteries, the basal tone is developed for vessel physiological pressure [841]. In addition to the average pressure, pulsatility might be involved in veins [842] and arteries [843].

Electrical coupling in the vessel wall associated with the vasomotor activity can be provided by connexins either between endothelial cells, or smooth muscle cells (homocellular coupling), and possibly endothelial cells and smooth muscle cells (heterocellular coupling). Ca^{++} can cross gap junctions [844]. Such processes can contribute to vasomotor response spreading. Many ion channels (Tables 8.8 and 8.9) and transporters (Table 8.10) do not seems to be modulated by cell tension.

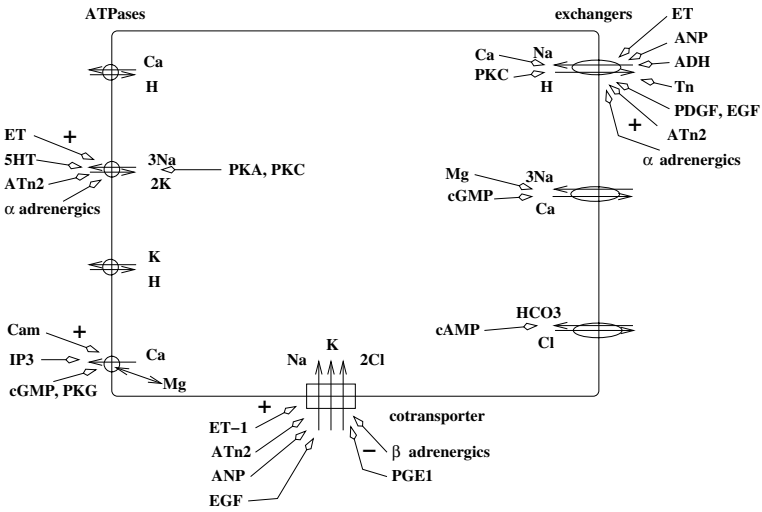

Figure 8.8. Interacting co-carriers of the vascular smooth muscle cell: primary active ion pumps and secondary active carriers (adapted from [832])

The vascular smooth muscle cell undergoes direct and indirect stimuli, essentially from its extracellular matrix, cells of the adjacent layer of the vessel wall, and blood flow. It then produces several types of substances (Table 8.11).

The glycoprotein elastin microfibril interface-located protein-1 (emilin-1) is secreted in the extracellular matrix. Moreover, emilin-1, co-expressed with transforming growth factor-β in vascular smooth muscle cells, is a negative regulator of transforming growth factor-β signaling. Emilin-1, once bound to proTGFβ1 in the extracellular space, indeed prevents the production of active TGFβ1 [849]. Emilin-1 regulate the blood vessel caliber and peripheral resistances, hence the blood pressure. In the absence of emilin-1, the blood vessel bore is reduced. The peripheral resistances and the blood pressure thereby increase.

8.3 Pericytes

The *pericytes*, which surround the capillaries, can also encircle precapillary arterioles and postcapillary venules. These periendothelial cells are rich in stress actin bundles. Differences in pericyte morphology and distribution among vascular bed types can be related to tissue-specific functions (heterogeneous functionality). The pericyte coating (wrapping degree, and pericyte density) depend on the perfused organ. The greater the pericyte density, the stronger the microvascular barrier. Among the types of pericytes, pre-capillary arteriolar, capillary, and post-capillary venular have different features. Pre- and post-capillary pericytes have α-actinin (SMC transitional cells), whereas capillary pericytes do not. Pericytes contain smooth muscle (like SMCs) and

Table 8.8. Ion channels and vasomotor tone with influence factors for involved channels (Sources: [845–848]).

Channel	Effect	Influence factors
	K^+ channels	
Inward rectifier (IRKC)	No significant role	
ATP-sensitive (ASKC)	No significant role	
Novel (NKC)	No significant role	
Voltage-dependent (VDKC)	Repolarization	PKC
Ca^{++}-activated (CAKC)	Repolarization	20 HETE (⊖)
	Cl^- channels	
Selective (Cl^- efflux)	No significant role	
Ca^{++}-activated	No effect	
Volume-gated	No effect	
	Stretch-activated channels (SAC) Non-selective cation channels (Na^+, K^+, Ca^{++})	

nonsmooth muscle (like endothelial cells) isoforms of actin and myosin, the former having higher levels in pre- and post-capillary pericytes, whereas the concentration of the latter is greater in capillary pericytes. A basal lamina

Table 8.9. Main features of certain SMC ion carriers.

	Potassium carriers
Inward rectifier	Mainly in arterioles Opening at resting potential Outward current
Delayed rectifier	Repolarization Slow activation beyond −30 mV Outward rectifying
ATP	Opening by low [ATP], by increase in [ADP], [GDP], and adenosine A1 receptors, H^+ Low basal activity Stimulation by cAMP-PKA
Ca^{++}-gated	Stimulation by cytosolic Ca^{++} and by depolarization High conductance Repolarization NO effector
	Calcium carriers
Voltage-gated	Influx High (L type) or low (T type) conductance Long (L type) or transient (T type) opening abundant in small arteries (L type)
Receptor-operated	Non-selective cation channel Effector of NAd, ATn, vasopressin, 5HT, histamine Linked to α1-adrenoceptor and ATPR
	Chloride carriers
Ca^{++}-gated	Efflux Modulation of basal potential
	Non-selective cation channel
	Permeability to Ca^{++}, Na^+, K^+ Stimulation by DAG Depolarization
	Stretch-sensitive cation channel
	Permeability to Ca^{++}, Na^+ Activation of voltage-gated Ca^{++} channel Depolarization

separates the endothelial cells and pericytes. However, tight and gap junctions can develop between the endothelial cells and pericytes. A basement membrane can also be found along the outer surface of the pericytes.

The pericytes, by their contractile function, can regulate capillary bore, and then the tissue perfusion, as well as transport from the blood via pericytic processes at interendothelial clefts. Furthermore, the pericytes mainly secrete vasoactive autoregulating substances and release structural constituents of the basement membrane and interstitial matrix.

The pericytes produce: (1) ECM structural proteins (collagen, fibronectin, laminin), (2) agents involved in clotting and vasculogenesis, such as tenascin,

Table 8.10. Membrane carriers with regulation factors for mechanosensitive carriers (PKA: cAMP-dependent protein kinase; PKG: cGMP-dependent protein kinase; Sources: [832, 848]).

Type	Regulation
Active transporters	
Na^+–K^+ ATPase	PKC, PKA, $[Na^+]_i$, $[K^+]_e$
Ca^{++} ATPase	Cam, PKG
K^+–H^+ ATPase	
Co-transporters	
Na^+–Cl^-–HCO_3^-	
Na^+–K^+-Cl^-	
Cl^-–HCO_3^-	
Exchangers	
Na^+–H^+	
Na^+–Ca^{++}	PKC, PKG

Table 8.11. Production of the vascular smooth muscle cell.

Contractile proteins	Actin, ABP, ASP, myosin
Adhesion molecules	Cadherin, integrin
ECM components	Elastin, collagens-1 and -3 Proteoglycans (PGE2, PGI2) Glycoproteins (emilin)
Growth factors	PDGF, M-CSF, G-CSF, GM-CSF MCP1 IL1, IL6, TNF

thrombospondin,[24] plasminogen activator inhibitor, (3) adhesion molecules, (4) vasomotor factors (endothelin-1), and (5) prostaglandins (TXA2, PGI2, PGE2, PGF2α).

The pericytes have plasmalemmal receptors for growth factors (e.g., epidermal growth factor receptor EGFR and platelet-derived growth factor receptor PDGFβR) and various other ligands (e.g., endothelin-1 and histamine receptors) [850]. The adenosine receptor A2R stimulates adenylyl cyclase activity. β-Adrenergic and muscarinic receptors have also been observed. Signaling leads to the activation of cAMP, transient increase in IP3, and Ca^{++} influx. In opposition to smooth muscle cells, the membrane potential of the pericyte does not significantly vary after stimulation by ET1, vasopressin, and acetylcholine.

Pericyte coverage is required during angiogenesis. Contacts between endothelial cells and pericytes may control vascular growth, via platelet-derived growth factor and transforming growth factors β, and endothelium functioning. In co-cultures, pericytes modulate EC proliferation and the converse.

Pericytes are recruited by endothelial cells that express PDGF-B to stabilize the vessel. Pericytes protect the endothelial cell, reducing their apoptosis rate.

Cerebral blood flow is strongly regulated to provide 20% of the body energy consumption, even though the brain accounts for only 5% of total weight, and to match the changing metabolic needs of specific brain regions. The cells that control the blood flow include the endothelial cell, smooth muscle cell, pericyte, neuron and astrocyte. Contraction and relaxation of the vascular smooth muscle cells decreases and increases the artery bore, respectively. Increase in intracellular calcium concentration in astrocytes regulates the arteriole caliber. In the absence of surrounding smooth muscle cells, blood flow through capillaries is, indeed, regulated by pericytes, which contain contractile machinery [851].[25] Groups of pericytes along capillaries and at capillary junctions of the central nervous system can constrict and relax according to local neuronal activity. Pericyte constriction can hence redirect the blood flow at the capillary level.

8.4 Vasomotor Tone

The vascular response, especially in arterioles, to changes in intravascular pressure p_i is vasoconstriction or vasodilation, whether p_i is elevated or reduced [852]. Blood flow regulates vessel caliber quickly by vasomotor tone changes,

[24] The thrombospondins interact with blood coagulation and anticoagulant factors. They are involved in cell adhesion, platelet aggregation, cell proliferation, angiogenesis, tissue repair, and tumor metastasis.

[25] About 65% noradrenergic innervation of the brain blood vessels ends near capillaries rather than near arterioles. Pericyte constriction is associated with a rise in intracellular calcium concentration.

later by stimulated gene expression and structural reorganization if loading is maintained, and chronically by wall remodeling (adaptative changes). Studies have been aimed at identifying the sequence of cellular events, from sensing to reaction, responsible for the vasomotor activity and SMC responsiveness, independently of neural and humoral factors. In particular, mechanical signals can be transformed into electrical cues by modulations of ion channel activity in endothelial cells and smooth muscle cells.

A *myogenic response*, independent of the vascular endothelium, couples SMC contraction or relaxation to SMC deformation with length-dependent changes in SMC contractility, or/and with possible changes in ion channel activity, and thus in cell polarization, or/and with possible modifications in cell metabolism [853]. Stretched smooth muscle cells respond by membrane depolarization [854, 855], the resting potential being determined by K^+ [846].

Stresses can act on: (1) exchangers and transporters, (2) plasmalemmal ion channels, and (3) membrane-bound enzymes which modulate activity of contractile proteins. Stretch-activated channel gating depends on stresses transmitted by the cytoskeleton. Stretch-activated calcium channels[26] (SACC), which modulates intracellular $[Ca^{++}]_i$,[27] can recruit voltage-gated calcium channels to rise $[Ca^{++}]_i$. Ligand-gated channels have been assumed to be in SMC membrane, on which act vasomotor factors. Voltage-dependent (VDKC) and Ca^{++}-activated (CAKC) K^+ channels, especially large conductance ones (LCCAKC),[28] provides repolarization that counteracts effect of SMC stretch. Consequently, stretching effect on SMC polarization is maintained in presence of inhibitors of CAKC, due to activation by Ca^{++}.

Deformations activate second messengers other than Ca^{++}. Phospholipid metabolism is linked to cytoskeleton activity.[29] Effects of PLC activation and release of IP3 and DAG are immediate via Ca^{++} influx [861]. IP3 triggers release of Ca^{++} from intracellular stores. DAG activates PKC, especially in the microcirculation [862]. Effects of PKC activation are then delayed. The phosphatidylinositol metabolism stimulated by mechanical factors enhances $[Ca^{++}]_i$ and causes a translocation of PKC from cytosol to membrane [863]. PKC enhances myogenic reactivity by modulating Ca^{++} availability [864]. Activated by DAG, PKC might cooperate with Ca^{++} for full contraction [865]. Mechanotransduction could also be mediated by cAMP. Repeated cyclic strain on cultured smooth muscle cells of coronary arteries reduces adenylyl cyclase activity and cAMP [866]. Decrease of cAMP level associated with activity reduction of adenylyl cyclase is induced by SMC cyclic stretching [867].

[26] Smooth muscle cells can directly contract in response to stretch after activation of its stretch-sensitive ion channels [856–858], contraction being maintained by a sustained deformation.

[27] EC membrane SACCs modulate $[Ca^{++}]_i$, from which depend syntheses of vasomotor substances.

[28] 20-HETE, a metabolite of arachidonic acid, can inhibit LCCAKC [859].

[29] Association of the actin-binding protein profilin and phosphoinositids inhibit PLC [860].

Table 8.12. Eicosanoids associated with blood circulation (taken from [868], with permission).

Type	Synthesis site	Activity
PGD2	Mast cell	Vasodilation Inhibition of TC and WBC aggregation Reduction in T-cell proliferation Decrease in Lφ migration Secretion of IL1a, IL2
PGE2	Heart	Vasodilation TC aggregation Reduction in T-cell proliferation Decrease in Lφ migration Secretion of IL1a and IL2 Enhancement of Bdk and His effects
PGF2α	Heart	Vasoconstriction
PGH2		Vasoconstriction TC aggregation
PGI2	EC	Vasodilation Inhibition of TC, WBC aggregation Reduction in T-cell proliferation Decrease in Lφ migration Reduced secretion of IL1a and IL2
TXA2	TC	Vasoconstriction TC aggregation Lφ proliferation
TXB2	TC	Vasoconstriction
LktB4	Mo, Bφ,Eφ, Nφ, mast cell	Increase in vascular permeability WBC chemotaxis and aggregation Secretion of INFγ, IL1, and IL2
LktC4	Mo, Bφ,Eφ, mast cell	Vasodilation Increase in vascular permeability Secretion of INFγ
LktD4	Mo, Eφ, mast cell	Vasodilation Increase in vascular permeability Secretion of INFγ
LktE4	Mast cell, Bφ	Vasodilation

After Ca^{++} influx, myosin light chain kinase activates myosin light chains to initiate contraction. Blood vessels yield autocrine and paracrine mechanisms to immediately react to hemodynamical changes. SMC function can be

modified by endothelial cells (Sects. 9.5.3 and 9.5.4). Early works proposed release of endothelium-derived constrictor (EDCF) and relaxing (EDRF) factors. Several endothelial-regulation mechanisms have been proposed. The wall shear stress activates G protein via membrane tension [869]. The directional response to flow forces is governed by cytoskeleton displacements that induce a redistribution of intracellular forces [870]. Biomechanical forces are transmitted by the membrane and the cytoskeleton[30] to the entire cell, and then to adjacent cells, the basal lamina, and the entire extracellular matrix. F-actin filaments, anchored in the cell and nucleus membranes via transmembrane proteins, and intercellular and cell-matrix adhesion proteins,[31] are the main transmission elements. Tensegrity[32] models have been proposed for active cytoskeleton reaction [872]. F-actin filaments are also required in transduction involving adenylyl cyclase and stretch-activated ion channels.

Hemodynamic forces imposed on EC wetted surface trigger the synthesis of either vasodilators, such as NO and prostacyclin, or vasocontrictors, such as endothelin-1[33] (local flow-induced regulation of the blood circulation, Table 8.12) and PGF2α. NO, PGI2, ET, and EDHF, indeed, are vasoactive substances released from the endothelium in response not only to humoral but also to mechanical stimuli to act on smooth muscle cells.

Stress increase causes SMC relaxation. These released molecules act locally on underlying smooth muscle cells to modulate the vasomotor tone [873]. Blood friction along the wall luminal surface is thus sensed by endothelial cells and transduced into molecular events. Several biochemical systems are involved, plasmalemmal ion channels,[34] receptor activation, reaction cascades and transcription regulation [874]. Several EC genes are regulated by WSS stimulation [875]. Because of WSS induction of several leukocyte-endothelial adhesion molecules that responds differently in the same cell exposed to the same stimulus, WSS response seems to be promotor specific, WSS transcriptional responses being mediated in presence of certain promotors but not others.

Pro-inflammatory cytokines increase the activity of GTP-cyclohydrolase, the rate-limiting enzyme for tetrahydrobiopterin production, a co-factor for NOS. Leukotrienes (Lkt) regulate smooth muscle tone and affect wall permeability, adherence and chemotaxis. Endothelial cells cannot synthesize leukotrienes but cooperate with neutrophils to metabolize leukotrienes. Prostacyclin is synthesized by endothelial cells in response to inflammatory mediators. Cultured endothelial cells metabolize arachidonic acid not only to prostaglandins, but also to hydroxyeicosatetraenoic acids (HETE) and epoxye-

[30] F-actin fibers align with flow direction.

[31] F-actin filaments bind to integrins by α-actinin, talin, and vinculin [871].

[32] Integrin-ECM interactions determine tensional integrity (tensegrity) of the cells.

[33] The endothelin, a strong vasoconstrictor, induces myocardium contraction, ANP secretion, and SMC division.

[34] Release of vasodilators is associated with K^+ channel activation in EC membrane.

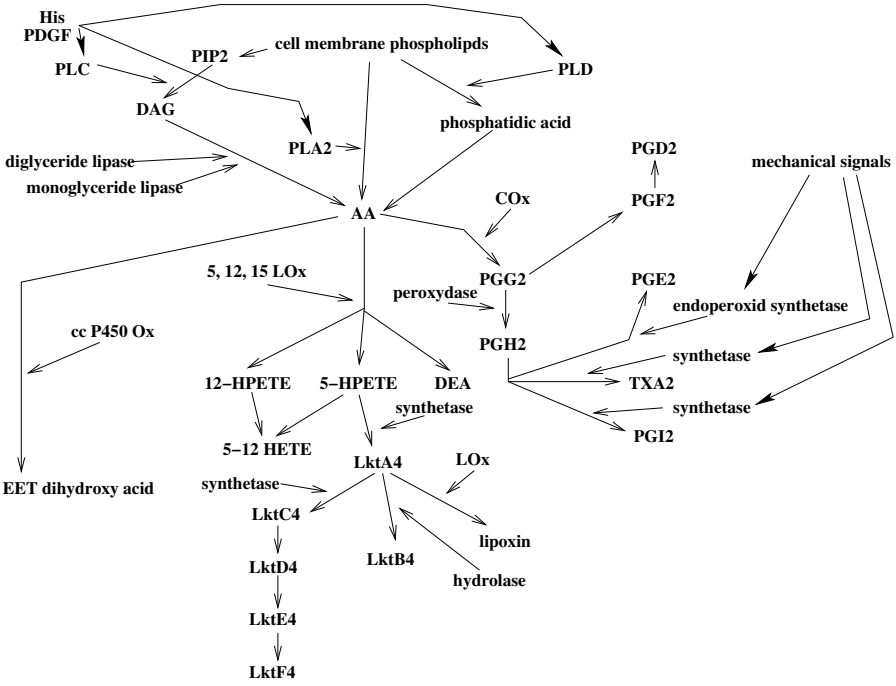

Figure 8.9. Arachidonic acid (AA) metabolism and stimuli (molecules, hemodynamic stresses, pressure, and WSS, and their modulation rates). AA is a constituent of membrane phospholipids, which is released from these phospholipids by phospholipase-A2. Many of the lipids involved as second messengers in cell signaling pathways arise from the arachidonic acid pathway. The three main AA-release pathways involve membrane-bound phospholipase-C (PLC), phospholipase-D (PLD) and phospholipase-A2 (PLA2). The three main AA-metabolism pathways include: (1) cyclooxygenase (COx), which leads to eicosanoids from prostaglandin G2 to H2, then, prostacyclin (PGI2) and thromboxane A2 (TXA2), etc.; (2) lipoxygenase (LOx), which forms hydroperoxyeicosatetraenoic acids (HPETE) and dihydroxyeicosatetraenoic acid (DEA), converted into hydroxyeicosatetraenoic acids (HETE), leukotrienes (Lkt) and lipoxins; and (3) cytochrome p450 epoxygenase (cc p450 Ox) manufacturing epoxyeicosatrienoic acid (EET) (Source: [876]).

icosatrienoic acids (EET; Fig. 8.9). Preloading isolated porcine coronary arteries with EET and DHET enhance endothelium-dependent relaxation. Stresses induce charge changes in subendothelial layer that can modify cell membrane polarization. Cultured endothelial cells are sensible to support medium charge and its frequency and modulation rate.

Table 8.13. Vasomotor substances.

Vasoconstrictor	Vasodilator
ET1	NO
TXA2	PGI2
PGF2α	EDHF/ EET, DHET
Up4A	Adrenomedullin
ATP	Acetylcholine
5HT	ANP
NPY	
20HETE	

8.5 Vasoactive Substances

Several substances, once bound to specific surface receptor, locally cause either SMC contraction or relaxation (Table 8.13). Among these substances, certain are released by the neighbor endothelial cells.

8.5.1 Vasodilators

Prostacyclin (PGI2) is another endothelium-derived vasodilator. *Endothelium-derived hyperpolarizing factor* (EDHF) can also be a prominent vasodilator activated by a cytochrome-P450 epoxygenase such as epoxyeicosotrienoic (EET) acid, which is a metabolite of arachidonic acid. EDHF production is damped by NO. When NO synthesis is impaired, alleviation of EDHF inhibition by NO, at least in part, maintains endothelial vasodilator function [877]. However, the precise nature of EDHF remains questionable [878].

The three main endothelial molecules, which generate vascular smooth muscle relaxation, nitric oxide, prostacyclin PGI2, and EDHF, involves fatty acid cycling. Endothelial nitric oxide synthase is reversibly palmitoylated. Palmitoylation allows eNOS cycling between the plasmalemma, where eNOS is inactive, and the cytosol, where it is active. PGI2 synthesis depends on a balance between PLA2-mediated liberation of AA from membrane phospholipids and acyl coenzyme-A synthase-mediated incorporation into membrane phospholipids of arachidonate substrates. Behind EDHF, arachidonic acid metabolites are involved because of vascular smooth muscle hyperpolarization induced by epoxyeicosatrienoic acids released from endothelial cells, which activate calcium-dependent potassium channels and calcium–potassium ATPase (Fig. 8.10).

In endothelial cells and vascular smooth muscle cells, cytochrome-P450 metabolizes arachidonic acid to vasoactive substances, producing epoxyeicosatrienoic acids (EET) and 20-hydroxyeicosatetraenoic acid (20HETE) [879]. Soluble epoxide hydrolase catalyzes the conversion of epoxyeicosatrienoic acids into dihydroxyeicosatrienoic acids (DHET). Cytochrome-P450

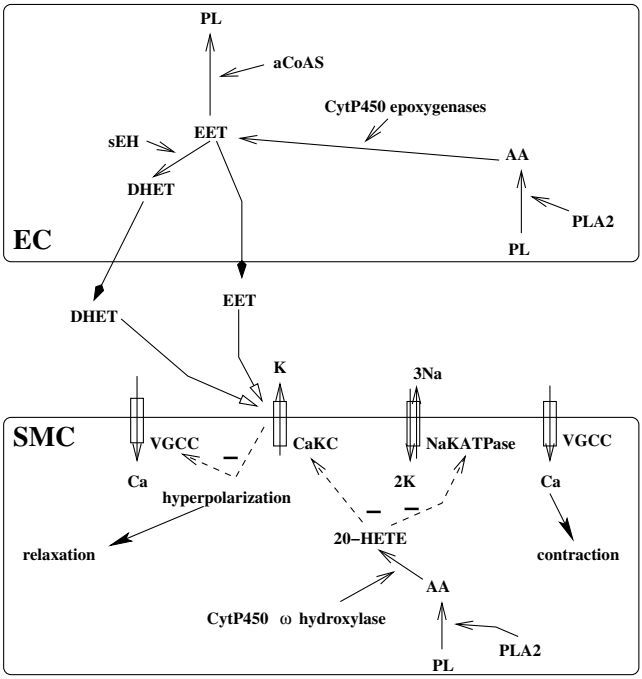

Figure 8.10. Cytochrome-P450 and arachidonic acid metabolism. Cytochrome-P450 vasoactive metabolites of arachidonic acid, eicosanoids such as epoxyeicosatrienoic acids (EET) owing to cytochrome-P450 epoxygenase and 20-hydroxyeicosatetraenoic acid (20HETE) using ω-hydroxylase, regulate the blood pressure. Soluble epoxide hydrolase (sEH) catalyzes the conversion of epoxyeicosatrienoic acids into dihydroxyeicosatrienoic acids (DHET). Activated by endothelin-1 or angiotensin-2 receptor, phospholipase-A2 releases arachidonic acid (AA) from plasmalemmal phospholipids (PL). Intracellular potential is negative with respect to the extracellular one. EETs and DHET released by the endothelial cell activate Ca^{++}-activated K^+ channels (CaKC) of the SMC plasmalemma, hence inducing a K^+ efflux and a cell hyperpolarization, which inhibits Ca^{++} influx via voltage-gated Ca^{++} channels (VGCC), leading to smooth muscle relaxation. On the other hand, 20HETE produced by the smooth muscle cell is a vasoconstrictor, hampering the activity of Ca^{++}-activated K^+ channels and Na^+-K^+ ATPase, thus preventing K^+ and Na^+ efflux which favors cell depolarization to activate L-type Ca^{++} channels, eliciting smooth muscle contraction. EETs are incorporated into plasmalemmal phospholipids by acyl coenzyme-A synthase (aCoAS; from [879]).

thus regulates blood pressure. Gαs and Src signaling activate epoxyeicosatrienoic acids. Epoxyeicosatrienoic acids act as autocrine and paracrine effectors, modulating ion transport and gene expression, leading to vasodilation/constriction, hindering inflammation and favoring fibrinolysis. In endothelial cells, cytochrome-P450 epoxygenase causes, independently of nitric oxide and prostacyclin, vasodilation of several vascular beds, particularly in

the heart and kidney, by the production and release of epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids (Fig. 8.10). In smooth muscle cells, cytochrome-P450 ω-hydroxylase generates 20-hydroxyeicosatetraenoic acid from arachidonic acid after calcium influx. The 20-HETE signaling cascade is activated by endothelin-1 and angiotensin-2. The vasoconstriction mediated by 20HETE, an antagonist to the Ca^{++}-activated K^+ channel, is attenuated by epoxyeicosatrienoic acids, agonists of the Ca^{++}-activated K^+ channel. Besides, acute increase in transmural pressure in vessels that express cytochrome P450-4A can stimulate stretch-activated Ca^{++} channels, and subsequently enhance 20HETE formation.

Moreover, products of cytochrome-P450 epoxygenase and ω-hydroxylase and derived reactive oxygen species resulting from NADPH consumption, are intracellular signal transducers for proliferation of vascular cells via extracellular regulated protein kinases-1 and -2 and angiogenesis. In the kidney, epoxyeicosatrienoic acids decrease sodium excretion and decrease renin release. The cerebral blood flow is regulated by the antagonistic effects of EET and 20HETE, EETs being produced by adjoining astrocytes. 20-HETE can reduce the vascular tone and dilate pulmonary arteries [880]. 20-HETE decreases the pulmonary vasoconstriction due to hypoxia. EET effects (vasodilation or constriction) on pulmonary vessels vary according to mechanical and chemical environment.

Epoxygenases of the arachidonic acid metabolism in the vasculature belong to cytochrome-P450-2B, -2C, and -2J. ω-Hydroxylases are members of the cytochrome-P450-4A and -4F subsets. Members of the cytochrome-P450 family are inhibited by NO. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are quickly incorporated into phospholipids by cardiomyocytes, endothelial cells, and platelets due to acyl coenzyme-A synthase (at least in endothelial cells).

Adrenomedullin activates the nitric oxide synthase. It induces vasodilation and possible hypotension. Adrenomedullin is a peptide similar to calcitonin gene-related peptide (CGRP), and hence belongs to the calcitonin/CGRP/amylin peptide family [881]. It is manufactured from a precursor, the preproadrenomedullin. The adrenomedullin gene is highly expressed in endothelial cells. It also functions in many cell types, such as vascular smooth muscle cells, cardiomyocytes, and blood cells. Adrenomedullin circulates in the plasma (picomolar concentration: 2–4 pM). Adrenomedullin has a wide range of actions. It regulates cellular growth and differentiation. It modulates hormone secretion (inhibition of the secretion of aldosterone, cortisol, and ACTH). It is involved in the regulation of fluid and electrolyte balance (diuresis and natriuresis). In isolated porcine ciliary arteries, adrenomedullin induces arterial relaxation via CGRP1 receptors. This vasodilation can be either mediated either by the release of EDHF and NO, and activation of calcium-dependent and ATP-dependent K^+ channels, or be independent of NO and EDHF [882]. Adrenomedullin increases the cellular cAMP level via

Gs activation. It modifies the intracellular amount of calcium and cGMP. Adrenomedullin enhances the cardiac contractility (inotropic effect).

The flow induces release of ATP, ACh, and substance-P from cultured endothelial cells [883]. Endothelial cells synthesize *acetylcholine*, a vasodilator [303]. *Adenosine diphosphate*, a P2Y ligand, produces a vasodilation. Endothelial P2X4 channels are strongly involved in flow-sensitive mechanisms that regulate local blood pressure and vascular remodeling [884].

Relaxin, a heterodimeric peptide hormone produced from pro-relaxin, is a renal vasodilator. It favors angiogenesis. Relaxin relaxes the uterine musculature. It inhibits collagen synthesis and enhances matrix metalloproteinase activity. Relaxin interacts with G-protein-coupled receptors RXFP1 (LGR7) and RXFP2 (LGR8) used by insulin-like peptide-3 and -7 (Table 8.14). Relaxin receptors exist in the heart, smooth muscle, connective tissue, and central and autonomous nervous systems.

Substance-P is a vasodilator due to the release of nitric oxide from the endothelium. It also acts as a neurotransmitter/neuromodulator. It belongs, with neurokinin-A[35] and neurokinin-B, to the tachykinin neuropeptide family, members of which bind to three known mammalian G-protein-coupled receptors NK1, NK2, and NK3, which activate phospholipase-C.

8.5.2 Vasoconstrictors

In addition to relaxing factors, the endothelial cells can produce contracting factors, which include superoxide anions [885], endoperoxides, and *thromboxane*

Table 8.14. Vasoactive peptide receptors and their main targeted G proteins (Source: [241]).

Type	Main transducer
	Galanin receptors
GalR1/R3	Gi/o
GalR2	Gi/o, Gq/11
	Neuromedin-U receptors
NMU1/2	Gq/11
	Relaxin peptide family receptors
RXFP1/2	Gs, Gi/o
RXFP3/4	Gi/o
	Tachykinin receptors
NK1	Gq/11 (substance-P>neurokinin-A>neurokinin-B
NK2	Gq/11 (neurokinin-A>neurokinin-B>substance-P
NK3	Gq/11 (neurokinin-B>neurokinin-A>substance-P

[35] Neurokinin-A is also known as substance-K. Neurokinin-A mediates contraction of the colon.

A2. The nucleotide *uridine adenosine tetraphosphate* (Up4A) vasoconstricts likely via P2X1 receptors (probably also via P2Y2 and P2Y4). Stimulation by adenosine triphosphate, uridine-triphosphate (UTP), acetylcholine, endothelin, and mechanical stress releases Up4A from endothelium [886].

Adenosine triphosphate, quickly released by the endothelium when the flow increases, is a vasoconstrictor, which binds to two purinergic receptor types, P2X and P2Y. However, ATP bound to endothelial P2X1 receptors induces a constriction followed by a vasodilation [301].

The wall shear stress enhances Bdk secretion [887]. Like the nitric oxide and endothelin, certain vasoactive substances can cause proliferation of endothelial cells and/or smooth muscle cells, such as ATP, angiotensin-2, substance-P.

Neuropeptide-Y (NpY) is a neurotransmitter. It augments the vasoconstrictor effects of noradrenergic neurons. It forms the lipostat with leptin and corticotropin-releasing hormone to reduce feeding and increases energy expenditure. NpY operates on a G-protein-coupled receptor.[36]

Neuromedin-U (NmU) is a neuropeptide acting on smooth muscle. It is widely distributed in the gut and central nervous system. Its activities include stimulation of smooth muscle, increase of blood pressure, alteration of ion transport in the gut, control of local blood flow, and regulation of adrenocortical function.[37] It binds to G-protein-coupled receptors NMUR1 (FM3) in peripheral tissues and NMUR2 (FM4) in specific regions of the brain.

8.6 Nervous Inputs

Activated α1-adrenergic receptors interact with Gαq, which signals via phospholipase-C leading to: (1) diacylglycerol, then activating protein kinase-C, and (2) inositol trisphosphate and calcium influx mainly from the endoplasmic reticulum (Fig. 8.11). β2-Adrenergic receptors induces relaxation of vascular smooth muscle cells (Table 8.15).

Smooth muscle myosin-2 consists of two heavy chains and two types of light chains. One part of the heavy chains fold into the globular head, which forms the motor domain, the other forms the myosin tail, which associates with others to produce the myosin filament. Contraction initiation and termination are switched by myosin phosphorylation by smooth muscle myosin light chain kinase, and dephosphorylation by myosin light chain phosphatase. The myosin light chain can also be phosphorylated by PKC, CamK2. Phosphorylation magnitude determines shortening velocity and tension development. MLCK isoforms of smooth muscle and non-muscle cells differ from the striated muscle MLCK. MLCK can be phosphorylated by different kinases, PKA, PKC, CamK2, and p21-activated protein kinase. MLCP is also phosphorylated

[36] Subtypes Y1 and Y5 stimulate feeding, whereas Y2 and Y4 favors satiety.
[37] NmU has anorexigenic effects.

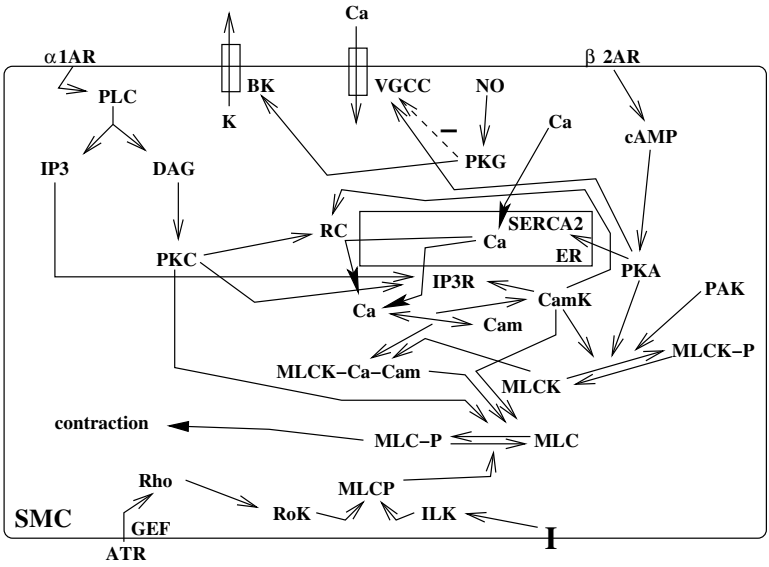

Figure 8.11. Contraction of the vascular smooth muscle cell and its regulation (BK: large-conductance Ca^{++}-activated K^+ channel; VGCC: L-type Ca^{++}-channel; I: integrin; Cam: calmodulin; GEF: GTP exchange factor; IP3(R): inositol trisphosphate (receptor); DAG: diacylglycerol; PKA, PKC: protein kinase-A, C; PLC: phospholipase-C). α1-Adrenergic receptors (α1AR) and β2-adrenergic receptors (β2AR) induce contraction via the PLC-PKC pathway and relaxation via the cAMP-PKA cascade, respectively. The myosin light chain kinase (MLCK) can be phosphorylated by different kinases: PKA, PKC, CamK2, and p21-activated protein kinase (PAK). The myosin light chain phosphatase (MLCP) can be phosphorylated by Rho kinase (RoK) and integrin-linked kinase (ILK). MLCK phosphorylation, which requires Ca-Cam, leads to activation, whereas MLCP phosphorylation leads to inactivation. Ca^{++} ATPase (SERCA2) is targeted by PKA, ryanodine channel (RC) with its regulatory proteins FKBP, and IP3R are activated by PKA, PKC, or Cam-dependent protein kinase-2. NO-induced PKG inhibits L-type Ca^{++}-channel and activates K^+ channel BK (Source: [804]).

(inhibited) by Rho kinase-2, ensuring a sustained vasoconstriction. Integrin-linked serine-threonine kinase ILK phosphorylates myosin light chain phosphatase, as well as the myosin light chain. The regulatory protein caldesmon forms a complex with actin, tropomyosin, and a Ca^{++}-binding protein. In the absence of calcium, caldesmon restricts the interaction of the myosin heads with actin. Calcium binding to Ca^{++}-binding protein relieves the inhibition. Calponin, which binds to actin and calmodulin, could enhance PKC-ERK1/2 signaling.

Smooth muscle-specific L-type Ca^{++}-channel Cav1.2b is phosphorylated by PKA, PKC, and CamK. Most of the L-type Ca^{++}-channels are packed with Na^+-Ca^{++}-exchanger NCX1, Na^+-K^+ pump, plasmalemmal Ca^{++} ATPase

and large-conductance Ca^{++}-activated K^+ channel BKCa. β-Adrenoceptor stimulation activates PKA, which in cooperation with AKAP, phosphorylates L-type Ca^{++}-channels. cAMP–PKA activity can be counteracted by nitric oxide and its effector PKG. PKG inhibits L-type Ca^{++}-channels, and activates BKCa and MLCP.

8.7 Wall Adaptability

SMC proliferation can be modulated by growth factors and other mediators, released from endothelial cells. Endothelial platelet-derived growth factor-A, which acts synergistically with endothelin-1, transforming growth factor-β, and nitric oxide are released by endothelial cells, the secretion level depending on the flow conditions. Angiotensin-2, formed by angiotensin-converting enzyme in a flow-dependent manner, is a growth factor for vascular smooth muscle cells. Both serotonin and thromboxane-A2, released by endothelial cells or aggregating platelets at sites of vascular injury, have a mitogenic effect from a given concentration via their respective specific receptors [888]. These substances can synergistically interact; SMC proliferation occurs at subthreshold concentrations.

Endothelium is able to adapt to ischemia. Indeed, endogenous NO contributes to CMC "hibernation" by reducing oxygen consumption and preserving calcium sensitivity and contractile function without an energy cost during ischemia [889]. Hypoxemia induces the production of PGE2 and LktC4 by cardiomyocytes. Delayed increase in NO production by eNOS is a mediator of endothelial preconditioning, with brief periods of ischemia-reperfusion, which induce delayed protection of coronary endothelial cells against reperfusion injury. [890].

Hypertension induces wall remodeling. The endothelial cells display structural changes associated with increased media thickness due to hypertrophy of the smooth muscle cells [891]. The amount of elastin does not change significantly, whereas the collagen concentration strongly increases. TGFβ levels

Table 8.15. Contraction and relaxation features in the cardiomyocyte and smooth muscle cell. The cardiomyocyte is characterized by fast, reversible calcium binding to cardiac troponin-C. Smooth muscle cell activity is regulated by reversible phosphorylation of myosin and/or actin components. Vasomotor tone depends on the activity ratio between MLCK and MLCP. β-Adrenergic receptor signaling increases intracellular calcium concentration in the cardiomyocyte, whereas its activity in vascular smooth muscle cell is independent of calcium.

	CMC	SMC
Contraction/relaxation	Fast	Slow
ATP consumption	High	Low
β-Adrenergic signaling	I+	Relaxation

produced by aortas of hypertensive rats increase up to threefold with respect to those of normotensive rats [892].

9

Endothelium

The endothelium constitutes the interface between the flowing blood and the deformable solid wall. The endothelium is a layer of connected and anchorage-dependent cells. The endothelium has several functions. It acts in molecule exchange between the blood and the vessel wall or the perfused tissues (Sect. 9.2). It controls flowing cell adhesion and extravasation (Sect. 9.3), especially for immune defense. It regulates coagulation and thrombolysis (Sect. 9.4). It regulates vasomotor tone and the growth and proliferation of vascular smooth muscle cells via the release of several compounds (Sect. 9.5). It is required in angiogenesis (Sect. 10.4.2).

Time-dependent hemodynamic stresses applied on and within the vessel wall (wall shear stress, axial and circumferential tensions within the wall) are implicated in: (1) the regulation of the vascular tone, which determines the vessel bore; (2) short-term wall adaptation and long-term remodeling because of their influence via cell signaling on cell growth, differentiation, migration, and apoptosis; and (3) vasculature diseases because they affect the cell functioning and transport processes. Endothelial cells detect hemodynamic stresses via mechanosensors, such as adhesion molecules (mainly integrins), ion channels, and plasmalemmal receptors (GPCRs and RTKs). Signaling pathways (MAPK, PKB, PKC, and ROS) augment the activity of transcription factors (activator proteins AP1 and AP2, cAMP response element, early growth response protein EGR1, and NFκB) with a magnitude that depends on the cell type (vascular region).

Fluorescent plant virus can be used to image small-bore vessels of the macrocirculation in deep tissues using fluorescence microscopy [893], as virus is incorporated in vascular endothelial cells. This technique identifies arterial and venous compartments, as fluorescent virus uptake in the veins occurs at a much higher rate than in the arteries.

9.1 Endothelial Cell

At the microscopic level, the wetted surface is wavy due to projective nuclei. The endothelium surface either from fresh arterial walls mounted on an appropriate holder and kept in physiological buffer or from cultures can be assessed by the scanning force microscope associated with a phase-contrast microscope [894]. The polygonal-shaped flattened cells (width $\sim$ 10–15 μm, length in the streamwise direction 60–100 μm) have no-flow and stretched flow-adapted configurations. The height variation has been measured along the endothelium with a maximum of 750 nm, representing the nuclear regions. Observed plasmalemmal granules and ring-like structures of various sizes have been assumed to be associated to the cytoplasmic plasmalemma layer rather than the outer one. Moreover, fibers can be seen, likely associated with actin filaments, which are bound to the cytoplasmic face of the plasmalemma. The cell membrane is covered by a thin glycocalyx. The between-cell space width ranges from 10 to 20 nm with tight and gap junctions. Myoendothelial junctions (MEJ), composed of connexins, allow signaling between endothelial cells and vascular smooth muscle cells. They thus lead to discontinuities in endothelial basement menbrane.

Endothelial cells contain Weibel-Palade bodies, long rod-shaped storage organelles (length 1–5 μm). These secretory and storage granules are filled with von Willebrand factors, which recruit platelets to the site of injury. The architecture of the Weibel-Palade body and tubular folding of the von Willebrand factor requires a low pH [895]. The tubules must not be disassembled prior to exocytosis. Once released, von Willebrand factors unfold rapidly and efficiently at neutral pH to trap circulating platelets by forming platelet-catching filaments (length $\sim$100 μm).

Endothelial cells monitor cell internal state and environment actions. Endothelial cells especially sense hemodynamic and hormonal stimuli and respond by secreting various mediators. Endothelial cells experience blood pressure, axial and circumferential tension from connecting endothelial cells, and blood friction on its wetted surface, the wall shear stress[1] (WSS). The applied forces are unsteady with noticeable spatial and temporal magnitude gradients as well as possible direction changes. The stress distributions in the membrane and cytosol affect EC functions.

As stated in the introduction, the endothelium is involved in: (1) blood-wall exchange control, (2) vasomotor tone modulation,[2] (3) coagulation regulation, (4) vessel wall growth and remodeling, and (5) in inflammation and

[1] The wall shear stress is the tangential force produced by blood moving along the endothelial surface. At the endothelial surface, most momentum and vorticity transports occur between fast-moving fluid particles and those slowed down by wall friction. The boundary layer develops. The wall shear stress depends on the local velocity gradient.

[2] Among its active functions, the secretion and modification of vasoactive substances induce contraction and relaxation of the vascular smooth muscle cells.

immune pathways, driving the leukocyte adhesion [896]. It responds by multiple molecule syntheses (Table 9.1, Fig. 9.1). In particular, endothelial adenosine triphosphate diphosphohydrolase hydrolyzes ADP and ATP into AMP. It thus has anti-thrombotic and anti-inflammatory activities.

Acetylcholine is synthesized and stored by choline acetyltransferase in endothelial cells of small brain vessels [897] and rat coronary arteries [898]. Serotonin and substance-P are observed in endothelial cells[3] [899]. Endothelial cells can also store serotonin, arginine–vasopressin, angiotensin-2, histamine, atrial natriuretic peptide, and even calcitonin gene-related peptide, neuropeptide-Y, and vasoactive intestinal peptide [825].

Endothelial cells express IL20 receptors. IL20 induces proliferation of endothelial cells and promotes angiogenesis.[4] Its activity is antagonized by IL10 [900].

Many proteins of the extracellular matrix are substrates of transglutaminase-2 (TG2) produced by endothelial cells. Transglutaminase-2 affects both matrix deposition and turnover. It promotes collagen-1 synthesis and resistance of the extracellular matrix to degradation by matrix metalloproteinase MMP1, but it downregulates the expression of collagen-3 and -4 [901].

Table 9.1. Examples of endothelial cell production.

Structure	Proteoglycans (hyaluronic acid, DS, KS, HS) Glycoproteins (Ln, FN, Tsp, vWF)
Growth	Growth factors (PGDF, GM-CSF, G-CSF, M-CSF) Growth mediators (5HT, TXA2, Ang2)
Motility	Chemokines (Lkt, HETE) Semaphorins, plexins
Vasomotor	ET, NO, PGI2, TXA2, EDRF, HETE, EET LKlF, adrenomedullin, ATn2, 5HT, ACh, ATP
Adhesion	Integrin, selectin, cadherin ICAM1, ELAM1 13-HODE, TxnIP
Inflammation	His, Bdk, Ang2
Coagulation	PAI1, PGI2, TM tPA, TXA2, vWF, FV, FIII, PAI
Hormone	Adiponectin

[3] Levels of serotonin and substance-P are similar in endothelial cells of femoral and mesenteric arteries of the rat. Substance-P is also localized in endothelium of rat coronary arteries [898].

[4] IL20 belongs to the interleukin-10 family. IL20 favors phosphorylation of ERK1/2, p38, and JNK.

Different structural types of heparan sulfate are secreted on the cell surface, such as syndecan and glypican, as well as in the basement membrane (thickness of 20–100 nm), such as perlecan, agrin and collagen-18 [902] (Fig. 2.6).[5] Several heparan sulfate proteoglycans have heparan sulfate chains only under specific circumstances, the part-time heparan sulfates, such as CD44, β-glycan, and testican. Heparan sulfates interact with manifold proteins, such as growth factors, cytokines, chemokines, proteases, lipases, and cell-adhesion molecules. The vascular endothelium can modulate inflammatory responses by changing the heparan sulfate structure, a given type of heparan sulfate being able or not to bind a given ligand. Heparan sulfate, in association with interacting proteins, inhibits SMC proliferation [903].

The functions of the endothelium are affected by the hemodynamics. The intracellular calcium concentration in cultured endothelial cells strongly depends on flow patterns.[6] Although oscillatory flow does not change $[Ca^{++}]_i$, steady and pulsatile flows increase $[Ca^{++}]_i$, with response dynamics that depend on the flow pattern [904]. The release of the substances active on vasomotor tone depends on shear stress applied on the endothelium (Sect. 9.5). Shear stress might also regulate the secretion of molecules involved in coagulation and fibrinolysis or inhibiting thrombosis (Sect. 9.4). High wall shear stress hinders leukocyte adhesion on the endothelium (Sect. 9.3). The endothelium adapts the vessel wall to the local flow pattern (Sect. 8.7).

Adrenomedullin is widely expressed throughout the body, but at highest levels in endothelial cells and vascular smooth muscle cells. Adrenomedullin circulates in the plasma at picomolar levels. Elevated plasma levels of adrenomedullin occur during pregnancy. Strongly vascularized tissues, such as lungs, heart, and kidneys release adrenomedullin during inflammation, hypoxia, sepsis, and cardiovascular diseases (myocardial infarction, heart failure, and atherosclerosis), particularly with hypertension. The adrenomedullin expression is regulated via estrogen-responsive elements Adrenomedullin type and concentration vary according to the genetic ground, especially the gender because the adrenomedullin gene is regulated by estrogen. The genetic ground then hinders or favors development of cardiac and renal damages, but in the latter case does not necessarily change the blood pressure [906].

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[5] These proteoglycans link to other basement membrane components, such as laminin, collagen-4, and entactin. Five types of heparan sulfates always carry heparan sulfate chains, the so-called full-time heparan sulfates. The full-time heparan sulfates on cell surfaces consist of four integral-membrane syndecans, and six glypicans, which are attached to the plasmalemma. The three ECM heparan sulfates are perlecan, agrin, and collagen-18.

[6] Cultured bovine aortic endothelial cells have been subjected to steady shear stress (0.02–7 N/m^2), non-zero mean sinusoidal shear stress, either with a strong mean component (4 +/− 2 N/m^2) or with a high unsteady component (2 +/− 4 N/m^2), and to oscillatory shear stress (+/− 2 N/m^2), using few flow frequencies (0.4, 1, and 2 Hz).

9.1.1 Endothelial Progenitor Cell

Endothelial progenitor cells or angioblasts can be isolated from blood [907]. Hemangioblast is a common precursor for hematopoietic and endothelial cells under the influence of growth factors. Hemangioblast leads to hematopoietic stem cells, which give birth to lymphoid and myeloid progenitor cells,[7] as well as vascular stem cells, precursors of endothelial progenitor cells and secondarily of endothelial cells on the one hand, and pericyte on the other hand. Endothelial progenitor cells activated by stimuli for tissue regeneration (vascular endothelial growth factor, granulocyte colony-stimulating factor, stroma-derived factor-1, and angiopoietin-1) are recruited from the bone marrow into the blood flow to be convected toward angiogenesis sites [908]. Two populations of endothelial progenitor cells exist, the early and late endothelial progenitor cells, with distinct growth patterns and secretion modalities of angiogenic factors. Circulating endothelial progenitor cells are defined by markers, including CD34, VEGFR2, and AC133. [8] Monocytes can also

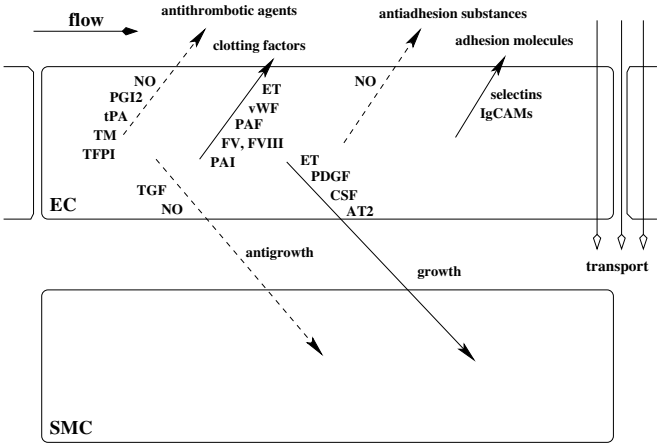

Figure 9.1. Effects of the endothelial cell on its environment. Endothelial cells regulate the endothelial permeability for plasma substances and adhesion of blood cells (promigration substances: selectins, IgCAMs; antimigration molecules: nitric oxide). They release clotting factors and anticoagulation agents (nitric oxide, prostacyclin, tissue plasminogen activator, thrombomodulin), growth regulators, either progrowth (angiotensin-2, platelet-derived growth factor, colony stimulating factors, and endothelin) and anti-growth molecules (nitric oxide, transforming growth factor-β), especially for the smooth muscle cells, and vasoactive substances (Source: [905]).

[7] Lymphoid progenitor cells differentiate into T, B, and NK lymphocytes; myeloid progenitor cells into granulocytes, monocytes, erythrocytes, thrombocytes, and dendritic cells.

[8] AC133 is a primitive hematopoietic stem cell marker, which is not expressed by mature endothelial cells. Both endothelial progenitor cells and mature endothe-

provide a source of endothelial progenitor cells, which do not proliferate, but release angiogenic growth factors.

9.1.2 Endothelium Types

High Endothelial Venules

High endothelial venules are specialized postcapillary venules of lymphoid tissues [909]. These venules serve as entry of blood-convected lymphocytes into lymphoid organs. Lymphocytes indeed migrate across high endothelial venules for immune surveillance. High endothelial venules are lined by quasi-cuboidal (plump) endothelial cells rather than flat, thin (except in the nucleus region) ones. These endothelial cells allow lymphocyte extravasation into tissues, using intercellular adhesion molecule-1 and CCL21 chemokines, which are upregulated during fever [910].

Lymphatic Endothelium

The lymphatic system has many functions. It conveys immune cells. It drains fluids from the interstitial spaces. It transports absorbed dietary lipids to metabolism sites. Lymphatic vessels develop from specialized venous endothelial cells. During embryogenesis, subpopulations of venous endothelial cells form lymphatic sacs in the region of the primitive subclavian, inferior vena cava, and iliac veins. These sacs then divide to create lymphatic networks. Mediators of embryonic lymphatic development include the transcription factor Prospero-related protein-1 (Prox1) and VEGF-C. Separation of lymphatic and blood microvasculature in the intestinal mucosa continues beyond fetal life.

Fasting-induced adipose factor (Fiaf),[9] produced by enterocytes of the small intestine, is required for separation between postnatal intestinal lymphatic and blood vessels [911]. Fiaf signaling implicates effector Prox1 in the postnatal intestinal lymphatic endothelium. However, lymphatico-venous partitioning also uses Prox1-independent pathways.

9.1.3 Endothelium Ion Channels

Ion channels are abundant in the plasma membrane of the nonexcitable endothelial cell [912]. They control ion influx necessary for production of vasoactive molecules, pro- and anti-coagulants, growth factors, etc., which are

lial cells express similar endothelial-specific markers, such as VEGFR2, Tie1, Tie2, and VE-cadherin.

[9] Fasting-induced adipose factor is also called angiopoietin-like protein 4. It inhibits lipoprotein lipase involved in the storage of triglycerides in adipocytes. It promotes endothelial cell survival. It reduces VEGF-induced microvascular permeability.

released for storage or immediate action. They are involved in the regulation of cell trafficking. Angiogenesis entails mediating ion channels.

Mechanosensitive (mechanogated) ion channels (MSIC) are required in mechanotransduction. Conformation of the closed state of the mechanosensitive ion channels and gating transition remain undefined. Ion channel status could be dictated by a coupling with a pathway involving integrins, the cytoskeleton, and G proteins. Opening of shear- or stretch-activated ion channels leads to Ca^{++} influx and stimulation of Ca^{++}-dependent signaling pathways.

Ca^{++}-influx channels are important for long-lasting responses, which are required for the release of various compounds (NO, vWF, PAF, tPA, and TFPI; Sect. 9.4). Members of the transient receptor potential (TRP)[10] family, transmembrane channels, can likely be involved. STRPCs include (1) receptor-activated cation channels[11] (RACC), which are commonly activated by phospholipase-C; (2) non-selective cation channels[12] (NSC); and (3) Ca^{++}-selective store-operated channels.[13]. Ligand-gated Ca^{++} channels, such as cyclic nucleotide-gated channels[14] (CNGC), P2X receptors,[15] and probably Na^{+}/Ca^{++} exchangers provide alternative routes for Ca^{++} entry in endothelial cell. Voltage-gated Ca^{++} channels is a potential candidate for a sustained Ca^{++} influx. The stretch-sensitive Ca^{++} channel, which is not

[10] TRP channel (TRPC) family comprises subfamilies, STRPCs (short), LTRPCs (long), and OTRPCs (osmotic), among others (Sect. 3.1.1). OTRPCs respond to changes in osmolarity. STRPCs contain several calmodulin-binding regions. STRPCs are activated by stimulation of PLC-coupled receptors.

[11] Endothelial Ca^{++}-permeable RACCs, activated by $[Ca^{++}]_i$ increase, provide a positive feedback on their own activation.

[12] The influx of Ca^{++} via Ca^{++}-permeable NSCs, activated by vasoactive agonists, depends on inositol trisphosphate. Constitutively open, nonselective Ca^{++}-permeable outwardly rectifying cation channels exist in both luminal and abluminal membranes of endocardial endothelial cells. There are superoxide-gated NSCs. Cyclic nucleotide-gated NSCs binds either cAMP or cGMP.

[13] Store-operated (SOC) or capacitative (CCE) Ca^{++} channels modulate Ca^{++} entry. Filled stores prevent Ca^{++} influx through store-operated Ca^{++} channels, whereas empty stores promote influx of extracellular Ca^{++}. EC highly Ca^{++}-selective agonist-activated channels are gated either by ATP and bradykinin or IP3. Ca^{++}-release-activated Ca^{++} channel (CRAC) is a SOC. VEGF-E activates CRACs.

[14] Cyclic nucleotide-gated channels are non-selective cation channels, opened by the direct binding of cyclic nucleotides, cAMP and cGMP. Although CNGC activity exhibits a small voltage dependence, CNGCs belong to the superfamily of voltage-gated ion channels. CNGC ligand sensitivity and selectivity, ion permeation, and gating are determined by the subunit composition of the channel complex. CNGC activity is modulated by Ca^{++}–calmodulin and phosphorylation. CNGC1 are found in arteries. NO-induced cGMP can activate CNGC2, which exerts a negative feedback on Ca^{++} entry.

[15] Purinergic ATP-gated receptor-channel complexes (P2X family) are involved in shear stress-induced Ca^{++} influx.

clearly identified, is inhibited by PKG. PMCA and SERCA Ca^{++} pumps are responsible for efflux and intracellular sequestration.

Mechanosensitive inwardly rectifying K^+[16] (IRKC), Ca^{++}-activated K^+[17] (CAKC), and probably voltage-dependent K^+ (VDKC) channels participate in the setting of the membrane potential, with volume-regulated anion (VRAC), Ca^{++}-activated Cl^- (CaClC), cystic fibrosis transmembrane conductance regulator channels (CFTRC), and Ca^{++}-impermeable NSCs. ATP-sensitive K^+ channels might be involved in mechanotransduction. The volume-regulated anion channel (VRAC), mainly permeable for Cl^-, is activated by shear stress. The annexin-2–p11 complex, which is part of the cortical cytoskeleton and involved in the formation of caveolae, is required for endothelial VRAC activation. Voltage-activated Na^+ channels are stimulated by PKC. Ca^{++}-dependent Cl^- channels, outward rectifiers, require ATP. Endothelial cell expresses other Cl^- channels, including high-conductance Cl^- channels, which are quasi-silent in intact cells, and cAMP-stimulated CFTRCs. Amiloride-sensitive Na^+ channels might be connected with water transport via aquaporin-4.

9.1.4 Glycocalyx

The glycocalyx forms a thin layer[18] between the circulating blood and the endothelium. It is a hydrated mesh of negatively charged proteoglycans, glycosaminoglycans,[19] glycoproteins, and glycolipids secreted by endothelial cells.

The glycocalyx is the first barrier to molecular transport from the flowing blood to the vessel wall, providing hydraulic resistance to mass transport through this sieve [913]. The transport conductance in the glycocalyx depends on the molecular size.[20] Both the hydrostatic and osmotic pressures act on transport across the glycocalyx.

At the basal state, the rheological properties of the glycocalyx might induce a lift that prevents cell adhesion [914]. In inflammatory sites, heparan sulfate on the surface of endothelial cells is a potential ligand for P-selectins (expressed by endothelial cells) and L-selectins (expressed by leukocytes), which are involved in initial attachment and rolling of leukocytes on the endothelium [915]. It also binds chemokines for stable adhesion of leukocytes on the endothelium.

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[16] IRK is a member of the Kir family.

[17] Different types of Ca^{++}-activated K^+ channels include maxi-K (BKCa), responsible for K^+ efflux, and intermediate-conductance (IKCa), which are inwardly rectifying, and small-conductance (SKCa) channels. BKCa channels are targeted by the endothelium-derived nitric oxide.

[18] The glycocalyx thickness is very difficult to determine accurately ($h \sim 100\,\text{nm}$ in the microcirculation and $h \sim 250\,\text{nm}$ in the arteries).

[19] Among the glycocalyx glycosaminoglycans, syndecans, and glypicans are bound to the cell membrane.

[20] The size of albumin and LDL is nearly 3.5 and 11 nm, respectively.

The glycocalyx, which is a polyelectrolyte coating, has been modeled as a semi-infinite, doubly periodic array of parallel charged cylinders [916]. Only the luminal layer part of the glycocalyx model markedly influences the transport.

9.1.5 Endothelial Fenestrae

Fenestrae (caliber 60–70 nm) exist in the capillary endothelium, where large molecule exchanges occur between flowing blood and perfused tissues. Fenestrae hence increase the endothelium permeability for water, electrolytes, and small macromolecules, especially in the nephron glomerulus, gastrointestinal tract, liver sinusoids, ocular choriocapillaris, and endocrine glands.

Fenestrae form an array characterized by regular spacing, the so-called sieve plate. The fenestra density in sieve plates can reach about 30 fenestrae per μm^2. The fenestra pore is made of 5- to 6-nm openings delineated by a diaphragm with radial fibrils from a central node. Fenestrae are composed of the diaphragm protein PV1, which is required for fenestra formation, as well as actin-filament remodeling [917].

9.2 Transendothelial Mass Transfer

The endothelium is a selective permeable barrier that regulates the transfer of molecules and cells from the blood to the underlying tissues. Transport across the endothelium depends on relative pressure and concentration gradients. Endothelial permeability can vary according to the state of the cytoskeleton (relaxation/contraction) with intercellular space narrowing/enlargment. Molecule transport across endothelial cells is affected by molecule characteristics: molecular size, charge, shape, and carbohydrate content.

9.2.1 Normal and Leaky Regions

The junctions between endothelial cells act also as a selective barrier to the egress of water and hydrophilic solutes from the circulation. The endothelium can be divided into two parts according to the state of endothelial cells and their junctions: (1) normal endothelial cells, the most numerous, with normal junctions; and (2) leaky endothelial cells, either mitotic or apoptotic endothelial cells of random location, with leaky junctions. The dynamic regulation of endothelial integrity relies on cell migration coordination and re-establishment of cellular junctions. These processes are regulated by the actin cytoskeleton, and thus on Rho GTPases and their effectors in particular.

Macromolecular permeability between endothelial cells is regulated by tight junctions, the most apical component of the intercellular cleft, and the thinest intercellular space, making intimate contacts between adjacent

endothelial cells. Most water and hydrophilic solutes that are small enough (lower than the albumin dimension) can cross the orifices of tight junctions in the endothelium clefts.

The spaces between several adjoining endothelial cells such as *tricellular corners*, where the borders of three endothelial cells meet, are more permeable regions because endothelial tight junctions are discontinuous at the corners [918]. Discontinuities are observed for occludin, ZO1, cadherin, and β-catenin at the tricellular corners [919]. Besides, 70% of neutrophil transendothelial migration occur at tricellular corners.

Leaky endothelial cells favor big molecule transport. Endothelium permeability is, indeed, enhanced when endothelial cells have high turnover rates such as in atherosclerosis [920]. Moreover, hypoxemia induces EC apoptosis, and thereby increases macromolecular transport across the vessel wall via leaky junctions. The normal junctions are modeled as circular section spaces around endothelial cells with a pore set in its central part, and leaky junctions as rings around leaky endothelial cells [921].

9.2.2 Transport Mechanisms

Two different transport mechanisms can be defined: (1) an intercellular transport through between-cell junctions for small molecules; and (2) an intracellular transport for macromolecules (Sect. 5.1). The highest transfer flux between the vascular lumen and extracellular medium occurs by vesicles (size 80 nm).[21] The vesicles can fuse between them and possibly with cytosol organelles to form tubules. Vesiculo–vacuolar organelles build channels through the cytoplasm. Membrane-bound tubules may create transendothelial channels for transfer of large plasma molecules [923]. Vesiculotubular structures specialize according to molecule types. Molecules can be modified during the transit. Many amino-acid transporters exist in endothelial cells. The y+ cationic amino acid transporter carry L-arginine, the substrate for nitric oxide [924].

Intracellular transport begins with receptor binding.[22] Once bound to their receptors, the molecules assemble in a patch that then bulges to form a vesicle. From the starting membrane granulation to its fusion with target membrane, the vesicle carries the molecules. Such types of shuttles also transport manufactured molecules between the different cell organelles: from the endoplasmic reticulum to the Golgi apparatus (Sect. 1.1), from which are launched vesicles

[21] Vesiculo–vacuolar organelles (VVO) formed by fusion of vesicles provide macromolecule transport of vascular endothelial growth factor, serotonin, and histamine, which can bind VVOs by means of receptors [922].

[22] Glucose is a hydrophilic compound that requires specific carriers for its transport into the cytosol. Glucose transporters GLUT are expressed in endothelial cells [925]. GLUTs are intrinsic transmembrane proteins with different tissue-specific isoforms. Cells have a continuous supply of glucose to be used either as a precursor of bigger molecules or an energy source by generating ATP through glycolysis.

for immediate extracellular release, vesicles for signal-dependent secretion, and digestive lysosomes.

The intracellular receptor-mediated transport is aimed at transfering molecules either to feed endothelial cell (*endocytosis*) or across the cytoplasm to supply the wall tissues with nutrients and growth factors from the blood (*transcytosis*). Endocytosis allows not only nutrient uptake, but also membrane maintenance. After internalization, main routes lead to degradation, recycling,[23] transient sequestration in endosomes, or transcytosis. Pinching off of vesicles requires dynamin. Vesicular traffic is controlled. Clathrin-mediated endocytosis, which involves actin, captures receptors, ligands and extracellular fluid. Caveolae-mediated endocytosis[24] is involved in receptor-mediated endocytosis and the transport of blood macromolecules. Caveosomes transport albumin across the endothelium. Vesicles used for intracellular transport are also involved in signal transduction. Caveolin of the caveolar membrane binds to certain signaling molecules and can be involved in angiogenesis and apoptosis.

Intersectins regulate membrane fission and fusion of endothelial caveolae. Actin and microtubules are implicated in raft-mediated endocytosis (Sect. 1.1). The cAMP–PKA pathway, which regulates cytoskeletal and adhesive protein function in endothelial cells, promotes the endothelium barrier function [926]. At the abluminal cell part, focal adhesions between endothelial cells and the basement membrane contribute for about 20% of permeability resistance [927].

The contraction of the EC cytoskeleton, inducing small gaps at cell junctions, can also explain increased extravasation of macromolecules [928]. Two myosin light chains (MLC), one structural and one regulatory, are bound to the myosin heavy chain (MHC). Ca^{++}–calmodulin-dependent MLCKs phosphorylate regulatory MLCs, which interact with F-actin filaments, myosin sliding along the actin filament generates the contraction. The endothelium permeability is enhanced by transient rise in $[Ca^{++}]_i$ induced by various mediators, which acts on the cytoskeletal arrangement and the quality of cell junctions. Besides, myosin-1-associated protein phosphatase balance by dephosphorylation of MLCs the phosphorylation effect of MLCKs. The phosphatase must then be inhibited. cAMP reduces MLC phosphorylation.

Nitric oxide augments cGMP concentration and consequently induces a cytoskeleton relaxation and reduces the endothelial permeability. Increase in $[Ca^{++}]_i$ activates endothelial nitric oxide synthase. Furthermore, Ca^{++}–calmodulin removes the inhibition by caveolin-1 on endothelial nitric oxide synthase.

[23] Expression of plasmalemmal receptors is regulated by recycling.

[24] Caveolae, invaginations of the plasma membrane that trap extracellular substances, are abundant in endothelial cells. Caveolae are main locations of PDGF receptors at the platelet surface.

The overall mass transfer is not significantly affected by cell shape, although the transport varies over the cell wetted surface, in a model of mass transfer coupled to a steady flow in a domain of height small enough to assume a homogeneous fluid (plasma flow in a diffusion boundary layer) [929].

9.3 Extravasation

Flowing cells interact between them, with the endothelium and with the subendothelial matrix in inflammation, healing, hemostasis and thrombosis via cell–cell and cell–ECM attachments. The leukocytes are the largest flowing cells, and must strongly deform to travel into the small blood vessels. Involved in body defense, once attached to the endothelium, they must cross tiny gaps between endothelial cells. The leukocyte distribution in small vessel lumen depends on the interaction with the surrounding erythrocytes susceptible to aggregation [930, 931].

Flowing cell adhesion to the endothelium starts by the penetration of the glycocalyx, mainly at the endothelium of post-capillary venules, where endothelial cells have weak tight junctions [932]. Circulating blood cells have adhesion receptors that enable the cells subjected to flow forces to adhere to the vessel wall. The cell-wall interaction is initially reversible. Once tethered to the wall, a cell forms an irreversible adhesion. Flowing cells thus undergo a sequential step extravasation, the kinetics of which is shear dependent. The leukocyte extravasation steps include tethering on the inflamed endothelium, rolling, activation of leukocytes by endothelium-bound chemokines, firm adherence of activated leukocytes to the endothelium, locomotion, diapedesis, and finally transendothelial migration (TEM) with degradation of the subendothelial basement membrane [933].

The endothelium can either favor or inhibit flowing cell adhesion on its wetted surface. Released NO can inhibit adhesion of cells involved in inflam-

Table 9.2. Extravasation-involved adhesion molecules (Source: [936]).

Extravasation step	Adhesion molecules
Capture tethering and rolling	Sialomucin, L-selectin, PSGL-1, α_4-integrin (leukocyte), E- and P-selectins, ICAM (endothelium)
Activation	GPCRs, chemoattractants
Tight binding arrest	β_2- and α_4-Integrins (leukocyte), L-selectin, ICAM (endothelium)
Endothelium crossing	VE-cadherin
Basal lamina crossing ECM migration	β_1- and β_2-Integrins ICAM

mation or coagulation. Endothelial cells continually produce 13-hydroxyoctadecadienoic acid (13-HODE), which confers to the normal endothelium resistance to platelet or monocyte adherence. Conversely, adhesion molecules attract leukocytes toward intercellular spaces for transmigration. During the first stage of extravasation, the cell interacts owing to integrins (Tables 2.8 and 9.2). The rolling step along endothelial cell is a prerequisite for adhesion. The cell then binds firmly to endothelial cells via chemical interactions.

Extravasation Steps

Rolling slows down circulating leukocytes and allows binding of chemokines (CC-chemokine ligand-5 and CXC-chemokine ligand-8) on inflamed endothelium to their G-protein-coupled chemokine receptors on leukocytes. Activated chemokine receptors trigger pathways of leukocyte integrin activation for firm adhesion of leukocytes. Interactions between β_2-integrins and intercellular adhesion molecule-1 and between $\alpha_4\beta_1$-integrins and vascular cell-adhesion molecule-1 are also required for leukocyte adhesion.

Rolling of flowing blood cells is affected by the hemodynamic forces applied at the wetted surface of the vessel lumen. The rolling mainly involves selectins. Hemodynamic forces can favor an extended conformation of certain domains of the selectin structure and thereby strengthen selectin–ligand bonds [934].[25]

During inflammation, resident macrophages produce cytokines, such as tumor-necrosis factor, which quickly induce expression of pre-formed P-selectins and E-selectins[26] on the wetted surface of endothelial cells. The interaction between P-selectins and leukocyte ligands, such as P-selectin glycoprotein ligand PSGL1, initiates rolling on the endothelium. The rolling is stabilized when the formation of new receptor-ligand bonds downstream balance the dissociation of bonds upstream. Rolling is further stabilized by L-selectin binding to heparan sulfate proteoglycans (HSPG) on endothelium wetted surfaces.[27]

Heparan sulfate proteoglycans participate in almost every stage of leukocyte diapedesis (Table 9.3). Like L-selectins, leukocyte integrins CD11b–CD18 bind to HSPGs. Heparan sulfate proteoglycans also present heparan sulfate-bound chemokines, such as CXC-chemokine ligand-8 produced by tissue-resident macrophages, to chemokine receptors on leukocytes. Chemokine

[25] Lectin and epidermal growth factor-like domains of P-selectin show both curved and extended conformations. The extended configuration increases the affinity of P-selectin for its ligand P-selectin glycoprotein-1. It also enhances rolling adhesion on L-selectin under shear flow.

[26] E-, L-, and P-selectin stand for endothelium-, lymphocyte-, and platelet-selectin, respectively.

[27] In vivo, the three selectin types interact with mucins produced by the endothelium. However, heparan sulfate proteoglycans are the dominant L-selectin ligand on the inflamed endothelium during acute inflammation.

binding activates leukocyte integrins, the leukocytes then forming a stable interaction with endothelial cells.

Adherent cell motion between the point of tight adhesion and the junction between endothelial cells requires synergistic actions of CD11a–CD18 ($\alpha_L\beta_2$) and CD11b–CD18 ($\alpha_M\beta_2$) with ICAM1 and ICAM2, respectively, and possibly of CD29 with VCAM. Neutrophils express CD11b and slighly CD29–CD49b, whereas lymphocytes express CD11a and CD29–CD49 [933]. The amine oxidase copper-containing-3 (AOC3),[28] expressed at the cell surface of endothelial cells, regulates leukocyte migration. Furthermore, the number of cells firmly adhering to the endothelium decreases. The cell squeezes to penetrate the cell junction and cross the basal lamina using *platelet endothelial cell adhesion molecule*-1 (PECAM1) and *endothelial junctional adhesion molecules* (JAM).

The transmigration takes 15 to 45 minutes. Once leukocytes have ended their transendothelial migration, they interact with chemokines presented by HSPGs (perlecan, agrin, and collagen-18) in the basement membrane. Leukocyte secrete various proteases, such as matrix metalloproteinases and heparanase. The degradation of the basement membrane results from a collaboration among endothelial cells, leukocytes, and platelets, as these three kinds of cells produce heparanase. Moreover, heparanase releases growth factors

Table 9.3. Main types of heparan sulfate proteoglycans (HSPG). The major plasmalemmal HSPGs are the glycosylphosphatidylinositol-anchored glypicans and transmembrane syndecans. Perlecan, agrin, and collagen-18 are the principal species in the extracellular matrix, especially basement membranes (Source: [902]).

	Location	Function
Syndecan-1	Monocytes, macrophages, neutrophils, eosinophils, lymphocytes, platelets, endothelial cells	L-Selectin ligand Chemokine receptor Signaling
Syndecan-2	Macrophages, endothelial cells	
Glypican	Endothelial cells, macrophages	
Perlecan	Basement membrane, interstitial matrix	Growth factor reservoir
Collagen-18	Basement membrane, interstitial matrix	L-selectin ligand Growth factor reservoir
Agrin	Basement membrane, interstitial matrix	Growth factor reservoir

[28] Amine oxidase copper containing-3 is also known as vascular adhesion protein-1 (VAP1). In AOC3-deficient mice, the rolling velocity of "polynuclear" leukocytes and lymphocytes on the endothelium in inflammation sites is greater than in wild-type mice [935].

bound to basement membrane proteoglycans. These growth factors contribute to angiogenesis and tissue remodeling.

Once stably arrested by their integrin ligands on the TNFα-activated vascular endothelium, leukocytes undergo a cytoskeletal remodeling to most often move to the cleft between endothelial cells, whereas resisting detachment from the vessel wall by the blood flow, and to cross the endothelium.

Activation of coupled Gαi-proteins by ligand-bound receptors is implicated in leukocyte extravasation in response to chemokine gradients.[29] Gαi-coupled receptors are required in rolling and adhesion of leukocytes recruited by chemotaxis on the wetted surface of the endothelium. However, the expression level of vascular cell adhesion molecule-1 and the α4-integrin–VCAM-1-dependent firm adhesion of lymphocytes on endothelial cells do not depend on Gαi signaling. However, Gαi2 in the vascular endothelium favors the diapedesis of firmly adherent lymphocytes [937]. Chemokine-stimulated G-protein-coupled receptors and their effectors, especially GTPases RhoA, Rac, Cdc42, and Rap, act on actin polymerization. In lymphocytes, Rac GTPases activation by integrins and chemokines depends on adaptors DOCK. T lymphocytes use two different chemokine-triggered actin processings: (1) a DOCK-dependent mechanism to move laterally along the endothelium, and (2) a DOCK-independent procedure to cross the endothelium [938].

Extravasation Modes

Leukocyte extravasation requires not only cell adhesion molecules and chemoattractants, but also plasmalemmal enzymatic reactions on both leukocytes and endothelial cells. *Ectoenzymes* (nucleotidases, cyclases, ADP-ribosyltransferases, peptidases, proteases, and oxidases), that have catalytic domains outside the plasmalemma, regulate the cell recruitment [939]. Ectonucleotidases (CD39 and CD73) and adenosine deaminase regulate ATP and adenosine concentrations. Adenosine inhibits adhesion molecules and decreases vascular permeability. Ecto-ADP-ribosyl cyclases (CD38 and CD157) and ectopeptidases (CD10, CD13, and CD26) mediate chemotaxis. Vascular adhesion protein-1 (VAP1 or AOC3), an ecto-oxidase expressed by endothelial cells, regulates leukocyte rolling, firm adhesion and transmigration.

Leukocytes use both paracellular and transcellular extravasations. Transcellular diapedesis is associated with endothelial transmigratory structure that surrounds and guides migrating leukocytes. This villosity-like structure is composed of intercellular adhesion molecules, particularly leukocyte integrins and endothelial IgCAMs [940]. T and B lymphocytes preferentially use the transcellular route. The intermediate filament networks of both lymphocytes and endothelial cells contribute to lymphocyte migration. The intermediate filaments are implicated in the distribution of adhesion molecules (ICAM-1

[29] Gαi1, Gαi2, and Gαi3 subunits are expressed in granulocytes, lymphocytes, airway smooth muscle cells, airway epithelial cells, and endothelial cells.

and VCAM-1 on endothelial cells and integrin β_{v1} on lymphocytes). The intermediate filaments of both endothelial cells and lymphocytes, indeed, form a dynamic anchoring structure at the between-cell contact loci. The initiation of this anchoring structure requires vimentin [941]. Vimentin in both the endothelial cell and lymphocyte stabilizes endothelial cell–lymphocyte interactions and reorganizes the intermediate filament network.

9.4 Hemostasis

Hemostasis[30] includes four components: (1) endothelium, (2) flowing platelets, (3) the plasma coagulation factors, and (4) the fibrinolytic molecules. Several processes are aimed at staunching the blood flow when a blood vessel is wounded: (1) vasoconstriction to reduce blood input in the damaged region; (2) primary hemostasis induced by platelets bound to collagen, which form the hemostatic plug within seconds after an injury; (3) coagulation (secondary hemostasis), i.e., a cascade of reactions involving coagulation factors down to fibrin production to generate a clot after several minutes; (4) repair, as the clot attracts and stimulates the growth of fibroblasts and smooth muscle cells; and (5) fibrinolysis leading to the clot dissolution.

Table 9.4. Antithrombogenic substances and clotting agents secreted by normal and damaged endothelium, respectively. Blood circulation is preserved by a balance between hemorrhage and thrombosis.

Antithrombotic agents	Clotting factors
Protein-C	Tissue factor
Protein-S	von Willebrand factor
Thrombomodulin	Multimerin
Heparan sulfate	Platelet activating factor
Antithrombin	Adhesion molecules
Tissue plasminogen activator	Fibronectin
U-Plasminogen activator	Collagen
NO	endothelin
13-HODE	Clotting factors V and VIII
PGI2	Factor IX- and factor X-receptors
PGE2	Plasminogene activator inhibitor-1 and 2
Lipoprotein-associated coagulation inhibitor	
Tissue factor pathway inhibitor	

[30] Hemostasis is a physiological process that stops hemorrhage. Hemostasis include several processes. Hence, coagulation participates with other mechanisms in arresting bleeding. Hemostasis is characterized by interactions between the vessel wall, platelets, coagulation factors and their inhibitors, and fibrinolytic proteins.

In normal vessels, endothelium prevents clotting (Table 9.4). Endothelial cells have anticoagulant properties because: (1) they form a barrier between the blood and its clotting factors and the vessel wall, and (2) they have thromboregulation function with anticoagulation activity (thrombin inactivation and profibrinolytic activity). Endothelial cells have plasmalemmal substances like *thrombomodulin*[31] (TM), *protein-C* (PC),[32] *lipoprotein-associated coagulation inhibitor* (LAC1),[33] *tissue factor pathway inhibitor* (TFPI), *protease-nexin* (PN),[34] and heparan sulfate. The majority of TFPI is bound to the endothelium, plasma and platelet pools accounting for less than half the vascular content.[35] TFPI reduces activation of FX, without abolishing it, in the

[31] On the surface of the endothelium, thrombomodulin interacts with multiple proteins to inhibit clotting and inflammation [942]. This proteoglycan, once bound to thrombin, promotes thrombin-mediated activation of protein-C, which has anti-coagulant, anti-apoptotic, and anti-inflammatory activities. With co-factor protein-S synthesized by endothelial cells, thrombomodulin activates protein-C which inhibits FV and FVIII, protein-C, and protein-S. Thrombomodulin forms a proteic complex activated by thrombin. The thrombin–thrombomodulin complex also activates TAFI, which inactivates vasoactive peptides such as complement C5a. Furthermore, thrombomodulin inhibits high mobility group box 1 DNA-binding protein (HMGB1), which has cytokine-like activity [943]. Thrombomodulin thus hampers overstimulation of the innate immune response. Thhrombomodulin–HMGB1 binding prevents signaling from the HMGB1 receptor, the receptor for advanced glycation end products (RAGE). Thrombomodulin secretion is shear-dependent.

[32] Thrombin, thrombomodulin, and endothelial protein-C receptor (EPCR) together activate protein-C. Both protein-C and activated protein-C interact with EPCR with a similar affinity. Both thrombin and activated protein-C cleave protease-activated receptor-1 (PAR1), but PAR1 cleavage by thrombin can be more efficient than that of activated protein-C. However, endogenous activated protein-C generated by thrombin on the endothelial cell surface has greater effects than exogenous activated protein-C. The PAR1 cleavage by thrombin yields thrombotic and inflammatory responses. Both endothelial protein-C receptor and protease-activated receptor-1, colocalized in membrane rafts of endothelial cell plasmalemma, are implicated in protein-C signaling [944]. Thrombomodulin, which is located with endothelial protein-C receptor and protease-activated receptor-1 in the same membrane raft, can recruit thrombin, then generate a thrombin–thrombomodulin complex to activate EPCR-bound protein-C. Anti-inflammatory and cytoprotective properties of activated protein-C are due to EPCR-dependent PAR1 cleavage by activated protein-C in endothelial cells. Anticoagulant function of activated protein-C results from degradation of activated clotting factors Va and VIIIa, hence preventing thrombin generation by activated protein C, which is stimulated by protein-S.

[33] LAC1 inhibits tissue thromboplastin.

[34] Protease nexin inhibits urokinase, thrombin, and plasmin. Protease-nexin resembles AT3, but at and near the cell surface.

[35] Platelets contain TFPI, the amount of which is estimated at 5–10% of the plasma level [945].

absence of antithrombin 3 (AT3). TFPI inhibits both FXa[36] and the tissue factor–VIIa complex by rapid formation of TFPI–FXa complex, a potent inhibitor of TF–VIIa complex [947]. Antithrombin-3 is a major plasma inhibitor of FXa. Antithrombin-3 also inhibits thrombin and FIXa. Endothelial cells inhibit platelet aggregation, releasing inhibitors such as prostacyclins[37] (PGI2) and NO. Endothelial cells activate fibrinolysis by binding the *plasminogene activator inhibitor* (PAI), which inhibits the fibrinolytic system. Plasminogene activator inhibitor and $\alpha 2$ antiplasmin ($\alpha 2$AP) are plasmatic enzymes that bind to fibrin and inhibit fibrinolysis. $\alpha 2$ antiplasmin also deactivates plasmin.

The blood can be exposed to clotting stimuli without initiating coagulation. Clotting is initiated on loci larger than a threshold size in the absence of fluid flow [948]. The magnitude of the threshold size can be described by the Damköhler number, which measures competition of reaction and diffusion.[38] Reaction produces activators at the site, and diffusion removes activators from the site.

9.4.1 Blood Coagulation

Upon vascular injury, intracellular materials from damaged cells are released and thus exposed to blood components. Extracellular RNA can promote blood coagulation [949]. It increases the activation of proteases of the contact pathway of blood coagulation, such as factors XII and XI. The von Willebrand factor is able to stop bleeding under high shear flow [950]. The reversible unfolding of this glycoprotein extends its length up to 100 μm, thereby providing a sufficient density of binding sites for collagen. Moreover, the conformational transition occurs in a very short time. The strong increased probability of bound von Willebrand factor favors the formation of a network for blood platelet adhesion.

The coagulation cascade takes place at the site of a vessel wall gap where platelets aggregate (Fig. 9.2). Several clotting factors are required (Table 9.5). Endothelial cells synthesize coagulation factors, *von Willebrand factor* (vWF) and *tissue factor*, which activate FVII. When the endothelium is damaged, blood must clot to halt bleeding. Blood clotting involves circulating platelets that are able to clump. The hemostatic process depends on the stable adhesion and aggregation of platelets with the subendothelial matrix molecules at the vessel injury site.

[36] FXa is an activator of TF–VII complex, which is blocked by AT3 in the presence of heparin [946].

[37] Prostacyclin release from endothelial cells is enhanced when they are subjected to pulsatile flow compared with steady flow.

[38] The Damköhler number, a measure of the rate of reaction of a molecule relative to diffusion, is also called the Thiole modulus. The Biot number is a measure of the rate of external transfer of a substance with respect to the diffusion rate.

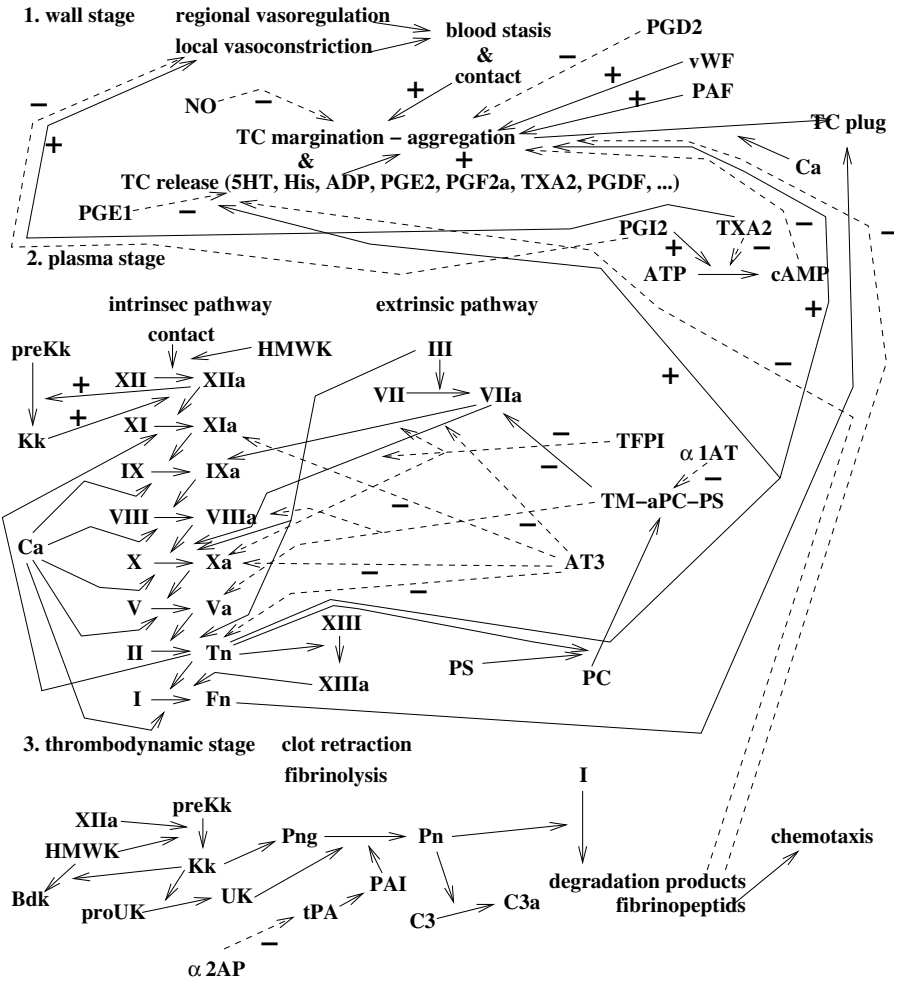

Figure 9.2. Coagulation and fibrinolytic events. The "contact phase" proteins include factors XII and XI, prekallikrein, and high-molecular-weight kininogen (HMWK).

Platelet adhesion receptor GP1bα,[39] which binds to von Willebrand factor,[40] can act independently of von Willebrand factor for platelet recruitment

[39] GP1b-5-9 receptor complex consists of four components: GP1bα, GP1β, GP5, and GP9. It allows interaction of resting platelets with activated leukocytes via Mac1 and activated endothelial cells via P-selectin. GP1bα can associate to thrombin, high-molecular-weight kininogen, coagulation factors XI and XII, and thrombospondin-1. Ligand-binding activates GP1b-5-9 receptor complex for calcium mobilization, cytoskeleton rearrangement, granule release, and activation of $\alpha_{II}\beta_3$-integrin, the main integrin implicated in platelet aggregation, which

under high shear stress in sites of exposed subendothelium after vascular injury and adhesion to the extracellular matrix and growing thrombus [951].

The coagulation cascade is a step-by-step complex process induced by blood vessel injury that produces a solid fibrin clot to cover the damaged blood vessel and stop hemorrhage.

`Primary hemostasis` refers to the plug formed by platelets at the site of injury. Various plasma clotting factors then respond to form fibrin strands that strengthen the platelet plug. Primary hemostasis is initiated when platelets adhere to collagen fibers, using a specific platelet collagen receptor GP Ia/IIa.[41] This adhesion is stabilized by vWF, which forms links between the platelet GP Ib/IX/X and collagen fibrils. The platelets are then activated and secrete granule contents into the plasma. Fibrinogen (Fng) links adjacent platelets by binding with GP IIb/IIIa. Primary hemostasis thus involves a set of adhesion receptors (GPs Ib, IX, X, integrin $\alpha_{11b}\beta_3$, etc.) and proteins (vWF, Cn, FN, Fng, Ln, TSP2, etc.). ADP, release from activated platelets, is a potent inducer of platelet aggregation (self-perpetuating process). PAF and Ad acting together relieve the effect of inhibitors of platelet aggregation.

`Secondary hemostasis` has two pathways, intrinsic and extrinsic, which join in a common pathway leading to fibrin (Fn) formation. The `intrinsic pathway` is characterized by the formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein (preKK), and FXII (Hageman factor). FXII cleaves into two fragments, one is fixed on the wound edge for molecule adhesion, the other becomes a protease that activates FXI, which, in turn, becomes a protease, etc. (activation cascade; Fig. 9.2) until fibrinogen is transformed into fibrin to form a clot. More precisely, FXII is activated in FXIIa. FXIIa converts FXI into FXIa, which activates FIX in FIXa. Reacting platelet surface phospholipids accelerate intrinsic coagulation. In the `extrinsic pathway`, FVII is activated by *tissue factor* (TF), which is released from cellular membranes during injury. FVIIa activates FIX and FX. The `common pathway` begins with activation of FX by FIXa and/or FVIIa. The process requires FVIIIa. Thrombin (Tn) is then produced.[42] FXa activates Tn, requiring FVa from FV, via a positive feedback loop by Tn itself like FVIII. Tn not only converts Fng to Fn but also activates FVIII, FV and their

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interacts with von Willebrand factor. Collagen receptor GP6 needs GP1bα for platelet adhesion.

[40] von Willebrand factor also carries coagulation factor VIII at sites of vascular lesion and protects it against proteolysis.

[41] Tethering and rolling phases of platelet adhesion depend on bindings between the platelet and vessel wall elements. The bond formation is firstly reversible with fast association and dissociation rates. vWF–GP bindings initiate activation of integrins $\alpha_{11b}\beta_3$ with slow rates of bond formation and destruction, and mediate irreversible adhesion [952].

[42] Thrombin binds to G-protein-coupled protease–activated receptors (PAR). Thrombin activation of PAR1 requires ADP to stimulate Gi.

Table 9.5. Coagulation factors (Source: [953]).

Coagulation factors	Targets
I (fibrinogen [Fng])	Fn
II (prothrombin)	I, V, VII, XIII, PC, platelets
III (tissue thromboplastin)	
IV (Ca^{++})	V, VIII, IX, X
V (proaccelerin)	X, II
VI (accelerin or Va)	
VII (proconvertin)	IX, X
VIII (antihemophilic factor A)	IX, X
IX (antihemophilic factor B) (plasma thromboplastic component)	X
X (Stuart-Prower factor) (prothrombin converting enzyme)	II
XI (PTA) (plasma thromboplastin antecedent)	XII, preKK
XII (Hageman or contact factor)	XIa, preKK, Fn
XIII (fibrin-stabilizing factor)	Fn
von Willebrand factor (vWF)	VIII, platelet
PreKK	XIIa, preKK, HMWK
HMWK	XII, XI, preKK
Antithrombin-3 (AT3)	IIa, Xa
Heparin cofactor II	IIa
Protein-C (PC)	V, VIII
Protein-S (PS)	PC
Plasminogen (Png)	Pn, Fn
α2-Antiplasmin	Pn
Prourokinase (ProUK)	Png
Tissue plasminogen activator (tPA)	Png
PAI1	Png, tPA
PAI2	Urokinase (UK)

inhibitor PC as well as platelets. Fibrin strands appear where the platelets adhere, change in shape and aggregate.

Coagulation is a controlled process. Co-factors and inhibitors are required for the coagulation cascade. Calcium and phospholipids, membrane constituents, are co-factors to the activation of FVII, FIX, FX, and FII. Such interactions are possible if vitamin K is acting on factor synthesis. Thrombin leads to a positive feedback of clotting by activating platelets and FXI and inhibits coagulation by stimulating protein C (aPC) via thrombomodulin binding. Activated protein-C binds to protein S and degrades FVa and FVIIIa.

Antithrombin is a serine protease inhibitor that degrades thrombin, FXa, FXIIa, and FXIa. Its action is enhanced by heparan sulfates and heparins. Tissue factor pathway inhibitor (TFPI) inhibits F VIIa-related activation of

FIX and FX. TFPI synthesis occurs in the vascular endothelium (which also expresses the tissue factor). Serotonin inhibits platelet release of ADP. PGE1 and fibrin degradation products inhibit platelet release. Furthermore, fibrin degradation products also impede platelet aggregation.

9.4.2 Fibrinolysis

Fibrinolysis occurs after coagulation during healing. It involves plasmin, which cleaves the fibrin clot. Plasmin is produced from plasminogen, which is synthesized in the liver, by tissue plasminogen activator[43] (tPA). α2-Antiplasmin and PAI inhibit tPA. Endothelial cells activate fibrinolysis by binding PAI. Activated protein-C deactivates PAI1. FXIIIa binds to fibrin and protects it from plasmin.

9.4.3 Thrombosis

Thrombi are formed in blood vessels and cardiac cavities from clotting factors using the same procedure as coagulation. The contact between the blood and an unusual material triggers the clotting reaction cascade, activating platelets quickly (in few seconds) adhering to the endothelial gap. Mural thrombi are dominantly composed of platelets. Platelets bind fibrinogen which forms fibrin strands, co-factors in platelet aggregation. Tissue factor and plasma fibronectin are also involved in thrombus growth.

When the blood flow is high enough, the microthrombi do not strongly adhere to the wall and can be destroyed by the fibrinolytic system. When the blood flow is low, the competition between activation and inhibition of coagulation favors thrombus formation. A thrombus can develop up to block the vessel lumen and then induce ischemia of the irrigated tissues. Thrombus portions can detach; they are then carried in the bloodstream as emboli (embolization). Emboli can block a vessel segment downstream from the thrombus site. Local or remote blockades cause infarction of the tissues normally irrigated by the corresponding blood vessels.

Platelet behavior with regard to the vasculature wall is also based on a balance between stimulation and inhibition of the different stages of the reaction chain of the coagulation. For instance, endoperoxids, precursors of prostaglandins, are used either by the platelet enzymes to produce thromboxane A2 (TXA2), which favors aggregation, or by the endothelium enzymes to synthesize PGI2 which inhibits platelet aggregation.

[43] When stroke occurs, glutamate is released by ischemic neurons. Glutamate induces an excess in receptor stimulation and a subsequent calcium ion influx. The latter leads to neuron death. tPA is produced by endothelial cells and neurons. tPA released by damaged neurons increases glutamate effects. Therefore, tPA administration after stroke must be carefully handled.

Type Ia phosphoinositide 3-kinase[44] PI3Kp110β isoform regulates the formation and stability of αIIbβ3-integrin adhesion bonds, involved in shear activation of platelets [954]. PI3Kp110β inhibitors have been developed that lead to defective thrombus formation.

9.4.4 Mathematical Modeling and Numerical Simulations

Mathematical multiscale[45] models of either clotting on a breach of the vessel wall or thrombosis after a rupture of an atherosclerotic plaque has been developed in the presence of a flow of an incompressible viscous fluid [955, 956]. In the explored fluid domain, the model set incorporates the platelets and coagulation factors, that are involved in the chemical reaction chain, each step being triggered by the activation of the corresponding zymogen into enzyme, and coagulation inhibitors. Compounds and platelet transport by convection and diffusion, are assumed to take place in a near-wall thin plasma layer. Competition occurs between the activation of the coagulation stages and removal by the flowing fluid of the clotting factors and cells away from the reaction site.[46] Numerical simulations use the immersed boundary method (Part II). Adhesion of platelets to the injured wall and cohesion between activated platelets are modeled by distributed elastic links.[47] Strain-dependent breaking of cohesive bonds between platelets and adhesive links between the platelet and the vessel wall are treated by a closure approximation of the evolution equations. The probability of platelet aggregation increases quickly after activation, remains nearly maximum for a significant time interval, and then declines [957]. The fibrinogen concentration and density in surface receptors strongly affect the time constant of platelet aggregation. The thrombin production depends on available binding sites. Thrombus growth, with possible vessel occlusion, and embolus shedding from the thrombus can be predicted by the stress field exerted by the moving fluid on the thrombus.

Interactions between mechanical factors introduced by the flowing blood and the biochemical agents with their multiple positive and negative feedbacks and regulators have also been mathematically modeled, not only during clot formation but also degradation [958, 959]. Clot reaction kinetics are affected by shear stresses. Shear stress-dependent platelet transport and activation, the

[44] The class I PI3Ks produce the second messengers phosphoinositide PI(3,4,5)P3 and PI(3,4)P2, and regulate platelet responses, such as the activation of integrin αIIbβ3 (platelet GPIIb-IIIa), a major platelet integrin that mediates platelet-vessel wall and platelet–platelet adhesive interactions through multiple ligands, including von Willebrand factor, fibrinogen, fibronectin, and CD40L. Platelets contain all class I PI3K isoforms.

[45] The model requires several spatial scales: the nanoscale of the coagulation factors, the microscale of the platelets, and the macroscale of the blood vessel.

[46] The flux of activated coagulation factors and platelets is flow dependent.

[47] Platelets aggregate assuming that bridges form isotropically, linear springs modeling the clusters of binding sites.

extrinsic pathway of coagulation leading to a viscoelastic clot and fibrinolysis[48] are represented by a set of coupled advection–diffusion–reaction equations, assuming a uniform distribution of platelets, reactants, and regulators in a shear-thinning viscoelastic blood for a given wall flux of stimulating agents.

9.5 Mechanotransduction

Any cell acts on its environments and vice versa. Mechanical forces, such as forces applied by adjoining cells or the surrounding matrix, as well as forces developed by a given cell on its matrix or contacting cells, are transmitted (Sect. 4.15). The soft extracellular matrix contains a three-dimensional fibrous mesh. Living cells not only sense applied forces and respond to received signals, but also evaluate the mechanical properties of their matrix, in particular the stiffness of the extracellular matrix.[49] Moreover, the cell can recognize a local membrane curvature and recruit small guanosine triphosphatases to the curved parts of the plasmalemma [960]. Fiber curvature can induce cell-membrane curvature. Besides, certain K^+ channels are opened by a convex curvature of the membrane.

Furthermore, endothelial cells have a wetted surface that undergoes flow forces. Blood vessel walls are permanently subjected to tension and shear. Mechanotransduction starts with mechanosensing and leads to a relatively quick mechanoresponse. Molecular sensing of forces are transduced into biochemical signals that drive cell responses to properly shape cells and tissues and trigger suitable cell functions. The force can act not only by its magnitude and direction but also by the loading rate.

Mechanical forces regulate cell function so that the vessel wall restores basal stress conditions (Fig. 9.3). The cell change its shape and adapts to the mechanical loading. The cytoskeleton of mural cells transmits and modulates stresses within the cell via cellular junctions and cell-ECM adhesion sites. Moreover, applied forces initiate mechanotransduction cascades leading to transcription factor activation and subsequent gene expression.

Mechanosensing occurs locally at the plasmalemma. It can be done by conformational changes in plasmalemmal proteins and can involve cytoskeletal filaments that propagate loadings down to the nucleus.[50] Force sensing can be processed by: (1) partial protein unfolding with gain or loss of binding sites or of enzyme function; (2) opening of mechanosensitive ion channels due to membrane tension or to stress applied by force-bearing filaments connected to channels; and (3) strained receptor–ligand interactions [960].

[48] Fibrinolysis occurs when fibrin concentration decays below a given threshold or the local shear stress reaches a critical value that depends on concentrations of platelets and fibrin.

[49] Different cell types respond differently to matrix rigidity.

[50] Cytoskeletal early responses take seconds to minutes.

The endothelium provides a link between blood flow and vessel responses, in particular the vessel caliber. Multiple kinds of mechanical stresses and various types of mechanical environments are associated with flow patterns and unsteadiness. The vessel wall is sheared by the moving blood particles on the one hand and stretched and compressed by the pressure applied by the blood. Mechanical stresses applied to the endothelium wetted surface are indeed normal (mostly pressure) and tangential (shear). Wall friction fluctuates in magnitude and in direction at a given location and from point to point. Pressure and tensile reactions also undergo quasi-periodic fluctuations. The stresses applied by the blood continuum[51] are transmitted throughout

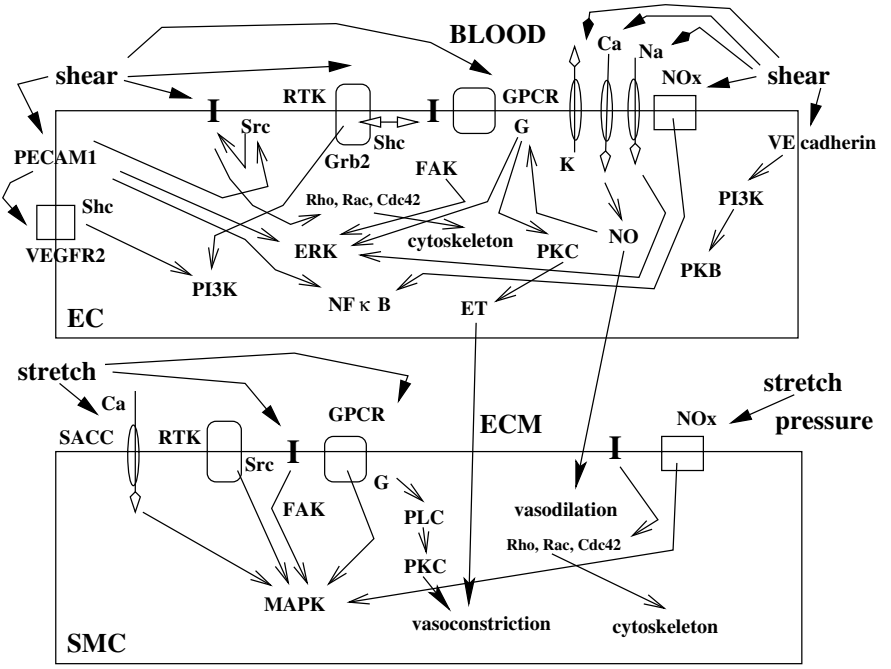

Figure 9.3. Effects of blood pressure (characterized by its large magnitude) on the vessel wall cells, and of three-dimensional shear (of much smaller magnitude than the applied pressure; but with large space gradients) on the wetted endothelial cells. Both forces undergo large amplitude oscillations during the cardiac cycle. Direction changes can also occur (flow separation, flow reversal during the diastole). I: integrin.

[51] When the flow scale is much greater than the circulating cell size, the blood can be considered as a continuum with given apparent physical properties, which depend on the microstructure of the concentrated suspension. The microstructure depends on the local stress field.

the wall continuum, which reacts. Intramural stresses are composed of axial, radial, and circumferential components[52] (Table 9.6).

The three following substances, *manganese superoxide dismutase*, *cyclooxygenase*-2, and *nitric oxide synthase*, which have atheroprotective activities (antioxidant, antithrombotic, and antiadhesive) react differently according to the type of fluid mechanical stimulus [962]. Mechanical removal of the endothelium by various means, keeping the functional integrity of the endothelium, abolishes the flow-dependent response (but not the reactions of the wall cells to acetylcholine and noradrenaline stimulations) [963]. The shear stress and pressure exerted on the wall by the blood generate a basal tone of smooth muscle cells in the absence of neurogenic and hormonal influences. Local metabolism and appropriate ion ($[Ca^{++}]$, $[Na^{+}]$) concentrations are required. The hemodynamic stresses act on the smooth muscle cells via stress transmission or compound release by the endothelial cells.

The EC membrane is the first wall component to bear stresses from the circulating blood.[53] The bulk cell content can be supposed to be a fluid-like

Table 9.6. Circumferential tension T_θ per unit length estimated by the Laplace law at a first approximation, assuming a membrane-like behavior ($T_\theta \sim hc_\theta \sim p_i R$).

Vessel	p_i (kPa)	R (mm)	T_θ (Pa)
Aorta Large arteries	13.3	10	133
Small arteries	11.3	2	22.6
Arterioles	8	0.25	2
Capillaries	4	0.01	0.04
Venules	2.7	0.25	0.7
Veins	2	3	6
Vena cava	1.3	15	20

[52] For a cat thoracic aorta at a pressure of 16 kPa, with an axial stretch ratio of $\sim$ 1.7 and an opening angle of $\sim$ 70 degrees in the unstressed state, intramural longitudinal and circumferential tensile Cauchy stress and radial compressive stress at outer and at inner wall have been found to be 95 and 71, 105 and 61, and -16 and 0, respectively [961].

[53] The membrane can be subdivided into three parts according to its neighborhood. A luminal part is subjected to hemodynamic stresses, with a segment protruding above the average cell height due to the deformed nucleus. The EC nucleus in the stress field is elongated, like the whole cell, in the streamwise direction. The abluminal part, which adheres to BM, and the sides, which have a given inclination and are connected to adjoining EC membranes, are subjected to stresses imposed by extracellular matrix and adjacent endothelial segments.

material containing solid particles and a cytoskeleton, wrapped into a solid-like membrane.[54] Membrane tension has been estimated to be three orders of magnitude higher than wall shear stress imposed by the flowing blood [964]. Membrane tension is affected by transmembrane static and osmotic pressures. It also at least partially controls osmotic pressures. Membrane tension influences not only the cytosol[55] and nucleus configurations by stress transmission, the cytoskeleton being moored to anchoring membrane proteins, but also the mass transport across the cell membrane, via pores, channels, or vesicles, and, thus, cell metabolism. Interactions thus occur between cell internal structures and messengers using electrochemical and biochemical processes.

Mechanical stresses alter structure and function at both cellular and molecular levels. Stresses can directly act on stretch-activated channels (SAC), which are sensitive to tension imposed on cell membrane[56] and on shear stress-activated K^+ channel (SSAKC) [965]. Mechanosensitive ion channels can be opened by membrane stretch. They convert external mechanical forces into electrochemical signals in the cell. Mechanical factors act not only on molecule transport from the flowing blood to the endothelium surface, but also on transendothelial transfer and through the wall directly by interface deformation. Stresses induce conformational changes of membrane proteins (ion channels, adhesion molecules, etc.), proteins associated with membrane proteins, and/or proteins associated with the cytoskeleton. These changes lead to biochemical responses.

Because arteries convey pulsatile flows, arterial walls are subjected to tensile strains, with steady and time-dependent components. High steady intraluminal pressures (~ 20 kPa) activate focal adhesion kinases. Phosphorylated FAKs bind to Grb2 and stimulate a pathway that involves extracellular signal-regulated kinase-1/2, Src and integrins [967]. However, FAKs are not implicated in cyclic stretches, although ERK1/2 is activated.

Steady pressurization[57] of isolated rat cerebral and mesenteric arteries leads to decreased cFos expression in the cerebral arteries subjected to 18.6 kPa compared with 10.6 kPa (control), but not in mesenteric arteries [968]. Stretch-mediated signaling by *elastin–laminin receptors* decreases cFos expression and subsequent cellular proliferation. Mechanosensitive signaling via elastin–laminin receptors hence depends on the artery type and/or perfusion territory (cerebral circulation vs. mesenteric circulation).

[54] EC membrane can be assumed to be a solid-like material because it resists applied stresses by deformation and not by flow.

[55] The configuration of the marginal fiber network may be imposed by the directions of principal stresses undergone by the membrane to which it adheres.

[56] Other stretch-sensitive ion channels are implicated in mechanotransduction and osmoregulation. They include stretch-inactivated K^+ channels, which have been described in neurons [966].

[57] Isolated arteries are subjected to 8.6 kPa during 2 h, then to a step pressure increase of either 10.6 or 18.6 kPa during 30 mn.

Shear stress and intraluminal pressure either promote or repress gene expression in the endothelial cells, a small fraction of targeted genes responding to both pressure and shear stimulation [969]. The two stimuli induce distinct gene expressions. Wall shear and stretch promote expression of transcription activators or repressors *cFos* and *cJun*, which bind DNA transcription promoters. Shear stresses also activate another transcription promoter, the shear stress response element [970]. Shear stress response elements are found in the promoter of platelet-derived growth factor-A and -B, tPA, TGFβ1, and ICAM1.

9.5.1 Transducers

Several molecules have been shown to be targeted during the mechanotransduction (Fig. 9.4). They are located: (1) either on the endothelium wetted surface; (2) on the lateral walls (cell–cell junctions); (3) on the abluminal membrane (cell–matrix junctions), or (4) within the cytoplasma. 3D conformational changes of membrane proteins induced by applied stresses might initiate the cell response. Mechanical transduction uses a set of messengers and leads to gene transcription.

Various mechanosensors detect stresses and strains applied to the endothelial cells (Fig. 9.4). Transduction surface elements include: (1) mechanosensitive ion carriers, especially stress-gated ion channels, (2) plasmalemmal receptors, such as receptor tyrosine kinases and G-protein-coupled receptors, and (3) adhesion molecules. Stimulated mechanosensors initiate different signaling pathways to trigger responses.

9.5.1.1 Plasmalemmal Receptors and Carriers

The density in pressure-activated cation channel increases in endothelium subjected to high pressure, with complete reversal by antihypertensive therapy [971]. Mechanosensitive potassium channels modulate plasmalemmal NAD(P)H oxidase (NOx) [972]. Stretch-activated Ca^{++} channels are located near integrins. Gαq proteins participate in flow-induced Ras activation [973]. Heparan sulfate, the dominant glycosaminoglycan of the glycocalyx, participates in mechanosensing for NO synthesis in response to shear stress [974]. Plasmalemmal microdomains can be implicated in mechanotransduction. Caveolae are sites for endothelial nitric oxide synthase, cyclooxygenase-2, and prostacyclin synthase. Caveolin-1 mediate shear stress effects by activating extracellular-regulated kinases ERK1/2, but not cJun NH2-terminal kinase, which can also be activated by the wall shear stress [975]. The integrin–cytoskeleton couple acts as a mechanosensor. Moreover, forces applied to endothelial cells induce assembly and extension of adhesive structures. Cell adhesion molecules are targeted for transmembrane signaling, using various biochemical cascades, such as focal adhesion kinase-Src and Rho pathways.

The wall shear stress acts on the wetted membrane via receptor tyrosine kinases [978, 979], caveolin-1 on extracellular signal-regulated kinase pathway [980], and guanine nucleotide-binding proteins [869].

The wall shear stress activates mechanosensitive K^+ channels involved in TGFβ1 production [981], Na^+ channels implicated in ERK1/2 activation [982], and Cl^- channels [983]. Voltage-gated K^+ inward rectifier channels (Kir2.1) of endothelial cells are modulated by shear stress [984]. Hyperpolarization precedes release of vasoactive substances. Kir2.1 activity can also be modulated by protein kinases PKA and PKC, tyrosine kinase Pyk, and phosphatases.[58] Shear current induction is stopped by channel dephosphorylation by phosphatase, which thus favors a shear-unresponsive channel state.

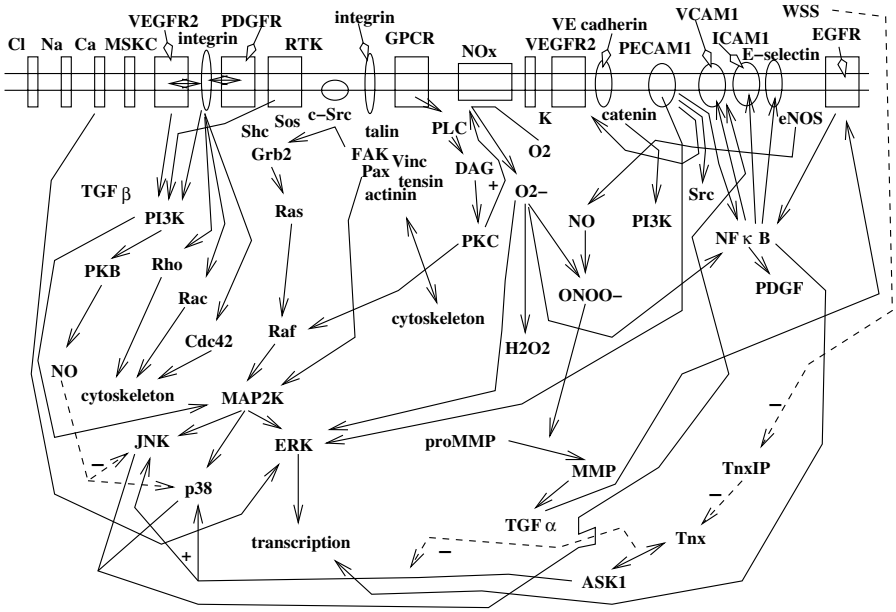

Figure 9.4. Biochemical pathways stimulated by applied pressure and shear. Initiation is done via: (1) ion channels; (2) growth factor receptors and others tyrosine kinase receptors (RTK); (3) G-protein-coupled receptors (GPCR); (4) membrane NADH/NADPH oxidase (NOx); and (5) adhesion molecules, mainly integrins, but also VE cadherins and IgCAMs. Effectors include small GTPases, PI3K, PKB, PKC, NO, FAKs, TGF, and O_2^- (O2−). Main targets are the transcription factors for cell and tissue remodeling (with possible degradation via MMPs after long-duration pressure rise), such as NFκB, the cytoskeleton, adhesion molecules, and ion channels (Sources: [362, 976, 977]).

[58] Protein kinase-A controls the links between Kir2.1 channel and its scaffold proteins AKAP and SAP. Pyk kinase is associated with SAP. Scaffold proteins allow formation of proteic complexes with ion channels, receptors, and enzymes to quickly tranduce signals.

9.5.1.2 Adhesion Molecules

The wall shear stress uses also adhesion molecules of the intercellular and the cell-matrix communications as transducer components (Fig. 9.4). Adhesion molecules, which link the endothelial cells either to neighbor endothelial cells or the basal lamina, transmit the sensed mechanical forces and adapt. Vascular endothelial cell cadherins, PECAM1 and integrins $\alpha_v\beta_3$ are particularly involved [976]. The stronger the changes in WSS amplitude and direction, the more sustained the activation. The pathways mediated by adhesion molecules comprises vascular endothelial growth factor receptor kinase (VEGFR2), which activates phosphatidylinositol 3-kinase, Src, ERK, JNK, p38, and PKB, as well as transcription factors, such as NFκB.

PECAM1 and VE-cadherin on the lateral membrane of the endothelial cell are targeted by shear. PECAM1 is phosphorylated when endothelial cells are exposed to WSS. This PECAM1 phosphorylation leads to ERK activation [985]. VE-cadherins of adherens junctions form a complex with β-catenins, phosphatidylinositol 3-kinase, and VEGF receptor-2 for signal transmission to PKB and BCL2 [986]. The interaction of α-catenin with VE-cadherin-bound β-catenin is regulated by phosphorylation of β-catenin shear-modulated phosphatase SHP2 associated with VE-cadherin complexes [987].

$\alpha_5\beta_1$ Integrins enhance shear stress-dependent cell migration [988]. $\alpha_v\beta_3$ integrins are involved in shear stress phosphorylation of VEGFR2 and its binding to adapter Shc [979], and in the shear stress activation of IκB kinases[59] and NFκB [990]. NFκB activation is mediated by time and magnitude changes in the wall shear stress. Ras GTPases and the inhibitor IκBα are involved (IKK intercompartmental transport being regulated by Ras GTPase) [991]. Last but not least, the cytoskeleton transduces force signaling to the different cell compartments [992].

9.5.1.3 Cell Junctions

Cell communications exist between: (1) neighboring endothelial cells, (2) adjoining smooth muscle cells, and (3) endothelial cells and smooth muscle cells through IEL fenestrae. Shear stresses imposed on monolayers of endothelial cells increase the hydraulic conductivity of the endothelium, acting on the paracellular transport by altering the state of the occludins of the tight junctions. After several minutes, the shear stress exposure increases occludin phosphorylation [993].

Cadherin-bound plakoglobin and Ca^{++} stabilize the cell adherens junction between cultured neighboring endothelial cells subjected to shear stresses [994]. The depletion in $[Ca^{++}]_e$, which causes the release of both cadherins and

[59] The inhibitors of NFκB (IκB α, β, and ϵ) inhibit by cytoplasmic retention the expression of NFκB-dependent genes, hampering the binding of p65 to DNA [989].

plakoglobin away from cell junctions and the inhibition of plakoglobin expression, induce dissociation of the endothelial layer subjected to flow. β-Catenin does not compensate for plakoglobin loss. Besides, PECAM1-mediated adhesion cannot ensure endothelium integrity under flow.

Stress fiber bundles in endothelial cells subjected to blood flow develop and align in the direction of the bloodstream. The cytoskeleton fibers associated with tight junctions between endothelial cells are reinforced and elongated [995].

9.5.2 Flow Chambers

To understand the flow-dependent functions of the endothelium and evaluate gene and protein expressions, EC cultures are exposed to stresses in flow chambers. In vitro effects of flow over cultured EC layer and cyclic stretch of the culture support have been investigated to study the responses of endothelial cells in well-defined mechanical conditions. Stresses applied on EC wetted or on abluminal surface affect: (1) cell shape and orientation[60] [996], as well as the cell ultrastructure[61] [998], (2) cell rheology [999, 1000], endothelial cells becoming stiffer, (3) cell proliferation, (4) cell metabolism and transport, and (5) cell adhesion to its support and matrix content.[62] Time estimations of EC responses to imposed stresses are given in Table 9.7.

Endothelial cells respond, in particular, to space and time changes in wall shear stress imposed at its luminal surface. WSS magnitude and orientation depend both on vessel axial and transverse configurations and flow pattern. Wall shear stress is equal to zero at separation and reattachment points. Change in direction of the WSS axial component occurs when the pulsatile flow generates a back flow in the whole vessel lumen or a layer near the wall. A WSS circumferential component appears when the vessel presents either a curvature of its axis and/or its walls (transition upstream from branching, taper, neighbor organ print, tube collapse, etc.). Cross variations in wall curvature, which are observed in collapsed tubes of uniform cross section, due to wall transverse bending, induce transverse gradient of the WSS axial component [1002, 1003]. Many investigations have been performed to explore either cell rheology or the behavior of mechanical stress-subjected cells. Flow chambers coated with a homogeneous confluent monolayer of EC cultures are used for EC exposure to laminar shear stresses [1004–1007]. A special flow chamber has been designed to expose endothelial cells to a shear stress that generates two forces applied at the cell inertia center: (1) a shear force that stretches the cell in the streamwise direction, e.g., the force arising from the

[60] The orientation of the cultured endothelial cell under flow conditions is a shear minimization process [997].

[61] EC elongation and orientation, as well as the remodeled cytoskeleton arrangment, reflect the local flow direction.

[62] The synthesis of junctional proteins increases. WSS influences the expression of growth factors, and thus wall remodeling, with its elastin and collagen content.

shear stress at the cell inertia center; and (2) a shear torque that twists the cell perpendicularly to the cell plane, induced by the WSS transverse gradient (WSSTG) [1008]. Four regions of the wetted cell culture were defined by these investigators: (1) region I near the contact point where both WSS and WSSTG are small; (2) region II of low velocity flow where WSS is low but WSSTG is significant; (3) region III with a change in wall curvature sign where both WSS and WSSTG are great; and (4) region IV, where WSS reaches its highest values but WSSTG is small. In region II, these authors discussed the role of torsion in the EC detachment both from the wall of the flow chamber and the cell layer, i.e., from the adjoining endothelial cells. WSS varies along the EC surface between the peripheral region and the central cell bulging zone associated with the nucleus. Atomic force microscopy has been used to study the shear distribution at a subcellular scale; steady flow was simulated over the EC surface [1009].

Endothelial cells exposed to shear stress using a shaker react according to their location [1010]. Orbital shear stress increased EC proliferation and adhesion molecule expression, whereas it reduced PKB phosphorylation and E-selectin downregulation in central endothelial cells exposed to lower shear stresses, with respect to peripheral endothelial cells. Studying the responses

Table 9.7. Mechanotransduction in cultured endothelial cells. Order of magnitude of response time (Sources: [998, 1001]).

Target	Ttime
K^+ channel SAC	$\mathcal{O}$(ms)
$[Ca^{++}]_i$ NO	$\mathcal{O}$(s)
IP3	$\mathcal{O}$(10 s)
Adenylyl cyclase PGI2, PGF2α	$\mathcal{O}$(mn)
PDGF ET tPA Pinocytosis Cell proliferation Cytoskeleton alignment	Hours
FN LDL metabolism Cell alignment	Day
Cn Cell growth	Days

of the endothelial cells to step flows, impulse flows, ramp flows, inverse ramp flows, and pulsatile flows,[63] it has been shown that the time derivative of the wall shear stress, and not the shear stress itself, is directly responsible for EC reactions [1011]. Both extracellular signal-regulated kinase (ERK1/2) phosphorylation and endothelin-1 release are attenuated when the duration of the pulse cycle decreases [1012].

In static conditions, the features of extracellular matrix affects cell adhesion and spreading, differentiation, migration, and apoptosis.[64] When endothelial cells are subjected to flow, the directionality of hemodynamic stresses governs their morphology, as well as their functioning and fate (survival/apoptosis).[65] In flow chambers, cultured endothelial cells on strips of fibronectin on silicone membrane react differently according to the flow direction with respect to strip axis. Pulsatile flow parallel to strip axis (pressure modulation rate of 1/3, after 12 hours under static conditions) cause cell elongation, increase in stress fibers and phosphorylated focal adhesion kinases, and apoptosis reduction [1013]. Pulsatile flow perpendicular to strip axis does not induce such changes. Constitutive GTPase RhoV14 augments the stress actin fiber formation and FAK phosphorylation,[66] and attenuates apoptosis under both static conditions and flow, whatever its direction (parallel or perpendicular to strip axis).

Support and perfusion media used in flow chambers can bring substances that can interfere with the cell response to the investigated stimulus. Consequently, experimental testing and result interpretation must be carefully handled.

9.5.3 Arginine and Nitric Oxide

Arginine (intracellular concentration 0.8–2 mM) is a component of proteins. Arginine is also a precursor of signaling molecules, NO, glutamate, and agmatine. L-Arginine decarboxylation by arginine decarboxylase forms agmatine.

[63] Step increment in shear stress from 0 to $1.6\,\mathrm{N/m^2}$ are followed by a sustained steady shear. Ramp shear is defined by a gradual increase in shear stress from 0 to $1.6\,\mathrm{N/m^2}$ over 2 mn, the loading being sustained afterward. Impulse flow means a a loading over 3 s of $1.6\,\mathrm{N/m^2}$. Pulsatile flows correspond to repeated impulse flows.

[64] Human umbilical vein endothelial cells have been cultured on strips of fibronectin on silicone membrane. On broader strips (width 30 and 60 μm), endothelial cells create actin stress fibers with anchoring spots of phosphorylated focal adhesion kinases and do no present significant apoptosis [1013]. On narrower strips (width 15 μm), endothelial cells develop few stress fibers and do no present phosphorylated focal adhesion kinases but significant apoptosis.

[65] The smaller the ability to form stress fibers and attachments with the extracellular matrix, the higher the apoptosis rate. Apoptosis leads to the disassembly of focal adhesion complexes and the assembly of actin into a peripheral ring.

[66] Rho GTPases cause FAK phosphorylation, which augments the onset of stress fiber formation.

Agmatine induces calcium uptake and stimulates nitric oxide synthase in endothelial cells [1014]. The extracellular pool of L-arginine must be mobilized and supply eNOS sites for NO production, which also depends on calcium ions. Agmatine is a ligand for imidazoline receptors and α2-adrenoreceptors.

Arginine behaves both as a substrate of biosynthesis (intracellular arginine) and as a receptor ligand (extracellular arginine), which initiates signal transduction. Arginine, like its products glutamate and agmatine, is able to bind to plasmalemmal receptors, possibly imidazoline receptors, which are nonadrenergic receptors, and α2-adrenoreceptors, which are G-protein-coupled receptors [1015].[67] Both imidazoline receptors and α2-adrenoreceptors activate eNOS, using calcium influx via phospholipase-C and second messenger.

Nitric oxide (NO) is produced by endothelial cells, smooth muscle cells, cardiomyocytes, and other cell types from L-arginine (L-Arg) by nitric oxide synthase. NOS catalyzes the conversion of L-arginine to NO and L-citrulline (Fig 9.5). There are two kindss of NOS: constitutive (cNOS) and inducible (iNOS). Ca^{++}–Cam-dependent NOS are found in endothelial cells (endothelial constitutive NOS [eNOS]) and smooth muscle cells. eNOS yields a basal release. iNOS is expressed in endothelial cells in the presence of cytokines (cytokine-inducible NOS).

Endothelial nitric oxide synthase is a plasmalemmal protein. eNOS can be confined to caveolae in endothelial cells by binding to caveolin, particularly caveolin-1 [1016]. Caveolin-1 thereby inactivates NOS. Caveolae thus act as temporal and spatial regulators of NO release in endothelial cells.[68] The continuous NO production is enhanced by multiple stimuli (ATP, acetylcholine, bradykinin, histamine, insulin, and substance P). Superoxide O_2^- and oxyhemoglobin (HbO_2) sequestrate NO.

Two signaling pathways can be involved in shear-mediated changes in NO production. The initial rapid G-protein- and Ca^{++}-dependent NO production phase depends on the rate of change of shear and not on the shear level. The subsequent lower rate G-protein- and Ca^{++}-independent NO production, which is shear level dependent, is involved in sustained NO release.

Nitric oxide is a vasodilator that inhibits vasoconstrictor influences (angiotensin-2 and sympathetic vasoconstriction). Acetylcholine and muscarinic agonists stimulate NO release from the endothelial cells [1017]. EDRF released from endothelial cells after ACh exposure could be a complex of NO. EDRF is

[67] Endothelial cells express a number of receptors (imidazoline, bradykinin, serotonin, adenosine A2 receptors, adrenoceptors, purinoceptors, muscarinic, histaminergic receptors, etc.). Bradykinin, histamine, acetylcholine, and adenosine enhance endothelium-dependent NO-mediated vasorelaxation. NO synthesis caused by acetylcholine is carried out via Gi, whereas NO production due to bradykinin is not coupled to Gi. A member of C-family of G-protein-coupled receptors GPCR-C6A bind arginine, lysine, and ornithine.

[68] Muscle-specific caveolin-3 inhibits eNOS in cardiomyocytes and nNOS in skeletal muscles.

more resistant than NO. It fastly reacts with the superoxide anion to produce short-life highly-reactive peroxynitrite.

Nitric oxide inhibits platelet and leukocyte adhesion to the endothelium. NO has an antiproliferative effect on endothelial cells and smooth muscle cells. NO acts via cyclic guanosine monophosphate, after binding to guanylyl cyclase (GCase), which is activated to produce cGMP from GTP. cGMP activates a kinase that subsequently inhibits calcium influx into smooth muscle cells and decreases Ca^{++}–Cam stimulation of myosin light chain kinase, thereby decaying smooth muscle tone.

Nitric oxide can be released from the endothelium by α2-adrenoceptor activation, serotonin, aggregating platelets, leukotrienes, adenosine diphosphate and bradykinin [1018]. Tissular hypoxia yields vasodilatation in various vessel types. Endothelial cells act as a local sensor for the O_2 supply regulation, increasing $[Ca^{++}]_i$ and releasing NO and PGI2 in response to hypoxia [1019].

The wall shear stress regulates NO release. Ca^{++} response to WSS depends on $[Ca^{++}]_e$ [1020]. WSS stepwise increase induces transient rise in $[Ca^{++}]_i$ [1021]. WSS-modulated $[Ca^{++}]_i$ is associated with NO metabolism via a receptor-triggered signaling cascade. The time gradients of the wall shear stress yield a transient high-concentration burst of NO release. Time shear gradients induce NO-mediated sustained ERK1/2 activation via G-proteins [1011]. Low concentrations of NO inhibit ERK1/2, whereas high levels of NO activate ERK1/2. These gradients also generate sustained productions of *reactive oxygen species*[69] (ROS), which act independently and synergetically with NO to mediate ERK1/2 activation.

Stress-activated NADPH oxidase produces reactive oxygen species in the vessel wall.[70] ROS interact with NO to produce peroxynitrite, which activates matrix metalloproteinases, leading to vessel wall remodeling [1023].

9.5.4 Endothelin

The endothelin is a potent vasoconstrictor secreted by endothelial cells. Three endothelin isoforms, ET1, ET2, and ET3 have been identified. ET1 is the single endothelin family member produced in endothelial cells stimulated by ischemia or shear stress. ET1 binds to two endothelin receptors ETR-A and ETR-B. Fixed on ETR-A of smooth muscle cells, it induces vasoconstriction. ETR-B, predominant ETR in endothelial cells, is dose- and PKC-dependently upregulated by the wall shear stress, and causes NO release.

Preproendothelin-1 is processed to the prohormone big endothelin-1, which is subsequently cleaved by endothelin-converting enzyme to produce endothelin. Endothelin acts locally. Locally secreted endothelin-1 binds to its G-protein receptors on smooth muscle cells. This binding leads to the formation

[69] The lipopolysaccharides (LPS) are also involved in the production of intracellular reactive oxygen species and the subsequent activation of signaling pathways.

[70] p47phox subunits of NADPH oxidase upregulate production of reactive oxygen species and matrix metalloproteinases.

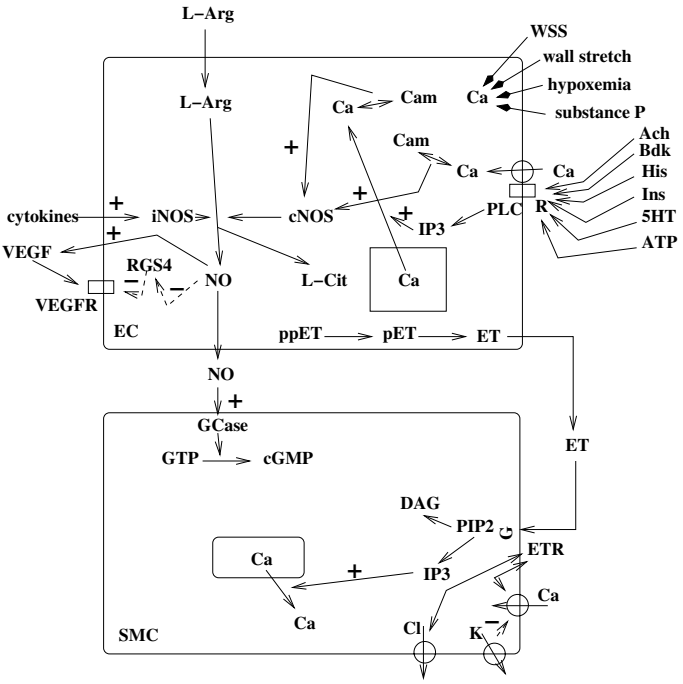

Figure 9.5. Nitric oxide and endothelin-1 synthesis, function, and regulation. NO, discovered in the endothelial cell, is produced by NO synthase from L-arginine. NO binds to its guanylyl-cyclase receptors and increases the intracellular level of cyclic guanylyl monophosphate (cGMP). cGMP stimulates the cGMP-dependent protein kinase, which inhibits intracellular calcium release by inhibiting phospholipase-C and inositol triphosphate in particular (Sources: [831, 1022]).

of diacylglycerol and inositol triphosphate. The latter stimulates Ca^{++} influx from the sarcoplasmic reticulum and causes vasoconstriction. Endothelin half-life ranges from 15 to 20 mn. There is no storage. Once released into blood circulation, ET is catabolized as soon as it reaches the lungs.

Like NO, endothelin has several biological actions. Vascular endothelium regulates vascular structure. Endothelin yields proliferation of endothelial cells. ET1 stimulates SMC proliferation. Endothelin thereby regulates extracellular matrix synthesis by stimulated vascular smooth muscle cells. Smooth muscle cells synthesize collagen-1 and -3 in particular. Although angiotensin-2 enhances collagen-3 synthesis (ET1 do not have significant effect), ET1 increases collagen-1 production, more efficiently than angiotensin-2 in cultured smooth muscle cells [1024].

ET1 acts on cardiac tissue. In human myocardium in vitro, endothelin exerts a positive inotropic effect via sensitization of cardiac myofilaments to calcium and activation of the sodium exchanger [1025]. The positive inotropic effect is associated with a prolongation of the duration of the action

potential [1026]; but endothelins induce coronary vasoconstriction and delayed negative inotropic effects, which balance and cancel the transient positive inotropic and chronotropic effects. Endothelin is also a growth factor for cardiomyocytes, inducing muscle-specific gene transcripts through the possible involvement of protein kinase-C activation or intracellular Ca^{++} mobilization [1027].

The endothelin release is shear dependent. The release of ET1 from cultures of endothelial cells varies with the duration and level of shear [1028]. Sustained exposure to low levels of shear (0.18 Pa) or brief exposure (< 1 h) to 1 Pa stimulate ET1 release via activation of protein kinase-C. Exposure to 0.6 to 2.5 Pa for less than 6 h inhibits ET1 release, implicating either NO and/or cGMP. Transient and long-term steady application of wall shear stress at physiological levels (0.1–3 Pa) up- and downregulates preproendothelin-1 (ppET1) mRNA, respectively [1029]. Inhibition of endothelial NO synthase prevents ppET1 mRNA downregulation by shear stress. Increasing degrees of long-term shear stress upregulate endothelin receptor B mRNA by NO and PKC mechanisms.

9.5.5 Other Mechanotransduction Effects

The endothelium reacts to mechanical forces by gene expression. Flow-induced gene expression (endothelium being subjected to pulsatile flow in vitro) can also be generated by other agents, such as tumor necrosis factor-α, then possibly involving ROSs, interleukin-1β, transforming growth factor-β, vascular endothelial growth factor, etc. These reactions, which are not flow specific, belong to general adaptive responses. However, the endothelial transcription factor *lung Kruppel-like factor* (LKlF/KlF2) is uniquely induced by flow [1030]. The lung Kruppel-like factor regulates several genes involved in the vascular tone in response to the local flow pattern. LKlF acts on the synthesis not only of nitric oxide, but also endothelin-1 and adrenomedullin. In steady and pulsatile flows, high shear stresses increase LKlF expression, whereas cyclic stretch has no effect [1031].

The small GTPase Cdc42[71] is activated by wall shear stress, activation being mediated by bonds between shear-stimulated integrins[72] and the extracellular matrix via PAR6/PKCζ [1032] (Fig. 9.4). Furthermore, in shear stress-stimulated endothelial cells, the small GTPase Rac1 is activated also from the integrin–ECM binding pathway.

Mechanical stress mediates p53-dependent SMC apoptosis via the p38 pathway [1033]. The endothelial cell subjected to the fluid stresses not only

[71] Cdc42 is implicated in cell polarity, inducing reorientation of the microtubule organizing center (MTOC) and Golgi apparatus toward the motion direction.

[72] WSS activates particularly integrins $\alpha_v\beta_3$, increasing integrin binding to the extracellular matrix, which inhibits the small GTPases Rho. This transient inhibition is required for cytoskeletal alignment in the flow direction.

secretes vasomotor subtances, but also acts on other local biological phenomena, such as inflammation (Sect. 10.5). The *thioredoxin interacting protein* (TxnIP)[73] is a stress-responsive protein that inhibits thioredoxin (Txn) activity. The thioredoxin binds to and inhibits the *apoptosis signaling kinase-1*[74] (ASK1). When Txnip binds to Txn, in the absence of shear for instance, it hinders Txn binding to ASK1 (Fig. 9.4). TNFα can then phosphorylate ASK1. Activated ASK1 stimulates MAP2K and its downstream pathway, p38 and JNK, which increase the expression of VCAM1, hence promoting leukocyte adhesion. Short (1 hour) and long (1 day) exposure to steady flow on rabbit and mice aortae and EC culture inhibits the TNF-ASK1-JNK/p38 pathway and TNF-mediated VCAM1 [977]. The wall shear stress decreases the Txnip concentration in endothelial and favors the binding of Txn to ASK1, subsequently hampering p38 and JNK inflammatory effects via VCAM1. TxnIP and Txn are then involved in mechanotransduction associated with TNF signaling in the endothelial cells and inflammation.

Oxidants release Txn from ASK1. This effect of the wall shear stress on the vascular endothelium is beneficial, with nitric oxide production and enhanced expression of antioxidant enzymes. The apoptosis-suppressive effects of wall shear stress are indeed mediated by *superoxide dismutase* (SOD) and NO synthase[75] [1034].

NO have thus both cytotoxic[76] and cytoprotective effects. NO protects the vessels against oxidative reactions, including antioxidant defenses such as glutathione, and activation of MAPK and inhibition of cytochrome-C release from mitochondria. The shear stress upregulates the expression of glutathione peroxidase (GPx1) mRNA in a time- and force-dependent manner in bovine aortic endothelial cells, and increased GPx activity[77] [1035]. The wall shear stress can then protect the vessel wall against oxidative stresses. Furthermore, NO inhibits the ASK1 activation of MAP2K [1036]. NO then modulates redox cell signaling [1037].

Smooth muscle cells migrate during atherosclerosis and neointimal hyperplasia. Hemodynamic forces stimulate endothelial cells to secrete SMC chemoattractants such as PDGF. SMC chemotaxis is associated with the activation of SMC extracellular signal-regulated protein kinase-1/2 [1038]. Physiological shear stresses stimulate PDGF-BB and IL1α secretion by endothelial cells and subsequent SMC migration, whereas lower levels of shear stress and cyclic strain have no effect.

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[73] Thioredoxin interacting protein is also known as vitamin-D upregulating protein-1.

[74] ASK1, a MAP3K, is also called apoptosis signal-regulating kinase

[75] SOD and NO, upregulated by WSS, hinder the caspase cascade in response to apoptosis-inducing stimuli.

[76] SOD converts superoxide anion to H_2O_2, which may lead to vessel wall inflammation.

[77] Glutathione peroxidase (GPx1) reduces H_2O_2 to H_2O.

T2-Tryptophanyl-tRNA synthetase (T2TrpRS), a natural fragment of TrpRS, inhibits flow-induced activation of PKB, ERK1/2, and NOS [1039]. T2TrpRS hinders EC elongation and alignment of stress fibers and microtubules in the flow direction. In addition, T2TrpRS is an antagonist of VEGF-induced angiogenesis. T2TrpRS binds at intercellular junctions of endothelial cells with VE-cadherin [1040], but complete TrpRS remains inactive.

9.6 HDL Effects on the Endothelium

HDLs decrease the lipid peroxidation of LDL, due to the presence of paraoxonase-1 (PON1) [1041]. Furthermore, HDLs affects several functions of the vascular endothelium [1042].

HDL acts on the vascular tone through the vasoactive substances released by the endothelium. HDL activates eNOS and the inducible COx2, releasing NO and PGI2. HDL triggers MAPK signaling via scavenger receptor SR-BI in endothelial cells. In vivo, the plasma concentration of HDL-C is used as a predictor of NO-dependent vasodilation and correlate with the plasma concentration of the stable PGI2 metabolite 6-keto PGF1α. Moreover, HDL might inhibit the secretion of ET1.

HDL plays a role in inflammation. HDL downregulates TNFα and consequently decreases the synthesis of cell adhesion molecules, reducing leukocyte recruitment and extravasation. HDL reduces the production of platelet activating factor. Furthermore, PAF is also degraded by HDL-bound enzymes, platelet-activating factor acetylhydrolase (PAF-AH), LCAT, and paraoxonase. HDL then prevents PAF-induced adhesion of leukocytes to the endothelium. However, HDL could lose its anti-inflammatory properties during inflammation, part of apoA-I, apoA-II, paraoxonase, and PAF-AH being removed.

HDL is involved in coagulation. HDL enhances the synthesis of antithrombotic NO and PGI2. HDL limits PAF level. HDL might inhibit the production of von Willebrand factor. HDL binds to tissue factor pathway inhibitor, hampering the extrinsic coagulation pathway. HDL enhances the anticoagulant activity of proteins-C and -S.

HDL stimulates the proliferation of endothelial cells, in association with phospholipase-C and intracellular calcium. HDL and sHDL protect cultured endothelial cells from TNFα-induced apoptosis (Fig. 4.12), inhibiting caspase-3. HDL suppress the mitochondrial pathway of apoptosis, activating PKB.

10

Tissue Growth, Repair, and Remodeling

Tissue growth is controlled and its processes are coordinated by mechanisms requiring signals and factors, such as transcription factors. During tissue development, cell proliferation must be tightly coordinated with possible differentiation to determine the correct configuration. Cell wall extensibility and junction communications are involved in tissue growth.

10.1 Growth Factors

Any tissue needs mechanical, electrical, structural, and chemical signals to grow into functional three-dimensional tissues. Interactions of cells with the extracellular matrix provide structural cues for normal cellular activity. Cell responses to various environmental signals are mediated by growth factors (Table 10.1). Many growth factors (GF) are molecules, often proteins but also lipids, which bind to receptors (Sect. 3.2.2) on the cell surface (Fig. 10.1), mainly to activate cellular proliferation and/or differentiation. The terminology currently used comes from the initial discovery context.

Many growth factors have a pleiotropic nature so they stimulate almost every cell types, whereas others are specific to particular cell types. The growth factors have functional redundancy, but they can have distinct effects. Their production and activation depend on the balance between inhibitory and stimulatory growth factors as well as on GF transport and storage capacities. Growth factors promote not only cell division, maturation, and functioning, but also tissue growth and remodeling. Growth factors act on: (1) growth factor producing cells (*autocrine effect*),[1] (2) neighboring cells (*paracrine ef-*

[1] Autocrine growth factor (AGF) is involved in autostimulatory growth control, in which a cell secretes a factor that binds to its receptor (AGFR), which is also expressed by the producing cell. This autogenous loop creates a substance that acts back on the cells that produce it.

Table 10.1. Growth factors associated with vascular and blood cells (Sources: [628, 629, 1043, 1044]).

Factor	Sources	Targets	Function
PDGF	EC, SMC, Nφ, TC, Mφ, FB	Nφ, Mo, EC, FB, SMC	Chemotaxis (Nφ, Mφ, FB), Angiogenesis, Cn synthesis Mitogenesis
EGF	TC, Mo, Mφ,	FB, EC,	Chemotaxis, mitogenesis Angiogenesis
FGF1, 2	Mo, EC, FB, Mφ	FB, EC, SMC	Chemotaxis (EC, FB), Angiogenesis, mitogenesis
Epo	Kidney	RBC	Erythropoiesis
TGFα, β	Mφ, T Lφ, EC, TC, FB	FB, EC, Mo, Lφ, SMC	Healing, anti-inflammatory, Angiogenesis, Cn synthesis Chemotaxis (Mφ, FB), ECM production, Cell–matrix interaction
IGF	Mφ, FB, TC	FB, EC, SMC	Cn synthesis, FB proliferation
TNF	Mφ, T Lφ, Nφ	Mφ, FBs	Angiogenesis
VEGF	Mφ, FB, SMC	EC	Angiogenesis
G-CSF	Mφ, EC	Nφ	Hematopoiesis
M-CSF	T Lφ, Mφ	Mo, Mφ	Hematopoiesis
GM-CSF	Mo, Mφ, T Lφ, EC, FB	Mφ, Nφ, FB	Immunity

fect),[2] (3) via cell interactions (*juxtacrine effect*), or (4) on distant cells after blood transport (*endocrine effect*). Growth factor–receptor complexes are better correlated with cell proliferation and angiogenesis than growth factor or receptor alone, pointing to auto- and paracrine loops [1045]. Cell growth is controlled by a balance between growth-promoting and growth-inhibiting factors. Concentration gradients of growth factors are required for cellular chemotactic responses.

Cytokines (κυτοσ: cavity [cell], κινεω: to move) are growth factors that modulate activities of immune cells. These immunomodulators are mainly secreted by leukocytes, either by lymphocytes (*lymphokines*) or by monocytes or macrophages (*monokines*). However, some cytokines are produced by almost the entire cell spectrum. Cytokines stimulate both the humoral and cellular

[2] Paracrine growth factor (PGF) influences the growth and functional activities of surrounding cells expressing the corresponding receptor. PGFs modulate the microenvironment and are involved in angiogenesis, stroma formation, modulation of immune response, activation of proteolytic enzymes, etc.

immune responses. Among lymphokines, *interleukins* (IL) are growth factors targeted to hematopoietic cells (Table 6.11). *Interferons* (Ifn) are glycoprotein cytokines produced by the cells of the immune systems in response to foreign agents (viruses, bacteria, parasites, tumor cells; Table 10.2).[3] Immune cells have reciprocal activations.[4]

The *epidermal growth factor* (EGF) activates, after binding to the ERBB family of receptors (Sect. 3.2.2.2) of responsive cells, the tyrosine kinase activity (phosphorylation of EGF receptor and other proteins). Epidermal growth factor promotes receptor dimerization and activation of the intracellular tyrosine kinase domain. Activated receptors phosphorylate each other on a various sites for enzymes or adaptors. EGF has proliferative effects, especially on fibroblasts.

EGF generates an initial rapid (20-mn) wave of transcription of a small number of immediate early genes (IEG), such as AP1 components Fos and Jun,

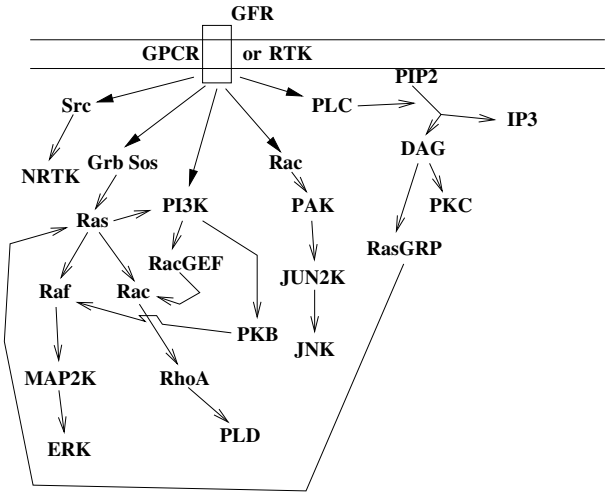

Figure 10.1. The growth factor receptor and biochemical cascades. Growth factor-bound receptor (GFR) activates adapter Grb coupled to the guanine nucleotide releasing factor Sos (Grb–Sos complex), and subsequently Ras, Raf, and mitogen activated protein kinase kinase (MAP2K) successively, and then extracellular regulated kinase (ERK) on the one hand, and Rac, RhoA, and PLD on the other hand (Source: [238]). Ligand-bound receptor also activates: (1) phosphatidylinositol 3-kinase (PI3K), protein kinase-B (PKB), (2) Src, (3) Rac and JNK, and (4) phospholipase-C (PLC).

[3] The heart particularly expresses the following growth factors: TNFα and -β, IL1α and -β, IL2–IL6, IL10, and Ifnγ.

[4] T lymphocytes synthesize lymphokines (GM-CSF, IL3, and Ifn) which stimulate macrophages. Afterward, macrophages produce monokines (IL1) which activate T lymphocytes.

which encode transcription factors for signaling responses. Certain proteins, such as Sprouty2 and Mig6 with a peak expression 60 to 120 mn after EGF stimulation (after EGF receptor degradation), generate a refractory period by inhibiting EGF receptors to avoid repetitive stimulation. The coordinate expression of delayed early genes (DEG) impedes the action of immediate early genes (Fig. 10.2). The delayed early genes have a peak expression 40–240 mn after growth factor stimulation. The epidermal growth factor ErbB signaling system includes genes that are co-expressed in feedbacks for signaling attenuation at specific nodes of the biochemical cascade. A node of the mitogen-activated protein kinase module of the EGF pathway is inhibited by dual-specificity MAPK phosphatases [1046]. Other inhibitors also determine the activation duration. These inhibitions are triggered by the signaling pathway, the activity of which is hampered. Among these inhibitors, there are transcription regulators and RNA-binding attenuators.[5]

The family of *platelet-derived growth factors* (PDGF) comprises four members, PDGF-A, -B, -C, and -D.[6] Platelet-derived growth factor is a potent activator of the PI3K pathway. PDGFs bind to two plasmalemmal receptor tyrosine kinases, PDGFRα and PDGFRβ. These receptors recruit and activate several proteins, such as PI3K, PLCγ, SHP2 phosphatase and Src kinases.

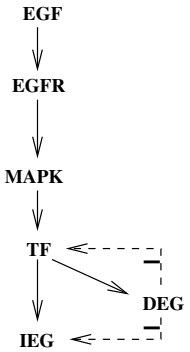

Figure 10.2. Negative feedback for signaling attenuation. EGF signaling pathway activates immediate early genes (IEG; first post-stimulation wave of transcription) via mitogen-activated protein kinases (MAPK) and transcription factors (TF). Activated delayed early genes (DEG; second post-stimulation wave of transcription) impedes the action of immediate early genes (Source: [1046]).

[5] Transcription attenuator most often forms a complex with and attenuates the action of a transcription activator that is activated earlier. Kruppel-like factor KLF6 and avian musculoaponeurotic fibrosarcoma oncogene MAFF are activated by EGF [1046].

[6] PDGF is involved in atherosclerosis.

PDGFRs also bind adapters NHERF1s[7] and NHERF2.[8] PDGF activates Rac GTPases in lamellipodial protrusions of migrating cells.

The *fibroblast growth factors* (FGF),[9] also characterized by tyrosine kinase activity, are particularly involved in embryonic development, angiogenesis and wound healing. FGF2 indeed promotes endothelial cell proliferation and formation of tube-like endothelial structures. The fibroblast growth factors signal via their tyrosine kinase receptors, which consist of four members (FGFR1,

[7] There are two types of sodium–hydrogen exchanger isoform-3 regulatory factors (NHERF): NHERF1 (also called ezrin–radixin–moesin-binding phosphoprotein-50 [EBP50]), and NHERF2 (also named sodium–hydrogen exchanger type 3 kinase-A regulatory protein [E3KARP]). NHERF1 and NHERF2 contain two tandem PDZ domains for the assembly of proteic complexes. NHERF thus interacts with various receptors, such as β2-adrenergic receptor and P2Y purinergic receptor. The regulatory co-factor of the sodium–hydrogen exchanger-3 (NHE3) mediate the regulation of the activity of ion transporters, in particular NHE3 by protein kinase-A. Na^+/H^+ exchanger isoform-3 regulatory factor-1 was indeed first found to be a co-factor in cAMP-associated inhibition of the renal brush border Na^+/H^+ exchanger NHE3. NHERF1, ezrin, and PKA form a complex that links the actin cytoskeleton and downregulates NHE3. Scaffold NHERF1 recruits various receptors, ion carriers, and other proteins to the plasmalemma. In particular cytosolic adapter NHERF1 binds to actin-associated proteins, such as ezrin, radixin, moesin, and tumour suppressor merlin (moesin–ezrin–radixin-like protein merlin is the product of the neurofibromatosis-2 tumor-suppressor gene). Membrane-associated ERMs regulate the structure and function of certain domains of the cell cortex. ERM Proteins associate with: (1) either directly the cytoplasmic region of membrane proteins, (2) or indirectly other membrane proteins via NHERFs. They also connect to filamentous F-actin. ERMs link to signaling molecules of the Rho pathway such as Rho guanine dinucleotide-dissociation inhibitor. Therefore, ERMs participate in microfilament–membrane attachment and Rho-signaling pathways. Binding sites for NHERFs, F-actin, and RhoGDI are masked in inactive monomers. The binding regulation of ERMs and merlin is carried out by phosphorylation in combination with acidic phospholipids such as phosphatidylinositol(4,5)bisphosphate [1047]. ERM Proteins then serve as a regulated linkage between plasmalemmal proteins and the cortical cytoskeleton. Attachment of plasmalemmal proteins to F-actin determines cell shape and is required in cell adhesion, motility, and transport associated with signaling pathways.

[8] NHERF2 is strongly expressed in the vasculature. NHERF2 interacts with the PDGF receptor, favoring its dimerization and subsequent activation of tyrosine kinase activity. NHERF2 leads to the formation of a cytoskeleton-associated signaling complex able to trigger the mitogen-activated protein kinase pathway.

[9] There are several kinds of fibroblast growth factors: FGF1, or acidic fibroblast growth factor (aFGF), and FGF2, or basic fibroblast growth factor (bFGF). Other proteins are similar to acidic and basic FGFs, the heparin-binding growth factors (HBGF) and the endothelium cell growth factors (ECGF). FGF1 is also called β-ECGF, ECGF-A, ECGF-B, ECGFβ, FGFα, and HBGF1. FGF2 is also named FGFβ and HBGF2.

FGFR2, FGFR3, and FGFR4).[10] The fibroblast growth factors interact with plasmalemmal heparan sulfates for signal transduction.

Fibroblast growth factor-19 set regulates glucose metabolism, bile acid synthesis, phosphate, and vitamin-D metabolisms. FGF21 is a liver-derived endocrine factor which stimulates glucose uptake in adipocytes upon binding the complex formed by FGF receptors and β-Klotho and activating MAPK cascade [1049]. Klotho confers tissue-specific activity to FGF.

The *transforming growth factor*-α (TGFα) secreted by activated macrophages and platelets binds to the EGF receptor (EGFR) and its own receptor. The TGFβ family includes the activin and inhibin proteins, Mullerian inhibiting substance (MIS), and bone morphogenetic protein[11] (BMP). TGFβ stimulates G-protein-coupled receptor kinase-2 (GRK2), which desensitizes G-protein-coupled receptors and inhibits TGFβ signaling (negative feedback loop) [1051]. TGFβ receptors have serine/threonine kinase activity. TGFβ is a growth inhibitor for endothelial cells, fibroblasts, and other cell types. TGFβ1 is synthesized by macrophages, lymphocytes, and endothelial cells, and TGFβ2 by keratinocytes among others.

The *insulin-like growth factor*-1[12] (IGF1) is produced in response to growth hormone. The IGF1 receptor is a tyrosine kinase. IGF2 is almost exclusively expressed in embryonic and neonatal tissues. Cardiac synthesis of insulin-like growth factor-1 is higher in athletes than sedentary control subjects, whereas the production of endothelin-1 and angiotensin-2 does not change [1052].

Vascular endothelial growth factor[13] (VEGF) stimulates angiogenesis. It increases NO activity, which stimulates endothelial proliferation via a protein kinase-G pathway. The vascular endothelial growth factor also permanently acts on endothelial cells to maintain the vasculature in a suitable state. Inhibition of vascular endothelial growth factor causes capillary regression associated with a endothelium fenestration [1053].

There are several VEGF isoforms, the VEGF family consisting of five members, VEGF-A, -B, -C, -D, and placenta growth factor (PLGF). The

[10] FGF23 regulates the sodium–phosphate co-transporter and enzymes of the vitamin D metabolism in the kidney. FGF23 specifically binds to FGFR1 owing to plasmalemmal protein Klotho [1048]. The interaction of Klotho and FGFR1(3c) indeed forms a specific FGF23 receptor.

[11] Several signals, such as bone morphogenetic protein (BMP), Wnt and FGF, are involved in heart development. The noggin, a bone morphogenetic protein antagonist, is transiently and strongly expressed in the heart-forming region during gastrulation [1050]. Cardiomyocytes from mouse embryonic stem cells (ESC) can be obtained by inhibition of BMP signaling.

[12] Insulin-like growth factor-1 is also named somatomedin-C.

[13] Vascular endothelial growth factor is also called vascular permeability factor. Vessel wall fenestrations induced by VEGF-A allow leakage of small molecules. Large substances are transported via caveolae, vesiculovacuolar organelles, and transendothelial pores. VEGF-induced permeability depends on nitric oxide.

VEGF receptor tyrosine kinases, which are encoded by the FLT gene family, have distinct functions in the regulation of the growth of blood and lymph vessels. A VEGF receptor is mainly observed on the lymphatic endothelial cells, which is activated neither by VEGF-A nor VEGF-B, but by VEGF-C [1054]. VEGF-C induces proliferation of the lymphatic vessels but not blood vessels. VEGF-D is also able to trigger growth of lymphatic vessels [1055].

Vascular endothelial growth factor receptors (VEGFR) have different functions according to the type [1056]. VEGFR1 is required for the recruitment of hematopoietic precursors and migration of monocytes. VEGFR2 and VEGFR3 are involved in the functioning of the endothelium of blood and lymph vessels, respectively. There are also co-receptors (VEGF-binding molecules without VEGF-induced catalytic function), such as heparan sulfate and neuropilins.[14] VEGFR1 can regulate VEGFR2 signaling. VEGFR1 is regulated by hypoxia-inducible factors. VEGFR2 is inhibited by phosphotyrosine phosphatases SHP1 and SHP2. VEGFR2 activates PLCγ and PI3K, and therefore the extracellular-signal regulated kinase cascade and protein kinase-B. VEGFR2 forms mechanosensory complexes with platelet–endothelial cell adhesion molecule-1 and vascular endothelial cadherin.

Vascular endothelial cells continuously perceived mechanical and chemical stimuli and coordinate the activity of the corresponding signaling pathways. Among chemical stimuli, VEGF quickly binds VEGFR2, recruiting in particular adapter Nckβ to trigger the MAPK pathway.[15] Mechanical stimuli, such as the wall shear stress, activate similar plasmalemmal targets (integrins and VEGFR2) and effectors (ERK and JNK). These convergent modules of the pathway triggered by mechanical and chemical stimuli then require insulation by different molecular complexes to keep their specificity, i.e., to possess divergent modules responsible for the specific response to different stimuli. VEGF, but not shear stress, induces the formation of a complex associating VEGFR2 and Nckβ (Fig. 10.3) [1057]. The wall shear stress activates ERK via VEGFR2 without recruiting Nckβ, but Cbl.[16] It bypasses VEGFR2 phosphorylation for JNK activation, but uses Src, small GTPases Rho, PI3K, and Rho kinases.

Semaphorins form a family of membrane-bound and secreted proteins, which signal via plasmalemmal receptors, plexins and neuropilins. Semaphorins-3 are involved in vascular development. Neuropilin-1 (Nrp1) is a co-

[14] Neuropilin-1 is expressed in arteries, neuropilin-2 in veins and lymphatics.

[15] Activated VEGFR2 interacts with various proteins, such as Grb2, Nck, PI3K, Shc, and Sos. Nckβ binds various receptor tyrosine kinases, such as EGFR and PDGFR. Nckβ also associates with other proteins, such as p21-activated kinase, thereby serving as a docking protein to expose the protein to its activators (Cdc42 and Rac1 for PAK). Nckβ can then lead to the stimulation of downstream effector of MAPK cascade, ERK, and JNK.

[16] Wall shear stress can stimulate ERK via manifold proteins, such as integrins, PECAM1, FAK, Src, and PKCϵ. Both integrins or PECAM1 can interact with VEGFR2.

receptor for semaphorin-3A and for vascular endothelial growth factor-A, acting with VEGFR2, both in vascular endothelial cells and smooth muscle cells.[17] Neuropilin-1 increases VEGF binding in both cell types and regulates VEGFR2 expression. Neuropilin-1 enhances VEGF signaling in endothelial cells whereas it hinders VEGF activity in smooth muscle cells. Neuropilin-1 is targeted by either heparan sulfate or chondroitin sulfate [1058].

Lymphatic endothelial cells express higher levels of *hepatocyte growth factor receptor* (HGFR) than blood vascular endothelial cells [1059]. HGF promotes the proliferation of lymphatic endothelial cells, their migration (which is partially mediated via α9-integrin), and the formation of lymphatic vessels.

Tumor necrosis factor-α acts synergistically with EGF and PDGF.[18] TNFα induces expression of several ILs. TNFβ[19] is able to kill different cell types and to induce terminal differentiation in others. TNFβ inhibits lipoprotein lipase in endothelial cells. It is mainly synthesized by T lymphocytes, in particular cytotoxic T lymphocytes (CTL).

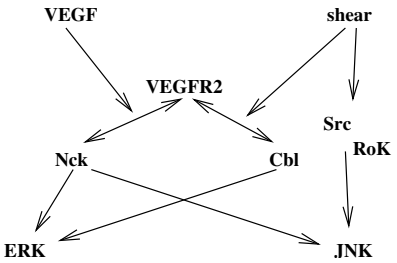

Figure 10.3. VEGF and insulation. Both VEGF and wall shear stress stimulate VEGFR2. Because of the presence of this convergent node, insulation by different molecular complexes is required for specificity. VEGF induces the formation of a complex associating VEGFR2 and Nckβ to activate its effectors, such as ERK and JNK., whereas wall shear stress leads to the recruitment of Cbl to stimulate ERK. The wall shear stress activates JNK owing to Src, Rho, PI3K, and Rho kinases (Source: [1057]).

Table 10.2. Interferons.

Interferon	Main sources	Principal targets
INFα, β	Mφ, Nφ	NK Lφ, Mφ
INFγ	TH1 &NK Lφ	Mφ, Nφ, NK Lφ

[17] The composition of Nrp1 glycosaminoglycan chains differs between endothelial cells and smooth muscle cells. These cell types then differentially respond to VEGF.

[18] TNFα is also named cachectin.

[19] TNFβ is also called lymphotoxin.

Interferons I and II have antiviral, antiproliferative, and immunomodulatory effects, activating the Janus activated kinase-signal transducer and activator of transcription signaling pathway, mitogen-activated protein kinase p38 cascade, and phosphatidylinositol 3-kinase cascade [348]. Ifns induce the expression of multiple genes. Certain genes are regulated by Ifns I and II, whereas others are selectively regulated by distinct Ifns. Interferons I (Ifnα, Ifnβ, etc.), produced by many cell types, including T and B cells, bind to a specific receptor (IfnAR) to yield antiviral activities. They stimulate both macrophages and NK cells. Ifnω is released by leukocytes at the site of viral infection or tumors. Interferons II (INFγ) are secreted by CD8+ T cells. INFγ is involved in the regulation of the immune and inflammatory responses. It potentiates the effects of Ifnα and Ifnβ. It promotes the presentation of antigen to CD4+ T helper cells. It also stimulates macrophages. Another class of Ifns corresponds to Ifnλ.

Sphingosine-1-phosphate (S1P), a lipid growth factor produced from phosphorylation of *sphingosine* (Sph) of the platelet membrane by Sph kinase, activates endothelial cells by *phospholipase-D* (PLD), independently of PKC and Ca^{++} [1060]. S1P is produced intracellularly[20] and is then secreted [1061]. Endothelial cells have an intracellular reserve of functional S1P1 in caveolae. S1P is a ligand for endothelial differentiation gene receptor (EDGR) coupled to G-protein and a mediator of EC response for locomotion, maturation, and angiogenesis (Fig. 10.4). S1P controls vascular permeability (barrier-enhancing effect) by cytoskeleton regulation associated with cortical actin polymerization, G-protein-dependent cascade, and Rho GTPases.[21] Moreover, S1P enhances assembling of adherens junctions, like adherens junctions and focal adhesions [1063, 1064], using Rho and Rac pathways. S1P-S1P1 tightens adherens junctions between endothelial cells, characterized by VE cadherins. S1P stimulates translocation of ABPs such as cortactin, which enhances actin polymerization (whereas ASPs like cofilin are inactivated) and MLCK for adhesion stabilization. S1P redistributes cadherin and catenin to the cortex, as these molecules are involved in adherens junctions.

[20] The lysophospholipids sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) regulate various cell functions [1061]. LPA is most often synthesized extracellularly in microvesicles shed from cell membranes. Similar to most small lipids in extracellular spaces, the newly generated and secreted lysophospholipids are bound by plasma proteins. Serine palmitoyltransferase generates sphingomyelin that is converted to ceramide by sphingomyelinase, itself transformed into sphingosine by ceramidase. Then two sphingosine kinases phosphorylate sphingosine to form S1P. S1P acts via G-protein-coupled receptors.

[21] The GTPases RhoA, Rac1, and Cdc42, are rapidly activated by WSS. RhoA can be activated within 5 mn after WSS stimulation, leading to cell rounding via Rho-kinase. Endothelial cells then elongate as RhoA activity returns to baseline and Rac1 and Cdc42, which are required for cell elongation, reach peak activation [1062].

Sphingosine-1-phosphate 1 (S1P1) receptor agonists cause sequestration of lymphocytes in secondary lymphoid organ. They hinder T-cell motility between medullary cords and lymphatic sinuses, mainly the migration through the endothelium of lymph nodes [1065].

10.2 Chemotaxis

Chemotaxis represents responses of cells according to a chemoattractant concentration gradient. G-protein-coupled chemoattractant receptors initiate the chemotaxis. Chemotaxis requires three main processes: (1) cell alignment along the chemoattractant gradient, (2) cell polarization, and (3) protrusion at the leading edge (cell front) and retraction at the trailing edge (cell back) of cytoskeletal elements, which all implicate small GTPases [1067]. Indeed in neutrophils, Rac mediates the protrusion of the pseudopod by stimulating F-actin assembly. RhoA controls the retraction of the cell back via actin–myosin contractility. Cdc42 at the front guides the response. The chemoattractant binds to its receptor and activates: (1) Rho kinase via RhoGEF and RhoA; (2) PI3K, which transforms phosphatidylinositol(4,5)bisphosphate into phosphatidylinositol(3,4,5)trisphosphate; and (3) Cdc42 via the PIX–PAK1–Cdc42 complex. Activated PI3K increases the concentration of phosphatidylinositol

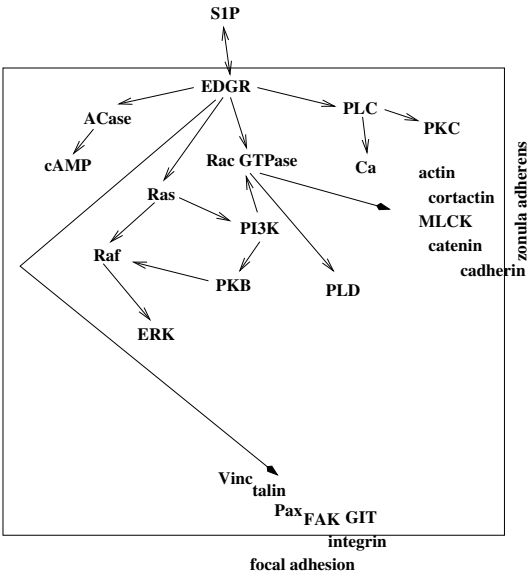

Figure 10.4. Sphingosine-1-phosphate effects on the endothelial cell. The sphingosine-1-phosphate (S1P) acts from G-protein-coupled receptors on adenylyl cyclase (ACase), Ras, phosphatidylinositol 3-kinase (PI3K), and phospholipase-C (PLC). S1P is strongly involved in cell adhesion (Source: [1066]).

trisphosphate at the cell front. PIP3 and activated Cdc42 cooperate for F-actin polymerization via Rac. Activated RoK binds and stimulates *phosphoinositide phosphatase* PTEN, which is located with RhoA and RoK to the back and sides of the motile cell [1068]. Besides, RhoA acts synergistically with PTEN to reduce the levels of the phosphorylated protein kinase-B.

Chemotaxis Model

The time gradient of the cell number depends on cell diffusion flux, then on cell diffusivity, chemotactic flux, cell proliferation, and death rates. The chemotactic flux depends on: (1) a chemotactic response function of the available cell number and of the chemoattractant concentration; and (2) chemoattractant concentration gradient [1069]. The time gradient of the chemoattractant concentration depends on the production and destruction rates as well as its diffusion flux. The Keller-Segel model is widely used for the chemical control of cell movement [1070]:

$$\begin{aligned} \frac{\partial \rho_p}{\partial t} &= \mathcal{D}_p \boldsymbol{\nabla}^2 \rho_p - \boldsymbol{\nabla} \cdot (\rho_p \kappa_s \boldsymbol{\nabla} c_c) + \kappa_d \rho_p, \\ \frac{\partial c_c}{\partial t} &= \mathcal{D}_c \boldsymbol{\nabla}^2 c_c + \kappa_p \rho_p - \tau^{-1} c_c, \end{aligned} \tag{10.1}$$

where ρ_p is the cell density, c_c the chemoattractant concentration, $\mathcal{D}_p$ the cell diffusivity, $\mathcal{D}_c$ the chemoattractant diffusivity, κ_d the cell mitosis rate, κ_p the

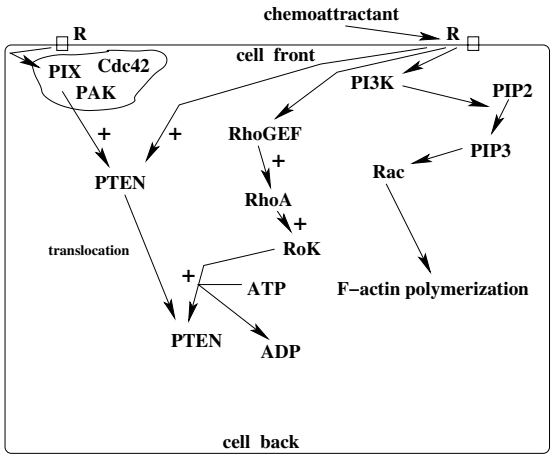

Figure 10.5. Chemotaxis. Chemoattractants activate RoK via RhoGEF and RhoA. Activated RoK binds and phosphorylates PTEN. PTEN localizes with RhoA and RoK to cell back and sides of the motile cell. Activation of the PI3K and Cdc42–PAK1–αPIX complex at the cell front (leading edge) further increases PTEN activity.

chemoattractant production rate, κ_s the cell sensitivity to chemoattractant, and τ the chemoattractant half-life.

A new formulation of the system of partial differential equations has been obtained by the introduction of a new variable and is approximated via a mixed finite element technique [1071]. More recently, a computational model for cell migration in 3D matrices has been developed [1072]. Effects due to matrix sterics and mechanics arising in 3D situations are exhibited. Current trends in taxis modeling rely on hyperbolic equations [1073] instead of the classical Keller-Segel model and parabolic equations [1074].

10.3 Therapeutic Repair

Therapeutic strategies have been recently developed either to promote revascularization of ischemic tissues and regenerate the myocardium or inhibit angiogenesis in certain body disorders.

10.3.1 Stem Cells and Regenerative Therapy

Stem cells can be used to stop and reverse damages caused by degenerative diseases or by perfusion defects. Among these, embryonic stem cells are the best targets but rise ethical issues, as adult stem cells are most often only able to form tissue of origin. Moreover, stem cells have been isolated from only some of the body organs (blood, brain, skeletal muscle, myocardium, skin). However, multipotent adult precursors could act as alternatives to embryonic stem cells if they have the same potential.

Stem cells modulate tissue formation, maintenance, and repair. Stem cells most often stay in relative quiescence, and reside in specialized microenvironments (niches). Stem cells can: (1) be self-renewing and thus be able to generate additional stem cells; and (2) differentiate into various progenitor cells.[22]. Stem cell can thus be involved in therapies which require repair, replacement, and regeneration.

Cardiac stem cells divide either symmetrically or asymmetrically.[23] The myocardium contains stem cell niches [1076]. Cardiac stem cells and supporting cells are connected by gap and adherens junctions. Integrins of the plasmalemma of primitive and committed cells within the niches attach these cells to the extracellular matrix proteins (fibronectin and laminin), which transduce mechanical signals for differentiation regulation.

[22] The transcription factors OCT4, SOX2, and NANOG coordinately contribute to stem cell pluripotency and self-renewal of embryonic stem cells, activating genes that maintain pluripotency and repressing those that are required for differentiation. Moreover, OCT4, SOX2, and NANOG have an autoregulatory loop to allow rapid responses to environmental stimuli [1075].

[23] Asymmetric division predominates. The replicating cardiac stem cell gives birth to one daughter cardiac stem cell and one daughter committed cell (cardiomyocyte, endothelial cell, or smooth muscle cell).

During the early stages of embryo development, the fertilized egg starts to divide into blastomeres. Embryonic stem cells (ESC), derived from the totipotent cells[24] of preimplantation embryos are pluripotent cells (Fig. 10.6).[25] Af-

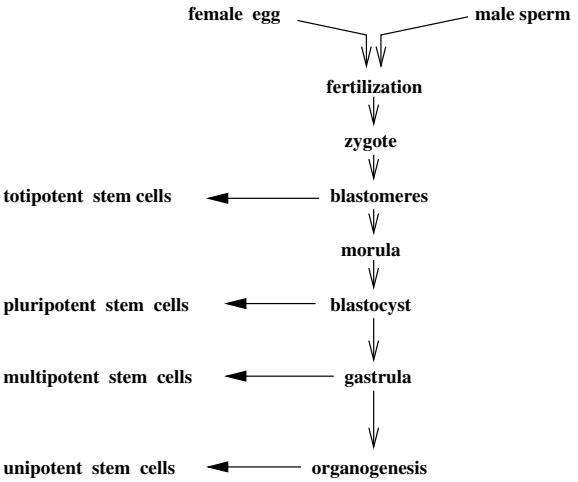

Figure 10.6. Different types of stem cells. The fusion between a female and a male gamete (fertilization) leads to the zygote and embryogenesis. After fertilization, a set of fast mitoses occurs (cleavage) with formation of blastomeres building the blastula. In mammals, the blastula is called the blastocyst. The morula corresponds to an organized cell set with external and internal cells. After the mitosis rate has slowed down, the blastomeres move (gastrulation), forming the gastrula with three germ layers: ecto-, endo-, and mesoderm.

[24] A single totipotent cell leads to the development of several hundreds cell types.

[25] Pluripotent stem cells either renew themselves continuously or form the various types of mature, functional, body cells. Embryonic pluripotent stem cells (ESC) derive at various stages of embryo development according to their type, embryonic germ cells occurring at an earlier phase than others. Human embryonic stem cells can be removed either from the blastocyst or morula, or from later stage embryos, using various isolation procedures. The optimal method consists in deriving pluripotent embryonic stem cells from the inner cell core of blastocysts, before implantation in the uterus (preimplantation blastocyst), i.e., 5 to 6 days after in vitro fertilization. The inner cell mass is then large enough (outer cells, the trophoblasts, differentiate into the placenta). A single blastomere can be extracted from the eight-cell (3-day) embryo for preimplantation genetic diagnosis. Cultured blastomeres prior to genetic analysis can divide, so the genetic check-up can be associated with the production of human embryonic stem cells without reducing embryo developmental potential. Human embryonic stem cells (HESC) are self-renewing pluripotent cells, that provide a source for cell replacement therapies in certain pathologies (e.g., infarction sites). HESCs generating cells are able to differentiate into sufficient quantities of a specific, transplantable, functional cell type. ESCs behave normally within a blastocyst but can induce

ter gastrulation, they give birth to multipotent cells, which are irreversibly programmed for a given tissue. They form: (1) the mesoderm, from which are derived connective tissue, muscles and the circulatory system; (2) the ectoderm (nervous system, skin, etc.); and (3) the endoderm (digestive tract, respiratory system, and endocrine glands). Multiple stem cell populations have been discovered from various adult tissues (hematopoietic stem cell, which can proliferate and differentiate to produce lymphoid and myeloid cell types, bone marrow-derived stem cells BMSC, which can differentiate into various unipotent cells, such as cardiomyocytes). The number in tissues is less than 1 for 10^4 cells.

Stem cells might regenerate the myocardium, especially after myocardium infarction and in ischemic cardiomyopathy, because the heart is a weakly regenerative organ. *Progenitor cells* secrete paracrine factors and might contribute to vasculogenesis and tissue repair and remodeling [1078]. *Endothelial progenitor cells* (EPC) circulate in the normal state, but the number is reduced and they can become dysfunctional in chronic diseases. Circulating progenitors contribute to cardiomyocyte repopulation only sligthly. Endothelial progenitors can induce myogenesis in vitro, and angiogenesis for O_2 supply. A time window exists for optimal cell therapy. Infusion of endothelial progenitor cells promotes neovascularization after ischemia. The protease *cathepsin L* (CathL), expressed in EPC and not strongly in endothelial cells, degrades the matrix, allowing invasion by EPC [1079]. In the heart, resident stem cells can lead to endothelial cells, smooth muscle cells, and cardiomyocytes [1080]. The heart contains various populations of *resident myocardial progenitors*, with different expression modes, which might differentiate into cardiomyocytes and endothelial cells; but they could correspond to an artifact from in vitro culture.

Although cardiogenic progenitor cells maintain myocardial turnover, they do not lead to heart regeneration. Consequently, injured adult hearts form scar without cardiomyocyte proliferation. Appropriate differentiation of stem cell and induction of the division cycle of resident cardiomyocytes can be stimulated with suitable factors. Cytokine (G-CSF) treatment improves infarction repair, but such a therapy can increase the mortality [1081]. Cell delivery can be done either by intracoronary infusion or direct intramyocardial injection during grafting or using endovascular procedures. Periostin activates

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tumors when they are ectopically transplanted. Cultured HESCs have the following main time-invariant characteristics: (1) in vitro proliferation on appropriate substrata (in animal-free conditions, every xenogeneic component being eliminated); (2) maintenance of a normal karyotype throughout long culture durations; (3) expression of the standard molecular markers of stem cells (stable expression of surface markers and transcription factors associated with an undifferentiated state); and (4) differentiation into cell lines of all three germ layers (ectoderm, endoderm, and mesoderm), with an epigenetic status (autosomal imprinting, X-chromosome inactivation), differently responding according to environmental stimuli [1077]. FGF signaling is strongly involved in the maintenance of a proliferative, undifferentiated state in HESC cultures.

plasmalemmal α_V, α_1, α_3, and α_5 integrins, and induces reentry of cardiomyocytes into the cell cycle [1082].

Skeletal myoblasts have in vitro proliferative capacity and in vivo ischemic tolerance. Moreover, the risk of aberrant differentiation is limited [1083]. Unfortunately, implanted myoblasts do not necessarily differentiate into excitable cardiomyocytes. Furthermore, skeletal muscle cells do not express the adhesion and junction proteins required for electromechanical coupling. Thereby, wall implantation of autologous myoblasts remains almost useless.

Transplantation of *bone marrow cells*, possibly enriched with *hematopoietic stem cells* leads to the development of coronary endothelium to a greater extent than the myocardium [1081]. Bone marrow cells also enhance infarct healing. Implanted HSCs differentiate into blood cells, but significantly not into cardiomyocytes.

Mesenchymal stem cells (MSC) of the stromal region (nonhematopoietic compartment) of the bone marrow produce growth factors and cytokines. They colonize sites of injury. Implanted MSCs decrease pathological wall remodeling. Bone marrow mesenchymal stem cells can differentiate into cardiomyocytes and vascular cells [1084], but they can have limited survival after transplantation in the myocardium [1085]. Furthermore, cell therapy may be ineffective or even hazardous in certain clinical settings and specific subgroups of patients [1086, 1087]. Bone marrow mononuclear cells have also been used to enhance systolic ejection.

Intramyocardial injection of adult bone marrow-derived stem cells has been proposed to repair damaged myocardium after acute infarction. The bone marrow-derived MSCs that overexpress gene Akt1 (PKB1) are better for cell therapy of acute myocardial infarction. The secretion of paracrine cytoprotective factor(s) from the stem cells exert cytoprotective effects on cardiomyocytes exposed to hypoxia [1088].

Embryonic stem cells differentiate into all cell types, especially cardiomyocytes organized in myofibers, when stimulated by suitable cardiac promoters; but ESCs can produce teratomas. Moreover, they can form ectopic pacemakers. Recently, a mixed ester of hyaluronan with butyric and retinoic acid (HBR) has been shown to act as a cardiogenic/vasculogenic agent in human mesenchymal stem cells isolated particularly from fetal membranes of term placenta. HBR primes stem cell differentiation into endothelial cells and cardiac cells [1089].

In summary, transdifferentiation have stimulated multiple clinical trials. Cardiac progenitors from the cardiac side population and from cardiomyogenic cells derived from adult bone marrow stromal cells are indeed able to differentiate into cardiomyocytes when interacting with cardiomyocytes. Regenerative cardiology trials most often use bone marrow cells and mesenchymal stem cells, because cardiac stem cell do not generate significant repair. However, the results of stem cell injection in hearts after heart attack at most show slight, transient benefit. Cell-treatment optimization hence remains challenging, taking into account the disease type, patient history, accompanying

therapy for the selection of the cell type, associated growth factors, delivery route, and administration timing.

10.3.2 Gene Therapy

Genes encode for the synthesis of proteins required for cell structures and functions. Gene therapy is aimed at correcting defective genes responsible for lesions and thus repairing diseased tissues. Normal genes are inserted within the genome to replace abnormal genes using a vector. Current vectors are genetically modified viruses, which unload their genetic materials with the therapeutic human gene into target cells. Nonviral procedures can also be used for gene transfer, especially liposomes.[26] Manufactured non-viral vectors, with low toxicity and immunogenicity, can efficiently enter into cells, but yield low gene expression.

The sonic Hedgehog[27] (Shh) pathway, which regulates heart development during embryogenesis, can preserve, after intramyocardial gene transfer in the adult animal heart the left ventricular function after both acute and chronic myocardial ischemia by enhanced neovascularization, as well as reduced fibrosis and apoptosis [1090].

10.3.3 Tissue Engineering

Tissue engineering[28] is the construction of living tissue. Bioreactors are devices used for the growth of tissues in an artificial environment that mimics the physiological conditions.

In vivo biomechanical (with flow and support loading in particular)[29] and chemicophysical conditions are created for in vitro cell conditioning and construction of blood vessel, heart valves, etc., with similar features to the native tissue ones. Mechanical loading with a conditioning protocol (cycle frequency and magnitude range) of tissue in formation in bioreactors leads to more homogeneous tissue and increased synthesis of extracellular matrix constituents than in the absence of dynamical loading. Smooth muscle cells cultured in a three-dimensional collagen-1 matrix, stimulated by PDGF and TGFβ and

[26] The liposome is an artificial lipid capsule with an aqueous core with therapeutic DNA, able to cross the cell membrane.

[27] Shh is a member of the hedgehog family of secreted glycoproteins. Shh acts via the patched 1/smoothened receptor complex to upregulate the transcription of the zinc-finger transcription factor Gli.

[28] Tissue engineering is a multidisciplinary topic that involves, in particular, cell and molecular biologists, biomaterial scientists, imaging specialists to develop materials that can replace or support diseased or damaged tissues and restore or improve tissue functions.

[29] Because mechanical forces regulate the physiological functioning of the cells, these influences must be reproduced in cell and tissue engineering.

by cyclic mechanical loading respond accordingly to the combination of biochemical and mechanical stimuli [1091]. The produced effects depend on the nature of the extracellular matrix.

In vitro investigations on cultured cells can give useful information, but they simplify the reality because important, more or less unknown, factors can be neglected, in particular the blood supply. Signaling associated with the local and regional needs as well as the state of the whole organism is neglected. By analogy with ecology, histological niches better define the cell status, living site, functioning, and interactions with the environment. Bioreactors must then replicate the niche. For example, endothelial cells are the niche component of the adult neural stem cell[30] and osteoblasts lining the inner bone surface are associated with hematopoietic stem cells.[31]

A scaffold made of natural and synthetic materials, which acts as a temporary extracellular matrix, with a nourishing supply bathed with growth factors is seeded by cells that thrive and adapt to produce engineered tissue to be implanted in the body. Synthetic biomaterials are thus developed for use as three-dimensional extracellular environments that mimic the regulatory characteristics of natural extracellular matrix and ECM-bound growth factors. Support biomaterials include self-assembling fibrillar networks and other protein polymers that present bioactive ligands and respond to cell signals. Such cues can lead to remodeling and developmental processes involved in tissue-specific differentiation with suitable structure–function relationships. Besides, bone marrow cultured in growth matrix can be used to yield angiogenesis. Moreover, mesoscale tissular modules can be assembled and associated with vascular supplies to form vascularized engineered tissues with significant viable cell densities [1094].

Tissue production depends on interactions between the growing tissue and its environment. The dynamic environment within bioreactors, in particular stimulation by mechanical stresses under control (such that cells are not damaged), like shear, is indeed known to significantly affect tissue growth and development.

Composite matrices can be used for heart valve engineering. Enzymatically decellularized porcine aortic valves that are impregnated with biodegradable polymer by a stepwise exchange provide a scaffold for heart valve tissue engineering with complete endothelialization [1095]. Ovine cell seeding of decellularized porcine heart valves (with almost complete preservation of the

[30] In the hippocampus, adult neural stem cells receives cues not only from the astrocytes [1092], but also from the endothelial cell [1093]. Endothelial cells, but not vascular smooth muscle cells, release factors that stimulate the self-renewal of neural stem cells, inhibit their differentiation, and enhance the neuron production, activating Notch pathway. The signaling between adult stem cells and support cells, indeed, involves the same molecular pathways than during embryonic development, such as Wnt, Notch, and TGFβ/BMP pathways.

[31] They bear a membrane protein jagged-1, which activates the Notch receptor of hematopoietic stem cells. Stimulated Notch pathway leads to HSC self-renewal.

extracellular matrix) is improved under pulsatile flow conditions [1096]. Preliminary numerical experiments have been carried out to study the content and orientation of collagen fibers[32] of the loaded aortic valve [1097]. In an isotropic cusp, the fiber orientation is driven by the principal strain directions. The remodeling depends on the transvalvar pressure, fiber stiffness, initial fiber direction (close to the principal strain directions in this work), and loading conditions.

Sheet-based tissue engineering, using fibroblasts and endothelial cells, without smooth muscle cells and exogenous biomaterials, can form cylindrical blood vessels, characterized by a composite multilayered wall with vasa vasorum [1099]. Cell sheet technology applied to a monolayer of adipose tissue-derived mesenchymal (non-hematopoietic and pluripotent) stem cells allows the repair of scarred myocardium after myocardial infarction in rat hearts [1100]. Engrafted mesenchymal stem cells prevent wall thinning in the scar area and improve the cardiac function.

Engineered heart tissue can form a thick myocardium after implantation on myocardial infarcts in immune-suppressed rats [1101]. When evaluated 28 days later, engineered heart tissue shows neither undelayed electrical coupling to the native myocardium, arrhythmia, nor dilation. Cardiomyocytes seeded onto mechanically conditioned perfused porous scaffolds can conduct action potentials and beat synchronously, but the scar hinders a proper integration of implanted cells.

Arterial stenoses and lumen blockage by clots locally or emboli remotely can be treated by bypass grafting surgery. Autologous grafts (internal mammary artery, saphenous vein) are used in most patients. Synthetic grafts can also be proposed. To reduce the failure rate of synthetic (polytetrafluoroethylene) grafts due to thrombosis and scar within graft lumen, biocompatible, durable biosynthetic grafts have been made. Synthetic tubes are coated by an adhesion matrix (fibronectin) and endothelial cells on its interior surface. The endothelial cells are genetically changed to over-express fibulin-5, in order to improve their adhesion on the matrix and to avoid flow-induced detachment [1102, 1103]. Because of their decayed capacity of proliferation, these modified endothelial cells are also genetically altered to over-express vascular endothelial growth factor. A coating of adhesion matrix on the tube exterior surface allows seeding of smooth muscle cells, which are also genetically altered to over-express adhesion factors and growth factors.

Mesenchymal stem cells can also be used for the construction of vascular grafts. Strain affects the differentiation (particularly in smooth muscle cells) and growth of mesenchymal stem cells, mechanotransduction effects depend-

[32] The cardiac extracellular matrix is mainly composed of collagenous fibers. The fiber network is involved in the distribution of forces generated in the heart. Studies on the collagen synthesis by cardiac fibroblasts show that the ratio of collagen 3 to collagen 1 increases in mechanically stretched cells [1098]. Collagen 3 mRNA levels are increased in response to cyclic mechanical stretch in 12 hours, whereas collagen 1 mRNA concentrations do not change.

ing on the orientation of cells with respect to the strain axis [499].[33] The implicated signaling pathway can be affected by the activity of the cytoskeleton and focal adhesions, both subjected to applied loading.

A better understanding of mechanotransduction processes can improve the tissue engineering setup. Numerical simulations, once validated, are important because: (1) they provide the fields of imposed stresses and (2) they can optimize the bioreactor design.

10.4 Vasculature Growth

Vasculogenesis defines the formation of capillary plexi from endothelial stem cells (embryological process). A primitive vascular network is formed during embryogenesis through the assembly of angioblasts. *Angiogenesis* is characterized by maturation of or generation from a primary vascular network. *Lymphangiogenesis* deals with the development of new lymph vessels. *Arteriogenesis* deals with formation of mature arterioles and arteries with smooth muscle cells, particularly for collateral development to bypass an obstructed artery.

Blood and the vessel wall are also involved in defense and repair processes. Both *chemotaxis* and *haptotaxis*[34] are involved during the tissue development. Various fibronectin-binding integrins can collaborate to yield cell adhesion and migration on fibronectin [1104].

Disruption of epithelial layers instantaneously generates lateral electric fields [1105], directed toward the wound center, with sustained outward electric currents. The lesion shunts the transepithelial potential difference. These electric fields trigger cell migration during wound healing. *Electrotaxis* is controlled by phosphatidylinositol 3-kinase-γ and tumor suppressor phosphatase and tensin homolog (PTEN) [1106].

10.4.1 Vasculogenesis

Mesodermal cells in the early embryo differentiate into endothelial precursors, the angioblasts, and aggregate. During vasculogenesis, angioblasts determine

[33] Cyclic uniaxial strain on elastomeric membranes coated by mesenchymal stem cells upregulates smooth muscle cell markers, such as α-actin, caldesmon, calponin and myosin, until the cells align perpendicularly to the strain axis. Microgrooves created in the elastomeric cell-culture membrane in the loading direction keep mesenchymal stem cells aligned parallel to the strain axis and change the cell behavior, with a production increase in calponin-1 and decrease in cartilage matrix markers. Microgrooves normal to the loading direction lead to attenuation in matrix remodeling and cell signaling.

[34] Haptotaxis corresponds to the adhesion gradient associated with the concentration of the constituents of the support medium, i.e., gradient in extracellular matrix density.

"blood pockets", which lengthen to form irregular capillaries.[35] Fusion of angioblast pockets form primary capillary plexi. The pipes connect each other in a non-hierarchical inhomogeneous network of primitive vessels. Once associated with the heart pump and blood flow, this network, which conveys moving blood, remodels with branching. A network of arteries, arterioles, capillaries, venules, and veins results. However, the dorsal aorta and cardinal vein are directly formed by assembly of angioblasts.

10.4.1.1 Vessel Formation Modes

The growth of blood vessels involves sprouting and branching. Certain endothelial cells at the distal end of each sprout, the tip cells, which strongly express VEGFR2, are selected for sprouting. Sprouting is controlled by the balance between angiogenic signals (VEGF, DLL4, and ephrin-B2) and antagonists (tight contacts with pericytes recruited by platelet-derived growth factor-B, certain extracellular matrix components, and VEGF inhibitors). Navigation cues are sensed by tip cells. The growing endothelial sprout is guided by attractive substances (netrins) and repulsive cues (semaphorin-3). Interactions between tip cells regulate the fusion of adjoining sprouts to form a continous lumen. Changes in the local balance between pro- and anti-angiogenic factors can lead to the elimination of new connections (pruning). The splitting of vessels through the insertion of tissue pillars (intussusception) expends the vascular network. Perfusion reduces hypoxia-induced angiogenic factors and promotes vessel maturation.[36] Bone marrow-derived circulating cells can be retained in the perivascular space due to stromal-derived factor-1 in response to VEGFA, and then enhances endothelial proliferation.

Intussusception,[37] a mechanism for non-sprouting angiogenesis, represents a peculiar way for expanding and modifying a vessel networks [1109]. Regions

[35] Vascular plexi, formed by aggregating angioblasts, remodel and mature into organized vascular networks of large and small, ramified or merged, vessels. Parts of vascular plexi contain blood islands. The outer cells of the blood islands give birth to endothelia, the inner cells to hematopoietic progenitors [1107].

[36] Maturation is related to the transition from a growing vascular bed to a functional network, characterized by stabilized vessels with mural cells and a basement membrane, valved veins, capillaries, and lymphatics with either fenestrations or tight junctions.

[37] Intussusception ("growth within itself"), corresponds to pillar formation for capillary genesis. It is observed in the rapidly expanding postnatal lung capillary bed. There are three modes of intussusception [1109]: intussusceptive microvascular growth, arborization, and branching remodeling. Intussusceptive microvascular growth is characterized by insertion of transluminal pillars, which induces a rapid expansion of the capillary plexus. Vessels are generated from the capillary plexus by intussusceptive arborization by perpendicular pillar formation in rows, which delineate future vessels. Pillar reshaping and fusions determine the new vascular entity. Pillars and folds are then formed in the capillary sheet, which separate the new vessels from the old capillary plexus. Intussusceptive branch-

of locally changed stress sensed by endothelial cells can trigger growth of septa, ridges, pillars, and folds. Intussusception predominantly occur in regions with accelerated blood flow.

10.4.1.2 Vasculature Compartments

Vessels through which blood flows with high and/or quick flow rates widen and vice versa. The local features of the hemodynamics thus lead to the formation of main perfusion vessels. Endothelial cells indeed respond to flowing blood forces. The vessel network is organized accordingly to neighboring tissue growth and structuration. Vessel positioning depends on traction/compression forces exerted by the growing tissues. The network progressively matures with arteries, capillaries, and veins [1108]. The direction of moving blood determines the differentiation in artery or vein, whether the lateral branch receive blood or provide it.

The arteriovenous differentiation is done via different processes. In arteries, Notch signaling is indirectly stimulated by VEGFA via VEGFR2 or neuropilin-1 and promoted by forkhead box transcription factors FoxC1 and FoxC2 [1110]. One the other hand, Notch pathway in the venous endothelium is suppressed by the nuclear orphan receptor COUP–TFII. Neuropilin Nrp1 is found in arteries, whereas Nrp2 is restricted to veins and lymphatic vessels. Arteriovenous differentiation is also controlled by hemodynamic factors. The specification of vascular cells in the different compartments of the blood vessel circuit (arteries, capillaries, and veins) is not only determined by applied mechanical forces and chemical factors (activators, inhibitors, and hypoxia) but it is also genetically programmed. Specific markers assigned to cells in each compartment can be detected before the onset of circulation [1107]. Vasculature development thus depends on a combination of intrinsic pre-patterning and extrinsic responses to environmental parameters.

10.4.1.3 Vessel Architecture and Cost Functions

Optimal design of vessel branching is based on cost functions that are the sum of the rate at which work is done on blood and the rate at which energy

ing remodeling adapts the branching angle and the bores of the branches of new supplying and draining vessels by insertion of transluminal pillars at branching points (abundant small holes are observed at branching sites). The combined cross-sectional area of the branches is greater than that of the stem: $R_t^p = R_{b1}^p + R_{b2}^p$ (subscripts t and b for trunk and branch) with $2 < p < 4$, p depending both on the trunk bore and the vessel type (supplying or draining). The area ratio is indeed significantly different in arteries and veins [1109]. The distribution of blood flow at each bifurcation depends on the asymmetry ratio between the two branches. Strong asymmetry can lead to vascular pruning (non-perfusion and removal of branches at bifurcations). The time needed to complete pillar formation ranged from 40 to 120 min.

is used, which are supposed to be proportional to the vessel volume for each vessel segment [1111]. Other cost functions have been proposed, based on the minimal total surface area of blood vessels, minimal total volume, or the minimal total wall shear force on the vessel wall, or minimal power of the blood flow.

10.4.2 Angiogenesis

Angiogenesis is the development of new branching vessels from existing vasculature. Angiogenesis thus involves the migration and proliferation of endothelial cells from pre-existing vessels. Fetal cells (trophoblasts, erythroblasts, leukocytes, hematopoietic progenitors, and mesenchymal stem cells) migrate in the maternal circulation during pregnancy. Fetal endothelial progenitors can then participate in maternal angiogenesis during pregnancy [1112]. After birth, angiogenesis participates in organ growth. During adulthood, angiogenesis in a healthy subject mainly occurs in the cycling ovary and the placenta during pregnancy. Otherwise, angiogenesis appears in trauma sites, wound healing and developing tumors.[38]

The vascular sprouts develop and build a structure. Angiogenesis is tightly coupled to tissue development to supply the growing tissue with oxygen and nutrients and remove its metabolic waste. The three-dimensional fractal-like network of branched vessel result from local growth gradients over small distances. Localized productions of growth factors promote tissue expansion and determination of the position of branching nodes. The process is adaptative, because vessel development, maturation, and regression co-exist. Local control of cell responses to stimuli, such as the regulation of the extracellular matrix, avoids disorganization.

Angiogenesis includes sequential events, beginning by increased capillary permeability, and endothelial cell and pericyte activation and hypertrophy. The following stages include basement membrane degradation, endothelial cell migration in the targeted extracellular matrix, endothelial cell proliferation, and capillary lumen formation. The later stages, with recruitment of pericytes, subsequent inhibition of endothelial proliferation, basement membrane reconstitution, and junctional complex formation, stabilize the new vessels. Bone marrow-derived pericytes are also recruited for angiogenesis, particularly after ischemia [1113]. Angioblasts are able to differentiate into both blood cells and endothelial cells.

Circulating endothelial progenitors contribute to angiogenesis, even during steady-state conditions in adult human beings. Moreover, the recruitment of myelomonocytic cells from the bone marrow to tissues can serve as a source of pro-angiogenic cytokines after ischemia. The coexistence of myeloid lineage

[38] Tumors are hypoxic at some stage because of high oxygen consumption and inadequate blood supply. In response to hypoxemia, tumor cells secrete angiogenic factors. Angiogenesis promotes tumor progression and metastasis.

progenitors able of endothelial differentiation and pro-angiogenic myeloid accessory cells then leads to two complementary mechanisms of angiogenesis. Vascular endothelial cells can differentiate from common myeloid progenitors and more mature granulocyte/macrophage progenitors [1114]. Bone marrow-derived progenitors of endothelial cells express CD31, von Willebrand factor, and Tie2, but not CD45, and the pericyte marker desmin and smooth muscle actin.

Numerous mechanisms are involved at various scales: chemical signaling and genetic response, cell interactions, and environmental stresses.[39] Computational simulations have been proposed to provide insights into structure–function relationships at all involved scales [1115]. Vasculogenesis is modeled as traction-driven remodeling of an initially uniform tissue in the absence of blood flow, and angiogenesis as a flow-driven remodeling of a porous structure.

The development of vascular trees includes adaptation to the wall stress field. Angiogenic molecules are generated in response to hypoxia and other stimuli. VEGF, *angiopoietins*, and integrins regulate the vessel caliber. The expression of the angiopoietin receptor Tie2 characterizes three cell types that have angiogenic activity: (1) endothelial cells, (2) Tie2-expressing monocytes and its hematopoietic progenitors, and (3) pericyte precursors of mesenchymal origin [1116].

Angiogenic vessels differ from mature vessels. The wall structure is not well organized. The interactions between endothelial cells and pericytes are impaired. The wall is leaky. Angiogenic endothelial cells have altered surface markers and adhesion molecules.

10.4.3 Surrounding Medium in Vessel Formation

Angiogenesis involves many growth factors and enzymes, as well as different cell types and the extracellular matrix. The behavior of endothelial cells, especially their migration and proliferation, and the formation of tubular structures is influenced by the extracellular matrix. Interactions between endothelial cells and surrounding cells, pericytes, and vascular smooth muscle cells, regulate not only vessel stabilization and remodeling, but also vascular formation. Intercellular communications are based on multiple molecules (transforming growth factor-β, angiopoietins, platelet-derived growth factor, sphingosine-1-phosphate, Notch ligands, etc.) [1117].

Endothelial cells express different vascular integrins involved in vasculogenesis and postnatal angiogenesis, which require vascular cell adhesion, migration, positioning, proliferation, and differentiation. Integrins are implicated as mediators of vascular formation and homeostasis [1118].

Plasmalemmal integrins interact with the extracellular matrix. Integrins promote cell attachment to the extracellular matrix and transduce signals

[39] Vascular network development is regulated by local interactions between vascular cells and hemodynamic conditions.

to the nucleus. In particular, V3-integrins are expressed on endothelial cells during angiogenesis. Integrin-mediated adhesion to the extracellular matrix activates the ERK pathway. The activation of extracellular signal-regulated kinase leads to *cyclin*-D1 induction, which triggers nuclear events. Cell division is controlled by the cyclins, the cyclin-dependent kinase (CDK), and the CDK-inhibitory proteins [1119] (Sect. 1.2.5).

Integrins are also required in VEGF signaling via GTPase Ras or PI3K. Integrin-binding protein *Lactadherin*[40] is involved in VEGF-dependent neovascularization in the mouse [1120]. Lactadherin interacts with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and alters VEGF-dependent PKB phosphorylation and neovascularization.

The *tenascin* (Ten) family of cell adhesion glycoproteins[41] is involved in vasculogenesis and in various normal and pathological processes of the mature life, such as wound healing and vascular diseases [1121]. In association with ECM proteins and plasmalemmal receptors, such as integrins, tenascins have contrary cellular functions, according to the mode of presentation, cell types, and differentiation states of the target tissues. Tenascins are regulated by growth factors, vasoactive peptides, ECM proteins, and mechanical factors [1121]. Tenascin-C[42] interacts with integrins, collagens, proteoglycans, and fibronectin, behaving as either an adhesive or an antiadhesive protein. It binds to annexin-2, a plasmalemmal receptor of endothelial cells [1122]. TenC gene expression is mechanosensitive.

Fibulins, a family of calcium binding proteins, interact with several matrix proteins, such as fibronectin. Fibulin-1 is associated with elastic fibers. It is also present in blood and is incorporated in fibrin clots. Fibulin-1 suppresses fibronectin-mediated adhesion and motility [1123].

A coupling exists between cell shape and proliferation, and cell division depends more on the degree of possible extension than the level of ECM binding. The actin cytoskeleton state and activity of the myosin thus play a role in cell and tissue growth. Laminin, fibronectin, and collagens-1, -3, -4, and -5 promote cell spreading. GTPase Rho can be involved in tension-dependent growth control, because it regulates cytoskeletal contractility. ECM-associated cytoskeletal mechanics can explain tension-driven tissue modeling. Besides,

[40] Lactadherin is also known as milk fat globule-EGF factor-8 (MFG-E8).

[41] Tenascins are ECM glycoproteins. They act via integrins, IgCAMs, phosphacan/RPTP, annexin II, fibronectin, and lectican. The tenascin family includes TenC, TenR, TenW, TenX, and TenY. Tenascin-C is implicated in tissue development and remodeling, particularly in vasculogenesis and wound healing. It also acts in vascular diseases and tumorigenesis. TenC promotes integrin-dependent protein clustering at focal adhesions, interacting with RTKs and activating ERK. TenR (also called restrictin or janusin) and TenW are involved in the development of the nervous system. Like TenC, TenX (or flexilin) is expressed in the heart and vascular smooth muscle cells. TenY is co-expressed with TenC in the lungs.

[42] Tenascin-C is also called cytotactin.

endothelial cell podosomes, sites of MMP concentration, are involved in endothelial cell migration and angiogenesis.

The derivatives of basement membrane proteoglycans, endostatin (fragment of collagen-18), and endorepellin (an inhibitor of angiogenesis derived from perlecan), have dual roles as structural constituents and functional regulators of tissue growth. They modulate the activity of growth factors. They can inhibit the growth of blood vessels and stabilize the basement membrane [1124].

Remodeling of the extracellular matrix by various molecules, especially extracellular and membrane-bound matrix metalloproteases is required during angiogenesis. The type of cleavage of matrix components can lead to opposite effects. Proteolysis of collagen-4 and -18, as well as plasminogen, releases angiogenesis inhibitors, whereas cleavage of collagen-4 by MMP9 stimulates migration of endothelial cells and angiogenesis.

Vessel formation requires a tuned balance between various interacting stimulatory and inhibitory signals. Thrombospondin (TSp) inhibits blood vessel formation. *Thrombospondins* TSp1 binds to integrins, matrix metalloproteinase-2, transforming growth factor TGFβ1, proteoglycans, and fibronectin. Vasohibin is also an endogenous inhibitor.

Angiogenesis requires localized destabilization of the extracellular matrix. However, transglutaminase-2 is downregulated in endothelial cells during capillary morphogenesis, to secondarily stabilize the underlying basement membrane [901]. Glutamate carboxypeptidase-2[43], expressed in endothelial cells, contributes to the remodeling of the extracellular matrix for angiogenesis. Glutamate carboxypeptidase-2 acts on β1-integrin signaling and activation of p21-activated kinase-1 [1125]. It also interacts with actin-binding filamin-A, leading to phosphorylation of p21-activated kinase-1.[44] Glutamate carboxypeptidase-2 thus participates in an autoregulatory loop. The tumor suppressor protein p53 activates the gene encoding collagen prolyl(4)hydroxylase. The latter is required for the extracellular release of collagen-derived peptides, such as endostatin and tumstatin, which inhibit angiogenesis [1126].[45]

[43] The transmembrane glutamate carboxypeptidase-2 is also named prostate-specific membrane antigen.

[44] Inhibition of p21-activated kinase-1 enhances the association of glutamate carboxypeptidase-2 with filamin A.

[45] p53 gene is inactivated in many cancers.

10.4.4 Mediators of Vascular Formation

The formation of new blood vessels, the patterning of the ramified network,[46] as well as the remodeling of existing blood vessels require the controlled migration and growth of various cell types by manifold substances, including guidance and growth factors, and their inhibitors (Table 10.3). Microvascular growth and remodeling, which is influenced by the mechanical factors (local stress field), are variable. These phenomena are indeed based on sprouting, non-sprouting (intussusceptive), and mixed angiogenesis.

Two genes, Hey1 et Hey2, play an important role in vessel formation [1127]. The combined loss of Hey1 and Hey2 do not affect initial vasculogenesis but subsequent development of major vessels. Notch components mediate the arterial gene program[47], the venous specification via the nuclear orphan receptor COUP–TF2[48], and the homeobox gene Prox-1 the lymphatic commit-

Table 10.3. Angiogenesis receptors and their ligands. Crosstalks between signaling effectors allow to coordinate their spatial and temporal activities.

Receptor	Ligand
VEGFR	VEGF (endothelial cell)
neuropilin	Semaphorin
PDGFR	PDGF (smooth muscle cell)
ERBB/EGFR	EGF
FGFR	FGF
TGFR	TGF
TNFR	TNF
Eph receptor	Ephrin
Tie	Angiopoietin
EDGR	S1P
Roundabout	Slit
Netrin	Uncoordinated-5
Galectin-1	Anginex

[46] The vascular network is locally composed of arteriolar branched and venular junctional networks interconnected by the capillary bed. The networks are afferent to and efferent from the irrigated tissues, or efferent from and afferent to the heart, respectively.

[47] Delta-like Dll4 is a Notch ligand (via the receptors Notch1 and Notch4) devoted to the specific development of the arterial endothelium.

[48] Chicken ovalbumin upstream promoter-transcription factor (COUP-TF) inhibits the activity of many transcription factors, such as nuclear receptors RAR, RXR, thyroid hormone receptors, and steroidogenic factor SF1. Transcription factor COUP-TF is able of binding to DNA either as a homo- or as a heterodimer. There are two types of COUP-TFs encoded by the NR2F1 and NR2F2 genes. COUP-TF2 is highly expressed in venous endothelium, not arterial endothelium. Transcription factor COUP-TF2 is also involved in formation of atrial-ventricular septum and coronary vessels.

ment[49] [1107]. The lymphatic system develops differently, most lymphatics differentiating from veins.

Nerve and vessel navigations to targets are subjected to a cooperative procedure, axonic and angiogenic signals guiding both vessels and axons. Ephrins, semaphorins, netrins, and slits[50] control vessel morphogenesis.

10.4.4.1 Semaphorins

During blood vessel genesis, endothelial cells produce semaphorins-3, which control integrin function for vascular reshaping [1128]. Sema3A expressed by endothelial cells of developing vessels inhibits endothelial cell migration at nascent adhesive sites of spreading endothelial cells, by interacting with integrins, like endostatin and thrombospondin. Neuropilin-1 is a membrane receptor for multiple ligands,[51] including semaphorins-3, which then regulate developmental process guidance [1129, 1130]. Sema3A and Sema3F influence vessel formation via neuropilin-1. Dimeric Sema3E targets plexin-D1 and is a chemorepellant of endothelial cells [1131]. Sema3E controls endothelial cell positioning and the patterning of the developing vasculature; but catabolized Sema3E can act as a chemoattractant. Semaphorins Sema4A, an activator of T-cell-mediated immunity, is also expressed in endothelial cells. Sema4A hinders endothelial cell migration and division stimulated by vascular endothelial growth factor, then preventing angiogenesis in vivo [1132]. Sema4A uses different receptors according to the cell type: plexin-D1 in the endothelial cells, with a lower affinity for plexin-D1 than Sema3E, whereas Sema4A, an activator of T-cell-mediated immunity, targets T-cell immunoglobulin and mucin domain-2 in T lymphocytes. Sema4D induces endothelial cell proliferation and tubule formation via plexin-B1 [1133]. Plexin-B1 activates tyrosine kinase Met. Sema4A also binds to Plexin-B1. Sema4A targets downstream effectors of PI3K in the VEGF/VEGFR2 pathway, suppressing phosphorylation of PKB and production of Rac-GTP, which promotes endothelial cell motility.

10.4.4.2 Netrins

Netrins[52] yield migration signals for axon guidance and for motion of other cells in the developing central nervous system. Axon-guidance functions are

[49] The homeobox gene Prox1 is expressed in certain endothelial cells that yield buds from veins to give birth to lymphatics.

[50] Slits are proteic chemorepellents in axon guidance and neuronal migration via the Roundabout receptor. Robo1 is expressed by vascular endothelial cells. Slit2 attracts endothelial cells.

[51] Neuropilin-1 and -2, which bind collapsin/semaphorin, are involved in axon guidance. They are also receptors for heparin-binding isoforms of VEGF-A. Both Sema–Nrp1 signaling and VEGF–Nrp1 signaling are required for heart development.

[52] Netrin is named after the sanskrit word netr, which means scout.

mediated by two families of receptors: (1) Uncoordinated-5 receptor (Unc5) family and (2) the deleted in colorectal cancer (DCC) and neogenin family. Calcium influx via plasma membrane Ca^{++} channels and cytoplasmic stores is required. High ratios of cAMP to cGMP favor attraction, low ratios repulsion. Netrins also regulate cell adhesion, motility, proliferation, differentiation, and survival in other biological tissues.[53] Netrins are guidance cues for endothelial cells and vascular smooth muscle cells during angiogenesis. Netrin receptor Unc5B in developing capillaries mediates repulsion. $\alpha_6\beta_4$ and $\alpha_3\beta_1$ integrins function as netrin-1 receptors in epithelial and endothelial cells [1134]. Activated integrins recruit and phosphorylate adapter Shc, which, in association with the Grb–Sos complex, activates Ras, then Raf, and mitogen-activated protein kinases. Shc also activates phosphatidylinositol 3-kinase and protein kinase-B.

Netrins activate small GTPases Rac, Rho, and Cdc42 (Table 10.4). Netrin-1 bound to DCC recruits extracellular signal-regulated kinases ERK1 and ERK2 into a DCC receptor complex. DCC also interacts with focal adhesion kinases and Src kinases. Netrin-1 acts a survival cue. Once bound to DCC and Unc5H, it activates caspase-3, which cleaves the death domains of these receptors, the latter activating apoptotic caspase-9. Moreover, Unc5B targeted by p53 mediates p53-dependent apoptosis until it binds netrin-1.

10.4.4.3 Ephrins

Angiogenesis is driven not only by an initiation program but also by a patterning program. Guidance molecules regulate patterning of blood vasculature during development. The navigation of migrating angiogenic components requires guidance signaling via ligand–receptor complexes, such as ephrins with Eph receptors, slit ligands with roundabout (Robo) receptors, semaphorins with plexin and neuropilin receptors, and netrins with plasmalemmal receptor deleted in colorectal carcinoma (DCC), and with neogenin and uncoordinated-5 receptors [1135, 1136].[54]

Table 10.4. Effectors of netrin-1 signaling (Source: [1134]).

GTPases	Rho, Rac, Cdc42
Kinases	FAK, Src, PLCγ, PI3K, PAK, MAPK, PKA, PKG
Transcription factors	ELK1, NFAT, p53

[53] Netrin-1 is particularly expressed in the developing pancreas, lungs, and mammary gland.

[54] EphrinB2–EphB4 signaling is involved in arteriovenous differentiation. There are four slit receptors, the Roundabouts (Robo). Robo4 and plexin-D1 are expressed by the endothelium. Semaphorin-3 inhibits endothelial cell motility via plexin-D1. Neuropilins, in competition with Sema3A, associate with VEGF receptors and allow binding of heavy VEGF isoforms to endothelial cell receptors.

Ephrin-A1 mediates TNFα-induced angiogenesis in vivo [1137]. Ephrin-B2 and its receptor EphB are arterial markers. Besides, the development of the vascular system is strongly affected by genetic mutations. EphB activates Src kinase via a cleavage of ephrin-B2 and production of intracellular peptide ephrinB2–CTF2 by presenilin-1–γ-secretase complex. EphrinB2–CTF2 binds Src, inhibiting its association with inhibitory Src kinase and allowing Src autophosphorylation [1138]. Activated Src phosphorylates ephrin-B2, inhibiting its processing by γ-secretase and triggering the recruitment of Grb4 to ephrin-B2. Grb4 controls actin dynamics and cell migration, thereby sprouting of endothelial cells.

Endothelial cells express guidance molecules for angiogenesis, such as EphB4 and its ligand ephrin-B2 [1139]. EphB4 is a negative regulator of blood vessel branching, leading to circumferential vessel growth rather than angiogenic sprouting and vessel interconnection. EphB4 and ephrin-B2 restrict migration of endothelial cells. Moreover, EphB4 reduces vascular permeability via angiopoietin-1–Tie2 activation at the endothelium–pericyte interface. EphB4 reverse signaling via ephrin-B2 represents the predominant signaling pathway, being independent of EphB4 RTK activity and EphB4 forward signaling.

10.4.4.4 Growth Factors

Many growth factors exert chemotactic and mitogenic activities on endothelial cells, smooth muscle cells, and fibroblasts, as well as control their metabolic functions. Examples of angiogenic factors acting on smooth muscle cells and/or endothelial cells are given in Table 10.5.

Certain growth factors (VEGF and angiopoietin-1) can act both on angiogenesis and vascular permeability. The transforming growth factor TGFβ stimulates extracellular matrix production to stabilize the structure of the new vessels. Tissue factor mediates angiogenesis in interaction with the clotting factor VIIa. TF–factor VIIa interactions promote the synthesis and secretion of VEGF. Endothelial cells, activated by vascular endothelial growth factor-A, assemble, and form tubes.

Nascent channels become covered not only by endothelial cells, but also by pericytes and smooth muscle cells. Pericytes and vascular smooth muscle cells stabilize small and large blood vessels, respectively. Pericytes differentiate from perivascular progenitor cells in the bone marrow. The differentiation of progenitors into pericytes and vascular smooth muscle cells is promoted by transforming growth factor-β1. However, the latter can lead to local destabilization and regression. Platelet-derived growth factor PDGF-BB

Netrin-1 acts on both the endothelial cells and smooth muscle cells of the vessel wall. It can have repulsive and attractive effects, according to the context. Neogenin is expressed in vascular smooth muscle cells, whereas Unc5 is expressed in endothelial cells.

and angiopoietin-1 recruit mural cells around endothelial channels. PDGF-B on the endothelial cell surface ensures suitable coverage of blood vessels by pericytes in the microcirculation and smooth muscle cells in the macrocirculation. PDGFRβ in pericytes and smooth muscle cells is required for guided migration and incorporation into the vessel wall, then for cell proliferation and maturation of the vasculature. PDGFRβ can cooperate with G-protein-coupled receptors S1PRs, sphingosine 1-phosphate being secreted by endothelial cells to get the appropriate number of mural cells and form adhesion sites between them and endothelial cells. Perivascular progenitors expressing the platelet-derived growth factor receptor-β (PDGFRβ) are recruited from the bone marrow to perivascular sites, particularly in tumors[55] [1140]. PDGF-B is required for recruitment of pericytes and maturation of the microvasculature. PDGF-A–PDGFRα recruit angiogenic stromal fibroblasts [1141].

The initiation of angiogenesis requires particularly fibroblast growth factor-2 and paracrine vascular endothelial growth factor. The growth factor activity favoring either cell survival or apoptosis depends on the activated signaling pathways. Both VEGF and FGF2, independently of VEGF, are survival factors for endothelial cells, whereas TGFβ1, which acts via Smad proteins and MAPKs, induces transient endothelial cell apoptosis.[56]

The growth factors, TGFβ1, FGF2, and VEGF, are often coexpressed in tissues in which angiogeness occurs. VEGF, via VEGFR2 and p38, favors, in the absence of TGFβ1 endothelial cell survival, and, in the presence of TGFβ1, cell apoptosis [1142]. During angiogenesis, apoptosis is required for pruning the forming vascular network. TGFβ1 promotes the expression of

Table 10.5. Examples of growth factors and cytokines involved in angiogenesis and arteriogenesis (Source: [1044, 1192, 1193]).

Molecule	Target
VEGF	EC
FGF	EC, SMC
PDGF	EC, SMC
TGF	EC (α⊕, β⊖), SMC, ECM
TNF	EC ⊖
EGF	EC
PD-ECGF	EC, SMC
MCP1	EC, SMC
IL1	EC, SMC
Angiogenin	EC
Angiopoitin-1	EC

[55] During tumoral angiogenesis, pericytes have morphological abnormalities.

[56] The transient apoptotic effect on endothelial cells of TGFβ1 is followed by refractoriness of the cells to TGFβ1-induced apoptosis.

FGF2 by endothelial cell, which upregulates the expression of VEGF and acts via ERK1/2.

Receptor tyrosine kinase signaling, especially via VEGF receptors and angiopoietin receptors Tie1 and Tie2, is regulated by tyrosine phosphatases, such as vascular endothelial protein tyrosine phosphatase (VEPTP). VEPTP dephosphorylates Tie2 and VE-cadherin, but not VEGFR2. VEPTP is expressed in both arterial (more strongly) and venous endothelium in embryos [1143]. VEPTP is highly expressed in the developing outflow tract of the heart and developing heart valves. Although it does not intervene in early stages of vasculogenesis, VEPTP mediates suitable remodeling of vascular plexi into large vessels and branched vascular networks by pruning, coalescence, sprouting, and intussusception. VEPTP continues to be expressed in adults in the vasculature and heart valves.

Vascular endothelial growth factors regulate the initial steps of vasculogenesis and angiogenesis. VEGFs are required in the earliest stages of blood vessel formation. Later, they are also implicated in association with other activators, such as angiopoietin, transforming growth factor-β, ephrin, and Notch. VEGF is a potent mitogen for endothelial cells derived from arteries, veins, and lymphatics [1144]. Moreover, VEGF stimulates cardiomyocytes. VEGF exerts its angiogenic effects via its specific receptors VEGFRs (Fig. 10.7).

Vascular endothelial growth factors also act on the development of the nervous system[57] and protect nerve cells under stress conditions, as a neurotrophic and angiogenic factor.

VEGF-A binds to two receptor tyrosine kinases on the plasmalemma of endothelial cells: (1) VEGFR1[58], and VEGFR2.[59] However, VEGF-A angiogenic effects are mainly mediated by VEGFR2. VEGF-A is required for chemotaxis and differentiation of endothelial precursors, as well as endothelial cell proliferation, migration, and assembly into vascular structures (sprouting and tube formation) and remodeling. Different VEGF-A isoforms regulate blood vessel growth and patterning.

Vascular endothelial growth factor-A stimulates the synthesis in cultured endothelial cells of various molecules involved in different angiogenesis stages, such as urokinase-type and tissue-type plasminogen activators (uPA, tPA), uPA-receptor (uPAR), and $\alpha_v\beta_3$ and ualpha$_v\beta_5$ integrins. VEGF-A also act on pericytes, in coordination with other growth factors, such as the platelet-derived growth factor-BB secreted by endothelial cells [1146].

Activation of VEGFR2 by VEGF stimulates the extracellular signal-regulated kinase and p38 pathways. Vascular endothelial growth factor-A

[57] In vitro, VEGF is implicated in the survival, proliferation, and migration of Schwann cells, astrocytes, and microglia [1145]. In vivo, VEGF is required for migration of certain motoneurons.

[58] VEGF receptor-1 is also called Flt1. VEGFR1 is implicated in chemotaxis and vascular permeability.

[59] VEGF receptor-2 is also named KDR or Flk1. VEGFR2 is involved via protein kinase-B and MAPK in endothelial cell proliferation and survival.

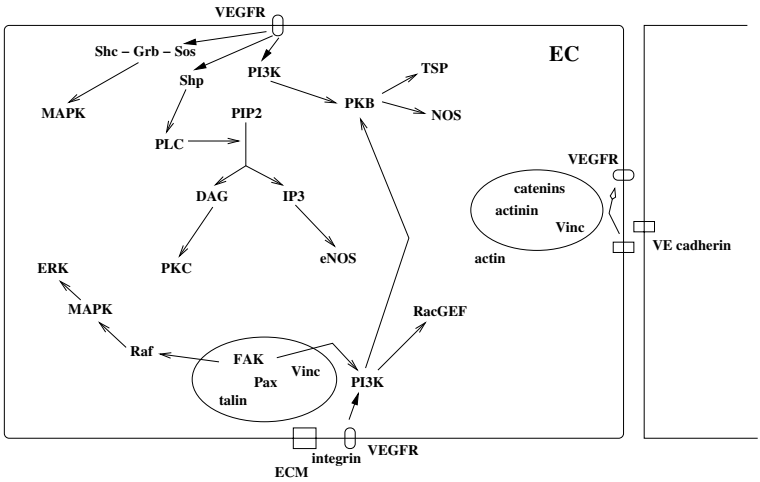

Figure 10.7. Vascular endothelial growth factor (VEGF) stimulates angiogenesis, particularly proliferation of endothelial cells (EC). VEGF binds to its receptors (VEGFR1, VEGFR2, and VEGFR3). It then activates cell type-dependent signaling cascade via Shc and Grb, tyrosine phosphatases Shp and PLCγ, phosphatidylinositol 3-kinase (PI3K), etc. Vascular endothelial cadherins (VE-cadherin) are involved in the adherens junctions between neighboring ECs. VE-cadherins interact with catenins, and subsequently with α-actinin and vinculin (Vinc), and the actin cytoskeleton. VE-cadherin acts with VEGFR2 to control the PI3K–PKB pathway. ECs are linked to the extracellular matrix (ECM) via integrins, such as $\alpha_v\beta_3$ and focal adhesion molecules, such as focal adhesion kinase (FAK), talin, paxillin (Pax), and Vinc (Source: [238]).

induces endothelial cell migration after actin filament assembly and stress fiber formation [1147], via a p38 pathway, activated by MAP2K3/6. MAPK-activated protein kinase-2, a downstream kinase of p38 cascade stimulated by VEGF-A, phosphorylates heat-shock protein 27, an actin-binding protein, which in its non-phosphorylated form, inhibits actin polymerization. Phosphorylated Hsp27 is released from capped actin filaments, thereby stimulating actin polymerization [1148]. MAPKAPK2 also phosphorylates (activates) LIM kinase-1,[60] which in turn inactivates cofilin, an actin-severing factor required in cell migration [1149].[61]

Vascular endothelial growth factor activates VEGFR2, Src-dependent phosphorylation of guanine nucleotide-exchange factor Vav2, and GTPase Rac. Activated GTPase Rac stimulates its effector p21-activated kinase, which phosphorylates VE-cadherin associated with VEGFR2. Phosphorylated VE-

[60] LIM kinase-1 is also phosphorylated by downstream kinases of the Rho GTPases, such as RoK and PAK.

[61] Cofilin phosphatases slingshot and chronophin dephosphorylates (activates) cofilin. Overexpression of LIMK1 suppresses cell motility [1150, 1151].

cadherin recruits and binds β-arrestin-2 involved in endocytosis via clathrin-coated vesicles. β-Arrestin-2 attracts Src near VE-cadherin for phosphorylation of cadherin–catenin complexes. VEGF hence promotes VE-cadherin endocytosis and disassembling of intercellular adherens junctions to disrupt the endothelium [1152].

VEGF-B binds to VEGFR1.[62] VEGFR1 mediates the recruitment of implicated bone marrow-derived cells. VEGFR1 is also involved in the induction of matrix metalloproteinases [1153] and in the paracrine release of growth factors from endothelial cells [1154].

Nitric oxide favors VEGF synthesis, which is also stimulated by hypoxia, via the transcription factor hypoxia-inducible factor-1α[63] (HIF1α) [1155] (Fig. 1.6). In cultured endothelial cells, VEGF induces expression of urokinase-type (uPA) and tissue plasminogen activators as well as plasminogen activator inhibitor-1 on the one hand and collagenase on the other hand, with a subsequent environment degradation to prepare the ground for angiogenesis. VEGF promotes the expression of urokinase receptor in endothelial cells. The interaction of uPA with uPAR mediates cellular invasion and tissue remodeling. VEGF also induces VCAM1 and ICAM1 production by endothelial cells. VEGF increases vascular permeability with possible plasma protein leakage and formation of a fibrin gel used as a substrate by endothelial cells. VEGF stimulates hexose transport in endothelial cells for increased energy demands during proliferation. G proteins in endothelial cells, particularly Gαq and Gα11, interact with and mediate intracellular signaling stimulated by VEGFR2 [1156].

VEGF expression stimulated by growth factors, mitogens, and hypoxia, is regulated by many transcription regulators. Hypoxia stimulates several transcription factors, such as hypoxia-inducible factor (mainly), cAMP-response element-binding protein, early growth response EGR1, metal-transcription factor-1, NFκB, and activating enhancer-binding protein AP1.[64] Transcription factors NFκB and AP1 are often activated by the same stimuli to target common genes. HIF targets NFκB, which stimulates AP1 subunit junB[65], the latter being required for basal and hypoxia-induced VEGF transcription activation [1157] (Fig. 10.8). The latter targets core-binding factor-β (CBFβ), which forms with Runt-related transcription factor Runx the core-binding transcription complex [1158]. CBFβ also play the role of a transcription fac-

[62] VEGF-B and placental-like growth factor are selective ligands of VEGFR1.

[63] Under hypoxic conditions, HIF1α is activated by PI3K–PKB and ERK pathways. HIF1α, with HIF1β, stimulate glycolysis and angiogenesis.

[64] The AP1 family is composed of dimeric protein complexes formed by products of Jun, Fos, and ATF gene families. Hypoxia upregulates AP1 expression, which activates target genes for endothelin-1 and PDGF-B in endothelial cells.

[65] Hypoxia-responsive transcription factor JunB is activated by numerous stimuli, such as inflammatory cytokines and NFκB. Other AP1 subunits cJun and cFos act as partners of VEGF response to hypoxia.

tor for enzyme synthesis, such as collagenase-3 or MMP13, which degrades the extracellular matrix, thus liberating space for angiogenesis.

10.4.4.5 Notch

Notch acts downstream from VEGF to regulate angiogenesis. The formation of endothelial tip cells at the leading edge of vascular sprouts is regulated by Delta-like ligand-4–Notch-1 signaling. Vascular endothelial growth factor promotes Notch signaling, which suppresses the formation of tip cells[66] [1159, 1160]. The Delta-like ligand-4–Notch pathway is specific to the vasculature. The Delta-like ligand-4 (Dll4), which triggers Notch signaling in the vasculature,[67] regulates proliferation and differentiation of endothelial cells and vascular development. The Dll4 expression in endothelial cells is controlled by vascular endothelial growth factor. Dll4 restrains excessive VEGF-induced vascular sproutings. Dll4-mediated Notch signaling hence acts as a negative regulator of VEGF-induced angiogenesis. Blockade of the interaction between Delta-like ligand 4 and Notch receptor prevents Notch signaling and inhibits efficient angiogenesis[68] in tumors resistant to VEGF inhibitors [1161, 1162]. The Dll4–Notch pathway intervenes during active vascularization rather than

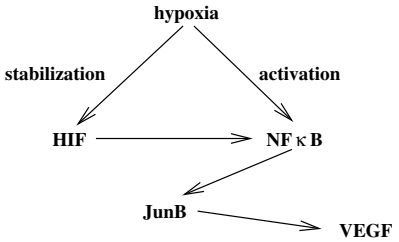

Figure 10.8. Hypoxia-responsive transcription factor JunB is activated by NFκB triggered by HIF for hypoxia-induced VEGF transcription activation (Source: [1157]).

[66] Specialized endothelial tip cells at the leading edge of vascular sprouts that form filopodia in response to guidance signals.

[67] Vascular-specific Notch ligand Dll4 is expressed during vascular remodeling. Dll4-mediated Notch signaling is restricted to the vascular system. It is involved in arteriovenous differentiation.

[68] Tumor growth rate is not correlated with tumor vascular density. Tumor perfusion could be improved by vasculature pruning. Dll4 blockade results in excessive angiogenesis. Normally, endothelial cell stimulations by multiple growth factors lead to cell proliferation with controlled degradation of the extracellular matrix, and migration using various chemoattractants and adhesion molecules. Endothelial cells then form a central lumen, and mature into a functional vessel. Neutralized Dll4 leads to defective cell fate, impairing formation of tubular structures by endothelial cells. Most of tumor vessels are not functional; they are either not perfused or convey a reduced blood flow.

in vessel maintenance [1162]. Dll4 is upregulated in tumor rather than normal vessels [1161].

10.4.4.6 Adrenomedullin

Adrenomedullin, as VEGF, endothelin-1 and -2, and angiogenin, is stimulated by hypoxia. Angiogenic adrenomedullin activates protein kinase-B, mitogen-activated protein kinase, calcitonin receptor-like receptor interacting with receptor activity-modifying proteins RAMP2/3, and focal adhesion kinases in endothelial cells, especially during acute ischemia [1163]. Moreover, adrenomedullin upregulates VEGF expression.

10.4.4.7 Regulators of G-Protein Signaling

Regulators of G-protein signaling shorten the duration of active Gαi and Gαq and acts on cell migration, proliferation, and mitogen-activated protein kinase activities [1164]. RGS4 inhibits MAPKs and VEGFR2 expression [1165]. Other stimulators of G-protein-coupled receptors (thrombin, angiotensin-2, endothelin-1, prokineticin-1 and -2, etc.) are also implicated.

10.4.4.8 Sphingosine-1-Phosphate

Sphingosine-1-phosphate released by platelets during blood clotting is a selective EC chemoattractant. It favors angiogenesis during healing [1166]. S1P activates EDGR1 and three subtypes of G-protein-coupled receptors on endothelial cells to regulate angiogenesis.

10.4.4.9 Phosphoinositide 3-Kinase and Phospholipase-C

Angiogenesis is characterized by stabilization and destabilization stages, selecting the vessel able to develop for suitable perfusion. Angiogenesis can be regulated at the level of plasmalemmal lipids. Phosphatidylinositol(4,5)biphosphate coordinates the angiogenesis phases. The angiogenic program is indeed driven by phosphoinositide-3 kinase and antagonist phospholipase-Cγ, the former promoting tube formation, the latter regression [1167]. Both substances compete for their common substrate, phosphatidylinositol(4,5)bisphosphate. PLCγ controls tube formation by reducing the level of PtdIns(4,5)-P2, which is targeted by protein kinase-C to produce diacylglycerol and inositol triphosphate, and which inhibits tyrosine kinases.

10.4.4.10 Protein Kinase-B

Protein kinase-B regulates vascular permeability and angiogenesis by fine tuning of signaling pathways in endothelial cells, including pro- and anti-angiogenic signalings [1168]. PKB has various roles at different stages of angiogenesis. The coronary angiogenesis is enhanced during the acute phase

of adaptive cardiac growth, implicating myocardial VEGF and angiopoietin-2 [1169].[69] On the other hand, neovascularization is reduced during pathological remodeling. Indeed, sustained PKB signaling causes microvascular malformations [1170]. Chronic PKB activation inhibits PI3K activity (PI3K-dependent effectors are required for full cardioprotection) [1171]. PKB short-term activity mediates hypoxia-induced expression of VEGF, whereas its prolonged activation decreases VEGF level. Protein kinase-B1 is required for migration and integrin-mediated adhesion of endothelial cells. PKB is also necessary in pericyte recruitment for vessel maturation. Protein kinase-B targets endothelial nitric oxide synthase. The eNOS pathway is implicated in vascular maturation. Protein kinase-B also controls the expression of thrombospondins TSp1 and TSp2, inhibitors of angiogenesis, by endothelial cells. Reduction in thrombospondin[70] TSp1 and TSp2 concentrations shift the PKB activity toward increased angiogenesis.

The zinc-binding matrix metalloprotease, a disintegrin and metalloprotease with thrombosspondin ADAMTS1, modulates angiogenesis, cleaving matrix-bound thrombospondins Tsp1 and Tsp2, and thereby releasing antiangiogenic polypeptidic fragments [1173]. ADAMTS1 mainly targets Tsp1 of the endothelial basement membrane.[71]

10.4.4.11 Angiopoietins

Angiopoietins, with its two kinds angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) bind endothelial cell-specific tyrosine kinase with domains Tie2. Ang1 is the major agonist for receptors Tie2. Angiopoietin-1 binding regulates blood vessel maturation [1174]. Paracrine angiopoietin-1 stabilizes newly formed blood vessels and decreases vascular permeability. Angiopoietin-1 tightens blood vessels via adhesions between the cells as well as between the cells and the extracellular matrix. The angiopoietin-1–Tie2 pathway favors the association of pericytes with the endothelium, reducing vascular leakage. Ang1 collaborates with VEGF during blood and lymph vessel development.

Autocrine angiopoietin-2 disrupts mature vessels and initiates vessel regression. Ang2, upregulated by hypoxia or VEGF, impedes the interaction between Ang1 and Tie2. Ang2 can activate Tie2 on some cells (agonist), whereas it blocks Tie2 activation on others (antagonist). Ang2 acts as a Tie2 agonist in lymphatic vessels, but as an antagonist in blood vessels [1175]. Therefore, angiopoietin-2 promotes angiogenesis according to the tissue and context.

[69] This transient signaling is associated with normal microvascular development, acute PKB activation of myristoylated PKB (myrPKB) being cardioprotective.

[70] Thrombospondins are involved not only in endothelial cell proliferation and apoptosis, but also in collagen matrix assembly and the maintenance of vascular integrity. TSp2 control the level of the matrix metalloproteinase MMP2 [1172].

[71] ADAMTS1 substrates also include aggrecan, versican, and nidogen. It is associated with catalytic modifiers, such as fibulin-1. Moreover, ADAMTS1 has a non-catalytic function, sequestrating VEGF.

10.4.4.12 Cyclooxygenase

Cyclooxygenase-2 favors expression of angiogenic factors such as VEGF and FGF2. It can inhibit endothelial cell apoptosis, stimulating intracellular anti-apoptotic pathways.

10.4.5 Angiogenesis-Targeted Therapies

Angiogenesis (and lymphangiogenesis) can be either promoted or reduced according to the disease type, arterial lesions or cancers. Blood (and lymph) vessel formation are regulated by interacting promoters and inhibitors (Table 10.6). Imbalance between these factors can lead to disease.

10.4.5.1 Angiogenesis Promotion

Therapeutic angiogenesis by administration of growth factor (VEGF, PDGF, FGF, and angiopoeitin) or gene therapy is aimed at treating artery diseases, especially coronary obstructions, by endogenous bypasses. Endothelial precursor cells can be seen in adult blood. However, they are less efficient than umbilical cord blood-derived cells. The transplantation of cord blood-derived EPCs enhances neovascularization [1179]. CD34+KDR+ cell transplantation increased capillary density in ischemic tissues, via VEGF-A release [1180].

10.4.5.2 Angiogenesis Inhibition

Most body tissues are irrigated by blood vessels, except cartilage, tendons, ligaments, the retina, and the cardiac valves. The *avascularity* of these tissues is needed for an appropriate functioning. Aberrant angiogenesis in the cartilage contribute to arthritis. Angiogenesis could be involved in degenerative tendon and ligament diseases. Angiogenesis in the retina is associated

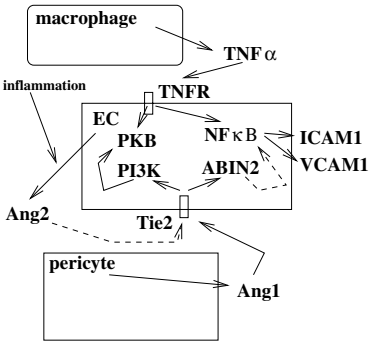

Figure 10.9. Angiopoietins are involved in angiogenesis and inflammation. They have antagonist actions (Source: [1176, 1177]).

with vision loss. The cornea expresses soluble VEGF receptor-1 (sVEGFR1 or sflt1), which binds to and sequesters corneal VEGF-A, inhibiting its activity even during hypoxia [1181].[72] In atherosclerosis, rheumatic valvular heart disease or infective endocarditis, cardiac valves express angiogenic factors leading to neovascularization. Thickening of the adult cardiac valves, which leads to congestive heart failure, such as in aortic stenosis, occurs by dysregulation of angiogenesis inhibitors, such as endostatin and chondromodulin-1 [674].

Whereas vascular endothelial growth factor receptor-2 in endothelial cells activates phospholipase-Cγ1 during angiogenesis, casitas-B-lineage lymphoma E3 ubiquitin ligase (c-Cbl) forms a complex with PLCγ1 and VEGFR2 and inhibits angiogenesis [1182].

Table 10.6. Stimulators and inhibitors of angiogenesis (Source: [1178]).

Stimulators	Inhibitors
Angiopoietin-1	Angiopoietin-2
Angiogenin	Thrombospondins TSp1, TSp2
Adrenomedullin	Cortisone
Vascular endothelial growth factor	Angiostatin
Platelet-derived growth factor	PTEN
Fibroblast growth factor	
Epidermal growth factor	
Erythropoietin	
Insulin-like growth factor IGF1	
Hepatocyte growth factor	
Transforming growth factor TGFα, TGFβ	
Tumor-necrosis factor TNFα	TNFα
Hypoxia-inducible factor HIF1, HIF2	Interferons Ifnα, Ifnβ
Interleukins IL1, IL4, IL8	IL10
Matrix metalloproteinases	Tissue inhibitors of matrix Metalloproteinases TIMP1, TIMP2
Tissue factor	Endostatin
Urokinase plasminogen activator	
Platelet-activating factor	
Nitric oxide	
Protein kinase-B	Protein kinase-B
Cyclooxygenase-2	
Prostaglandins PGE1, PGE2, PGI2	

[72] sVEGFR1 also binds VEGF-B and placenta growth factor. sVEGFR1 forms heterodimers with VEGFR2. Avascularity could be maintained by multiple redundant mechanisms associated with many anti-angiogenic molecules which exist in the cornea. But, neutralization of sVEGFR1 alone abolishes corneal avascularity. sVEGFR1 also regulates the availability of VEGF-A in cyclic vascularization and embryonic sprouting.

Limitation in angiogenesis is provided by protein fragments, such as *angiostatin* (fragment of plasminogen), released by tumor-infiltrating macrophages, and endostatin, which specifically inhibits EC proliferation. The angiogenesis inhibitor angiostatin, which acts via its receptor angiomotin, is specific for endothelial cells. *Angioinhibins* and other factors prevent angiogenesis, either by inhibiting EC proliferation (PF4, Ifnα and γ, and thrombospondin-1) [1183], hampering EC migration (PEDF and IL4), or EC proliferation induced by other mediators (FGF2 and IL). Uncontrolled growth is prevented by growth factors, cell–cell and cell–ECM contacts, and mechanical forces.

Inhibition of angiogenesis is useful to treat cancers. Angiogenesis is a regulated process necessary for tumor growth beyond 1 to 2 mm^3 (the tissue oxygen diffusion limit is 100–200 μm, i.e., which corresponds from 3 to 5 cell layers around a blood vessel), locoregional invasion, and metastasis. Tumor endothelial cells divide up to about 40 times more frequently than normal cells. They overexpress integrins $\alpha_v\beta_3$ integrin, E-selectin, endoglin, endosialin, and VEGF receptors.

The turnover of tumor endothelial cells is much greater than in normal endothelium. Activated endothelial cells express specific markers, like certain adhesion molecules and growth factors. Drugs can target activated endothelial cells. Numerous anti-angiogenic compounds exist, including inhibitors of: (1) ECM remodeling, such as MMP inhibitors[73] (TIMP1–TIMP4) [1184], (2) adhesion molecules, in particular antagonist of $\alpha_v\beta_3$ [1185], (3) activated endothelial cells, (4) angiogenic mediators or receptors, and (5) EC intracellular signaling. Therapies aimed at struggling against tumor angiogenesis must target a set of growth factors and mediators to be successful. Depletion in VEGF, in IL8, the expression of which is regulated by nuclear factor-κB, and in hypoxia-inducible factor-1 reduces tumor angiogenesis [1186].

Angiogenesis inhibitors can be used as single agents or in combination with cytotoxic drugs. Treatments that target multiple angiogenic mechanisms can increase the effectiveness of antiangiogenic therapy. Direct angiogenesis inhibitors hinders endothelial cell ability to form new vessels. Indirect angiogenesis inhibitors block either the production of angiogenic factors or signaling pathways. Mixed angiogenesis inhibitors include kinase inhibitors, epidermal growth factor receptor inhibitors, inhibitors of cyclooxygenase, inhibitors of Ang2, etc.

Certain microtubule-disrupting agents used in cancer therapy, such as combretastatin A4 phosphate[74] (CA4P), have additional antitumor effects, inducing tumor vasculature regression, acting on the VE-cadherin–β-catenin–PKB pathway, thus hampering between-cell junctions, cell migration, and anchor-

[73] Activation of metalloproteinases 9 leads to the release of soluble KIT ligand, which promotes the proliferation and motility of circulating endothelial precursors.

[74] CA4P binds to tubulin. CA4P selectively target endothelial cells, but not smooth muscle cells.

age [1187]. CA4P not only blocks the formation of a capillary network, but also induces the regression of unstable tumor neovessels.

Angiogenic factors released by tumor cells promote activation, proliferation, and migration of endothelial cells to the tumor tissue for neovessel formation. Tumor cells produce angiogenic factors, such as VEGF-A, VEGF-C, FGF2, placental-like growth factor, angiopoietins, and interleukin-8. Tumors recruit not only neighboring vascular endothelial cells but also bone marrow-derived circulating endothelial precursors, which express VEGFR2, as well as pro-angiogenic hematopoietic cells with VEGFR1. Tumor cells secrete stromal cell-recruitment factors, such as PDGF-A, PDGF-C, or transforming growth factor-β. Tumor-associated endothelial cells synthesize PDGF-B, which promotes recruitment of pericytes after activation of PDGFRβ. Angiogenic factors such as stromal cell-derived factor SDF1, which can recruit bone marrow-derived angiogenic cells, are also released by stromal cells (fibroblasts, and inflammatory and immune cells). Tumor-associated fibroblasts produce hepatocyte growth factor and heregulin. Because PDGF-A is involved in recruitment of angiogenic stroma cells that produce angiogenic factors, PDGF and VEGF inhibitors can be combined for tumor treatment.

Galectin-1 (Gal1), expressed in endothelial cells of various tumors for vessel guidance and growth, is a receptor for anginex, an angiogenesis inhibitor [1188]. Galectins are located in the nucleus, cytoplasm, or extracellular matrix according to the cell type.

Promyelocytic leukemia (PML) tumor suppressor inhibits angiogenesis in ischemic and neoplastic tissues.[75] PML prevents the synthesis of hypoxia-inducible factor-1α, interacting with the mammalian target of rapamycin (mTOR), thereby hampering mTOR association with cytoplasmic GTPase Rheb [1189].

Vascular endothelial growth factor inhibitors destroy most of the tumor vasculature, but the remaining structural components of the local vasculature, the basement membrane and pericytes,[76] allow the rapid growth of new vessels once VEGF inhibitor administration is stopped [1190].

10.4.6 Lymphangiogenesis

The primary lymph sacs and the primary lymphatic plexus appear in 6- to 7-week-old human embryos. The first lymphatic endothelial cells sprout from the embryonic veins, then migrate and form lymphatic sacs. The lymphangiogenic tissues mature by sprouting, branching, and remodeling.

[75] Promyelocytic leukemia, which regulates tumor-suppressor transcription factors, is lost in various types of human cancers.

[76] Many pericytes survive after VEGF inhibitor administration. However, the pericyte phenotype reversibly changes during treatment, with a downregulation of the expression of α-smooth muscle actin. This change reverses when the treatment is stopped.

The transcription factor prospero-related homeobox Prox1 is required during the initial lymphatic development. Prox1 regulates lymphatic endothelial cell differentiation. Sprouting, growth, survival, migration, and proliferation of lymphatic endothelial cells need vascular endothelial growth factors VEGF-C and VEGF-D, as well as the receptor VEGFR3 and neuropilin-2.[77] Nrp2 expressed in lymphatic vessels can interact with VEGFR3 and bind to VEGFC and VEGFD, the latter being less important than the former. Fibroblast growth factor-2, platelet-derived growth factor-B and hepatocyte growth factor also stimulate lymphatic vessel growth [1191].

Tyrosine kinase Syk and adapter SLP76 control the separation of the lymphatic and blood vessels. Both limit the number of lymphatico-venous anastomoses. Forkhead box FoxC2, angiopoietin-2, and ephrin-B2 are involved in the differentiation of terminal lymphatics and collecting lymphatics, as well as in the formation of valves. Forkhead transcription factor FoxC2 is then implicated in the differentiation between lymphatic capillaries and collecting valved lymphatic vessels. Angiopoietin receptors Tie1 and Tie2 are expressed by lymphatic endothelial cells. Angiopoietin-2 is required in patterning of the lymphatic network and SMC recruitment to the collecting lymphatics. EphrinB2 is involved in the remodeling of the lymphatic vasculature and valve formation. In the absence of Ephrin-B2, pericytes and vascular smooth muscle cells does not stably associate with blood vessels and can migrate and cover lymphatics.

Podoplanin, a transmembrane glycoprotein highly expressed in lymphatic endothelial cells, could play a role in lymphatic patterning. Integrins are also involved. One bound to matrix fibronectin, $\beta 1$ integrin interacts with VEGFR3. Integrin-$\alpha 9$ binds VEGF-C.

10.4.7 Arteriogenesis

Once the lumen of a main artery narrows too much, the lumen of a small artery increases to form a collateral to maintain the blood flow. Arteriogenesis is initiated by the monocyte chemoattractant protein-1 (MCP1). MCP1 has indeed been found to stimulate the formation of a collateral circulation on arterial occlusion [1194]. Arteriogenesis thus involves inflammation with monocyte recruitment. Various substances are also required at different stages of arteriogenesis, among these, TGFβ, PDGF, FGF2, GM-CSF, and TNFα. Attracted monocytes produce fibronectin and proteoglycans as well as proteases to remodel the extracellular matrix. These inflammatory cells then produce growth factors to stimulate EC and SMC proliferation.

[77] Neuropilin-2 is a semaphorin receptor of the nervous system and lymphatic capillaries.

10.5 Inflammation

The immune system responds to aggression by inflammation, a defensive event cascade. Inflammation include: (1) effector and cell delivery to the involved region, (2) limitation of the damage spreading by a physical barrier, and (3) tissue repair. The major inflammatory events include: (1) an immediate local increase in blood supply with vasodilation upstream and vasoconstriction downstream from the affected region; (2) a decrease in blood velocity for cell adhesion; (3) an increase in local capillary permeability, resulting in plasma leakage in affected tissues and creating interstitial space congestion (the exudated fluid has a higher protein content than the fluid that is normally filtrated by the capillary wall); (4) an increase in expression of endothelial adhesion molecules[78] for cell adhesion and extravasation; and (5) an influx of phagocytic cells into damaged tissues.[79] Later, lymphocytes enter the inflammation site.

Margination allows flowing leukocytes to move away from the bloodstream. Capture or tethering represents the first contact of a leukocyte with the activated endothelium. The leukocytes migrate across resting endothelium if a chemoattractant is present (chemotactic transmigration). A number of adhesion molecules have been implicated in transmigration (PECAM1, ICAM1, VE-cadherin, CD11a/CD18, CD47, and VLA4; Table 10.7). P-selectin on endothelial cells is the primary adhesion molecule for capture and rolling initiation.[80] The main leukocyte ligand for P-selectin is P-selectin glycoprotein ligand-1 (PSGL1). L-selectin exhibits an important role in capture as well. The selectin family of transmembrane adhesion receptors mediates this rolling process.[81]

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[78] Endothelial cells, activated by released IL1 and TNF, express VCAM, ICAM1, E-selectin, and L-selectin. Mast cells release histamine and prostaglandin, macrophages TNFα and IL1.

[79] Neutrophils, followed by macrophages, are the first leucocyte type to migrate.

[80] Once leukocytes are captured, they may transiently adhere to the endothelium and begin to roll.

[81] P-selectin is the most important selectin involved in rolling. P-selectin glycoprotein ligand-1 on all lymphocytes, monocytes, eosinophils, and neutrophils allows these cells to bind to endothelial P-selectin and, then, to roll along the endothelium. During rolling, bonds are formed at the leading edge of the rolling cell and broken at the trailing edge. L-selectin and E-selectin also take part in the rolling process. L-selectin is much less efficient than P-selectin in mediating the rolling process, but is necessary for the normal inflammatory response in capturing leukocytes and initiating rolling. E-selectin could be responsible for slow rolling interactions and possibly the initiation of firm adhesion. CD18 integrins participate in leukocyte deceleration and arrest. Neutrophils express small amounts of other integrins, including $\alpha_4\beta_1$, which may be important. $\alpha_L\beta_2$ Integrin is the most important integrin in firm leukocyte adhesion, whereas $\alpha_M\beta_2$ seems to be important in neutrophil activation and phagocytosis. Both $\alpha_L\beta_2$ and $\alpha_M\beta_2$

Inflammation involves mediators released by the damaged tissue, generated by plasma enzymes and produced by the leukocytes. Released histamine is a vasodilator that increases capillary permeability. Cell membrane arachidonic acid pathway leads to the production of leukotrienes, which are vasoconstrictors, and prostaglandins, which are either vasoconstrictors or vasodilators; both are chemoattractants for neutrophils (Table 8.12, Fig. 8.9). Platelet-activating factors induce platelet aggregation and attract neutrophils. Other chemokines, such as IL8, monocyte chemoattractant protein, attract flowing leukocytes to the inflammation site. Once entered the inflammation site, phagocytes release inflammatory cytokines, such as IL1, IL6, and TNFα. IL1 and TNFα not only stimulate adhesion molecules, but also activate macrophages and initiate production of growth factors required for healing and inflammatory mediators. These cytokines induce the production of acute-phase proteins by the liver, C-reactive protein and mannan-binding lectin, and increase hematopoiesis. Acute-phase proteins trigger formation of the membrane attack complex and release of *complement* components, such as fragment C3b of complement component-3.[82] Phagocytosis also leads to the production of inflammation mediators, like NO, peroxides, and oxygen radicals, which are toxic to possible microorganisms. Fibroblasts form connective tissue.

Table 10.7. Adhesion molecules involved in leukocyte migration from vessels during inflammation (Source: [123, 130]). The random contact is followed by rolling to a stop involving selectins. Sticking and extravasation require integrins.

Extravasation step	Adhesion molecules
Rolling	CD15, CD62, CD162 GAGCAM1
Stopping	$\alpha_4\beta_1$, β_2, $\alpha_4\beta_7$ Integrins ICAM, VCAM1, MadCAM1 CD11a/CD18
Transmigration	β_2 Integrins ICAM1, VCAM1, PECAM1

Integrins can bind to ICAM1 and ICAM2. $\alpha_4\beta_1$ Integrin binds to endothelial VCAM1.

[82] The complement system is an important component of innate immunity. Activation of the complement system produces derived products that contribute to pathogen elimination; but inappropriate stimulation of the complement system leads to inflammatory diseases. Complement component-3 (C3), once activated, forms fragments. The C3b fragment cements the assembly of convertases for C3 and C5 activation. C3 cleavage and activation of C3 allows binding with various molecules. The proteolysis of C3b gives birth to other fragments, such as C3c.

The tumor necrosis factor modulates cellular responses via the mitogen-activated protein kinase and the nuclear factor-κB signaling pathways to activate gene transcription. Another regulatory mechanism of TNF-dependent MAPK signaling exists [1195]. *T-cell protein tyrosine phosphatase* (TCPTP) is involved in the hematopoiesis and negatively regulates inflammation. TCPTP selectively regulates the TNF-induced MAPK signaling, suppressing the activation of MAPKs induced by TNF, without acting on the NFκB pathway. TCPTP interacts with the adaptor protein TRAF2, and inactivates Src tyrosine kinases, modulating TNF-mediated inflammation. Besides, TNF is a potent inducer of interleukin-6. TCPTP-deficiency leads to: (1) enhanced ERK1/2, but not p38, signaling and (2) higher IL6 levels. Src kinases are involved in the elevated TNF-induced MAPK signaling observed in TCPTP-deficient cells. TCPTP appears to selectively regulate the TNF-dependent MAPK signaling pathway by inhibiting Src kinases. However, in vivo regulation of SFK-induced ERK1/2 activation by TCPTP in hematopoietic cells appears to be cell-type specific.

The *inflammasome*, a cytosolic protein complex, is made of precursors of pro-inflammatory caspases. These caspases cleave the precursor of interleukin-1β. Active IL1β causes a potent inflammatory response. Inflammasome contains NALP1, NALP2, and NALP3,[83] which links to the adaptor protein apoptosis-associated speck-like protein (ASC). ASC recruits pro-inflammatory caspase precursors, which are activated by NALP3 [1196, 1197].

The D6 chemokine receptor is required in inflammation [1198]. In case of D6 deficiency, an excess concentration of residual chemokines induces an inflammatory pathology. Chronic inflammation, characterized by numerous CD4+ T lymphocytes and macrophages, can lead to fibrosis and granuloma.

10.5.1 Angiogenesis and Inflammation

Vascular endothelial cells are nodes in the body's defense system, being involved in angiogenesis and inflammation, two linked processes. There are, indeed, common regulators of angiogenesis and inflammation, such as angiopoietin-1 and -2, which hinder and enhance inflammation, respectively [1176] (Fig. 10.9). The angiopoietin–Tie2 pathway regulates vessel maturation and quiescence. Angiopoietin-1 is secreted by pericytes and activates the tyrosine kinase receptor Tie2[84] expressed by adjoining endothelial cells (paracrine Tie2 activation) to stabilize newly formed blood vessels. Angiopoietin-2 released by endothelial cells is an antagonist for Tie2 (autocrine Tie2 stimulation) on vascular endothelial cells,[85] which destabilizes mature blood vessels and an

[83] NALP3 is also named cryopyrin.

[84] Signal transduction by Tie2 activates the PI3K–PKB pathway. Stimulated Tie2 interacts with the adaptor protein ABIN2 and blocks NFκB, implicated in the synthesis of the tissue factor and adhesion molecules ICAM1 and VCAM1.

[85] Angiopoietin-2 sensitizes endothelium for TNFα-mediated inflammation and upregulates adhesion molecules ICAM1 and VCAM1 on endothelial cells.

agonist of Tie2 on lymphatic endothelial cells. The quiescent endothelium is not affected by local perfusion of tumor necrosis factor α, whereas tumor vasculature is disrupted [1177]. Angiopoietin-2 antagonize the anti-inflammatory effect of angiopoietin-1 by enhancing leukocyte extravasation.

10.5.2 Inflammatory Diseases

The migration patterns of activated immune cells depend on the stimulus features. Innate bone marrow-derived immune cells react to inflammation signals, whereas naive thymus-derived T lymphocytes poorly respond to such cues. Neutrophils are often twinned to T helper-1 cells in inflammatory infiltrates.[86] Eosinophils participate in inflammations implicating T helper-2 cells.[87] Monocytes, which differentiate to give birth to macrophages or dendritic cells, are often involved in inflammatory lesions.

Inflamed endothelium undergo changes in blood molecule permeability and flowing cell adhesiveness. Chemoattractants trigger leukocyte adhesion to endothelial cells and guide leukocyte migration and positioning. They mainly act via G-protein-coupled receptors. Homing receptors are involved in the cell migration.[88] Except T and B lymphoblasts, which express adhesive integrins, circulating leukocytes have inactive integrins. Leukocyte activation leads to integrin which is able to bind to specific endothelial ligands. Integrin activation by endothelial chemokines occurs very quickly (few ms). Combinations of chemokines and GPCRs (Gi protein–RhoA and Rap1 pathways mainly) activate endothelium adhesion under shear. L-selectin, expressed on most circulating leukocytes, initiates leukocyte extravasation in venular endothelium in inflammation sites. P- and E-selectins are inducibly produced by stimulated endothelial cells to attract neutrophils, eosinophils, monocytes, natural killer cells, and T and B lymphocytes. Moreover, L-selectin on circulating leukocytes can also bind surface molecules (such as P-selectin glycoprotein ligand 1) on wall-adherent leukocytes, enhancing the extravasation rate.

Diapedesis requires transient disassembly of endothelial junctions. Rac, Rap1 and RhoA GTPases, focal adhesion kinases, protein kinase-C, and phosphatidylinositol 3-kinase promote leukocyte extravasation. Extravasation depends on basal lamina composition: leukocytes cross vascular basement membranes that contain laminin-8.[89] The interstitial matrices remodel during in-

[86] TH1 inflammation is characterized by tissue infiltration of interferon γ-secreting CD4+ and CD8+ T lymphocytes and activated macrophages.

[87] Allergic inflammation is characterized by infiltration of TH2 cells. Eosinophils and mast cells secrete interleukins-4, -5, and -13.

[88] CCR4 and 8 might be involved in the displacement of T helper-2 cells, CXCR3, CXCR6 and CCR5 in the T helper-1 recruitment. Eosinophil uses CCR3 at sites of allergic inflammation, as well as certain TH2 cells, and monocytes CCR2 at sites of TH1 inflammation. Tissue-specific homing receptors, such as CCR10 for the skin and CCR9 for the gut explain the specificity of leukocyte recruitment.

[89] Leukocytes cannot migrate through basal lamina with laminin-10.

flammation with an increased production of fibronectin, tenascin, collagen, and sulfated proteoglycans. Proteases and glycosaminoglycan-degrading enzymes are upregulated shortly after transendothelial migration by inflammatory cells to penetrate the extracellular matrix.

The strong neutrophil inflammatory response can damage host tissues.[90] Any anti-inflammation therapy must target the infiltrated immune cells translated in effectors of inflammatory diseases without too much disturbing the migration and function of leukocytes required in immunity. Effective therapies must target specific immune cells and focus on peculiar biochemical mechanisms involved in chemotaxis and cell motion.[91] TH1 cell recruitment in TH1 inflammation disorders, such as atherosclerosis, can be targeted.

10.6 Healing

The repair of damaged tissues involves blood cells and molecules to seal the vessel wall gap off, clean the wound, and reconstruct the tissues. Four interconnected plasma enzyme systems produce mediators: (1) the kinin, (2) complement, (3) coagulation, and (4) fibrinolytic systems . Tissue damage activates FXII, which triggers the kinin and clotting cascades. Bradykinin causes vasodilation and increased permeability. Cell-bound bradykinin also promotes histamine production. The fibrinolytic system leads to plasmin synthesis. Plasmin degrades the fibrin clot and activates the *complement* system.

The wound healing process has three phases: inflammatory, proliferative, and maturational phases. The inflammatory phase (`phase I`) is characterized by hemostasis and inflammation. Blood flow is regionally regulated. Exposed collagen activates the clotting cascade and initiates the inflammatory phase. Damaged cells release vasoconstrictors, thromboxane-A2 and PG2α, and wounded vessels immediately constrict to reduce hemorrhage. Hemostatic plug occurs that will be transformed into a stable clot. Platelets, the first responding cells, release chemokines, such as PDGF[92] and TGF,[93] which initiate healing. Involved cytokines and chemokines also include IGF, EGF,[94] and FGF.[95] Fibronectin, fibrinogen, histamine, serotonin, and vWF are also

[90] Inflammatory diseases can target any body tissue, but they mainly damage peculiar organs or tissues, such as the skin, intestine, central nervous system, skeleton articulations, respiratory conduits, and arteries.

[91] Blockade of neutrophil chemokine receptors CXCR1 and CXCR2 hinders the neutrophil flux.

[92] PDGF is chemotactic for fibroblasts, neutrophils, and macrophages. Once attracted to the wound site, PDGF then activates macrophages and induces fibroblast proliferation.

[93] TGF is chemotactic for monocytes, lymphocytes, and fibroblasts. TGF regulates matrix proteins, collagen, proteoglycans, fibronectin, and matrix degrading proteases, and their inhibitors.

[94] EGF, produced by macrophages, induces epidermal regeneration.

[95] FGF1 and FGF2 are involved in wound healing. FGF2 stimulates angiogenesis.

recruited. These mediators control bleeding through clot formation. Platelet degranulation activates the complement cascade, specifically C5a, a chemoattractant for macrophages, neutrophils, and mast cells, which also increases the wall permeability of vicinity vessels and stimulates leukotrienes LktC4 and LktD4. Capillary vasodilatation is later induced by local histamine release, and inflammation cells migrate to the wound-healing locus. The early infiltrated neutrophils, attracted by proteolytic degradation products of fibrin, complement components C3a and C5a, platelet factor 4 and PDGF, scavenge cell debris and foreign bodies.[96] The neutrophils produce elastase and collagenase.

The proliferative phase (`phase II`) follows. The next cells present in the wound are leukocytes, particularly macrophages. Attracted monocytes differentiate into macrophages. The macrophages also phagocytose and kill bacteria that have been opsonized by binding specific antibody and complement fixation products from serum exudate. The macrophages also phagocytose cell debris. Enzymes and cytokines are secreted by macrophages: collagenases, ILs, and TNF (which stimulate fibroblasts and promote angiogenesis), and TGF. Lymphocytes participate in the late inflammatory infiltrate. Fibroblasts, which have migrated from the vicinity under chemotaxis, produce ground matrix and then collagen to build a new extracellular matrix.

Cell migration and signal release lead to tissue reconstruction (`phase III`). The remodeling is characterized by a balance among synthesis, deposition, and degradation. The connective tissue is able to receive water (edema), minerals, molecules, and cells. The edema region is invaded by various substances and cells to become an inflammatory granuloma. Granulation tissue is formed, composed of a matrix of fibrin, fibronectin, glycosaminoglycans with proliferating endothelial cells, and fibroblasts mixed with macrophages and lymphocytes. Epithelialization occurs early in wound repair. Angiogenesis, stimulated by TNFα, is marked by EC migration and capillary formation. New capillaries deliver nutrients to the healing region. Fibroblasts differentiate. Wound extracellular matrix, composed of collagen and elastin fibrils interspersed with glycosaminoglycans, polysaccharides, and proteoglycans, fulfills a structural and regulatory role in cell behavior by contact or as a reservoir of growth factors. Fibroblasts transform into myofibroblasts with contractile features. During the final maturational phase, the tissues undergoes contraction. Collagen turnover is increased. Remodeling of the collagen fibers with degradation by matrix metalloproteinase manufactured by macrophages and fibroblasts among others, and with synthesis stimulated by cytokines, such as TNFα and IL1, is associated with re-orientation of collagen fibers.

[96] Local decontamination deals with bacteria destruction. The bacteria are phagocytosed and contained within phagosomes, where they are killed by reactive oxygen species, such as superoxide, hydroxyl free radicals, and hydrogen peroxide.

Simple models of wound healing are based on conservation equations dealing with cell density, ECM density, concentration in stimulators, and inhibitors of cell division [1069]. The change rate in cell density depends on the cell migration flux and production and loss rates. The change rate of ECM density depends on its production and degradation rates and on its deformation rate. Cell–ECM interaction is introduced by a force balance in which the body forces are in equilibrium with the divergence of the traction stress exerted by the cells on the extracellular matrix and the divergence of the resistive stress of the extracellular matrix, which is composed of an elastoplastic and a viscous component. The time gradient of substance concentration depends on the chemical diffusion flux and its production and activity rates. The role of the blood flow in the transport of cells and compounds is ignored.

10.7 Vascular Tissue Remodeling

The wall of the heart and the blood vessels bear stresses applied by the flowing blood. The wall reacts, and then strengthens in high stress regions. The short-term wall adaptation can indeed lead to long-term remodeling when abnormal stress magnitude and oscillation amplitude during the cardiac cycle are sensed. Integrative model incorporates behaviors at various length and time scales in order to efficiently describe structure-function relationships of physiological systems.

10.7.1 Myocardium Remodeling

Acute myocardial infarction leads to necrosis of cardiomyocytes and other cells. The inflammatory response triggers the migration of platelets, neutrophils, macrophages, monocytes, and other inflammatory mediators to the infarction site, where matrix metalloproteinases (MMP) degrade components of the extracellular matrix. Regions of fibrosis are formed as collagen is deposited. The fibrotic areas of infarctions do not have the contractile function, without regional myocyte regeneration, appropriate ECM formation, and angiogenesis.

The heart contains very rare cardioblasts (with a decreasing number with age), susceptible to divide[97] and mature after birth, for self-regeneration, especially after a heart attack [1199]. CMC proliferation, from cardiomyocytes, resident stem cells, endothelial cells, fibroblasts, or migrated hematopoietic stem cells, in areas adjacent to the infarcted zone can be a regeneration source. Adequate input in growth factors is necessary to stimulate the myocardial regeneration with needed angiogenesis and ECM formation to avoid maladaptive remodeling of the myocardium.

[97] Unlike stem cells, cardiac progenitor cells undergo a finite number of divisions, but become fully specialized cardiomyocytes.

Embryonic stem cells lead to two types of cardiovascular progenitors that express VEGFR2 [682]. The first type of VEGFR2+ cells act as *hemangioblasts*, giving birth to hematopoietic cells and endothelium. The second type gives rise to cardiomyocytes, endothelium, and smooth muscle cells. The pathways (Wnt, TGFβ1, bone morphogenetic factors and their endogenous antagonists, fibroblast growth factors, etc.), which regulate the cardiac development, could be used for stem cell-based cardiac repair.

Granulocyte colony stimulating factor (G-CSF) participates in heart regeneration after myocardial infarction. G-CSF activates the Janus tyrosine kinases and the signal transducer and activator of transcription (JaK–STAT pathway) in cardiomyocytes, mediating the phosphorylation of STAT3 and JaK2 [1200]. G-CSF has an anti-apoptotic effect, with increased levels of the anti-apoptotic proteins BCL2 and BCLxL. However, G-CSF stimulates the angiogenesis but not the proliferation of cardiomyocytes.

Lipopolysaccharides and postischemic reperfusion activates myocardial p38 and nuclear factor-κB, which lead to TNF production by the cardiomyocytes [1201]. TNF depresses myocardial function by NO-dependent and sphingosine-dependent mechanisms. TNF can induce cardiomyocyte apoptosis.

Tumor-necrosis factors and lipopolysaccharides induce distinct biological responses although they use the same signaling pathway with the effector nuclear factor-κB, via TNF receptor-1 and Toll-like receptor-4, respectively. Positive and negative feedbacks are involved that determine the timing of transcription factor activity and control the expression of genes mediated by the same transcription factor [1202, 1203]. TNF-dependent activation of IKK reaches its peak between 5 and 15 minutes and then shows oscillatory behavior if the stimulus is continued, whereas LPS-mediated signaling induces a slower biphasic IKK response (a small initial followed by a larger increase and slow attenuation).

Cardiac hypertrophy is induced by sustained pressure overload. Multiple hypertrophy signaling pathways are triggered by pressure: calcineurin–NFAT, PI3K–PKB, and ERK1/2. The calcium-dependent phosphatase *calcineurin* influences the growth and gene expression of the myocardium. Calcineurin binds to *calsarcins*.[98] Calsarcin-1 negatively modulates the functions of calcineurin [1204]. The absence of calsarcin-1 enhances the cardiac growth response to pressure overload and favors hypertrophy.

Vasoactive substances such as angiotensin-2 are secreted by the overloaded myocardium. Angiotensin-2 induces superoxide anion production in cardiomyocytes and heart hypertrophy. Cardiac hypertrophy is mediated by myocardial oxidative stress, Rac1 GTPase, and NADPH oxidase [1205].

Cardiac hypertrophy caused by chronic hypertension is associated with fibrosis, such as in myocardium infarction. Fibrosis due to hypertension involves reciprocal interactions between stimulatory and inhibitory factors, resulting

[98] Calsarcin family are proteins of the sarcomeric Z disc (Sect. 7.7.4).

in increased deposition of collagens-1 and -3 within the adventitia of coronary arteries (perivascular fibrosis), which progressively extends.

10.7.2 Vessel Wall Remodeling

Blood vessels are subjected to mechanical forces that are implicated in vascular development, adaptation, and genesis of vascular diseases. Vessel caliber changes depend on a combination of wall wetted surface and intramural stresses. Blood pressure changes quickly induce tissue remodeling. Hypoperfusion reduces lumen caliber and media mass, whereas hyperperfusion increases these two quantities. Chronic increases in arterial blood flow lead to vessel enlargement, and WSS reduction to physiological values.

Arteries enlarge in response to increased blood flow and wall shear stresses. Gaps in the internal elastic lamina have been observed in arteries exposed to high blood flow, whereas EC proliferation assures a continuous lining [1206]. Artery enlargement and adaptive remodeling is associated with FGF2 expression which acts on endothelial cells and smooth muscle cells [1207]. Conversely, upregulation of endothelin-1 and downregulation of nitric oxide can initiate and mediate the remodeling after blood flow reduction.

Step pressures induce a bore increase followed by a reactive SMC contraction (quick adaptation). Delayed slow structural changes are characterized by SMC proliferation. The time required by internal vessel radius variations after flow rate changes in large arteries is few days to weeks [1208]. Wall remodeling is characterized by a rapid first stage of geometrical changes. Besides, hypertension attenuates endothelial-to-endothelial or smooth muscle-to-endothelial communications in skeletal muscle resistance arteries [1209]. Wall distensibility is more progressively restored. The mechanical changes occur during the second stage of wall remodeling.

Under sustained hypertension, the wall thickness of segments of the descending thoracic aortas increases in hypertensive dogs with respect to normotensive dogs, with a concurrent prestress reduction and an axial stiffening increase [1210]. Wall thickness of rat thoracic aortas correlates significantly with systolic blood pressure [1211]. The wall elastic moduli of hypertensive rats becomes equal to the normal values after a relatively long time.

Rapid changes in stiffness of the basilar artery occurs during hypertension, with increased collagen content in the media and adventitia and smooth muscle cells in the media, within 2 weeks after the initiation of hypertension, using an aortic coarctation model [1212].

Hypoxia-induced pulmonary hypertension leads to remodeling of pulmonary vessels and right ventricular hypertrophy. Pulmonary vessel remodeling is characterized by wall thickening, collagen deposition and infiltration by pulmonary macrophages.

Vascular smooth muscle cells participate, in response to hypertension, to geometrical and rheological adaptation, characterized not only by SMC proliferation but also migration, with myogenic adjustment. Vascular smooth

muscle cells migrate from the media into the intima, proliferate, synthesize extracellular matrix, and form the neointima. Hypertension induces change in vasomotor tone and wall circumferential stress [1213].

Flow-dependent remodeling is more pronounced in muscular arteries than in elastic arteries. These regional differences may be associated with the local flow pattern and the quantity of smooth muscle cells and ECM components.

The arterial wall layers non-uniformly thicken during induced hypertension [1214]. The inner wall layers thicken more in the acute phase of hypertension, whereas the outer layers are thicker than the inner layers when the vessel is subjected to long-term (up to 56 day) hypertension. The medial collagen content quickly increases during the acute hypertension phase and slowly later. The elastin level slightly and steadily increases. Vascular smooth muscle tone rapidly rises and nearly returns to control levels.

Hypertensive remodeling is characterized by wall accumulation of collagens-1, -3, and -4 to counteract wall distention, but it yields wall stiffness. However, released matrix metalloproteinases, especially MMP9 [1215], lead to wall degradation with subsequent enhanced wall distensibility.

Nitric oxide is a predominant mediator of vasodilation and remodeling in response to increased wall shear stresses. NO release from endothelial cells exposed to excessive shear triggers a set of events, including growth factor induction and MMP activation, which together contribute to restructuration of the vessel wall [1216]. Moreover, MMP degradation can overcome concomitant ECM synthesis. Although the elastic content remains normal, fragmentations in medial elastic laminae induce vessel tortuosity.

Platelet-derived growth factor regulates the tyrosine phosphorylation of signaling proteins, such as the PDGF receptor and the phospholipase-Cγ1, via intracellular production of H_2O_2 in particular. PDGF is especially involved in smooth muscle cell proliferation and migration during vascular remodeling.

The *peroxiredoxin-2* (Prx2), a peroxidase that eliminates endogenous H_2O_2 in response to growth factor signaling, is a negative regulator of PDGF signaling, especially during vascular remodeling [1217]. Neointimal layers of injured carotid arteries of mice lacking Prx2 are thicker than those of wild-type mice. Prx2 is involved in PDGF-dependent neointimal thickening, atherosclerosis, and other lesions of blood vessels.

The *urokinase* (uPA), involved in functioning of the smooth muscle cells (proliferation, adhesion, and migration[99]), participates in wall remodeling via the uPA receptor (uPAR). uPAR activation induces its association with

[99] SMC migration involves activation of the Janus kinase Tyk2, phosphatidylinositol 3-kinase, and the Rho kinase pathway.

platelet-derived growth factor receptor PDGFRβ,[100] leading to PDGF-independent PDGFRβ activation of its tyrosine kinase activity [1218].

The expression of *P2X4 receptors* in the vessel wall strongly increases after balloon injury, with a much higher density in the media, a very high density in the enlarged neointima, and a ten-time density increase in the endothelium [302]. Activated P2X4 receptors can thus be involved in neointimal proliferation. Examinations of blood vessels used for coronary artery grafts show that levels of P2X4 are much lower in arterial vessels than vein samples. Therefore, artery grafts are more suitable than venous grafts to prevent restenosis and atheroma. P2Y2 expression also increases in balloon-injured endothelium and neointima, but without no discernible differences between the different examined vessels.

In a dynamical adaptation model of the vessel wall under hypertension, the time evolution of the vessel geometry (external χ_e and internal χ_i perimeters, wall thickness h, vessel length L) has been described, assuming a thick-walled tube made of non-linear elastic incompressible material [1219]. The geometrical quantities are expressed as functions of the respective stresses in normal (superscript n) and hypertensive (superscript h) states. For example, the remodeling rate equation of the wall thickness is given by: $dh/dt = (1/\tau_h)[(\breve{c}^h_\theta(t) - \breve{c}^h_\theta)/\breve{c}^n_\theta]$, where τ_h is the corresponding time constant and $\breve{c}_\theta$ the wall-averaged circumferential stress. The model predicts, in particular, that the vessel caliber increases up to a maximum and, then, decreases to a limit value 1 month later. The mechanical properties vary during the remodeling. The constitutive equations must then include a growth-stress relationship in addition to the usual stress–strain relationship. Bending experiments of excised vessel samples have been proposed to determine the rheology constants of the wall layers during the layer structure-dependent remodeling [1220]. However, in vitro data may be very far from in vivo values.

10.8 Growth Modeling

In planar cultures on matrices rich in collagen-4 and laminin, the endothelial cells form clusters and pull on the matrix, generating tension lines that can extend between the cell aggregates. The matrix eventually condenses along the tension lines, along which the cells elongate and migrate, building cellular rods. The rate of change in cell density is equal to the balance between the convection and the strain-dependent motion. The inertia being negligible, the forces implicated in the vasculogenesis model include the traction exerted by the cells on the extracellular matrix, cell anchoring forces, and recoil forces of

[100] The two PDGFRα and β are expressed in smooth muscle cell, but PDGFRβ expression is stronger. uPAR interacts also with integrins, the LDL receptor-related protein, the G-protein-coupled receptor formyl peptide receptor (FPRL1, or lipoxin A4 receptor LXA4R), the endothelium-derived growth factor receptor (EDGFR).

the matrix [1221]. A mathematical model has been carried out using a finite difference scheme to study the role of the mechanical and chemical forces in blood vessel formation, and to simulate the formation of vascular networks in a plane [1222]. The numerical model assumes: (1) traction forces exerted by the cells onto the extracellular matrix, (2) a linear viscoelastic matrix, and (3) chemotaxis. The equation set is composed of: (1) a convection–diffusion–reaction equation that describes the cell density, (2) a conservation equation for extracellular matrix density, (3) a traction–displacement equation associated with extracellular matrix organization, which contains a non-linear term due to cell traction saturation at high cell densities, leading to two scalar equations for the force and displacement vector components (2D problem), and (4) diffusion–reaction equation for the chemotactic molecule concentration. Spontaneous formation of networks can be explained via a purely mechanical interaction between cells and the extracellular matrix.

Chemotaxis alone is not sufficient to generate tissue formation, with cell proliferation and ECM formation. During vessel sprouting, mechanical forces can help in the formation of well defined vascular structures. The modeling can take into account cell–cell interactions and feeding [1223]. A model, which is based on the Navier-Stokes equations in steady state and a simple mechanistic tissue response, can predict bifurcation formation and microvessel separation in a porous cellular medium [1224]. The tissue is remodeled according to the tangential shear stress; the convection is approximated by simple non-diffusive heuristics at each remodeling step.

Whereas angiogenesis cannot be explained by parabolic models, numerical simulations based on hyperbolic models of chemotaxis mimic migration of endothelial cells on matrigel and the formation of networks that lead to vasculature [1225, 1226].

When tissues remodel, zero-stress state of the vessel is modified. Wall remodeling implies changes in rheological properties, and consequently, if the elastic behavior is expressed in terms of a strain-energy function (SEF), the material constants in the function must be updated. Moreover, the constitutive equation must include not only stress history but also material history due to wall restructuration.

The vessel lumen subjected to sustained increments in blood flow enlarges. The arterial wall thickens in response to sustained increases in blood pressure. Walls remodel, mainly to restore the stress field toward their homeostatic values. Flow-induced changes involved in long-term vascular tissue growth and remodeling have been studied using the continuum approach and motion decomposition [1227, 1228]. A homogenized, constrained mixture theory is used to develop a 3D constitutive law that takes into account the three primary load-bearing constituents (smooth muscle cell, collagen, and elastin) with time-varying mass fractions due to the turnover of cells and extracellular matrix fibers during the wall remodeling under a varying stress field. The turnover of constituent i is described by its total mass evolution, introducing two evolution functions for production and degradation rates. Besides,

axial extension quickly increases the length of a carotid artery and the rate of turnover of cells and matrix, the turnover rates correlating with the stress magnitude. Numerical simulations show that moderate (15%) increases in axial extension generate much greater axial stress than circumferential stress augmentation induced by marked (50%) rise in blood pressure [1229]. A 2D constrained mixture model, based on different constitutive relations shows that the turnover of cells and matrix in altered configurations is effective in restoring nearly normal wall mechanics.

Concluding Remarks

Part I chapters give the necessary knowledge associated with cell behavior, especially when the cell reacts to various stimuli, particularly mechanical stresses. The magnitude and direction of these mechanical stresses applied by the flowing blood on the wetted surface of the endothelium as well as within the vessel wall varies during the cardiac cycle. The heart generates unsteady flows in a network of blood vessels characterized by complicated architecture and variable geometry both in space and time. Vessel geometry varies over short distances. The vascular network of curved blood vessels is composed of successive geometrical singularities, mainly branchings. Three-dimensional blood flow is thereby developing, being conveyed in entrance length (App. C.7). Moreover, blood vessels are deformable. Changes in transmural pressure (the pressure difference between the pressure at the wetted surface of the lumen applied by the moving blood on the vessel wall and the pressure at the external wall side, which depends on the activity on the neighbor organs) can also affect the shape of the vessel cross-section, especially when it becomes negative. More generally, the change in cross-section shape is due to taper, possible prints of adjacent organs with more or less progressive constriction and enlargment, and adaptation to branching (transition zone). These changes can induce three-dimensional blood motions displayed by virtual transverse currents, even if the vessel is considered straight (Part II). Local changes in the direction of stress components are caused by flow separation and flow reversal during the cardiac cycle. Flow separation is set by an adverse pressure gradient when inertia forces and blood vorticity are high enough, especially in branching vascular segments. Due to the time-dependent feature of blood flow, the flow separation region spreads over a variable length during the cardiac cycle and can move. The location and variable size of the flow separation region depends on the flow distribution between the branches, which can vary during the cardiac cycle. Flow reversal occurs during the diastole of the left ventricle in elastic arteries, such as the aorta, and most of the muscular arteries, such as brachial and femoral arteries (but not in the carotid arteries). Flow reversal can be observed either in a region near the wall, more

or less wide with respect to the position of the local center of vessel curvature, or in the entire lumen.

This book also provides a survey of flow modeling and simulations in the cardiovascular system. The basic knowledge of fluid mechanics necessary to understand blood flow behavior is given in part II. However, the Navier-Stokes equations, the set of partial differential equations (PDE) developed in the framework of continuum mechanics that governs the fluid flow is introduced in the following. Like any mathematical model, Navier-Stokes equations link the physical quantities that are involved in the studied process (both independent and dependent variables, time, space coordinates, temperature, pressure, velocity, energy, cross-section area, etc.) using equations which describe the system behavior. The mathematical model has a value only when realizable measurements validate the model, i.e., the model generates verifiable predictions.

The Navier-Stokes equations correspond to the mathematical formulation of basic physical principles, especially the conservation of mass and momentum. Any fluid motion is indeed characterized by three principles: (1) the matter is neither lost nor created; (2) the variation rate of momentum of any fluid part is equal to the resulting forces (sum of all applied forces) on this fluid part; and (3) the energy is neither lost nor created.

Conservation Equations

Mass and momentum conservation equations are obtained from the analysis of the evolution of involved quantities in an infinitesimal control volume, the so-called fluid particle. The following differential operators are used: (1) the gradient operator $\boldsymbol{\nabla} = (\partial/\partial x_1, \partial/\partial x_2, \partial/\partial x_3)$, (2) the divergence operator $\boldsymbol{\nabla}\cdot$, and (3) the Laplace operator $\boldsymbol{\nabla}^2 = \sum_{i=1}^{3} \partial^2/\partial x_i^2$. The gradient $\boldsymbol{\nabla} p$ of scalar p is a vector of component $(\nabla p)_i = \partial_i p$. The gradient $\boldsymbol{\nabla}\mathbf{v}$ of vector $\mathbf{v}$ is a second order tensor with component $(\nabla \mathbf{v})_{ij} = \partial_i v_j$.

The first basic postulate states that matter is neither destroyed nor created, meaning that any matter finite volume neither disappers nor becomes infinite. The second basic postulate states that matter is impenetrable; any matter element does not share the same location with another element (*continuity axiom*).

The elements in presence are: (1) the mass change rate $\dfrac{\partial}{\partial t}\displaystyle\int_{V_e} \rho \, dV$, and (2) the mass flux across the surface Γ_e of the infinitesimal control volume V_e, of unit normal $\widehat{\mathbf{n}}$ outward oriented $-\displaystyle\int_{\Gamma_e} \rho \mathbf{v} \cdot \widehat{\mathbf{n}} \, dA$.[1] Using the divergence theorem (Gauss formula),[2] the equation of mass conservation, or the so-called equation of continuity, is obtained:

[1] The scalar product of two vectors provides a scalar: $\mathbf{v} \cdot \mathbf{n} = \sum_{i=1}^{3} v_i n_i$.

[2]
$$\int_V \nabla \cdot (\rho \mathbf{v}) \, dV = \int_\Gamma (\rho \mathbf{v}) \cdot \widehat{\mathbf{n}} \, dA.$$

$$\partial_t \rho + \nabla \cdot (\rho \mathbf{v}) = 0. \tag{10.2}$$

The equation of momentum conservation of a moving fluid of mass density ρ, of dynamic viscosity μ, of kinematic viscosity $\nu = \mu/\rho$, which is conveyed with a velocity $\mathbf{v}(\mathbf{x}, t)$ ($\mathbf{x}$: Eulerian position, t: time) is obtained from the balance of surface and remote forces applied to the fluid particle.

The elements in presence are: (1) the body forces $\displaystyle\int_{V_e} \mathbf{f}\, dV$, (2) the pressure forces $\displaystyle -\int_{\Gamma_e} p\hat{\mathbf{n}}\, dA$, (3) the viscous forces $\displaystyle\int_{\Gamma_e} \mathbf{C}\hat{\mathbf{n}}\, dA$, and (4) the inertia forces $\displaystyle\int_{V_e} \rho \frac{D\mathbf{v}}{Dt}\, dV$, where $\dfrac{D\mathbf{v}}{Dt} = \partial_t \mathbf{v} + (\mathbf{v} \cdot \nabla)\mathbf{v}$.[3] Using the Gauss formulas,[4] the momentum conservation is obtained:

$$\rho(\partial_t \mathbf{v} + (\mathbf{v} \cdot \nabla)\mathbf{v}) = \mathbf{f} - \nabla p + \nabla \cdot \mathbf{C}. \tag{10.3}$$

The Navier–Stokes equations governing an unsteady flow of an incompressible (ρ constant) fluid in the absence of body forces are then given by:

The divergence $\nabla \cdot \rho\mathbf{v}$ of the vector $\rho\mathbf{v}$ is the scalar $\sum_{i=1}^{3} \partial_i \rho v_i$.

[3] The convective inertia term $(\mathbf{v} \cdot \nabla)\mathbf{v}$ is a vector of component i magnitude given by:

$$\Big((\mathbf{v} \cdot \nabla)\mathbf{v}\Big)_i = (\sum_{j=1}^{3} v_j \partial_j) v_i.$$

The convective inertia term can also be expressed either in Cartesian coordinates by the contraction product of the velocity vector and of the velocity gradient tensor $\mathbf{v}\boldsymbol{\nabla}\mathbf{v}$, or by the divergence of the tensorial product of the velocity vector by itself $\nabla \cdot (\mathbf{v} \otimes \mathbf{v})$ when the fluid is incompressible ($\nabla \cdot \mathbf{v} = 0$; $\mathbf{v} \otimes \mathbf{w}$ being a second order tensor of component $(\mathbf{v} \otimes \mathbf{w})_{ij} = v_i w_j$, and $\nabla \cdot (\mathbf{v} \otimes \mathbf{v})$ a vector of component j $\sum_{i=1}^{3} \partial_i v_i v_j = \sum_{i=1}^{3} v_i \partial_i v_j + v_j \sum_{i=1}^{3} \partial_i v_i$). The convective inertia term is also equal to:

$$\nabla(\mathbf{v} \cdot \mathbf{v})/2 \; - \mathbf{v} \times (\nabla \times \mathbf{v}).$$

The velocity curl has components $(\nabla \times \mathbf{v})\hat{\mathbf{e}}_i = \partial_j v_k - \partial_k v_j$, $i \neq j \neq k$ using circular permutation.

[4]
$$\int_V \nabla p\, dV = \int_\Gamma p\hat{\mathbf{n}}\, dA.$$
$$\int_V (\nabla \cdot \mathbf{T})\, dV = \int_\Gamma \mathbf{T}\hat{\mathbf{n}}\, dA.$$

The divergence $\nabla \cdot \mathbf{T}$ of tensor $\mathbf{T}$ is the vector of component:

$$(\nabla \cdot \mathbf{T})\hat{\mathbf{e}}_i = \sum_{i=1}^{3} \partial_i T_{ij} \hat{\mathbf{e}}_i.$$

$$\nabla \cdot \mathbf{v} = 0,$$
$$\rho(\mathbf{v}_t + (\mathbf{v} \cdot \nabla)\mathbf{v}) = -\nabla p_{\mathrm{i}} + \mu \nabla^2 \mathbf{v}. \tag{10.4}$$

The Navier–Stokes equations are associated with initial and boundary conditions be solved.

When the inertia term $\dfrac{D\mathbf{v}}{Dt}$ is neglected, i.e., the diffusive term $\mu\nabla^2\mathbf{v}$ is predominant, the momentum conservation equation is called the *Stokes equation*:

$$\nu\nabla^2\mathbf{v} - \nabla p_{\mathrm{i}}/\rho = 0. \tag{10.5}$$

The matrix $\mathbf{A}$ of the coefficients $\{a_{ij}\}$ of the second order derivatives $\partial_i\partial_j\mathbf{v}$ $(i, j = 1, 2, 3)$ is diagonal. $\det(\lambda\mathbf{I} - \mathbf{A}) = (\lambda - \nu)^3 = 0$. The eigenvalue $\lambda = \nu$ is positive and the Stokes equation belongs to the elliptic equation class of the set of partial derivative equations. Any point of the fluid domain receives information from all neighboring points (all directions) in the context of diffusive process. Downstream events thus intervene.

When the viscous term $\mu\nabla^2\mathbf{v}$ is neglected, i.e., $\dfrac{D\mathbf{v}}{Dt}$ becomes the major equation term, the momentum conservation equation is called the *Euler equation*:

$$\frac{D\mathbf{v}}{Dt} + \nabla p_{\mathrm{i}}/\rho = 0. \tag{10.6}$$

The Euler equation belongs to hyperbolic equation class. Informations travel in one direction given by the velocity field. The downstream region does not influence flow.

A predominant convective inertia term $(\mathbf{v} \cdot \nabla)\mathbf{v})$ brings a hyperbolic feature, even in the presence of temporal inertia. The time inertia $\partial_t\mathbf{v}$ brings a parabolic feature.

Flow Boundary Conditions

Boundary conditions, as well as initial conditions, are required to solve the set of partial derivative equations. The boundary conditions are applied at the computational domain surface, i.e., when carrying out flow computations at the wall, entry and exits of the vasculature model (Tables 10.8, 10.9, and 10.9).

The boundary of the fluid domain Ω is partioned into three surfaces: the entry cross-section Γ_1, the exit cross-sections Γ_2 and the vessel wall Γ_3.[5] The classical no-slip condition is applied to the rigid vessel wall. A time-dependent uniform injection velocity $\mathbf{v}_\Gamma(t)$ can be prescribed, at least, at the inlet, which is obtained from the Fourier transform of in vivo MR flow signals.[6] At the

[5] Γ_3 is the fluid-structure interface, i.e., the moving boundary when the deformable wall is taken into account.

[6] Most often the spatial resolution of in vivo velocity measurements is not high enough to provide velocity distribution at vessel ends, especially at the domain

outlet cross-sections,[7] either the pressure is set to zero or, better, a stress-free condition is commonly applied.

Table 10.8. A first family of boundary conditions for the Navier-Stokes equation. The boundary Γ of the fluid domain Ω is partioned into three parts Γ_i. The association of two conditions in certain cases is necessary for a well-posed problem.

Boundary part	Condition type	Examples
Γ_1	$\mathbf{v} = \mathbf{v}_{\Gamma_1}$	No slip at wall
		Injection velocity
Γ_2	$\mathbf{v} \times \mathbf{n} = \mathbf{v}_{\Gamma_2} \times \mathbf{n}$	Pressure condition
	and $p = p_{\Gamma_2}$	(for Stokes problem)
	or $p + (1/2)\lvert\mathbf{v}\rvert^2 = p_{\Gamma_2}$	(for Navier-Stokes problem)
Γ_3	$\mathbf{v} \cdot n = \mathbf{v}_{\Gamma_3} \cdot \mathbf{n}$	Jet
	and	
	$\nabla \times \mathbf{v} \times \mathbf{n} = \boldsymbol{\omega}_{\Gamma_3} \times \mathbf{n}$	

Table 10.9. A second family of boundary conditions for the Navier-Stokes equation. Vector $\mathbf{u}_\tau$ represents the projection of $\mathbf{u}$ on the tangent plane.

Boundary part	Condition type	Examples
Γ_1	$\mathbf{v} = \mathbf{v}_\Gamma$	Wall adhesion
		Velocity distribution
Γ_2	$-\nu \dfrac{\partial \mathbf{v}}{\partial n} + p\mathbf{n} = 0$	Free exit
Γ_3	$\alpha \mathbf{v} \cdot \boldsymbol{\tau}_i - \nu \frac{\partial \mathbf{v}}{\partial n} \cdot \boldsymbol{\tau}_i = \beta \frac{\mathbf{v} \cdot \tau_i}{\lvert\mathbf{v}_\tau\rvert}$	Slip condition
	$i = 1, 2$ on Γ_3	(turbulence model)
	and $\mathbf{v} \cdot \mathbf{n} = \mathbf{v}_{\Gamma_3} \cdot \mathbf{n}$	

entry of greater cross-section. In absence of measurements, the inlet velocity profile is provided by the Womersley solution, which implies a fully developed flow without body forces in a long smooth straight pipe of circular cross-section, a set of properties never encountered in blood circulation and vessel geometry. Such inlet BC is thus not more appropriate than time-dependent uniform injection velocity. However, the uniform velocity condition is associated with high wall shear at the entrance susceptible to induce larger flow separation if an adverse pressure gradient is set up, in particular in the transition zone between trunk and branches. Furthermore, high entry wall vorticity diffusion and convection in the vessel may raise the swirling amount found in the explored volume. Besides, the cross mesh must limit velocity discontinuity between zero Dirichlet wall and inlet boundary conditions.

[7] The outlet sections must be perpendicular to the local vessel axis and be short straight pipe exits to avoid a pressure cross gradient.

Dimensionless Form of Navier–Stokes Equations

The Navier–Stokes equations can be transformed into a dimensionless form using appropriate scales for the length ($L^\star$), the time ($T^\star$), the velocity ($U^\star$), and the pressure ($\rho {U^\star}^2$):

$$\tilde{t} = t/T^\star,\ \tilde{x} = x/L^\star,\ \tilde{u} = u/U^\star,\ \tilde{p} = p/(\rho {U^\star}^2).$$

The dimensionless differential operators are then expressed by:

$$\widetilde{\boldsymbol{\nabla}} = L^\star \boldsymbol{\nabla}, \widetilde{\boldsymbol{\nabla}^2} = {L^\star}^2 \boldsymbol{\nabla}^2.$$

The dimensionless Navier-Stokes equations are hence obtained:

$$St \frac{\partial \tilde{\mathbf{v}}}{\partial \tilde{t}} + (\tilde{\mathbf{v}} \cdot \tilde{\boldsymbol{\nabla}})\tilde{\mathbf{v}} = -\widetilde{\boldsymbol{\nabla}}\tilde{p} + \frac{1}{Re}\widetilde{\boldsymbol{\nabla}^2}\tilde{\mathbf{v}},$$

where St and Re are the Strouhal and Reynolds numbers (App. C.3).[8] The dimensionless Navier–Stokes equations suitable for bends of uniform curvature in a single plane can be found in [1230].

Solving Procedures of Navier–Stokes Equations

The analytical solution of any PDE provides the exact solution of the problem. However, the Navier-Stokes equations can be analytically solved only in few cases. Two analytical solutions are well known in physiology. The Poiseuille flow deals with the steady fully developed laminar flow in a straight rigid pipe with a circular cross-section (App. C.5), the Womersley flow with the unsteady flow in the same type of conduit (with either rigid or purely elastic wall) driven by a sinusoidal pressure gradient (App. C.6). Other analytical

Table 10.10. Additional boundary conditions for the Navier-Stokes equation.

Boundary part	Condition type	Examples
Γ_{in}	$\mathbf{u} = \mathbf{u}_\Gamma = cst$ $\nabla \times \mathbf{u} \cdot \mathbf{n} = \boldsymbol{\omega}_\Gamma \cdot \mathbf{n}$	One-dimensional potential flow associated with wall shear $\boldsymbol{\omega}_\Gamma = \begin{cases} \boldsymbol{\omega}_w, \\ 0 \text{ elsewhere.} \end{cases}$

[8] The Reynolds number in a single-direction fully developed flow is the ratio of inertia force $\propto \rho(\partial v/\partial z)$ to viscous forces (shear variation with the radial position, normal to the streamwise direction z) $\propto \mu \dfrac{\partial}{\partial n}(\partial v/\partial n)$. The ratio $\dfrac{\rho {U^\star}^2/L}{\mu U^\star/{L^\star}^2} = U^\star L^\star/\nu$ is dimensionless, being a force ratio.

solutions are illustrated by viscosimeter flows, particularly the Couette flow between two coaxial cylinders, one rotating, whereas the other is fixed.[9]

The features of the vasculature do not allow an analytical solution of the Navier-Stokes equations with available mathematical tools. Approximation methods are then used for a numerical solution and computational models are developed (App. D). The space-time continuum substitution by the problem discretization using a mesh leads to large number of algebraic equations; but these equations comprise simple operations (addition, multiplication, etc.). The numerical methods, indeed, rely on a sample of nodes distributed in the computational domain and a sample of instants. The numerical methods replace differential operators by other operators, in the simplest case, by difference operators. The gradients of the physical quantities are substituted by formulas giving the difference in quantity between adjoining nodes of the spatial mesh and two instants of the temporal mesh. The stability of certain numerical schemes is ensured by adequat conditions, such as the Courant-Friedrichs-Lewy condition, which prescribes the space (Δx) and time (Δt) steps suitable for the flow velocity in each mesh node at each time. The fluid particle cannot move over more than one mesh element of size Δx during the time step Δt.

[9] Between two fluid slices steadily moving in a single direction with velocity magnitudes v and $v + dv$, the shear stress $\tau = -\mu(du/dn)$ (n: direction normal to the streamwise direction). The dynamic viscosity thus has the dimension of the ratio of a force per unit surface area to a speed per unit length, i.e., $M.L^{-1}.T^{-1}$ (App. C.2).

A

Endocrine System and Hormones

The endocrine system, composed of many scattered endocrine glands with feedback loops between them, secrete hormones for communication between body organs, to coordinate their activities (Table A.1). The three groups of hormones according to the chemical structure include steroid hormones, amino acid derivatives, and peptide hormones.

The hormones are released into the blood and circulate to reach their distant specific targets, binding to receptors. Two main types of hormones are synthesized in the endocrine system: (1) steroid and (2) non-steroid hormones. Non-steriod hormone receptors are plasmalemmal proteins that generally act via second messengers within the cell. The receptors of steroid (glucocorticoids, mineralocorticoids, androgens, and estrogens) and thyroid hormones are located within the cell.

A.1 Endocrine Organs

Among endocrine glands, certain tissues belong to organs with other functions than hormone secretion, such as the digestive tract, kidneys, liver, gonads, and heart. The endocrine system also functions with the nervous system. The endocrine system accomplishes multiple tasks, including control of body homeostasis, tissue growth, reproduction, and responses to the surrounding medium. The endocrine system provides a chemical connection from the hypothalamus.

The hypothalamic–pituitary–adrenal axis is activated by multiple stimuli. Cues from various cerebral regions lead to the production by the paraventricular nucleus of the hypothalamus of vasopressin and corticotrophin releasing factor, which coordinates the hypothalamic–pituitary–adrenal axis. Corticotrophin releasing factor activates CRH receptors in anterior pituitary, releasing adrenocorticotropic hormone. ACTH targets its receptors in the adrenal cortex, thus increasing the synthesis of glucocorticoids.

A.1.1 Hypothalamus

The hypothalamus produces corticotropin-releasing hormone (CRH), dopamine, endomorphines, gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), somatostatin, and thyrotropin-releasing hormone (TRH).

Corticotropin-releasing hormone[1] is responsible for adrenocorticotropic hormone[2] (ACTH) secretion. Dopamine is a neurotransmitter, activating dopamine receptors, and a neurohormone which inhibits the release of prolactin. Dopamine acts on D, β1, β2, and α1 G-protein-coupled receptors. D1-like (D1, D5) family of dopamine receptors (Table A.2), once activated, increases cAMP level (excitatory action), whereas D2-like (D2–D4) receptor activation reduces cAMP amount (inhibitory effect). Gonadotropin-releasing hormone activates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Growth hormone-releasing hormone[3] stimulates the

Table A.1. The endocrine system and various types of hormones.

Production site	Hormones
Hypothalamus	CRH, dopamine, endorphins, GnRH, GHRH, adenylyl cyclase-activating polypeptide, oxytocin, neuropeptide-Y, somatostatin, TRH
Posterior pituitary	Vasopressin, lipotropin
Anterior pituitary	GH, ACTH, TSH, LH, FSH, prolactin, MSH, endorphins, lipotropin
Pineal gland	Melatonin
Thyroid gland	T3, T4, calcitonin
Parathyroid gland	PTH
Adrenal medulla	Adrenaline, noradrenaline, adrenomedullin
Adrenal cortex	Aldosterone, cortisol, DHEA
Heart	Natriuretic peptides, adrenomedullin
Adipose tissue	Leptin, adiponectin
Kidney	Renin, erythropoietin, thrombopoietin, calcitriol
Liver	IGF1, thrombopoietin, erythropoietin
Pancreas	Glucagon, insulin, somatostatin, VIP
Stomach	Gastrin, ghrelin, melatonin
Duodenum	Gastrin, CCK, GIP, secretin, motilin
Ileum	CCK, enteroglucagon, GIP
Ovary	Estradiol, progesterone, inhibin, activin
Testis	Testosterone, AMH, inhibin
Placenta	hCG, hPL, estrogen, progesterone

[1] Corticotropin-releasing hormone is also termed corticoliberin.
[2] Adrenocorticotropic hormone is also defined as corticotropin.
[3] Growth hormone-releasing hormone is also known as somatocrinin.

growth hormone (GH) secretion, in opposition to somatostatin.[4] Somatostatin also inhibits the release of thyroid-stimulating hormone (TSH)[5] and gastrointestinal hormones. Thyrotropin-releasing hormone[6] stimulates the release of thyroid-stimulating hormone and prolactin. Endorphins, or endomorphines, are endogenous opioids. Hypothalamic nuclei also produce neuropeptide-Y, which among other functions, modulates the sympathetic nervous system in the control of blood pressure, by a potentiation of noradrenaline-induced vasoconstriction, using G-protein-coupled receptors. The hypothalamic neuropeptide, adenylyl cyclase-activating polypeptide is a vasorelaxant. Oxytocin is made in cells in the supraoptic nucleus and paraventricular nucleus of the hypothalamus.

Orexins-A and -B, or hypocretins-1 and -2 because they are incretins synthesized in the hypothalamus, are neuropeptide hormones, that regulate energy expenditure and sleep/wakefulness and maintain readiness for activity. Orexin neurons are activated during wakefulness and inhibited during sleep. Orexin neurons regulate nuclei in the brain stem, which controls sleep and wakefulness. Both G-protein-coupled orexin-1 and -2 receptors are involved.[7] Orexin neurons also link the arcuate nucleus to regulate feeding. Orexin production is inhibited by leptin and activated by ghrelin and hypoglycemia (orexigenic activity). Orexins stimulate sympathetic system. Intracerebroventricular injection of orexins increases blood pressure and heart rate [1232].

Neuropeptide-B (NpB) and *neuropeptide-W* (NpW) found in anterior pituitary gland and hypothalamus, respectively, bind to G-protein-coupled receptors NpBWR1 (GPR7) and NpBWR2 (GPR8) in the central nervous system and adrenal gland. NpB and NpW modulate inflammatory pain and feeding behavior. They also regulate the release of hormones, such as corticosterone, prolactin, and growth hormone.

The corticotropin releasing factor is implicated in different responses to stress. The corticotropin releasing hormone and CRH-related peptides, the urocortins (Ucn1, Ucn2, and Ucn3), activate two members of the B1 family of G-protein-coupled receptors, CRHR1 and CRHR2.[8] These compounds modulate the functions of the central nervous system (appetite, addiction, hearing, and neurogenesis). They also target the endocrine, cardiovascular, reproductive, gastrointestinal, and immune systems.

[4] Somatostatin is also designated as growth hormone-inhibiting hormone.

[5] Thyroid-stimulating hormone is also named thyrotropin.

[6] Thyrotropin-releasing hormone is also called thyroliberin or protirelin.

[7] Orexin-1 receptor (OX1R) has a greater affinity for orexin-A than for orexin-B. Orexin-2 receptor (OX2R) has similar affinity for orexin-A and -B.

[8] The B1 family of GPCRs includes receptors for growth hormone releasing factor, secretin, calcitonin, vasoactive intestinal peptide, glucagon, glucagon-like peptide-1, and parathyroid hormone. CRH has a higher affinity for CRHR1 than for CRHR2. Urocortin binds CRHR2 with a much higher affinity than CRH.

Table A.2. Receptors of hormones of hypothalamo–pituitary axis and pineal gland, their main targeted G proteins, and order of ligand potency when available (Source: [241]).

Type	Main transducer	Ligand (potency order)
Hypothalamus hormones		
GnRHR1	Gq/11	Gonadotrophin-releasing hormone, GnRH1>GnRH2
GnRHR2	Gq/11	GnRH1<GnRH2
GHRHR	Gs	Growth hormone-releasing hormone
TRHR1/2	Gq	Thyrotropin-releasing hormone
CRFR1/2	Gs	Corticotropin-releasing hormone
SST1–SST5	Gi	Somatostatin
V1a/b	Gq/11	Oxytocin/vasopressin
V2	Gs	Oxytocin/vasopressin
OT	Gq/11, Gi/o	Oxytocin/vasopressin
D1	Gs/s,Gs/olf	Dopamine
D2/D3/D4	Gi/o	
D5	Gs	
Y1–Y6	Gi/o	Neuropeptide-Y, peptide-YY, pancreatic polypeptide-PP
VPAC1/2, PAC1	Gs	Vasoactive intextinal peptide, adenylyl cyclase-activating peptide, growth hormone-releasing factor
Pituitary hormones		
MC1–MC5	Gs	Melanocortin set (MSH, ACTH)
TSH	Gq, G12, Gs, Gi	Thyroid-stimulating hormone
FSH	Gs	Follicle-stimulating hormone
LH	Gq/11, Gs, Gi	Luteinizing hormone
Pineal gland hormones		
MTR1–MTR3	Gi/o	Melatonin

Table A.3. Examples of neuropeptide receptors and their main targeted G proteins (Source: [241]).

Type	Main transducer
Neuropeptide-B/W receptors	
NPBW1/2	Gi/o
Neurotensin receptors	
NTS1/2	Gq/11
Orexin receptors	
OX1/2	Gq/11

A.1.2 Hypophysis

The pituitary gland, or hypophysis, is connected to the hypothalamus by the infundibulum. It is composed of two regions, the anterior lobe, or adenohypophysis, and the posterior lobe, or neurohypophysis. The *adenohypophysis* synthesizes: (1) the adrenocorticotropic hormone, (2) gonadotropic hormones (LH, FSH), (3) growth hormone, (4) prolactin, and (5) thyroid-stimulating hormone. These hormones target (1) the cortex of the adrenal glands, (2) gonads (ovaries and testes), (3) bones, muscles, and other organs, (4) mammary tissues, and (5) the thyroid, respectively. *Growth hormone*[9] is synthesized by somatotropes in the anterior pituitary. It has direct effects on protein, lipid, and glucid metabolisms and indirect effects via insulin-like growth factor-1 (IGF1) secreted by the liver and other tissues in response to growth hormone.[10] Hypothalamic growth hormone-releasing hormone (GHRH) stimulates the synthesis of the growth hormone. Somatostatin produced by several body tissues inhibits growth hormone release (negative feedback loop), whereas gastric ghrelin stimulates growth hormone secretion. *Prolactin* promotes the growth of the mammary glands during pregnancy and lactation after birth. Melanocyte-stimulating hormones (MSH) stimulate the production of melanin.

The *neurohypophysis* secretes the antidiuretic hormone (or vasopressin), the oxytocin, and the lipotropin. *Vasopressin* enhances water and salt reabsorption by the nephron (Part II). In the pituitary gland, oxytocin binds neurophysin. *Oxytocin* is released during orgasm and labor. Oxytocin causes contraction of smooth muscle cells in the uterus wall and ducts of mammary glands. It is also a neurotransmitter in the brain.

A.1.3 Epiphysis

The *pineal gland*, or epiphysis cerebri, located between the lateral thalami, contains pinealocytes, which synthesize *melatonin*. Melatonin is an antioxidant, controlled by circadian rhythm. Melatonin is anti-gonadotropic, inhibiting the secretion of LH and FSH in the anterior pituitary. The production of melatonin is stimulated by darkness and inhibited by light. The precursor to melatonin is the neurotransmitter serotonin, itself derived from tryptophan. Melatonin receptors are G-protein-coupled receptors, mainly found in the suprachiasmatic nucleus of the hypothalamus, anterior pituitary, and retina.

A.1.4 Thyroid and Parathyroids

The thyroid gland in the neck produces the thyroid hormones, *thyroxine* (T4; about 95% of active thyroid hormones), and *triiodothyronine* (T3). The

[9] Growth hormone is also called somatropin or somatotropin.

[10] The growth hormone activates the proto-oncogene cFos, using the JaK–STAT and Ras–MAPK pathways (Sects. 3.2.9 and 4.3.2.4).

thyroid hormones increase the basal metabolic rate and sensitivity to catecholamines. They regulate protein, lipid, and glucid metabolism. Thyroid-stimulating hormone stimulates the secretion of T3 and T4. *Calcitonin*, also secreted by the thyroid gland, reduces calcemia. Furthermore, calcitonin lowers phosphate levels in the plasma. Calcitonin binds adenylyl cyclase-coupled receptor-activity modifying protein.

The calcitonin family of peptides includes calcitonin, amylin, adrenomedullin, and calcitonin gene-related peptides (CGRP; Table A.4). *Receptor-activity modifying proteins* (RAMP), proteins with a single transmembrane domain, determine the functional specificity of calcitonin gene-related peptide receptors [1234]. RAMPs interact with G-protein-coupled receptors of class 2.[11] The calcitonin receptor does not require RAMP to translocate to the plasmalemma and to bind calcitonin. *Calcitonin gene-related peptide* is a neuropeptide and potent vasodilator.

The parathyroid glands secrete *parathyroid hormone* (PTH), which regulates the blood calcium level, in opposition to calcitonin. The parathyroid hormone increases calcemia by enhancing calcium release by bones, calcium reabsorption by nephrons, and calcium absorption by the intestine. PTH also decreases phosphate concentration in the blood. Parathyroid hormone receptor-1 binds both parathyroid hormone and parathyroid hormone-related protein[12] (PTHRP). This ligand-bound G-protein-coupled receptor activates

Table A.4. Receptors of calcitonin, amylin, CGRP, and adrenomedullin, with their main targeted G proteins. The gene CALCR codes for calcitonin receptor; gene CALCRL codes for calcitonin receptor-like receptor. (Source: [241]).

Type	Main transducer	Ligand
CTR (CalcR)	Gs, Gq	Calcitonin, calcium, NO
AmyR1 (CalcR + RAMP1)	Gs	Amylin, calcium, NO
AmyR2 (CalcR + RAMP2)	Gs	
AmyR3 (CalcR + RAMP3)	Gs	
CGRPR (CalcRL + RAMP1)	Gs, Gq	Calcitonin gene-related peptide
AMR1 (CalcRL + RAMP2)	Gs	Calcitonin gene-related peptide
AMR1 (CalcRL + RAMP3)	Gs	Adrenomedullin

[11] G-protein-coupled receptors of class 2 include receptors for calcitonin, parathyroid hormone, glucagon, and vasoactive intestinal peptide/pituitary adenylyl cyclase activating polypeptide. CGRP receptors are formed by RAMP1 and the calcitonin receptor-like receptor (CRLR). Adrenomedullin receptor is generated by the assembling of RAMP2 or RAMP3 with CRLR. The association between calcitonin receptor (CtR) and RAMPs yields amylin receptors (AmyR). RAMP1, RAMP2, and RAMP3 associated with CtR lead to AmyR1, AmyR2, and AmyR3, respectively.

[12] Parathyroid hormone-related protein controls the cell life (proliferation, differentiation, and apoptosis) and the development of several tissues.

both adenylyl cyclase and phospholipase-C, and secondarily protein kinases PKA and PKC (Sects. 4.5 and 4.3). The cAMP–PKA pathway is predominant. Parathyroid hormone receptor-2 coupled to adenylyl cyclase binds PTH.

A.1.5 Pancreas

The pancreas has exocrine regions, which secrete digestive enzymes, and endocrine zones, the pancreatic islets, which release glucagon and insulin. Insulin and glucagon have opposite effects on the hepatic control (glucose storage and delivery) of glycemia. *Insulin* is synthesized by the pancreatic β cells. Insulin increases the synthesis of glycogen and fatty acids, as well as amino acid and potassium uptake. Furthermore, it decreases proteinolysis, lipolysis, and gluconeogenesis. *Glucagon* is produced by the pancreatic α cells. It converts glycogen into glucose in the liver and releases the latter into the blood. In the pancreatic islets, α cells secrete glucagon in response to low glycemia, whereas β cells release insulin in response to high glycemia. The glucagon receptor mediates the actions of glucagon on carbohydrate metabolism in the liver and insulin release by the pancreatic β cells. Glucagon G-protein-coupled receptors, positively and negatively regulated by glucose and glucagon, respectively, increase the intracellular cAMP level. The insulin receptor is composed of two extracellular insulin-binding α and two transmembrane β subunits. The β subunits have cytoplasmic ATP-binding and tyrosine kinase domains. Insulin binding induces autophosphorylation of β subunits, activating the catalytic activity of the receptor. Several intracellular proteins serve as substrates for the insulin receptor, such as insulin receptor substrate-1. Binding of insulin to receptors on such cells causes fusion of cytoplasmic vesicles containing glucose transporters GluT4 with the plasmalemma and insertion of the glucose transporters into the membrane. In the absence of insulin, glucose transporters are removed into the cytoplasm. Certain substances can alter the conformation of the cytoplasmic kinase domain or bind to modulator binding sites of the insulin receptor.

Amylin is also produced by pancreatic β cells. It inhibits food intake and postprandial glucagon secretion [1235]. It also opposes insulin activity in skeletal muscles, stimulating glycogen degradation.

A.1.6 Adrenal Glands

The adrenal glands, located over the kidneys, comprises an outer cortex and inner medulla. The adrenal medulla is regulated by the hypothalamus via a nervous electrochemical command (sympathetic nerves). The adrenal medulla secretes two hormones, adrenaline (or epinephrine) and noradrenaline (or norepinephrine). *Adrenaline* is implicated in the short-term stress reaction. In particular, it increases heart rate and stroke volume, constricts arterioles of the skin and gut, and dilates arterioles in leg muscles. Adrenaline acts on

α1, β1, and β2 receptors (Sect. 4.5). *Noradrenaline* acts mainly on α1 receptors. Noradrenaline is a hormone secreted from the adrenal medulla, and also a neurotransmitter of noradrenergic neurons released during synaptic transmission. As a stress hormone, it is involved in contexts that need attention and impulsion. It activates the sympathetic nervous system, increasing heart rate and muscle readiness, releasing energy from lipid stores.

The adrenal medulla, as well as the lungs, heart, kidneys, gastrointestinal tract, spleen, thymus, endocrine glands, brain, and vascular endothelial and smooth muscle cells produce adrenomedullin. *Adrenomedullin* is produced by cleavage of preproadrenomedullin. Adrenomedullin belongs to a regulatory peptide family that includes calcitonin gene-related peptide, amylin and calcitonin. The adrenomedullin (selective) receptors, derived from the calcitonin receptor-like receptor, increase cAMP level, according to the mode of interaction with receptor-activity modifying proteins.[13] Adrenomedullin has paracrine effects in the vasculature, rising cAMP levels in vascular smooth muscle cells and nitric oxide production in endothelial cells. Adrenomedullin is a potent vasodilator, and thus a potent hypotensive peptide. Moreover, adrenomedullin protects endothelial cells from apoptosis. Its receptor is involved in angiogenesis, as adrenomedullin promotes angiogenesis. Adrenomedullin also acts on vascular tone and permeability. Adrenomedullin enhances sodium excretion either by directly acting on nephron function or inhibiting aldosterone secretion. Adrenomedullin receptor expression is upregulated in cardiac hypertrophy secondary to hypertension.

Adrenomedullin acts via: (1) calcitonin gene-related peptide receptors (CGRPR), and (2) specific adrenomedullin receptors (AMR). The calcitonin receptor-like receptor (CRLR), a B-GPCR homologue to calcitonin receptor, combines with the transmembrane domain receptor activity modifying proteins (RAMP) to form a functional receptor.[14] The coexpression of CRLR with RAMP1 constitutes CGRP1 receptor, and CRLR with RAMP2 or RAMP3 yields adrenomedullin receptors (AMR) [1236]. Two AMR subtypes exist, AMR1, composed of CRLR and RAMP2, and AMR2, made of CRLR and RAMP3 [1237]. The cytosolic receptor component protein (RCP) binds to CGRPR and acts as a downstream regulator. RAMP1 exerts a dominant effect over RAMP2 to produce CGRP receptors rather than AMR in cells able to express both. The major signal transduction pathway is activated by AMR and CGRPR targets via Gs protein and adenylyl cyclase. CGRPR activation also increases intracellular calcium level.

The adrenal cortex produces *corticosteroids*, a class of steroid hormones with three subsets: (1) the *glucocorticoids* (cortisol), which regulate glucid, lipid, and protid metabolism, and are anti-inflammatory; (2) the *mineralocorticoids* (corticosterone and aldosterone), which control the concentrations of

[13] RAMP1 generates CGRP receptors from CRLR, whereas RAMP2 and RAMP3 produce selective adrenomedullin receptors.

[14] Neither RAMPs nor CRLR function in the absence of the other protein.

sodium and potassium; and (3) the *gonadocorticoids* in small amounts in both sexes, androgens (dehydroepiandosterone [DHEA] and androstenedione) and estrogens. The inner regions of the adrenal cortex, the zona fasciculata and zona reticularis, synthesize glucocorticoids and adrenal androgens, glucocorticoid production being dominant, whereas the outer zona glomerulosa manufactures aldosterone. Adrenocorticotropic hormone (ACTH) released by the hypothalamus regulates the cortisol production. Plasmalemmal ACTH receptors activate adenylyl cyclase. A feedback loop controls the circulating levels of corticotropin releasing hormone, ACTH, and cortisol. Angiotensin-2 stimulates plasmalemmal receptor coupled to phospholipase C of zona glomerulosa cells.

A.1.7 Gonads

The gonads not only produce sperm and ova, but also *steroid hormones*, androgens and estrogens. *Inhibins*, which inhibit FSH synthesis, are counteracted by *activins*. Inhibins are synthesized in the ovary. There are four activin genes. The activities of activins are regulated by inhibitors *follistatins*.[15] Follistatin can bind heparan sulfate proteoglycans, like many growth factors, which rely on heparan sulfate for their signaling. Activins participates in LH-stimulated androgen synthesis in the ovary and testis. Activin also enhances spermatogenesis. Activin is produced not only in the gonads, but also in other organs, such as the pituitary gland, placenta, etc. *Anti-Müllerian hormone* (AMH) is secreted during embryogenesis to prevent the development of the mullerian structures (vagina, uterus and cervix, and oviducts). AMH is produced in women after puberty whereas it decays in men during puberty. Activins, inhibins, and AMH belong to the transforming growth factor-β family.

Estrogens (estradiol, estriol and estrone) are steroid hormones present in both men and women, but at higher levels in women of reproductive age. They promote the development of female secondary sex characteristics, and regulate the menstrual cycle. *Progesterone* is a steroid hormone produced in the adrenal glands, gonads, and brain, which is involved in the menstrual cycle, pregnancy, and embryogenesis. *Androgens* are steroid hormones that control the development and maintenance of male characteristics. Adrenal androgens constitute a subset of androgens. Androgens are estrogen precursors. Androgens promote protein synthesis. *Testosterone* has virilizing (maturation of the sex organs) and anabolic (muscle growth and bone maturation) effects. Gonadal steroid hormones, testosterone, progesterone, and estradiol are produced by the testes and ovaries under the control of follicle stimulating hormone and luteinizing hormone by the pituitary on the one hand and gonadotropin-releasing hormone by the hypothalamus on the other hand. Low levels of circulating sex hormones reduce feedback inhibition on GnRH synthesis (long negative feedback loop), leading to elevated FSH and LH. FSH and LH act via the cAMP/PKA pathway after binding to plasmalemmal receptors.

[15] Follistatins belongs to a protein group with noggin, gremlin, and chordin

A.1.8 Other Endocrine Organs

Other organs, the heart, thymus, digestive tract, and placenta produce hormones. *Thymosin*, produced by the thymus gland, is involved in the development of the immune system. The placenta is a temporary endocrine gland. Human chorionic gonadotropin (hCG), produced during pregnancy, maintains progesterone production. Human placental lactogen (hPL; or chorionic somatomammotropin) increases the production of insulin and IGF1, as well as insulin resistance.

The heart manufactures *natriuretic peptides* (Part II). The kidneys produce *renin* (Part II), *erythropoietin* (Sect. 6.3), and *calcitriol* (vitamin-D3). Calcitriol increases the calcium absorption in the gastrointestinal tract.

The digestive tract with its serial organs (stomach, duodenum, jejunum, ileum) secretes various hormones acting via receptors (Table A.5). *Cholecystokinin* (CCK; or pancreozymin) causes the release of digestive enzymes from the pancreas and bile from the gallbladder. *Enteroglucagon* is derived from preproglucagon. *Gastrin* stimulates secretion of hydrochloric acid and pepsin for food digestion. *Glucose-dependent insulinotropic peptide* (GIP) induces insulin secretion. *Motilin* increases gastrointestinal motility and stimulates the production of pepsin. *Secretin* is produced in the S cells of the duodenum crypts of Lieberkühn. Secretin stimulates the secretion of bicarbonate from the liver, pancreas, and duodenal Brunner glands to buffer the acidic chyme. It also reduces acid secretion from the stomach by inhibiting gastrin release from G cells. It enhances the effects of cholecystokinin. It promotes growth and maintenance of the pancreas. *Vasoactive intestinal peptide* (VIP) stimulates the secretion of water and electrolytes in the intestine, dilates intestinal smooth muscles, activates pancreatic bicarbonate secretion, and inhibits gastrin effects. It can act on the circadian rhythm. VIP also induces coronary vasodilation and has positive inotropic and chronotropic effects.

Neuromedin-B and *gastrin releasing peptide* (GRP) of the bombesin family stimulates gastrin release from G cells. They form with cholecystokinin a negative feedback to stop eating. They activate three G-protein-coupled receptors BBR1, BBR2, and BBR3. Activated bombesin receptors stimulate tissue growth, smooth muscle contraction, and secretion in particular. Neuromedin-B (brain) and *neuromedin-C* (digestive tract) elicit amylase and insulin release.

Neurotensin is a neuropeptide found in several organs, such as the brain and intestine. It regulates luteinizing hormone and prolactin release. Epidermal growth factor and transforming growth factor-α stimulate synthesis in cultured rat hepatocytes, which is inhibited by transforming growth factor-β [1238]. Insulin, vasopressin, or angiotensin-2 cooperate with low concentrations of epidermal growth factor, but, this amplifying effect does not occur with neurotensin-related peptides, such as kinetensin or neuromedin-N. Neurotensin acts as a co-mitogen, like insulin, vasopressin, and angiotensin-2, which can regenerate the liver.

A.2 Adipocyte

Adipocytes (adeps: fat) store lipids for restriction periods. Adipose tissues have an important growth potential, then require angiogenesis (Sect. 10.4.2). However, excess inputs develop adipose tissues, increasing cell size and number, and augment the occurrence of cardiovascular diseases. Lipid accumulation in adipocytes disturbs adipokine secretion (Table A.6), impairs insulin signaling, and dysregulates cell functioning.

Table A.5. Receptors of digestive tract hormones and related substances and their main G-protein transducers. Glucagon-like peptide-1 (GLP1), of short life duration, binds to a G-protein-coupled receptor, and potentiates insulin secretion in response to food intake (Source: [241]).

Type	Main transducer	Ligand
Ghrelin	Gq/11	Ghrelin
Secretin	Gs	Secretin
CCKR1	Gq/11, Gs	Cholecystokinin, gastrin
CCKR2	Gs	
APJ	Gi/o	Apelin
BBR1–BBR3	Gq/11	Gastrin-releasing peptide, neuromedin-B/C
GlucagonR	Gs	Glucagon
GLPR1/R2	Gs	Glucagon-like peptide-1/2
GIPR	Gs	Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide)

Table A.6. Adipocyte production. Adipokins regulate food intake, and thereby energy homeostasis.

Adipokines	Leptin, adiponectin, resistin
Cytokines	Tumor necrosis factor-α, interleukin-6
Inflammatory reactants	Serum amyloid A, pentraxin, lipocalin, ceruloplasmin, macrophage migration inhibitory factor
Angiogenic factors	Vascular-endothelium growth factor, monobutyrin
Lipogenic factors	Acylation-stimulating protein
Matrix components	Collagen-4
Proteases	Adipsin
Miscellaneous	Osteonectin, stromolysin

The adipokines (or adipocytokines) belong to a group of cytokines (between-cell communication, i.e., paracrine function) secreted by adipose tissues and other organs. They can also function as hormones (endocrine function). The adipokine family include leptin, adiponectin, resistin, visfatin, and retinol binding protein-4. Adipokines reduce fatty acids in non-adipose tissue cells.

Adipocytes also secrete angiotensinogen, adipocyte differentiation factor, interleukin-6, tumor necrosis factor-α, and plasminogen activator inhibitor-1, as well as others mediators, such as nitric oxide, prostaglandins, acylation-stimulating protein, adipsin (complement-D). Visceral adipose tissues seem to have up to five times the number of PAI1-producing stromal cells compared with subcutaneous adipose tissues [1239].[16] Circulating PAI1 level is correlated with accumulation of visceral fat.

Adipocytes and resident macrophages synergistically secrete TNFα and IL6, particularly in obesity. Obesity and insulin resistance increase cardiovascular risk by dyslipidemia, hypertension, glucose dysmetabolism, and mechanisms that implicate adipokines, cytokines, and hypofibrinolytic factors [1240]. Adipocytes release certain compouds that alter glucose and lipid metabolism, blood pressure, coagulation, and fibrinolysis, and lead to inflammation.

Dyslipidemia in obesity is characterized by increased concentrations of VLDLs and LDLs, and decreased levels of HDLs. Hepatic overproduction of VLDL is a consequence of hepatic steatosis. In insulin-resistant states of obesity,[17] dyslipidemia is characterized by an increased concentration of smaller, denser LDLs, after increased lipolysis by hepatic lipase [1240]. These LDLs are more exposed to oxidation. They are mostly targeted by macrophage scavenger receptors rather than the normal LDL receptor.

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[16] plasminogen activator inhibitor-1 is a marker of hypofibrinolysis.

[17] Insulin resistance leads to: (1) impaired glucose uptake, particularly in myocytes, in hepatocytes, and in adipocytes; (2) impaired LDL receptor activity, with delayed VLDL clearance; and (3) inability to suppress hepatic glucose production and release of non-esterified fatty acids from hypertrophic adipocytes. The level of non-esterified fatty acids increases owing to decreased lipolysis, fatty acid oxidation, low levels of adiponectin (the latter favoring fatty acid oxidation), stress-induced adrenergic stimulation, and inflammation. Increased levels of non-esterified fatty acids cause lipotoxicity, impair endothelium-dependent regulation of vasomotor tone, increase oxidative stress, and have cardiotoxic effects. Reduced lipoprotein lipase activity decays the clearance of triacylglycerol-rich lipoproteins. Impaired lipolysis of triacylglycerol-rich lipoproteins decreases the transfer of apolipoproteins and phospholipids from triacylglycerol-rich lipoproteins to HDL, thus reducing HDL concentration. Furthermore, delayed clearance of triacylglycerol-rich lipoproteins facilitates CETP-mediated exchange between cholesterol esters in HDL and triacylglycerols in VLDL. Besides, the degradation rate of ApoB100, which regulates VLDL secretion, is decreased in insulin resistance. In the cardiovascular system, insulin resistance is associated with inhibition of the PI3K pathway and overstimulation of the growth factor-like pathway.

A.2.1 Adiponectin

Adiponectin (necto: to bind) is synthesized mainly by adipocytes. It is also expressed by skeletal myocytes, cardiomyocytes, and endothelial cells. Adiponectin circulates in blood at high concentrations (5–10 mg/ml). Adiponectins exist as a low-molecular-weight full-length trimers and globular cleavage fragments. The full-length trimer can dimerize to form a middle-molecular-weight hexamer, which can oligomerize to form a polymer. Full-length adiponectin stimulates AMPK phosphorylation (activation) in the liver, whereas globular adiponectin yields this effect in skeletal myocytes, cardiomyocytes, and hepatocytes.

Adiponectin binds to its G-protein-coupled receptors, adipoR1 and adipoR2 (Table A.7). T-cadherin could act as a co-receptor for the middle/high-molecular-weight adiponectin on endothelial cells and smooth muscle cells, but not for the low-molecular-weight trimeric and globular forms [1241, 1242]. Activation of adipoR1 and adipoR2 by adiponectin stimulates the activation of peroxisome-proliferator-activated receptor-α, AMP-activated protein kinase, and p38 mitogen-activated protein kinase. Stimulation of AMP-activated protein kinase in the liver and skeletal muscle strongly affects fatty acid oxidation and insulin sensitivity.

Adiponectin affects gluconeogenesis and lipid catabolism. Adiponectin hinders atherosclerosis (Part II). Adiponectin favors insulin activity in the muscles and the liver via activated AMPKs (Table A.8). PPARγ upregulates the adiponectin expression and reduces the plasmatic TNFα concentration. TNFα, produced in adipose tissues, prevents adiponectin synthesis. TNFα phosphorylates insulin receptors, and hence desensitizes insulin signaling.

Adiponectin has dominant anti-inflammatory features, thus anti-atherogenic and antidiabetic properties (Table A.9). Adiponectin regulates the expression of both pro- and anti-inflammatory cytokines. It suppresses the

Table A.7. Receptors of adipocyte hormones and their main transducers. Adiponectin avoids G protein (Source: [241]).

Type	Main transducer	Ligand
AdipoR1	AMPK, MAPK	Adiponectin
AdipoR2	AMPK, MAPK	

Table A.8. Effects of adipokines on glycose level.

Decrease	Increase
Adiponectin	Resistin
Leptin	RBP4
Omentin	TNFα, IL6
Visfatin	

synthesis of tumor-necrosis factor-α and interferon-γ and favors the production of anti-inflammatory cytokines, such as interleukin IL10 and IL1-receptor antagonist by monocytes, macrophages, and dendritic cells. Adiponectin increases the synthesis of tissue inhibitor of metalloproteinase in macrophages via IL10 [1243].

Adiponectin inhibits the expression of adhesion molecules via inhibition of TNF and NFκB. Adiponectin thus also impedes endothelial-cell proliferation and migration [1242]. Adiponectin suppresses endothelial cell apoptosis [1244]. Adiponectin also hampers foam-cell formation.

A.2.2 Leptin

Leptin ($\lambda\epsilon\pi\tau o\sigma$: thin), mainly produced by adipocytes in response to high lipid levels, regulates satiety. Leptin, indeed, represses food intake and promotes energy consumption.[18] Leptin can be co-expressed with growth hor-

Table A.9. Adipocytokines and their activity in inflammation and immunity (Source: [1242]).

Adipokin	Inflammatory and immune effects
Adiponectin	Anti-inflammatory ($\downarrow$ endothelial adhesion molecules, $\downarrow$ phagocytosis, T-cell responses, $\downarrow$ B-cell lymphopoiesis, $\downarrow$ NFκB, TNFα, IL6, IFNγ, $\uparrow$ IL1RA, IL10) Pro-inflammatory ($\uparrow$ CXCL8 in presence of lipopolysaccharide)
Leptin	Pro-inflammatory ($\uparrow$ TNFα, ROS, IL6, IL12, chemotaxis, neutrophil activation, thymocyte survival, lymphopoiesis, T-cell proliferation, NK-cell function, $\uparrow$ TH1 response, $\downarrow$ TH2 activity)
Resistin	Pro-inflammatory ($\uparrow$ endothelial adhesion molecules, $\uparrow$ NFκB, TNFα, IL1β, IL6, IL12)

[18] Adipose tissues are aimed at storing energy. Adipocytes saturated with lipids can lead to lipid accumulation in other tissues, reducing their functioning. Adipose tissues act as endocrine organs, secreting adipokines. Leptin is detected by the arcuate nucleus in the hypothalamus. Increased arcuate nucleus activity inhibits the production of neuropeptide-Y in the paraventricular nucleus, thereby reducing feeding.

mone in somatotropes of the anterior pituitary.[19] Leptin circulates in the blood (at a concentration of a few ng/ml) and in the cerebrospinal fluid, crossing the blood–brain barrier to regulate food intake by the hypothalamus.

Leptin interacts with six types of receptors (LepRa–LepRf). LepRb is found in the hypothalamus, especially in the satiety center.[20] Leptin hampers the activity of neurons expressing neuropeptide-Y and agouti-related peptide (AgRP), and favors the activity of neurons expressing α-melanocyte-stimulating hormone.

Leptin receptors are widely distributed on endothelial cells and vascular smooth muscle cells. Leptin stimulates mitogen-activated protein kinases and phosphatidylinositol 3-kinase. Leptin induces SMC proliferation and migration [1245]. Leptin also favors platelet aggregation and promotes angiogenesis [1246]. Leptin intervenes not only in angiogenesis but also in hematopoiesis, upregulating the expression of vascular endothelial growth factor via activation of NFκB and PI3K [1241]. Leptin also favors the production of nitric oxide synthase-2, and thereby reactive oxygen species.

Leptin stimulates AMP-activated protein kinase, which decreases ATP-consuming anabolism and increases ATP-manufacturing catabolism. Leptin decreases insulin levels by inhibiting proinsulin synthesis and reducing secretion. In myocytes, leptin improves insulin sensitivity and reduces intracellular lipid levels by direct activation of AMP-activated protein kinase combined with indirect inputs to the central nervous system. In the liver, leptin also enhances insulin sensitivity.

Leptin has dominant pro-inflammatory effects (Table A.9). Leptin binds to its receptor OBRb and activates: (1) the mitogen-activated protein kinase pathway (p38 and ERK), and (2) signal transducer and activator of transcription STAT3, thus producing pro-inflammatory cytokines TNFα, and interleukins IL6 and IL12 in monocytes and macrophages. Leptin favors activities of monocytes, macrophages and natural killer cells [1242]. Leptin stimulates neutrophil chemotaxis and the production by neutrophils of reactive oxygen species. Leptin stimulates the production of IgG2a by B lymphocytes. Leptin increases IL2 secretion by T lymphocytes.

A.2.3 Resistin

Resistin is synthesized by adipocytes and other cells of adipose tissues, as well as by myocytes, pancreatic cells, and macrophages. Resistin circulates

[19] Somatotropes have leptin receptors. Leptin can thus be an autocrine or paracrine regulator.

[20] Leptin receptor is expressed at low levels in manifold tissues and at high levels in the mediobasal hypothalamus, particularly in the arcuate nucleus, ventromedial nucleus, and dorsomedial nucleus. Activation of leptin receptors in the hypothalamus represses orexigenic pathways involving neuropeptide Y and agouti-related peptide and stimulates anorexigenic pathways involving pro-opiomelanocortin and cocaine and amphetamine-regulated transcript [1241].

in high-molecular-weight hexamer and low-molecular-weight complex [1242]. The resistin synthesis is affected by pituitary, steroid and thyroid hormones, adrenaline, endothelin-1, and insulin. Resistin could reduce glucose uptake by muscles, adipose tissues, and the liver, affecting insulin sensitivity.

Resistin activates phosphatidylinositol 3-kinase and members of the mitogen-activated protein kinase family, p38 and ERK. Resistin has dominant pro-inflammatory features. Resistin increases the production of tumor-necrosis factor and interleukins IL1beta, IL6, and IL12 by various cell types via NFκB-dependent process. IL1, IL6, and TNF upregulates resistin expression. Resistin upregulates the expression of adhesion molecules, such as vascular cell-adhesion molecule-1 and intercellular adhesion molecule-1, as well as CCL2 by endothelial cells. It also favors the release of endothelin-1 by endothelial cells.

A.2.4 Other Adipokines

Visfatin has an insulin-like activity because it binds to and activates insulin receptor at binding site different from the insulin one [1247]. It thus favors glucose uptake. Besides, visfatin inhibits neutrophil apoptosis.

Visceral adipose tissue-derived serine protease inhibitor (vaspin) suppresses the production of tumor-necrosis factor, leptin, and resistin [1242].

Omentin, an insulin sensitizer made by stromal vascular cells within the fat pads, enhances glucose uptake [1241].

Retinol-binding protein-4 (RBP4) impairs insulin action on the liver and muscles [1241]. Retinol-binding protein-4 contributes to insulin resistance.

Insulin resistance is also associated with lipolysis and release of non-esterified fatty acids into the circulation [1241]. Circulating non-esterified fatty acids reduce glucose uptake by adipocytes and myocytes, and promotes glucose release by hepatocytes. Transient increases in non-esterified fatty acid levels, such as acute changes after a meal, enhance insulin secretion, whereas chronic elevations associated with insulin resistance, reduce insulin secretion by the pancreas.

B

Coagulation Factors

***Antithrombin*:** glycoprotein produced by the liver, which inactivates several enzymes of the coagulation cascade (factor X, factor IX and thrombin.

Factor V (FV)**:** a component (cofactor) of the prothrombinase complex, with Ca^{++} and factor Xa, which accelerates the conversion of prothrombin to thrombin. Factor V also is an anticoagulant cofactor, acting in concert with protein S and activated protein C in the inactivation of factor VIIIa. Eighty percent of its circulating pool is located in the plasma, the remainder is stored in platelet α granules, in complex with its carrier protein, multimerin. The major site of synthesis is the liver. Factor V deficiency characterizes Owren's disease.

***Factor VII*:** a plasma protein activated by tissue thromboplastin to form factor VIIa, which then catalyzes the activation of factor X.

***Factor VIII*:** the antihemophilic factor, part of factor VIII/von Willebrand factor complex, produced in the liver and a co-factor in factor X activation. This activation is enhanced by thrombin. Lack in FVIII causes hemophilia A.

***Factor IX*:** once activated (FIXa), forms a complex with factor VIII and calcium on PF3 to activate factor X. Factor IX deficiency results in hemophilia B (Christmas disease).

***Factor X*:** a glycoprotein activated into factor Xa by both the intrinsic and extrinsic pathways. Its synthesis in the liver requires vitamin K. It cleaves prothrombin, with calcium, phospholipid, and factor V as a cofactor. Factor Xa is inactivated by protein-Z-dependent protease inhibitor.

***Factor XI*:** once activated (FXa), it activates factors IX to IXa. Deficiency of factor XI signs hemophilia C.

***Factor XII*:** activated by contact with the subendothelial surface. With prekallikrein, it serves as the contact factor for coagulation initiation. Kallikrein activates factors XII to XIIa. Deficiency of factor XII is also called the Hageman trait, with increased incidence of thromboembolic disease.

***Factor XIII*:** a fibrin-stabilizing plasma enzyme activated by thrombin and Ca^{++} to form factor XIIIa. It stabilizes fibrin polymer (clot).

***Fibrinogen*:** (coagulation factor I) plasma glycoprotein cleaved by thrombin to form fibrinopeptides A and B. It is synthesized in the liver.

***Fibrin*:** (factor Ia) converted from fibrinogen by thrombin. Fibrin incorporates platelet aggregrates and thrombin, and is cross-linked by factor XIII to form a stable hemostatic plug.

High-Molecular-Weight Kininogen (HMWK)**:** protein of the blood coagulation cascade as well as the kinin-kallikrein system. HMWK participates in the intrinsic pathway of coagulation, as a co-factor for the activation of kallikrein and factor XII. It is also required for the activation of factor XI by factor XIIa. HMWK is a precursor of vasodilator bradykinin.

***Kallikrein*:** potent vasodilator that increases vascular permeability. Prekallikrein is the precursor of kallikrein. Kininogens, peptides of body fluids, are activated into kinins. High-molecular-weight kininogen (HMWK) is split by plasma kallikrein to produce bradykinin, and low-molecular-weight kininogen (LMWK) by tissue kallikrein to produce kallidin. Kinins are involved in inflammation, blood clotting, complement reactions, etc.

***Plasmin*:** degrades plasma proteins involved in fibrin clots (fibrinolysis). Plasmin also activates collagenases, certain mediators of the complement system. It cleaves fibronectin, thrombospondin, laminin and von Willebrand factor.

***Plasminogen*:** released form of plasmin, activated by tissue plasminogen activator, urokinase plasminogen activator, thrombin, fibrin, and factor XII. It is inactived by α2-antiplasmin or plasmin inhibitor.

Plasminogen Activator Inhibitor (PAI) PAI1 inhibits tissue plasminogen activator and urokinase. PAI2 is secreted by the placenta.

Platelet Activating Factor (PAF)**:** a phospholipid formed by platelets, basophils, neutrophils, monocytes, and macrophages. It is a potent platelet aggregating agent. PAF also acts on vascular permeability and smooth muscle contraction. PAF binds to a specific G protein-coupled receptor. PAF synthesized by activated endothelial cells remains on the cell membrane and mediates adhesion of leukocytes.

Platelet Factor 3 (PF3)**:** a lipoprotein of platelet membrane and granules required for activation of the extrinsic clotting pathway.

Platelet Factor 4 (PF4)**:** a chemokine stored in platelet α granules, chemoattractant for monocytes and granulocytes. It can form a complex with chondroitin sulfate released from platelets by thrombin. PF4 inhibits endothelial cell proliferation but stumulates platelet aggregation.

Platelet Factor 5 (PF5)**:** fibrinogen

Platelet Factor 6 (PF6)**:** fibrinolysin/plasmin inhibitor

Platelet Factor 7 (PF7)**:** cothromboplastin

Platelet Factor 8 (PF8)**:** antithromboplastin

Platelet Factor 9 (PF9)**:** factor XIII

Platelet Factor 10 (PF10)**:** serotonin

***Protein C*:** an anticoagulant, which is a vitamin-K-dependent serine protease activated by thrombin. Activated thrombin, with protein-S, degrades factors V and VIII.

***Protein S*:** has two forms in the blood circulation, free (co-factor), and complex bound to complement protein C4b.

***Protein Z*:** vitamin-K-dependent glycoprotein of the coagulation cascade.

***Prothrombin*:** (coagulation factor II) a plasma protein converted to thrombin by a prothrombin activator complex consisting of FXa, FV, phospholipid, and Ca^{++}. Prothrombin is produced in the liver and is post-translationally modified in a vitamin-K-dependent reaction.

***Prothrombinase complex*:** formed by calcium, phospholipid, and factor V and Xa, cleaves and activates prothrombin to thrombin.

***β-thromboglobulin*:** a platelet protein released when platelets aggregate.

***Thromboplastin*:** composed of protein and phospholipid, present in tissues, platelets, and leukocytes. Tissue thromboplastin (factor III) serves as a cofactor with factor VIIa to activate factor X.

***Thrombin*:** (factor IIa) enzyme that converts fibrinogen to fibrin. It is produced by the enzymatic cleavage of prothrombin by activated factor X. The activity of factor Xa is enhanced by binding to activated factor V (Va), to form the prothrombinase complex.

Tissue Factor Pathway Inhibitor (TFPI)**:** a serine-protease that counteracts tissue factor (TF or factor III), which is mainly membrane-bound and initiates the extrinsic coagulation. TFPI co-localizes in endothelial cells with caveolin, urokinase-type plasminogen activator receptor, and glycosphingolipids. Two forms of circulating TFPI exist, a free and a lipoprotein-bound TFPI.

Tissue Plasminogen Activator (tPA)**:** activates plasminogen, leading to fibrinolysis.

Urokinase (uPA)**:** converts plasminogen into plasmin. It is inhibited by plasminogen activator inhibitor 1 and 2. Urokinase also binds uPA receptor (uPAR) and enhances mitosis and cell migration.

von Willebrand Factor (vWF)**:** a plasma glycoprotein, is involved with factor VIII in hemostatic plug formation.

C

Basic Mechanics

Mechanics is based on: (1) expedients, such as ideal objects, material particles, rigid bodies, and continua; (2) scalar, vectorial or tensorial quantities; (3) concepts, including forces, moments, works, and energy; and (4) theories accompanied by mathematical relationships between physical causes and material deformation and motion. Classical mechanics describe the macroscopic state of an object. Statics study the stability of rigid objects. Object equilibrium is treated with respect to the acting forces. Kinematics ($\chi\iota\nu\eta\mu\alpha$: motion) investigate body motion independently of causes, using space- and time-independent variables and velocity- and acceleration-dependent variable. Kinetics ($\chi\iota\nu\epsilon\omega$: to move) describe the movement of a set of particles characterized by a mass, using the force concept. Dynamics ($\delta\upsilon\nu\alpha\mu\iota\varsigma$: force, power) relates applied external forces[1] to the body kinematics. Rheology deals with response to loading and unloading of more or less complex materials. It is aimed at proposing constitutive laws, i.e., relationships between strains and stresses. Strength of materials explores the internal effects of loadings applied to the object. It is aimed at computing body displacements, deformations, and stresses in all body points under various types of loadings.

There are some basic principles. (1) Space is assumed to have a proper existence independent of the matter (contrary to the general relativity). It is associated with a reference triedron $(O; \hat{\mathbf{e}}_x, \hat{\mathbf{e}}_y, \hat{\mathbf{e}}_z)$, in which point O is the origin. The time is considered absolute, independently of the observer. (2) Matter is supposed to be made of particles of infinitesimal size with respect to motion but much larger than molecule size. These particles are assembled to form the body of finite dimensions. The assembling constitute a continuum. Mathematically, it defines a domain Ω bounded by a boundary Γ.

The pressure in any point of the fluid domain is the sum of: (1) hydrostatic pressure measured in the fluid at rest; of (2) additional pressure due to the pump action (which is not the dynamic pressure $\rho v^2/2$); and (3) the head loss

[1] Internal twin forces cancel each other by the action–reaction principle, and hence do not contribute to body motion.

generated by friction in the conduits. Hydrostatic pressure can be considered as the reaction that balances gravity in a fluid at rest (although these two pressures do not belong to the same classe of forces, gravity inducing a body force on the fluid particle, and pressure forces being surface forces).

Deformations and motions in a given time interval $[0, t_\star]$ can be defined by mapping, one-by-one correspondence, between the initial configuration Ω_O and the displaced, more or less deformed configuration $\Omega(t)$. The configuration of any object is the set of position of its material particles, associated with the physical features of the body in this configuration. Any displacement of a material point of a body is a combination of translation and rotation.

C.1 Kinematics

The Lagrangian description analyzes motion of material particles that continuously fill a moving domain Ω in a reference frame incorporating space and time. Material particles are labeled by their initial position $\boldsymbol{\chi}$ ($\{\chi_\ell\}_{\ell=1}^3$ (X, Y, Z), $\mathbf{x}_0$ being any initial position). The Lagrangian description of motion hence follows the history of N material particles $\{\chi^{(k)}\}_{k=1}^N$ of the material domain, labeled by their material coordinates $\boldsymbol{\chi}$. The variations in physical quantities attached to each material particle considered isolated are described during the motion. The Lagrangian description thereby takes a displacement picture with a very long exposure time. Any quantity g is given by:

$$\mathsf{g} = G_L(\boldsymbol{\chi}, t).$$

In particular, $\mathbf{x} = X_L(\boldsymbol{\chi}, t)$ (X_L: position function, $\boldsymbol{\chi}$: label, $(\boldsymbol{\chi}, t)$: set of Lagrangian variables). The motion image is complete when $\mathbf{x} = X_L(\boldsymbol{\chi}, t)$ is known. The Lagrangian velocity $\mathbf{v}_L$ of a selected fluid particle is given by: $\mathbf{v}_L(\boldsymbol{\chi}, t) = \dfrac{\partial X_L(\boldsymbol{\chi}, t)}{\partial t}$.

Motion with Lagrangian analysis is described by particle trajectory. Trajectory is the path followed by a labeled material particle. The mapping X_L of the motion of a fluid particle, $\mathbf{x} \mapsto \boldsymbol{\chi}(\mathbf{x}, t)$, allows each fluid particle to move from its initial position to its destination position at instant t. The trajectory of any fluid particle can be described by the function $\mathcal{X}(\boldsymbol{\chi}, t) \equiv \mathcal{X}(\mathbf{x}_0, t_0; t)$ (more precisely, $\mathcal{X}(\boldsymbol{\chi}, \mathbf{v}_L, t)$).

The Eulerian description deals with the whole spatial domain Ω occupied by the fluid during its displacement. The Eulerian description of motion is focused on the history of space points of the fluid domain (loci of motion of fluid particles). The Eulerian description is aimed at studying the distribution at a given time of physical quantities in the instantaneous configuration $\Omega(t)$ occupied by the continuum at this instant (kinematic quantity field picture with very short exposure time):

$$\mathsf{g} = G_E(\mathbf{x}, t).$$

The Eulerian velocity of any fluid particle crossing the site $\mathbf{x}$ is given by: $\mathbf{v}_E \equiv \mathbf{v}(\mathbf{x},t) = V_L(X_L^{-1}(\boldsymbol{\chi},t),t)$. Knowledge of the initial configuration is not necessary.

Passage of the Lagrangian to the Eulerian description is done via a Jacobian transformation matrix ($\mathbf{J}_{LE}$) of component J_{LEij}, and conversely a Jacobian transformation matrix ($\mathbf{J}_{EL}$) of component J_{LEij}:

$$J_{LEij} = \partial x_i/\partial\chi_j, \quad J_{ELkm} = \partial\chi_k/\partial x_m.$$

$$\begin{pmatrix} \partial_X G_L \\ \partial_Y G_L \\ \partial_Z G_L \\ \partial_t G_L \end{pmatrix} = \begin{pmatrix} \partial_X x & \partial_X y & \partial_X z & 0 \\ \partial_Y x & \partial_Y y & \partial_Y z & 0 \\ \partial_Z x & \partial_Z y & \partial_Z z & 0 \\ \partial_t x & \partial_t y & \partial_t z & 1 \end{pmatrix} \begin{pmatrix} \partial_x G_E \\ \partial_y G_E \\ \partial_z G_E \\ \partial_t G_E \end{pmatrix}$$

In particular, the particle derivative, or total derivative, is the derivative associated with a selected fluid particle, and is given by:

$$\mathbf{a} = \frac{D\mathbf{v}}{Dt} = \frac{\partial\mathbf{v}}{\partial t} + (\mathbf{v}\cdot\nabla)\mathbf{v}.$$

The time derivative of $\mathbf{J}$ is given by

$$\frac{\partial}{\partial t}\mathbf{J}(\mathbf{x},t) = \mathbf{J}(\mathbf{x},t)(\nabla\cdot\mathbf{v})(\boldsymbol{\chi}(\mathbf{x},t)).$$

Also

$$\frac{\partial \mathrm{det}\mathbf{J}}{\partial t} = (\mathbf{v}\cdot\nabla)\mathrm{det}\mathbf{J} - \frac{D\mathrm{det}\mathbf{J}}{Dt}.$$

The *local acceleration* is the rate of time change in velocity of a fluid particle at a given position, the *convective acceleration* the rate of space change in velocity of a fluid particle caused by the displacement of this fluid particle with the given heterogeneous velocity field.

C.2 International System Units

The international system is based on fundamental units. The physical units are either independent and form the MKS system, or are derived from a relation between fundamental quantities. The dimension of any quantity is defined by the basic system mass-length-time-temperature M.L.T.Θ.[2] The primary quantities of the international system, their definitions, and their dimensions are given in Table C.1. The physical quantities that most often intervene are: (1) geometrical, (2) kinematic, and (3) dynamic. Moreover, the physical system is described by a set of properties (density, diffusivity, compressibility, elasticity, thermal conductivity, etc.). The sets of derived physical quantities are shown in Tables C.2, C.3, and C.4.

[2] For instance, any force $\mathbf{f} = m\mathbf{a}$ has the dimension $M.L.T^{-2}$.

Pressure Units

In the international system of units (SI), meter, kilogram, and second are the units of length, mass, and time, respectively. However, old pressure units are still used in physiology. Pressure conversion factors are given in Table C.5.

Table C.1. International system of fundamental quantities.

Physical quantity	Unit
Length	Meter (m)
Mass	Kilogram (kg)
Substance quantity	Mole (mol)
Time	Second (s)
Temperature	Kelvin (K)
Electric intensity	Ampere (A)
Light intensity	Candela (cd)

Table C.2. Physical quantities, their dimensions and units.

Geometrical quantities		
Surface area	L^2	m^2
Volume	L^3	m^3
Angle		rad
Solid angle		Steradian (sr)
Vergence	L^{-1}	Dioptry δ
Temporal quantities		
Frequency	T^{-1}	Hz
Thermal quantities		
Temperature	Θ	K
Calorific capacity	$L^2.T^{-2}$	$J.kg^{-1}$
Heat capacity	$L^2.T^{-2}.\Theta^{-1}$	$J.kg^{-1}.K^{-1}$
Thermal conductivity	$M.L.T^{-3}.\Theta^{-1}$	$W.m^{-1}.K^{-1}$
Molar entropy		$J.K^{-1}.mol^{-1}$
Fluid properties		
Concentration	$mol.L^{-3}$	$mol.m^{-3}$
Density	$M.L^{-3}$	$kg.m^{-3}$
Specific weight (ρg)	$M.L^{-2}.T^{-2}$	$N.m^{-3}$
Dynamic viscosity	$M.L^{-1}.T^{-1}$	Pl or $Pa.s$
Kinematic viscosity	$L^2.T^{-1}$	$m^2.s^{-1}$
Elastic modulus	$M.L.T^{-2}$	$N.m^{-2}$
Surface tension	$M.T^{-2}$	$m.s^{-2}$

C.3 Main Flow Dimensionless Parameters

Dimensional analysis groups together the influence factors in dimensionless ratios. The formulation of the dimensionless equations depends on the choice of variable scales ($\cdot^\star$). The dimensionless equations exhibit a set of governing dimensionless parameters that have a suitable physical meaning for the problem (Table C.6). The phenomenological analysis gives the order of magnitude of the different terms of dimensionless equations. Scaling takes place in dimensionless coefficients allocated to the corresponding terms. Models are devised using geometrical and dynamical similarity.

In a vessel cross-section that is not circular, hydraulic diameter is introduced: $d_h = 4A_i/\chi_i$ where A_i is the cross-sectional area of the duct lumen and χ_i is the wetted perimeter. Different types of forces act on every fluid particle (ρ: fluid density, μ: fluid dynamic viscosity). (1) Remote body forces generated by a potential such as the gravity ($\rho\mathbf{g}$) or an electromagnetic field such as in MRI, are currently neglected. The other forces are fluid-particle surface forces. (2) Pressure forces (p) result from the pressure gradient in the streamwise direction (applied to the downstream [D] and upstream [U] faces of the hexahedral infinitesimal control element taken as the fluid particle). Adjacent north [N], south [S], west [W], and east [E] particles in the two directions normal to the local flow direction induce pressure forces on the fluid particle, which prevent particle rotation, although a torque results from particle shearing, which provides vorticity. (3) Shear forces ($\propto \mu V^\star/L^{\star^2}$) are caused by the friction between a fluid particle and the pipe wall when the particle is close

Table C.3. Physical quantities, their dimensions and units (cont.).

Kinematic quantities		
Velocity	$L.T^{-1}$	$m.s^{-1}$
Acceleration	$L.T^{-2}$	$m.s^{-2}$
Angular velocity	T^{-1}	$rad.s^{-1}$
Angular acceleration	T^{-2}	$rad.s^{-2}$
Volume flow rate	$L^3.T^{-1}$	$m^3.s^{-1}$
Mass flow rate	$M.T^{-1}$	$kg.s^{-1}$
Dynamic quantities		
Force	$M.L.T^{-2}$	N
Stress	$M.L^{-1}.T^{-2}$	$N.m^{-2}$
Pressure	$M.L^{-1}.T^{-2}$	Pa
Momentum	$M.L.T^{-1}$	$kg.m.s^{-1}$
Work, energy	$M.L^2.T^{-2}$	J
Torque	$M.L^2.T^{-2}$	$N.m$
Angular momentum	$M.L^2.T^{-1}$	$kg.m^2.rad.s^{-1}$
Moment of inertia	$M.L^2$	$kg.m^2$
Power	$M.L^2.T^{-3}$	W $(J.s^{-1})$

to it and between adjacent particles, acting in the flow opposite directions when they are caused by slower moving particles. (4) Inertia forces, temporal ($\propto \rho V^\star \omega$) and convective ($\propto \rho {V^\star}^2 / L^\star$) are reactions to fluid motion. Values of the main dimensionless parameters in the arteries are given in Table C.7.

Dean number $\mathrm{De} = (R_\mathrm{h}/R_\mathrm{c})^{1/2}\mathrm{Re}$ in laminar flow through curved vessels is the product of the square root of the vessel curvature ratio $\kappa_\mathrm{c} = R_\mathrm{h}/R_\mathrm{c}$ by the Reynolds number (R_h: hydraulic radius, R_c: curvature radius). De is then proportional to the ratio of the square root of the product of convection inertia by centrifugal forces to viscous forces. The dynamical similar-

Table C.4. Other physical quantities, their dimensions and units, used in electrical analogs (lumped parameter models) and experiments.

Electricity		
Intensity	I ($M^{1/2}.L^{3/2}.T^{-2}$	A
Eectricity quantity	$I.t$	C ($A.s$)
Electric charge	$M^{1/2}.L^{3/2}.T^{-1}$	
Electric potential	$M.L^2.T^{-3}.I^{-1}$	V ($W.A^{-1}$)
Electric field	$M^{1/2}.L^{1/2}.T^{-1}$	$V.m^{-1}$
Resistance	$M.L^2.T^{-3}.I^{-2}$	Ω
Conductance	$M^{-1}.L^{-2}.T^3.I^2$	S or $A.V^{-1}$
Capacity	$M^{-1}.L^{-2}.T^4.I^2$	F or $C.V^{-1}$
Inductance	$M.L^2.T^{-2}.I^{-2}$	H or $V.A^{-1}.S$
Magnetism		
Magnetic field		$A.m^{-1}$
Magnetic flux	$M.L^2.T^{-2}.I^{-1}$	Wb ($V.s$)
Magnetic flux density	$M.T^{-2}.I^{-1}$	T ($V.s.m^{-2}$)
Optics		
Light flux	$le.sr$	lm
Luminance	$le.L^{-2}$	$cd.m^{-2}$
Lighting	$le.sr.L^{-2}$	lx
Acoustics		
Intensity		$W.m^{-2}$

Table C.5. Conversion table for pressure units

	Pascal (Pa)
1 cm of water	98.04
1 mm of Hg	133
1 mb	102
1 dyn/cm^2	0.1

ity of a steady laminar motion of an incompressible fluid through a rigid smooth bend, with a small uniform planar curvature, is found to depend upon De, introduced by Dean (1927, 1928). For fully developed turbulent flow, the friction factor depends on the Ito number Ito $= (R_\mathrm{h}/R_\mathrm{c})^2\mathrm{Re}$ (Ito, 1959). The Dean number usually cannot be calculated because of the complex curvature of the vessel axis, which varies continually in the three spatial directions.

***Mach number*:** ratio of the fluid speed to the propagation speed $\mathrm{Ma} = V_q/c$. The wave speed c depends on the involved deformable parts, fluid compressibility χ, and vessel distensibility D ($\chi \ll D$ even for inhaled air). The usual speed is the speed of sound. In blood circulation, Ma is related to the propagation speed of pressure waves. The Mach number defines convection regimes associated with vessel compliance (collapsible vessels).

***Nusselt number*:** $\mathrm{Nu} = \mathtt{h}L^*/lg_T$ is involved in heat transfer between a solid or a fluid and a moving mono- or polyphasic fluid ($\mathtt{h}$: convection coefficient, λ_T: thermal conductivity).

Péclet number (Pe)**:** involved in convection exchanges, is the ratio of the convection mass transport to diffusion mass transport $\mathrm{Pe} = L^*V^*/D = \mathrm{ReSc} = L^*V^*/\alpha_T = \mathrm{Re} \times \mathrm{Pr}$ (D: molecular diffusivity, α_T: thermal diffusivity). Pe is a Reynolds-like number based on molecular or thermal diffusivity rather than momentum diffusivity.

***Prandtl number*:** ratio of kinematic viscosity to thermal diffusivity ν/α_T.

***Reynolds number*:** $\mathrm{Re} = V^\star L^\star/\nu$ ($\nu = \mu/\rho$, $V^\star \equiv V_q$: cross-sectional average velocity, $L^\star \equiv R$: vessel radius) is the ratio between convective inertia and viscous effects applied on a unit of fluid volume. *Re* is also the ratio between the momentum diffusion time scale and the convection characteristic time $\mathrm{Re} = (R^2/\nu)/(R/V)$. In pulsatile flows, both mean $\overline{\mathrm{Re}} = \mathrm{Re}(\overline{V_q})$ and peak Reynolds numbers $\widehat{\mathrm{Re}} = \mathrm{Re}(\widehat{V_q})$, proportional to the mean and peak cross-sectional average velocity, respectively, can be calculated. Re controls flow pattern transition. $\mathrm{Re}_\delta = \mathrm{Re/Sto}$ is used for flow stability study (δ: boundary layer thickness). Branching pulsatile flows are cur-

Table C.6. Examples of flow dimensionless ratios. ρ: fluid density, μ: fluid dynamic viscosity, L: pipe length, d_h: tube hydraulic diameter, R: duct radius, R_c: conduit axis curvature, δ: boundary layer thickness, ω: angular frequency, $\overline{V_q}$, $\widehat{V_q}$: mean, peak cross-sectional average velocity, u_w: wall velocity, λ: wavelength, c: wave speed, p: pressure, t: time, K: pipe-wall bending stiffness.

Length ratios	L/d_h, R/R_c, δ/R, $V_q/(R\omega)$, $\widehat{V_q}^2/(R_\mathrm{c}R\omega^2)$, $u_w/(R\omega)$, $u_w^2/(\omega\nu)$, R/λ, etc.
Time ratios	$R\omega/\overline{V_q}$, $\delta\omega/\widehat{V_q}$, etc.
Velocity ratios	$\widehat{V_q}/\overline{V_q}$, $V_q(t)/c(p(t))$, etc.
Force ratios	Re, De, St, Sto, $L(dV_c/dt)/V_c^2$, $\mu L/(R_0^2(K\rho)^{1/2})$, etc.

Table C.7. Dimensionless parameter values in the arteries at rest with $f = 1\,\mathrm{Hz}$.

Blood vessel	Radius	V_q (cm/s)			Re			St			Sto
	(mm)	Mean	Peak	Min	Mean	Peak	Min	Mean	Peak	Min	
Ascending aorta	10	20	70	−20	500	1750	500	0.31	0.09	0.31	12.5
Descending aorta	10	20	60	−10	500	1500	250	0.31	0.10	0.62	12.5
	3	10	50		75	375		0.19	0.04		3.8
	2	7	30		35	150		0.18	0.04		2.5
	1	7	20		18	50		0.09	0.03		1.3

rently based on stem peak Reynolds number $\widehat{\mathrm{Re}}$ calculated from $\widehat{V_q}$, on the trunk radius R and on blood kinematic viscosity.[3] In bend flows, a secondary motion-associated Reynolds number can be introduced, using the velocity scale $V_2^\star$, when centrifugal forces $\rho V^2/R_\mathrm{c}$ are balanced by local inertia effects $\rho\omega V_2^\star$) $\mathrm{Re}_2 = V^2R/(\omega\nu R_\mathrm{c}) = V^2/(\omega\nu) \times \kappa_c = \mathrm{Re}/\mathrm{St}$. The wall shear Reynolds number is defined by $u_*\delta/\nu$

***Schmidt number*:** ratio of kinematic viscosity to molecular diffusivity of the specy $\mathrm{Sc} = \nu/D$. It provides the ratio of the viscous boundary layer to the concentration boundary layer.

***Stokes number*:** $\mathrm{Sto} = R(\omega/\nu)^{1/2}$ (also called Witzig-Womersley number) is the frequency parameter of pulsatile flows (ω: pulsation of flow oscillation). *Sto* is the square root of the ratio between time inertia and viscous effects. The Stokes number is a Reynolds-like number for periodic flow (local acceleration replaces convective acceleration). The Stokes number is proportional to the ratio between vessel hydraulic radius and Stokes boundary layer thickness ($\mathrm{Sto} \propto R_\mathrm{h}/\delta_S$) and to the ratio between momentum diffusion time and cycle period ($\mathrm{Sto} \propto ((R^2/\nu)/(1/\omega)) \equiv (T_\mathrm{diff}/T)^{1/2}$).

***Strouhal number*:** $\mathrm{St} = \omega L^\star/V^\star$ is the ratio between time inertia and convective inertia ($\mathrm{St} = \mathrm{Sto}^2/\mathrm{Re}$). In quasi-periodic flow in a branching vessel region, the peak Strouhal number is based on the trunk peak cross-sectional average velocity: $\mathrm{St} = \omega R/\widehat{V_q}$. The Strouhal number is proportional to the ratio between the steady and Stokes boundary layer thicknesses ($\mathrm{St} = \delta/\delta_S$). The dimensionless stroke length ($\widehat{V_q}/(R_\mathrm{h}\omega)$), which is also the ratio of the length scale of the axial displacement of a fluid particle to the vessel radius or the ratio between flow cycle and convection time scale, is the inverse of the Strouhal number. In the aorta at rest, $\widehat{V_q}/R_\mathrm{h}\omega = 11.1$ with the following value set: $f = 1\,\mathrm{Hz}$, $R_\mathrm{h} = 10\,\mathrm{mm}$, $\widehat{V_q} = 0.7\,\mathrm{m/s}$. Bend flows depend, for small values of the frequency parameter, on the Strouhal number for the secondary motion (velocity scale

[3] The blood kinematic viscosity $\nu = 4 \times 10^{-6}\,\mathrm{m^2/s}$ when it is assumed to be Newtonian.

$V_2^\star$), when centrifugal forces $\rho V^2/R_c$ are balanced by local inertia effects $\rho\omega V_2^\star$, $\mathrm{St}_2 = (\omega^2 R R_c)/V^2$. In turbulent periodic flows, $\mathrm{St} = R\omega/u'$ is the ratio of the time scale of turbulent fluctuations R/u' (u': turbulent intensity) to the flow period. The turbulent Strouhal number $\mathrm{St} = \omega R/\overline{u}_*$ can also be defined by the time mean friction velocity $\overline{u}_*$.

The *Helmholtz number* $\mathrm{He} = \omega L^\star/c$, used to estimate whether fluid compressibility must be taken into account ($\mathrm{He} \ll 1$: the compressibility is ignored), is a Strouhal-type number.

***Taylor number*:** based on the Stokes boundary-layer thickness δ_S, $\mathrm{Ta} = V^2\delta_S^3/(R_c\nu^2) = V^2/(\omega^{3/2}\nu^{1/2}R_c)$ is used as a stability parameter in bends. Ta is also equal to $V/(R_c\omega)^2 \times R_c(\omega/\nu)^{1/2} = \mathrm{Sto} \times \mathrm{St}_2^{-1}$ or to $\mathrm{Re}_\delta^2 \times \delta_S/R_c$.

C.4 Similarity and Phantom Experiments

A physical system is described from a set of physical quantities. Any independent physical quantity is defined by its sign with respect to a reference, its magnitude, nature (scalar, vector, tensor), etc. The physical quantity either can be directly measured or expressed owing to other measurable variables. The physical quantities are expressed in a reference unit system. The physical units allows us to quantify the values of the physical quantities with respect to reference values associated with the unit system. The system of units includes fundamental (primary), arbitrarily chosen, and derived (secondary) units. The concept of dimension (App. C.2) designates the nature of the physical quantity and not the measure. Certain physical dimensions are fundamental,[4] others are derived from the fundamental dimensions using a physical relation or definition.[5]

C.4.1 Phenomenological Analysis

The order of magnitude, a situation assessed by observations, is defined by a factor of ten or so.[6] In addition, physical units are usual defined by a factor 1000.[7]

[4] Fundamental units, such as mass (M), substance quantity (mol), length (L), time (T), temperature (Θ), electric intensity, light intensity, etc., do not have any relationship among them.

[5] For example, the force , which can be expressed as the product of mass by acceleration (two quantities of different physical features), has the dimension MLT^{-2}.

[6] Two quantities have the same order of magnitude when they differ by a factor much less than 10.

[7] Small dimension values are defined by milli (m; 10^{-3}), micro (μ; 10^{-6}), nano (n; 10^{-9}), and pico (p; 10^{-12}), and large values by kilo (k; 10^3), mega (M; 10^6), giga (G; 10^9), and tera (T; 10^{12}).

Phenomenological analysis is aimed at determining the dominant physical terms in a complex physical situation and the factors that can be neglected. Scales ($\bullet^\star$) are chosen from the equation according to the physical context, as well as from initial and boundary conditions. Scales operate in the coefficient assigned to each equation term. In other words, the dimensionless variables ($\tilde{\mathtt{g}}$) and their time and space derivatives, whatever the derivative order, are $\mathcal{O}(1)$.[8]

When the scales have been selected, it is assumed that the evolution of the physical quantities can be described as if the quantities vary at the chosen scales.

C.4.2 Dimensional Analysis

The *homogeneity principle* states that an equation is dimensionally homogeneous, all equation terms having the same dimension. Dimensional analysis is aimed at determining the ℓ independant dimensionless parameters formed by p physical quantities among the m involved variables, expressed by n fundamental units $\{\pi_i(\{\mathtt{g}_j^{p_j}\}_{j=1}^{p})\}_{i=1}^{\ell}$. The dimensional analysis consists of: (1) listing the involved quantities in the explored physical process, (2) determining independant variables to be included in dimensionless parameters specific to the investigated problem, and (3) selecting relations between these dimensionless parameters.

The dimensionless parameters (App. C.3) can be determined using the Vaschy-Buckingham theorem π. This theorem states that $\ell = m - n$ dimensionless parameters exist, which have a physical meaning. The single problem expression that connects the m physical quantities is the following:

$$\mathtt{f}(\mathtt{g}_1, \cdots, \mathtt{g}_m) = 0.$$

The single function $\mathtt{f}$ can be expressed by an arbitrary relation Φ of $m - n$ dimensionless products $\{\pi_i\}$:

$$\Phi(\pi_1, \cdots, \pi_{m-n}).$$

In fluid mechanics problems that implicate three ($n = 3$) fundamental units (M,L,T) and nine ($m = 9$) physical quantities,[9] the number of dimensionless parameters is equal to 6.[10]

[8] In particuler, the time derivatives $\tilde{\mathtt{g}}_t$ and $\tilde{\mathtt{g}}_{tt}$ are $\mathcal{O}(1)$, such that $L^\star/T^\star$ and $L^\star/T^{\star 2}$ give the magnitude order of the velocity and acceleration, $L^\star$ being the magnitude order of the distance covered during the time scale $T^\star$.

[9] These physical quantities are domain length (L), tube radius (R), fluid density (ρ) and viscosity (μ), driving pressure (Δp), flow rate (q), velocity (V_q), wave speed (c), and flow frequency (ω).

[10] The involved dimensionless parameters are shape factor L/R, the Reynolds number $V_q R/\nu$, the Strouhal number $\omega R/V_q$, the Stokes number $R(\omega/\nu)^{1/2}$, the Euler number $\Delta p/(\rho V_q^2)$ and the Mach number V_q/c.

Another application of the dimensional analysis is the model design. In the case of physiological conduits, the flow behavior in these vessels is deduced from experiments in large-scale models to get a good spatial resolution of the fields of the explored variables. In the model, each quantity is enlarged in the same proportion relative to its value in the original system. Dimensionless parameters are similarity criteria.

C.4.3 Similarity

The similarity defines geometrical, kinematic, and dynamic scales, i.e., the reduction (for small-scale models) or enlargement (for large-scale models) coefficients, between the real system and physical model (phantom).

C.4.3.1 Geometric Similarity

A geometric similarity exists between the real system (subscript r) and the physical model (subscript m) when there is point-by-point mapping between the two domains. The ratio between homologous dimensions defines the scale ($\lambda_L = L_m/L_r$) of the geometric similarity. The ratios between surface areas and volumes hence are λ_L^2 and λ_L^3, respectively.

C.4.3.2 Kinematic Similarity

A kinematic similarity exists between the real system and the physical model when homologous fluid particles occupy homologous positions at homologous times. The trajectories of homologous fluid particles are identical. The velocity ratios ($\lambda_v = \lambda_L/\lambda_t$) of homologous fluid particles are equal. The acceleration and flow rate ratios are given by $\lambda_a = \lambda_L/\lambda_t^2$ and $\lambda_q = \lambda_L^3/\lambda_t$, respectively.

C.4.3.3 Dynamic Similarity

A dynamic similarity exists between the real system and the physical model when homologous regions of the fluid domains are subjected to homologous forces. Therefore, the general law of dynamic similarity states that: the ratio of inertia forces between the physical model and the real system $F_{Im}/F_{Ir} = \lambda_M\lambda_L/\lambda_t^2 = \lambda_\rho\lambda_A\lambda_v^2$. The driving pressure ratio is given by $\dfrac{\Delta p_m}{\Delta p_r} = \lambda_\rho(\lambda_L/\lambda_T)^2 = \lambda_\rho\lambda_\nu^2/\lambda_L^2$. In a periodic flow, the ratio of the frequency parameters gives: $\lambda_L^2 = \lambda_T\lambda_\nu$.

In the case of a steady flow supposed to not be affected by body forces, the two main forces are convective inertia and friction. These two terms are proportional to $\rho V^\star 2/L^\star$ and $\mu V^\star/L^{\star 2}$, respectively. The ratio between these two forces in presence is the Reynolds number $Re = V^\star L^\star/\nu$. When Re is small, viscosity dampens any disturbances of small magnitude and changes

in mechanical quantities are regular. The flow is laminar. When flow velocity increases above a critical threshold, which depends on the nature of the pipe, inertial forces are dominant. Velocity fluctuations can be amplified and transported. Inertia forces are responsible for an energy transfer from flow structures of high magnitude to small-scale, small-amplitude motion components. At very high velocities, the flow loses its time and space regularity. The flow is turbulent. The Reynolds number can also be expressed as $Re = (V^\star/L^\star)/(\nu/L^{\star 2})$, i.e., as the ratio between momentum diffusion time scale and convection time scale. The Reynolds number hence gives a comparison between two terms of the same physical species (analogical ratio). At small Re, the flow disturbances are attenuated by diffusion before being convected in the flow.

When fluids steadily flow in two pipes characterized by geometric similarity, both flow velocity and pressure depends on space and the Reynolds number. When geometric similarity exists, the dynamic similarity is ensured by equality of the Reynolds numbers at homologous sites. The Reynolds similarity law is only valid for a steady flow of an incompressible fluid in the absence of significant body forces. Moreover, initial and boundary conditions must be similar.

Others dimensionless parameters must be taken into account, such as the Mach number $Ma = V_q/c$ in the case of gas flow, when the flow velocity V_q becomes relatively high with respect to the propagation speed c of the pressure waves, although the flow remains subcritical. Moreover, thermal phenomena arise in flows of compressible gases and an energy equation must enter in the flow assessment. Prandtl numbers $Pr = (\mu c_p)/\lambda_T = \nu/\alpha_T$ (ratio of momentum diffusivity to thermal diffusivity), heat capacity ratios, and wall temperature must be identical.

In the case of free-surface flows, the dynamic similarity requires both Reynolds and Froude conditions. The Froude number $Fr = V^\star/(L^\star g)^{1/2}$ gives the ratio between convective inertia ($\rho V^\star 2/L^\star$) and gravity (ρg) forces.

The dimensionless parameters that govern the flow have an identical values in both the real system and the model. Most often, the dynamic similarity is observed for the main factors, the minor ones varying in a small interval in order to satisfy a good approximation.

C.4.3.4 Material Similarity

Material similarity deals with similarity in structure and composition. Similar materials are such that the mass ratio is proportional to the volume ratio, hence to λ_L^3.[11] The homomorphy refers to material similarity between two bodies of similar geometry with homologous parts made of the same material.

[11] Thereby, the primary variable mass depends on the other primary variable length (allometric relation), the density being the same ($\rho_m = \rho_r = \rho$).

C.4.3.5 Temporal Similarity

The ratio of homologous times between two homomorphic systems is proportional to the ratio of geometric similarity with a power that depends on the problem. When the flow is dominated by viscous effects, the time ratio $\lambda_T = \lambda_L^2/\lambda_\nu$ (λ_ν: ratio of the viscosities). When the flow is dominated by elastic forces, $\lambda_T = \lambda_L/(\lambda_E/\lambda_\rho)^{1/2}$. When the flow is dominated by surface tension forces, $\lambda_T = (\lambda_L^3\lambda_\rho/\lambda_{st})^{1/2}$.

C.4.4 Allometric Analysis

The Borelli law (1680) states that the energy ratio of muscular exercise between two homomorphic systems (two similar mammals) is proportional to the volume ratio.

Allometric analysis consists of normalization by body features ($\mathtt{f}_B$) (weight ($\mathtt{w}$), height (H), mass, volume) to compare subjects of different body size within the same species, like those at various growth level, or same-age animals of different species.

The exponential function between the structural or functional quantity $\mathtt{g}$ and the body weight $\mathtt{g} = \kappa_1\mathtt{w}^{\kappa_2}$ leads to a useful log-transformed version $\ln \mathtt{g} = \ln \kappa_1 + \kappa_2 \ln \mathtt{f}_B$.

C.5 Poiseuille Flow

Poiseuille flow is used as a reference for comparison as well as a simplification in many models dealing with relationships between the flow rate and the pressure drop in a network composed of several vessel generations. This flow type cannot be observed in physiological vessels because it corresponds to a fully developed, steady, laminar flow of a homogeneous, incompressible, Newtonian fluid in a long, straight, cylindrical, pipe with rigid, smooth wall and with a uniform circular cross-section [1249]. The fluid particle flows with straight paths in concentric layers parallel to the pipe wall. The velocity profile is invariant. The pressure drop, balanced by the viscous effects, varies linearly with the distance along the duct. This conditions allows an analytical solutions of the Navier-Stokes equation.

$$u = \frac{1}{4\mu}\frac{\Delta p}{l}(r^2 - R^2) = u_M\left(1 - \left(\frac{r}{R}\right)^2\right) = 2, V_q(1 - \tilde{r}^2) \ ,$$

$$q = -\frac{\pi}{8\mu}\frac{\Delta p}{l}R^4 \ , \quad V_q = q/(\pi R^2) = \frac{1}{8\mu}\frac{\Delta p}{l}R^2, \quad u_M = 2V_q \ ,$$

$$\frac{du}{dr} = -2u_M r/R^2 = -\frac{\Delta p}{2\mu l}r \ , \quad \Delta p = \mathtt{R}q \ ,$$

$$\mathtt{R} = \frac{8\mu l}{\pi R^4}G = \frac{\Delta p}{l} = \frac{32\mu}{d^2}V_q \ , \quad \Lambda = \frac{64}{\mathrm{Re}} \ ,$$

$$\tau_w = -\mu\left(\frac{du}{dr}\right)\bigg|_{r=R} = -\mu\left(\frac{\Delta p}{2\mu l}r\right)\bigg|_{r=R} = -\frac{R\Delta p}{2l}, \quad \tau_w = -4\mu\frac{V_q}{R} \ ,$$

$$\tau_w = C_f\rho\frac{V_q^2}{2} \ , \quad C_f = \Lambda\frac{l}{d} = \frac{\Lambda}{4} = \frac{16}{\mathrm{Re}} \ .$$

C.6 Womersley Flow

Consider a fully developed laminar flow of a homogeneous incompressible Newtonian fluid in a horizontal, cylindrical, uniform, straight pipe of circular cross-section, of smooth rigid wall [1250]. The pulsatile flow is composed of a steady component ($\overline{V_q}$) and a sinusoidal modulation, with a given circular frequency (ω) and amplitude ($V_{q\sim}$): i.e., a non-zero mean sinusoidal flow ($\widehat{V_q} = \overline{V_q} + V_{q\sim} = \overline{V_q}(1 + \gamma_u)$, $\gamma_u = V_{q\sim}/\overline{V_q}$: amplitude ratio or modulation rate). This an example of analytical solution of the Navier-Stokes equation.

The velocity field is $\mathbf{v} = \mathbf{v}(r, t)$ ($v \equiv v_z$).

$$v_{,t} = -\frac{1}{\rho}p_{,z} + \nu\frac{1}{r}\partial_r(ru_{,r})$$

let $G_p = -p_{,z}$ be the constant pressure gradient,

$$G_p = \overline{G_p} + G_{p\sim}\exp\{\imath\omega t\} \ .$$

With the dimensionless quantities $\tilde{v} = v/V$ ($V = R/T$), $\tilde{r} = r/R$ and $\tilde{p} = p/(\rho V^2)$, the equation becomes:

$$\tilde{v}_{,\tilde{t}} = \frac{V}{R}\widetilde{G}_p + \frac{\nu}{R^2}\left(\tilde{u}_{,\tilde{r}\tilde{r}} + \frac{1}{\tilde{r}}\widetilde{u}_{,\tilde{r}}\right) \ .$$

The decomposition of the equation in a real steady part and an imaginary unsteady part leads to the following system ($V/(R\omega) = 1$):

$$\overline{\tilde{v}}_{,\tilde{t}} = \frac{V}{R}\overline{\widetilde{G}_p} + \frac{\nu}{R^2}\left(\overline{\tilde{u}_{,\tilde{r}\tilde{r}}} + \frac{1}{\tilde{r}}\overline{\widetilde{u}_{,\tilde{r}}}\right) \ ,$$

$$\imath\tilde{v}_{\sim,\tilde{t}} = \widetilde{G}_{p\sim} + \mathrm{Sto}^{-2}\left(\tilde{u}_{\sim,\tilde{r}\tilde{r}} + \frac{1}{\tilde{r}}\tilde{u}_{\sim,\tilde{r}}\right) \ .$$

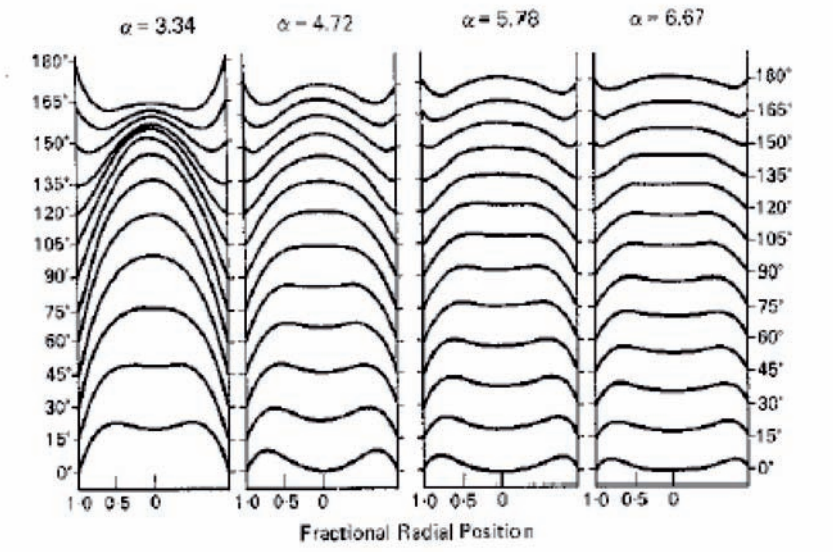

Figure C.1. Velocity profiles of the Womersley solution for different values of the Stokes number. The phase lag rises and the amplitude decays when the frequency increases (from [1251]).

With the variable change $w = \hat{\tilde{v}} + \imath\widehat{\tilde{G}_p}$, the equation of imaginary part becomes:

$$w_{,\tilde{r}\tilde{r}} + \frac{1}{\tilde{r}} w_{,\tilde{r}} - \imath \mathrm{Sto}^2 w = 0.$$

Notice that the term $(-\imath\mathrm{Sto}^2)^{1/2}\tilde{r}$ represents a new variable, a Bessel equation is obtained, which leads to the solution:[12]

$$\widetilde{v}_{\sim} = \imath\widehat{\widetilde{G}_{p\sim}} \left(\frac{J_0(\imath^{3/2}\tilde{r}(\omega/\nu)^{1/2})}{J_0(\imath^{3/2}\mathrm{Sto})} - 1 \right).$$

The higher the frequency parameter, the greater the distorsion of the velocity profile (Fig. C.1).

C.7 Entry Steady Flow in a Straight Pipe

The entry, or entrance, length Le has been first defined, for a steady laminar flow in a long straight cylindrical conduit of circular cross-section and smooth rigid impermeable wall, with uniform injection velocity, for instance,

[12] $J_0(\imath^{3/2}\tilde{r}(\omega/\nu)^{1/2}) = 1 + (\imath/2^2)(\tilde{r}^2\omega/\nu) - 1/(2^2 4^2)(\tilde{r}^2\omega/\nu)^2 + \imath/(2^2 4^2 6^2)(\tilde{r}^2\omega/\nu)^3 + \mathcal{O}[(\tilde{r}^2\omega/\nu)^4]$.

as the pipe length from which the deviation of the velocity distribution from the Poiseuille (subscript P) distribution[13] is less than 1% [1252]. The viscous effects have then pervaded the whole tube lumen. The inlet length has also been defined, for an easier record, as the distance through which the developing maximum velocity, which is the centerline velocity in a long straight tube, reaches 99%[14] of the peak velocity of the fully developed flow: $v_{\max}/v_{\mathrm{P}\max} = 0.99$ ($v_{\mathrm{P}\max} = 2\,V_q$: maximum of the Poiseuille velocity distribution). There is a huge between-author variability in the value of the entry length for a single and simple flow, which leads to the Poiseuille flow.

Boussinesq (1891) provided the following value of the dimensionless length $\widetilde{\mathrm{Le}}$ through which the velocity is redistributed approximately into a parabolic profile:[15]

$$\widetilde{\mathrm{Le}} = \frac{\mathrm{Le}}{d\mathrm{Re}} = 0.065, \tag{C.1}$$

where Re is the Reynolds number based on the tube hydraulic diameter d. However, this value is overestimated, and the following value is proposed:[16]

$$\widetilde{\mathrm{Le}} = 0.015\ , \tag{C.2}$$

when $200 < \mathrm{Re} < 2500$. When $\mathrm{Re} < 200$, $\mathrm{Le}^+ = \mathrm{Le}/d = 1.2$.[17]

Equations are also proposed as the following ones:

$$\mathrm{Le}^+ = \kappa_1 \mathrm{Re} + \frac{\kappa_2}{\kappa_3\,\mathrm{Re} + 1}\ , \tag{C.3}$$

where κ_1, κ_2, and κ_3 are constant (e.g., [1268, 1269]),[18]

[13] $u_{\mathrm{P}}(r) = 2V_q(1 - (r/R)^2)$ (V_q: cross-sectional average velocity, r: radial distance from the tube axis, R: tube radius).

[14] The same threshold is used to define the boundary layer thickness δ, as the distance from the wall where $u(z, \delta) = 0.99\,V_\infty$.

[15] An approximate analysis from the estimation of the boundary layer thickness gives a similar result: $\delta \sim 2(\nu z/V_q)^{1/2}$, therefore $\mathrm{Le}/(d\mathrm{Re}) = 0.0625$. A value of 0.06 is given in many textbooks (e.g., [1253–1255]). Normalization quantities $R\mathrm{Re}_d$ ($\mathrm{Re}_d = V_q d/\nu$) and $R\mathrm{Re}_R$ ($\mathrm{Re}_R = V_q R/\nu$) rather than $d\mathrm{Re}_d$ have also been used. Besides, the boundary layer thickness is estimated by a function of the characteristic length $R\mathrm{Re}_R$. The boundary layer is the flow region in which the friction forces $\propto \mu V_\infty/\delta^2$ are balanced by the inertia forces $\propto \rho V_\infty^2/R$. The balance yields the approximative formula for the boundary layer thickness $\delta \sim R\mathrm{Re}_R^{-1/2}$.

[16] The fluid mechanics literature shows great between-author data variability, e.g., 0.01 [1251], 0.028–0.030 [1256–1262] and 0.04 [1263, 1264]. The last value is equal to the inlet length in a straight channel with flat parallel walls of width w: $\mathrm{Le}/(w\mathrm{Re}_w) = 0.04$ [1265, 1266]. A range is sometimes provided to take into account between-author variability of the dimensionless entry length (e.g., [1267]: $\widetilde{\mathrm{Le}} \in [0.03\text{–}0.06]$, $100 < \mathrm{Re} < 2000$).

[17] The Reynolds number threshold of 200 is author-dependent, a value of 100 is often found.

[18] [1268] gives the following values: $\kappa_1 = 0.056$, $\kappa_2 = 0.6$, $\kappa_3 = 0.035$ and [1269] - for Le and not Le^+ - $\kappa_1 = 0.061$, $\kappa_2 = 0.72$, $\kappa_3 = 0.04$.

$$\mathrm{Le}^{+} = \kappa_1 + \kappa_2 \,\mathrm{Re}\,, \tag{C.4}$$

where κ_1 and κ_2 are constant (e.g., [1270])[19].

C.8 Dispersion in Fluid Flows

Consider the brief injection (δ-input) of a tracer in a vessel. Although the tracer concentration is uniform at the entrance of the test section, convected tracer concentration axially stretches, when it is observed a short time after the tracer bolus injection. The concentration spreading in the channel is due to the development of the velocity profile. The tracer bolus spreads as an evolving Gaussian curve with the traveled distance, with a peak concentration $c_{\max} \propto \mathrm{Pe}^{-1/2}$. The dispersion induced by convection is damped by transverse molecular diffusion, which homogenizes tracer distribution, a blunt distribution being observed at long time. This molecular diffusion involves axial dispersion-related diffusivity[20] $\mathcal{D}_d$:

$$c_t = \mathcal{D}_d \, c_{zz}.$$

Axial mixing, the so-called *Taylor dispersion*, which involves the convection, then differs from pure diffusion. The higher the diffusivity, the lower the dispersion.

C.9 Porous Medium

Different liquids can mix in a porous solid. Conversely, a porous medium can separate mixtures (filtration). Particles that are bigger than the pore size or molecules that bind to the solid walls are stopped. Besides, velocity fluctuations are damped by the wall friction in the porous medium.

Consider the brief injection of a tracer in a homogeneous porous solid. The transit times of the different tracer molecules across the porous solid differ; the concentration-time curve is Gaussian. Longitudinal and transverse *dispersions* are not equal (anisotropy). Tracer dispersion is associated with molecular diffusion. The *Taylor dispersion*, associated with the coupling of molecular diffusion to convection, is not involved in porous media. Indeed in a porous body, the speed of a given molecule in the pore center is relatively high, but the speed can be reduced in the following pore, the molecule coming into contact with the wall or close to it, and conversely.

Low speed flow through a rigid porous medium is described by the Darcy law: $\nabla p = -(\mu/\mathcal{P}_D)\breve{v}$ ($\mathcal{P}$: darcy permeability) and high speed flow by the Forchheimer law $\nabla p = -(\mu/\mathcal{P}_D)\breve{v} - \kappa\rho v^2$, which both relate pressure (p) to volume-averaged velocity ($\breve{v}$). Flow through a deformable porous body deforms the material and deformation affects the flow.

[19] $\kappa_1 = 0.59$, $\kappa_2 = 0.056$.

[20] The usual molecular diffusion refers to any kind of random processes.

C.10 Turbulence

Turbulent flow is characterized by a random three-dimensional process with statistical independency between spatially and temporally distant observations. This non-linear phenomenon, with an important convection inertia at high Reynolds number induces convective mixing and strong energy dissipation. The vortivity field generates intense fluctuations of the velocity curl and creation and destruction of vortices, with energy transfer between large and small flow structures. The additional momentum transport associated with Reynolds stress requires turbulent dynamic viscosity μ_T.

Turbulent Flow Characteristics

1. The unstable flow pattern is characterized by very high Reynolds number values, i.e., strong inertia or an important diffusion time scale. The value for which flow disturbances are maintained is given by the critical Reynolds number. Its value depends on the conduit geometry (axis curvature), wall surface roughness, flow development state, time-dependent or independent regime, and wall rheology. Moreover, it depends on the care with which experiments are performed. A critical Reynolds number of 10^5 can be found for a transitional flow in long straight ducts of smooth rigid wall and uniform circular cross-section, hence much larger than the value (~ 2300) proposed by O. Reynolds (1842-1912).
2. Turbulences are random processes. The turbulent flow velocity field is unpredictable because of the non-uniqueness of the solution of the Navier-Stokes equations and its sensibility to initial and boundary conditions.
3. Turbulence is a non-linear phenomenon, because inertia is a predominant term in Navier-Stokes equations. Additionally, the pressure gradient is also non-linear.
4. Turbulence is a three-dimensional motion, with fluctuations of the three components of the velocity vector. However, velocity fluctuations can appear with a zero mean.
5. Turbulence is defined by a vorticity field, with creation and destruction of swirls owing to a deformation velocity field. The vorticity field is characterized by large fluctuations.
6. Turbulence involves an energy transfer from small structures to large ones and conversely. According to the Richardson model (1933), there is an energy cascade from large to small scales.
7. Turbulence can be decomposed into mean flow and fluctuating motion, when the flow is apparently disorganized with complex interacting eddies but with constant time-averaged velocity and pressure.

$$\mathbf{v}(\mathbf{x}, t) = \overline{\mathbf{v}}(\mathbf{x}) + \mathbf{v}'(\mathbf{x}, t).$$

A coupling exists between the two velocity fields, the mean field depending on the fluctuationg one. Hence, when a periodic phenomenon, such as

ventilation, includes a random component, phase-average analysis leads to the following expression of any physical quantity ($\mathtt{g}$):

$$\mathtt{g} = \bar{\mathtt{g}} + \mathtt{g}',$$

with $\mathtt{g}' \equiv < \mathtt{g}'^2(t) >^{1/2} = \Big(\frac{1}{N}\sum_{k=1}^{N} \mathtt{g}'^2(t+kT)\Big)^{1/2}$.

Turbulence intensity is defined by the root mean square value of the fluctuation components: $(\overline{v'^2})^{1/2}$,

$$\Big(\frac{1}{3}\sum_{i=1}^{3}(\overline{v'^2}_i\Big)^{1/2}.$$

For each component i, $(i = 1, 2, 3)$, the turbulence intensity can be expressed with respect to the mean flow velocity component: $\dfrac{(\overline{v_i'^2})^{1/2}}{\overline{v}_i}$.

The Reynolds stress component $-\rho\overline{v_i' v_j'}$ characterizes the turbulent transfer of momentum, i.e., the transport of momentum $-\rho v_i'$ with velocity v_j'.

8. Turbulence has a mixing capacity. Turbulence is a source of fluid particle dispersion, with a higher transfer rate than with the molecular diffusivity alone.
9. Turbulence implicates energy dissipation, with transformation of the turbulence kinetic energy into heat, essentially associated with friction at the level of small structures. The notion of turbulent dynamic viscosityμ_t is then introduced.
10. Turbulence belongs to the continuum theory. The Kolmogorov scale L_K, the smallest turbulence length scale (at which eddies disappear), is characterized by a growing role of the molecular viscosity (friction energy is higher the small eddy energy), remains higher than the molecule mean free path (λ).
    The Knudsen number $Kn = \lambda/L_K$. Because $Re = uL_K/\nu \equiv c\lambda/\nu \sim 1$ $Kn, \sim (\nu/uL_K)(u/\bar{u})(\bar{u}/c) \sim (u/\bar{u})Ma$. Usually, $(u/\bar{u}) \sim 10^{-2}$, therefore, $Kn, \sim 10^{-2}\, Ma$.
    Kolmogorov (1941) introduced a scale range such that small scales do not depend on geometry and large scales are not affected by viscosity. Any quantity is a function of an intermediate length scale $\mathtt{\Lambda}$ according to a power law: $\mathtt{g} = f(\mathtt{\Lambda}^p)$.

C.11 Head Loss

Any flow is characterized by friction between fluid particles and the fluid and solid walls. Denote $\mathtt{h}$ the mean total head in any cross-section, Λ, the head

loss coefficient per unit length, and C_f the friction coefficient. The relationship between the head loss coefficient and the friction coefficient in a cylidrical pipe is the following:[21] $\Lambda = 4\,C_f$. The head loss in a segment of uniform cylindrical pipe conveying a steady fully developed flow is given by:

$$\Delta H = H_{in} - H_{out} = p_{g_{in}} - p_{g_{out}}/(\rho g),$$

where p_g is the generating pressure.

When $< C_f >$ is the friction coefficient averaged over the length L, then:

$$< C_f > \frac{L}{d} = 3.435\Big(\frac{L}{dRe}\Big)^{1/2}, \quad 10^{-5} < \frac{L}{dRe} < 10^{-3}.$$

The Darcy-Weisbach equation is associated with a flow that is steady in average[22] and fully dveloped in a uniform tube,

$$\Delta p_g = \Lambda \frac{L}{d_h}\rho\frac{V_q^2}{2} = \Lambda\frac{L}{d_h^3}\rho\frac{8q^2}{\chi_m^2}, \quad DeltaH = \Lambda\frac{L}{d_h}\frac{V_q^2}{2g}.$$

The Fanning equation uses the friction coefficient:

$$\frac{dp_f}{dx} = \frac{2C_f\rho V_q^2}{d}.$$

Head loss can be expressed with the resistance coefficient K_R rather than the head loss coefficientΛ. In a straight pipes, when the fluid is incompressible:[23]

$$\Delta p = K_R \mathtt{w}\frac{V^2}{2g},$$

where $\mathtt{w}$ is the specific weight.

The Hagen-Poiseuille formula gives the value of the head loss coefficient in a laminar steady fully developed flow in a straight pipe: $\Lambda = f/Re$, where f is the friction shape factor. When the pipe wall is rigid and smooth, and the cross-section circular $f = 64$. The head loss coefficient in turbulent flow can be calculated using either the Blasius formula when $Re < 10^5$, $\Lambda = 0.316/Re^{1/4}$, or the Karman-Prandtl-Nikuradse formula when $Re > 10^5$, $\frac{1}{\sqrt{\Lambda}} = 2\ln Re\sqrt{\Lambda} - 0.8 = 2\log\frac{\sqrt{\Lambda}}{2.51}$. Similarly, several empirical formulas give the value of the friction coefficient with respect to the Reynolds number. The Blasius formula gives $C_f = 0,079\,Re^{-1/4}$, $3000 < Re < 10^5$, the Knudsen–Katz formula $C_f = 0,046\,Re^{-0,2}$, and the Nikuradse formula (1932) $\frac{1}{\sqrt{C_f}} = 4\,\ln(Re\sqrt{C_f}) - 0,40$, $3000 < Re < 3.10^6$.

[21] $C_f = 2\tau_w/(\rho V_q^2)$.

[22] The flow is steady on average when the distribution of temporal fluctuations in velocity and pressure are the same whatever the cross-section.

[23] The resistance coefficient is expressed as $K_R = \Lambda(L/d)$ according to Darcy, or $K_R = \Lambda(L/R_h)$ according to Fanning.

Geometry changes (sudden or abrupt narrowing or enlargment, direction change, branching, merging, obstacle, etc.) induce an increase in mechanical energy loss:

$$\Delta p = \zeta \rho \frac{V_q^2}{2},$$

where ζ is the singular head loss coefficient.

In a bend with a curvature angle θ, the head loss can be estiamted using the Weisbach equation:

$$\Delta H = \frac{1}{2}\rho V^2 \Big(0.9457 \sin^2 \frac{\theta}{2} + 2.047 \sin^4 \frac{\theta}{2}\Big).$$

L Prandtl[24] proposed the following expression for the bend head loss coefficient Λ_c with respect to that in the straight duct Λ_s:

$$\Lambda_c = 0.37\, \Lambda_s [(R_c/R)^{0.05} Re/2]^{0.36},$$

when $40 < (R/R_c)Re < 1000$ (R_c : curvature radius) When $(R/R_c)Re < 40$, $\Lambda_c = \Lambda_s$. According to Ito[25], in a turbulent flow:

$$\Lambda_c = \Lambda_s [Re(R/R_c)^2]^{0,05},$$

when $It \equiv Re(R/R_c)^2 > 6$.

In a branching region, head loss can be estimated by:

$$\Delta p = \Big(\Lambda \frac{L}{d} + {}_i^j K\Big) \mathtt{w} \frac{V^2}{2g}.$$

The relationship between pressure drop Δp through the pipe and volume flow rate q of conveyed fluid can be assessed from the Darcy-Weisbach equation in a straight pipe for the steady laminar pattern:

$$\Delta p = \frac{1}{2}\rho \frac{64}{Re} L \frac{V_q^2}{d_h} = \frac{128}{\pi} \mu \frac{L}{d_h^4} q.$$

The pressure–flow relationship appears to be linear in straight pipe conveying a laminar steady flow. In addition, the pressure decays in a non-linear manner in the entry length of a straight pipe, whereas the pressure drop becomes contant in a fully developed flow.

In curved ducts and branching segments, the pressure–flow relationship is non-linear [1271]. However, the relation between axial pressure difference and flow rate can be linear for certain ranges of the flow rate. In addition to the longitudinal pressure difference, a transverse pressure difference appears in any cross-section of the bend between the inner and outer edges (with respect to the local center of tube curvature). The latter, which depends on the curvature angle and curvature ratio (R_h/R_c), also varies non-linearly with the flow rate.

[24] Fuhrer durch die Strömungslehre, 1949, p. 159.

[25] Trans. ASME, 81, p. 123-124.

C.12 Rheology Glossary

Consider a body elementary particle[26] that undergoes a displacement $\mathbf{u}$. This position change of material points can induce a configuration variation measured by the strain tensor[27] $\mathbf{E}$ which expresses translations and rotations. The displacement $\mathbf{u}$ is generated by forces, the effects (compression, elongation, shear) of which are measured by the stress tensor[28] $\mathbf{C}$.

***Breaking Strength*:** load corresponding to a maximum extension required to produce material rupture (1D test framework).

***Bulk Modulus*:** parameter which quantify the reaction of a material to a volume change when it is subjected to a given load ($B = p/(dV/V)$). The bulk modulus then refers to the ability of a material to resist a uniform, small expansion.

***Complex Viscoelastic Moduli*:** apply a sinusoidal shear on a material:

$$\begin{aligned} e^* &= \hat{e}\exp\{\imath(\omega t)\} = C^*(\omega)c^*(t)\ , \\ c^* &= \hat{c}\exp\{\imath(\omega t+\varphi)\} = G^*(\omega)e^*(t)\ , \\ G^*(\omega) &= \Re[G(\omega)]\ + \imath\Im m[G(\omega))] = G'(\omega) + \imath G''(\omega)\ , \end{aligned}$$

where $G^*(\omega)$ is the complex shear modulus, $G'(\omega)$ the *storage modulus* and $G''(\omega)$ the *loss modulus*.

In the case of 1D sinusoidal traction tests, the analysis being based on a Voigt phenomenological model, $E(\omega) = E_{\text{dyn}} + \imath\omega\eta$, where E_{dyn} is the dynamic elastic modulus and $\omega\eta$ the loss modulus. A complex incremental elastic modulus can be defined:

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[26] This infinitesimal volume element of the continuum is assimilated to a material point, which is also called an infinitesimal control volume, the smallest analysis volume, which contains a large number of material molecules. There is a one-to-one relation between the material point and its spatial position.

[27] The deformation is a measure of change in size, shape, and volume. The elastic and plastic deformations are reversible and irreversible, respectively. There are: (1) lineic deformation, change in length per unit length, (2) shear deformation, angular shift with shape change due to tangential stresses, and (3) volumic deformation, volume change per unit volume. A loading applied at time t on an unstressed body produces either instantaneous or delayed deformation. The residual deformation is a deformation that persists after loading withdrawal. The permanent deformation is a limit toward which the residual deformation tends when $t \to \infty$.

[28] The stress is a measure of forces resulting from internal reactions between body elementary particles due to sliding, separation, and compaction induced by external stresses. The internal resistance forces to the deformation result from normal and tangential forces, continuously distributed, with variable magnitude and directions, which act on elementary surfaces across the entire material. When the loading is quickly applied, it can affect the process via stress and strain wave propagation.

$$E^*_{\text{dyn}} = E_{\text{inc}}(R_e)\exp\{\imath\varphi\}\ ,$$

where φ is the phase lag between the imposed sinusoidal pressure of amplitude Δp and the resulting radial excursions of magnitude ΔR_e. Therefore, $E_{\text{dyn}} = E_{\text{inc}}(R_e)\cos\varphi$ and $\omega\eta = E_{\text{inc}}(R_e)\sin\varphi$.

***Compliance*:** refers to cross-sectional area variations due to pressure changes at a given vessel station $C = (\partial A/\partial p)$.

***Constitutive Law*:** relation between the stress and strain tensors. It must agree with experimental data for a large loading range. It must contain a minimal number of independent constants, which have a physical meaning and can be easily calculated.

***Creep* at constant stress:** when a stress is suddenly applied and maintained constant for a long time (step function), the strain gradually increases. When the stress is removed, either the strain does not come back or goes back slowly to its original value.

***Displacement Decomposition*:** Any displacement at any time can be decomposed into a uniform translation, a rigid rotation and a deformation, with respect to the reference frame (Cauchy-Stokes theorem):

$$\mathbf{u}_{P_2} = \mathbf{u}_{P_1} + \mathbf{du} = \mathbf{u}_{P_1} + \boldsymbol{\Omega}\times\mathbf{dr} + \mathbf{E}\cdot\mathbf{dr}.$$

Distensibility (specific compliance)**:** refers to cross-section deformation $D = (\partial A/\partial p)/A = \mathtt{C}/A$.

***Elasticity*:** property that enables the material to resist the deformation by the development of a resisting force.

***Elastic Modulus*:** ratio of the applied stress, or reacting stress, to the resulting deformation. A material is linearly elastic in a given loading range if the elastic modulus remains constant, the stress being proportional to the strain ("*ut tenso, sic vis*"; Hooke law: $E = C_{ii}/E_{ii}$).

***Extensibility*:** refers to 1D loading.

***Generalized Newtonian Model*:** in shear-thinning fluid flows, the extra-stress tensor, characterized by $\mathbf{T} = 2\mu(T,\dot{\overset{\bullet}{\gamma}})\mathbf{D}$ ($\mathbf{D} = (\nabla\mathbf{v} + \nabla\mathbf{v}^T)/2$) is given by $\mathbf{T} = 2\mu(T,\imath_2(\mathbf{D}))\mathbf{D}$ ($\imath_2(\mathbf{D}) = (\text{tr}(\mathbf{D})^2 - \text{tr}(\mathbf{D}^2))/2$).

***Hysteresis*:** successive loading-unloading cycles show a loop, ascending and descending branches not being superimposed. Sinusoidal inputs are currently used: $\varepsilon(t) = \bar{\varepsilon} + \varepsilon_\sim \sin\omega t$, $c(t) = \bar{c} + c_\sim \sin(\omega t + \varphi)$. The loop shape depends on the mean loading value $\bar{\varepsilon}$, loading amplitude $\Delta\varepsilon$, loading rate $\dot{\varepsilon}$, and loading history $\mathcal{H}(\varepsilon)$: $\int_{-\infty}^{t}\varepsilon(t-\tau)\,d\tau$ ($E = E(\bar{\varepsilon},\Delta\varepsilon,\dot{\varepsilon},\mathcal{H}(\varepsilon))$.

***Incremental Elastic Modulus*:** the elastic modulus is considered constant in small loading range, the non-linear stress-strain relationship being constructed into small intervals (piecewise constant elastic modulus). For a point of the outer surface of the vessel wall, which is easy to observed experimentally, Bergel (1961) proposed the following formula:

$$E_{\text{inc}}(R_i) = \frac{2(1-\nu_P)R_i^2 R_e}{R_e^2 - R_i^2}\frac{\Delta p}{\Delta R_e},$$

and for a point of the wetted surface, more or less easy to target by medical imaging:

$$E_{\text{inc}}(R_e) = \frac{(1+\nu_P)R_i}{R_e^2 - R_i^2}\big((1-2\nu_P)R_i^2 + R_e^2\big)\frac{\Delta p}{\Delta R_e} \ .$$

***Isotropic Material*:** the material properties are independent of direction.

$$E = 2G(1+\nu_P) \ , \qquad \nu_P = (3B-2G)/\big(2(3B+G)\big) \ ,$$
$$B = E/(3(1-2\nu_P)) \ , \qquad G = E/(2(1+\nu_P)) \ .$$

***Memory Effect*:** the behavior of certain material depends not only on loading applied at the observation time t, but also on the previously imposed stresses. The history of a physical variable g is the set of values taken by g during previous times: $\mathsf{g}(t)_{-\infty}^{t} \to \int_{-\infty}^{t} \mathsf{g}\, d\tau$. An influence function can be introduced ($\mathcal{H}$). Soft biological materials undergo at any instant stresses (deformations) that depend on the stress (strain) magnitude at that time, the loading rate, and the loading history.

***Orthotropic Material*:** a material that has at least two orthogonal planes of symmetry, within which material properties are independent of direction.

***Poisson Ratio*:** ratio of the relative contraction in the transverse direction j to the relative deformation in the direction i of the applied load $\nu_P = E_{jj}/E_{ii}$ $(i \neq j)$. 1D extension is characterized by: (1) a longitudinal lengthening $e_\ell = \Delta L/L$, and (2) a transverse shortening $e_t = \Delta d/d = -(\nu_P/E)c$ (transverse strain-to-axial strain ratio).

***Preconditionning*:** initial period of adjustment to loading. The cyclic loading response reaches a quasi-steady state after several succeeding cycles (adaptation period), probably due to matrix reorganization.

***Prestress*:** biological tissues in the physiological state are not unstressed. Once excised, they shrink (tethering effect of the surrounding tissues). Once axially cut, blood vessels widen.

***Pseudoelasticity*:** an approximative splitting description of the stress-strain relationship associated with cyclic loadings.

***Relaxation Function*:** incorporates the response to stress history. Y.C. Fung proposed a decomposition into a reduced relaxation function, a normalized function of time, and an elastic response that depends on the strain.

***Reference State*:** state that is commonly determined according to the physiological requirements. Consequently, it does not correspond to an unstressed state. For an artery, it is defined by $p_i = 13.3\,\text{kPa}$, knowing that in an artery deconnected from the vessel network $L/L_{\text{in vivo}} \sim 0.9$, and in an excised artery $L/L_{\text{in vivo}} \sim 0.6$–$0.7$.

***Shear Modulus*:** quantifies the resistance of a material to a shape change caused by a shear, keeping a constant volume (shear stress-to-shear strain ratio $G = C_{ij}/E_{ij}$, $i \neq j$). The shear modulus usually indicates the ability of a material to resist a small isovolume shape distortion. Application of

equal and opposite tangential surface stresses $\mathbf{c}$ at opposite faces of a control volume induces sliding with an angle α, without rotation due to normal stresses applied by adjacent particles ($G = |\mathbf{c}|/\tan\alpha$).

***Strain*:** There are several definitions of strains. The *stretch ratio* in the direction of the applied 1D stress is the relative displacement, i.e., the ratio of the length change to the unstressed length $\lambda = L/L_0$. The *engineering strain* for an uniaxial loading is the stretch ratio minus one $\varepsilon = \Delta L/L_0 = \lambda - 1$. Another strain measure refers to the deformed configuration $\varepsilon' = \Delta L/L = 1 - \lambda^{-1}$. Quadratic strains can be easily incorporated in strain energy densities. The *Green-St. Venant strain* is defined by $\varepsilon_G = (L^2 - L_0^2)/(2L_0^2) = (\lambda^2 - 1)/2$ and the *Almansi-Hamel strain* by $\varepsilon_A = L^2 - L_0^2/(2L^2) = (1 - \lambda^{-2})/2$. The *natural strain* $\varepsilon = \ln\lambda$ allows us to easily handle successive loadings because the resulting strain is the sum of the constitutive strain measures.

The static deformation of cylindrical orthotropic vessels generated by internal pressurization and uniform axial extension is described by $\lambda_z = L/L_0$, $\lambda_r = R/R_0$, $\lambda_\theta = \overline{\chi}/\overline{\chi}_0 = \lambda_r$[29] (perimeter associated with the wall neutral line).

Let $\mathbf{F} = \partial\mathbf{x}/\partial\mathbf{x}_0$ be the deformation gradient tensor ($\imath_1, \imath_2, \imath_3 = det\mathbf{F}^2$). Using the polar decomposition theorem, $\mathbf{F}$ can be expressed by the product of the right $\mathbf{U}$, or left $\mathbf{V}$, stretch tensor and the rotation tensor $\mathbf{R}$ ($\mathbf{R}^T\mathbf{R} = \mathbf{R}\mathbf{R}^T = \mathbf{I}$): $\mathbf{F} = \mathbf{R}\mathbf{U} = \mathbf{V}\mathbf{R}$.

The right and left *Cauchy-Green Deformation Tensors* are associated with dilation/compression and shearing actions: $\mathbf{S}_r = \mathbf{F}^T\mathbf{F} = \mathbf{U}^T\mathbf{U}$ and $\mathbf{S}_l = \mathbf{F}\mathbf{F}^T = \mathbf{V}\mathbf{V}^T$ respectively. The *Biot-Finger strain tensor* $\mathbf{B} = \mathbf{S}_l^{-1} = \mathbf{F}^{T^{-1}}\mathbf{F}^{-1}$. The *Green-Lagrange strain tensor* and *Almansi strain tensor* are given by: $\mathbf{G} = (\mathbf{S}_r - \mathbf{I})/2 = \mathbf{F}^T\mathbf{D}\mathbf{F}$ and $\mathbf{A} = (\mathbf{I} - \mathbf{B})/2$.

Strain Energy Density (elastic potential)**:** a function of the strain invariants if the elastic material is homogeneous and isotropic.

***Stress*:** The stresses are forces per unit surface area producing a deformation. The stress tensor components C_{ij} are conveniently expressed in an orthogonal basis $\{\hat{\mathbf{e}}_i\}_{i=1}^3$. In any material point $P \in \Omega$, a second order stress tensor $\mathbf{C}(P)$ exist (combination of the stresses acting on the faces of the infinitesimal control volume), such that the local force per unit surface area $\mathbf{c}(P, \widehat{\mathbf{n}}) = \mathbf{C}(P)\widehat{\mathbf{n}}$ [Cauchy theorem, 1822].[30] The diagonal elements (C_{ii}) represent normal (tensile) stresses and the others (C_{ij} $(i \neq j)$) the tangential (shearing) stresses.

$$\mathbf{c} = \mathbf{C}\cdot\hat{\mathbf{n}}\ , \quad c_i = C_{ij}n_j \quad \forall i, \forall j,\ i, j = 1, 2, 3. \qquad \text{(Cauchy formula)}$$

[29] λ_θ can be defined as: $\lambda_\theta = (\pi/(\pi - \theta))\lambda_r$ when the opening angle θ is known [1272].

[30] $\begin{pmatrix} c_1 \\ c_2 \\ c_3 \end{pmatrix} = \begin{pmatrix} C_{11}\ C_{12}\ C_{13} \\ C_{21}\ C_{22}\ C_{23} \\ C_{31}\ C_{32}\ C_{33} \end{pmatrix} \begin{pmatrix} \hat{n}_1 \\ \hat{n}_2 \\ \hat{n}_3 \end{pmatrix}$.

Stress is defined with respect to either the reference (*Lagrange-Piola* stress $C_{Lii} = f_i/A_0$) or to the deformed (*Cauchy-Euler* stress $C_{Cii} = f_i/A$) configuration. The *Kirchhoff* stress is defined by $C_{Kii} = C_{Lii}/\lambda_i$ or $C_{Kii} = (\rho_0/\rho)C_{Cii}/\lambda_i^2$.

Stress Relaxation at constant strain**:** when a strain is suddenly applied and maintained constant for a long time (step function), the induced stress decreases after reaching its maximum.

Tensile Strength (yield point)**:** tension at which a stretched material cannot go back to its original configuration and undergoes an irreversible plastic deformation (the material yields, with breaking of links between its constituents).

Tensor Decomposition**:** The velocity gradient tensor $\mathbf{L} = \boldsymbol{\nabla}\mathbf{v}$ ($\dot{\mathbf{F}} = \mathbf{LF}$, $(\boldsymbol{\nabla}\mathbf{v})_{ij} = \partial v_i/\partial x_j$) can be decomposed into a symmetric tensor $\mathbf{D}$, the *deformation rate tensor*, and a antisymmetric tensor $\mathbf{W}$, the *rotation rate tensor* or the *vorticity tensor*:

$$\boldsymbol{\nabla}\mathbf{u} = \mathbf{D} + \mathbf{W} = 1/2(\boldsymbol{\nabla}\mathbf{u} + \boldsymbol{\nabla}\mathbf{u}^T) + 1/2(\boldsymbol{\nabla}\mathbf{u} - \boldsymbol{\nabla}\mathbf{u}^T).$$

Thixotropy**:** the response of a thixotropic material depends on the body structure changes, and consequently on the loading rate, duration of the unstressed period, and loading duration with respect to the body-kinetic time scale.

Viscocity**:** material property dealing with resistance to deformation and motion.

Relative Viscocity of a suspension**:** ratio of the suspension viscocity to the suspending fluid (plasma) viscocity.

Volume Dilation**:** $dV/V = \boldsymbol{\nabla} \cdot \mathbf{u} = E_{ii}$

Phenomenological Models in Rheology

Rheology models are made by the assembling of elementary elements, springs and dashpots, which are associated

- either in parallel with the following rules:
  - the imposed net stress is the sum of the stresses ($c = \sum c_b$) applied to each branch (subscript b),
  - the undergone deformation is identical in each branch and equal to the net deformation ($e = e_b$);
- or in series with the following rules:
  - the imposed net stress is wholly borne by each element i: ($c = c_i$),
  - the net deformation is the sum of the deformation undergone by each element ($e = \sum e_i$).

The rheological features of the viscoelastic models are obtained using well-defined procedures (Table C.8).

Table C.8. Rheology tests. Viscoelastic materials.

	Static test		Sinusoidal loading
	Creep	Relaxation	Harmonic shear
Input	$c = \text{cst.}$	$e = \text{cst.}$	$c = \hat{c}\exp\{\imath\omega t\}$
Function	$\phi(t) = e(t)/c$	$\psi(t) = c(t)/e$	$e = \big(G'(\omega) - \imath G''(\omega)\big)\hat{c}\exp\{\imath\omega t\}$

***Maxwell model*:** is constituted by the association in series of a spring (subscript S) and a dashpot (subscript D):

$$\dot{u} = \dot{u}_{\mathrm{S}} + \dot{u}_{\mathrm{D}} = C\dot{c} + c/\mu$$
$$c = c_{\mathrm{S}} = c_{\mathrm{D}} \ ,$$

($C = 1/E$: elastic compliance).

Viscoelastic materials are often assumed to behave like simple Maxwell models, with their material constants E and μ, and the following rheological law:

$$(\mu/E)\dot{\mathbf{c}} + \mathbf{c} = \mu\dot{u} \ .$$

Constitutive laws of three-dimensional bodies are derived from the phenomenological one-dimensional model:

$$(\mu/E)\overset{\triangledown}{\mathbf{C}} + \mathbf{C} = 2\mu\mathbf{D},$$

where the upper convected time derivative of the stress tensor $\mathbf{C}$ is given by:

$$\overset{\triangledown}{\mathbf{C}} = D\mathbf{C}/Dt - (\nabla\mathbf{v})\mathbf{C} - \mathbf{C}(\nabla\mathbf{v})^T,$$

$D\cdot/Dt$ and $\nabla\mathbf{v}$ being the substantive derivative and the tensor of velocity derivatives respectively.[31]

***Voigt or Kelvin-Voigt model*:** is constituted by the association in parallel of a spring and a dashpot:

$$u_{\mathrm{S}} = u_{\mathrm{D}} = u \ ,$$
$$c = c_{\mathrm{S}} + c_{\mathrm{D}} = (1/C)u + \mu\dot{u} \ .$$

[31] The lower convected time derivative is:

$$\overset{\triangle}{\mathbf{C}} = \dot{\mathbf{C}} + \mathbf{C}(\nabla\mathbf{v}) + (\nabla\mathbf{v})^T\mathbf{C} \ .$$

Standard linear model (Boltzmann model)**:** is constituted by the association in parallel of a spring and a Maxwell model (subscript M):

$$u_{\mathrm{S}} = c_{\mathrm{S}}/E,$$
$$\dot{u}_M = c_M/\mu + \dot{c}_M/E_M$$

Rheological data of these simple models are summarized in Tables C.9, C.10 and C.11.

Table C.9. Constitutive laws of the simplest rheology models for a stress step ($c(t) = c_0 h(t)$).

Model	Relation $c(e)$	Viscosity	Creep	Stress relaxation
Hooke	$c = Eu$	$-$	$-$	$-$
Newton	$c = \mu\dot{u}$	$+$	$+$	$-$
Maxwell	$\dfrac{c}{\mu} + \dfrac{\dot{c}}{E} = \dot{u}$	$-$	$+$	$-$
Voigt	$c = Eu + \mu\dot{u}$	$+$	$-$	$-$

Table C.10. Creep function for a unit stress step $\mathtt{f}(t)$ in simple rheology models ($\cdot_{\mathrm{M}}$: Maxwell model component of the Boltzmann model; Source: [1272]).

Maxwell	$\left(C + \dfrac{t}{\mu}\right)\mathtt{f}(t)$
Voigt	$C(1 - \exp\{-t/\mu C\})\mathtt{f}(t)$
Boltzmann	$C\left(1 - \left(1 - \dfrac{\mu C_{\mathrm{M}}}{\mu C(1 + C_{\mathrm{M}}/C)}\right)\exp\{-t/(\mu C(1 + C_{\mathrm{M}}/C))\}\right)\mathtt{f}(t)$

Table C.11. Relaxation function for a unit strain step $\mathtt{u}(t)$ in simple rheology models ($\cdot_{\mathrm{M}}$: Maxwell model component of the Boltzmann model; Source: [1272]).

Maxwell	$\exp\{-t/\mu C\}\mathtt{u}(t)/C$
Voigt	$\mu\delta(t) + \mathtt{u}(t)/C$
Boltzmann	$C^{-1}\left(1 - \left(1 - \frac{\mu C(1+C_{\mathrm{M}}/C)}{\mu C_{\mathrm{M}}}\right)\exp\{-t/(\mu C(1 + C_{\mathrm{M}}/C))\}\right)\mathtt{u}(t)$

D

Numerical Simulations

> " *La pensée mathématique forme la base de l'explication physique. [The mathematical thought forms the basis of physical explanation]* " (G. Bachelard)

From the ancient times, people have invented means to compute (Babylonian charts, abaci, etc.). Computers are required tools for numerical calculations. The computer was first used in a heuristic (tentatively used to discover; ερινακζω: help to fecundate) manner by J. von Neumann and S. Ulam for pure mathematics problems. Since the mid-twentieth century, numerical simulations have been carried out.

Numerical simulations yield evolution in time and space of physical quantities with good resolution, which cannot be obtained by measurement. The numerical model thus allows a better understanding of the implicated phenomena of the investigated physical problem. The computational model also allows us to study the role played by the influence parameters, all other variables being kept constant.

> " *Dans le cas très fréquent où le nombre des variables en présence est considérable... la règle capitale à suivre... est de s'astreindre à laisser systématiquement invariable dans chaque expérience tous les facteurs sauf un seul...[In the very frequent case with a huge number of involved quantities... the chief rule to follow... is to force oneself in every case to keep constant all factors except a single one...]* " (Le Chatelier)

D.1 Numerical Model

The qualitative description of the physical problem of interest relies on a mathematical model usually defined by partial differential equations that link

the relevant variables. The set of partial differential equations is associated with initial and boundary conditions. Normally, the boundary conditions are required for a unique solution and initial conditions for the distribution of the involved quantities at the initial time. The mathematical model is determined using assumptions and approximations of the physical reality.

When the equation set cannot be analytically solved, approximation schemes are used. The discretization of the partial differential equations leads to a linear algebraic system solved by a suitable method [1273–1277]. In the case of the finite element method, the continuum is discretized into a set of polygons on its surface and polyhedra in its volume.

Numerical modeling includes several stages. The mathematical model associated with the physical problem corresponds to a boundary-value problem:

$$\mathcal{L}\mathbf{u} = \mathbf{f},$$

where $\mathcal{L}$ is a differential operator, $\mathbf{u}$ is the real-value solution of the problem and $\mathbf{f}$ a given function. In continuum mechanics, the partial differential equations result from equilibrium of fluxes and forces in an infinitesimal control volume. The problem is well-conditionned when a small relative variation in quantity only causes a slight relative change in the results.

The mathematical framework is analyzed by functional analysis. The existence, uniqueness, and essential properties of the solution are provided at least for a simplified model. The numerical model builds and analyzes a discrete approximation:

$$\mathbf{A}\mathbf{u}_h = \mathbf{f}_h,$$

where $\mathbf{A}$ is the approximation matrix, and $\mathbf{u}_h$ the vector of N unknowns or degrees of freedom associated with the solution representation after the domain discretization. Subscript h refers to the mesh, h being the characteristic space step. Vector $\mathbf{f}_h$ contains the discretization of $\mathbf{f}$ and the boundary conditions. Although linear systems can be solved by direct methods, the resolution of the discret problem is most often based on iterative procedures (Jacobi, Gauss-Seidel, GMRES, and Newton methods). A sequence $\{\mathbf{u}_h\}_1^N$ of estimates of $\mathbf{u}_h$ is thus generated. There is a convergence of iterations when:

$$\lim_{n\to\infty} \mathbf{u}_h^n = \mathbf{u}_h.$$

D.2 Approximation Methods

Numerical analysis yields an approximate solution of the problem with a given precision and finite number of elementary operations. Various approximation methods include: (1) deterministic techniques, such as finite difference methods, finite volume methods, finite element methods, spectral methods,[1] par-

[1] The basis functions of the spectral methods are global, whereas they are local in the finite element methods, being associated with mesh nodes.

ticle methods,[2] and cellular automata;[3] and (2) stochastic methods, such as Monte-Carlo algorithms.[4] The choice depends on the problem nature and the domain geometry. Whatever the selected method, the approximation convergence must be ensured:

$$\lim_{h \to 0} \mathbf{u}_h = \mathbf{u}.$$

Numerous sources of error occur during the different modeling steps: (1) truncature and round-off errors,[5] (2) iteration errors, (3) approximation errors, and (4) modeling errors (errors on values of input data, errors on the determination of equations, errors on hypotheses, errors due to mesh, etc.):

$$u_{calc} - u_{reel} = \begin{cases} u_{calc} - u_h^n & (1) \\ + & \\ u_h^n - u_h & (2) \\ + & \\ u_h - u & (3) \\ + & \\ u - u_{reel} & (4) \end{cases}$$

Successive steps of modeling and simulation propagate errors. The conditioning estimates the sensitivity with respect to the variations of a given variable. Bad conditioning can occur for certain variation intervals of the variable. Stability defines the sensitivity of the numerical procedure with respect to round-off errors. A numerical scheme is stable in the absence of error amplification.

D.3 Basic Techniques in Discretization

The approximate solution u_h in a finite-dimensional space is computed rather than the prediction u of the behavior of the explored complicated medium. The

[2] The studied system is considered as a set of moving particles, the motion of which is described by their position labeled with respect to a frame, velocity, mutual interaction forces, and possible momentum exchanges. In each mesh element, the averaged particle density is taken into account, not the large number of particles.

[3] Cellular automata have been introduced by J. von Neumann and S. Ulam to model auto-organization processes in biological systems. This deterministic technique links each mesh node to a discrete state, with takes only few values, sometimes two (states either excited or at rest). They have been used to simulate certain hydrodynamic processes.

[4] Monte-Carlo methods have been proposed by S. Ulam and N. Metropolis. Computations generate elementary random processes and select the phenomena with sufficient physical realizability, particularly ensuring conservation principles.

[5] Any number is represented by a finite bit (information binary unit) number. The computational writing approximate numbers with the fixed precision of significant digits. Moreover, computations of certain functions yield approximate results.

small parameter h is assumed to tend toward zero when the system dimension tends to infinity, i.e., toward the continuum. The computation is based on a set of notions and numerical concepts: (1) linkers, such as *interpolations*, between the finite-dimensional space and the continuum; (2) *consistency* which states that the approximate state is closer to the real state when the characteristic grid spacing h tends to zero; (3) *stability*, which states that u_h remains in a bounded set when h tends toward zero, and is associated with error damping when numerical computation proceeds; and (4) *convergence* which states that the numerical procedure produces a solution that approaches the exact solution as the grid spacing h is reduced (u_h is close enough to u). Several methods can be used.

D.3.1 Finite-Difference Method

The differentiation operators are replaced by difference operators derived from truncations of Taylor series. The finite-difference method substitutes the continuum by a mesh of regularly spaced nodes.[6] A finite-difference discrete system is determined as a function of linear combinations of translations of the unknown function.

The derivatives are discretized using the Taylor formulas:

$$u_{i+1,j,\ell} = u_{i,j,\ell} + \Delta x \frac{\partial u}{\partial x}_i + \frac{(\Delta x)^2}{2} \frac{\partial^2 u}{\partial x^2}_i + \mathcal{O}[(\Delta x)^3],$$

$$u_{i-1,j,\ell} = u_{i,j,\ell} - \Delta x \frac{\partial u}{\partial x}_i + \frac{(\Delta x)^2}{2} \frac{\partial^2 u}{\partial x^2}_i + \mathcal{O}[(\Delta x)^3],$$

The difference between these two formulas leads to the *central difference*:

$$\frac{\partial u}{\partial x}_i = (u_{i+1,j,\ell} - u_{i-1,j,\ell})/2\Delta x + \mathcal{O}[(\Delta x)^2],$$

for an order 2 approximation. The sum of the two Taylor formulas leads to:

$$\frac{\partial^2 u}{\partial x^2}_i = (u_{i+1,j,\ell} - 2u_{i,j,\ell} + u_{i-1,j,\ell})/(\Delta x)^2 + \mathcal{O}[(\Delta x)^2].$$

The first Taylor formula can give the *forward difference*:

$$\frac{\partial u}{\partial x}_i = (u_{i+1,j,\ell} - u_{i,j,\ell})/\Delta x + \mathcal{O}[(\Delta x)],$$

the approximation of order 1 is less good. The second Taylor formula can give the *backward difference*:

$$\frac{\partial u}{\partial x}_i = (u_{i,j,\ell} - u_{i-1,j,\ell})/\Delta x + \mathcal{O}[(\Delta x)].$$

Many finite-difference schemes cannot be applied to the computation of discontinuous problems. Furthermore, the mapping of the computational domain onto the physical one must be regular for sufficiently accurate truncated Taylor formulas.

[6] The mesh is such that $x_i = i\Delta x$, $y_j = j\Delta y$, and $z_\ell = \ell\Delta z$ for node (x_i, y_j, z_ℓ).

D.3.2 Finite-Volume Method

The finite-volume method introduces the notion of fluxes across the cells that compose the computational domain. Several techniques have been implemented (cell-centered and vertex-centered technique) whether the primal mesh provides the partition or the flux derivation is obtained on a dual partition. A typical element of a two-dimensional staggered mesh is displayed in Fig. D.1. The finite-volume method guarantees conservation of fluid variables in each control volume.[7] Numerical schemes which have *conservativeness* ensure global conservation of fluid variables (throughout the entire computational domain), by means of consistent expressions for fluxes of these variables across the cell edges (2D space) or faces (3D space) of adjacent control volumes. Handling of the relative magnitude of convection and diffusion allows *transportiveness*.

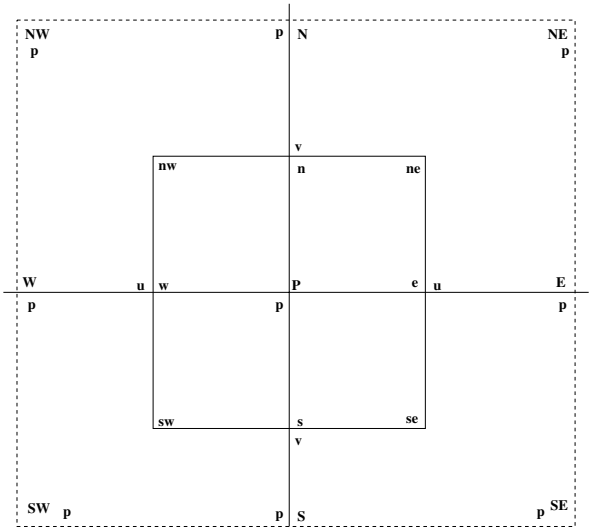

Figure D.1. Two-dimensional control volume in finite-volume method. Upper-case and lower-case letters stand for nodes at which pressure (p) and velocity components (u, v) are stored, respectively. The "velocity nodes" are staggered with respect to the storage locations of all other physical variables. The pressure is stored at the cell center and each velocity component at corresponding mid-edge (normal to the velocity component).

[7] The conservation of a fluid variables, such as velocity, within a control volume is ensured by the balance between the processes that increase and decrease this variable. The time rate of the variable change in the control volume is equal to the sum of the net flux of the variable due to convection across the control volume, of the one due to diffusion, and the net rate of creation or vanishing inside the control volume.

In an orthogonal curvilinear coordinate system, $\{x_i\}_{i=1}^3$ (x_3 : axial coordinate), $\{h_i\}_{i=1}^3$ the variable scaling factors allows to map the Cartesian coordinates to the curvilinear coordinates ($h_i = |\partial \mathbf{r}/\partial x_i|$). The curvature radii of of lines $x_1 = cst$ et $x_2 = cst$ are given by $1/R_{c_j} = (1/(h_1 h_2))\partial h_i/\partial h_j$.

The continuity equation is written as:

$$\frac{1}{h_1 h_2}\frac{\partial}{\partial x_1}(\rho h_2 u_1) + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_2}(\rho h_1 u_2) + \frac{\partial}{\partial x_3}(\rho u_3) = 0.$$

The transport equation of the physical quantity $g(\mathbf{x})$ is given by:

$$\frac{1}{h_1 h_2}\frac{\partial}{\partial x_1}(\rho h_2 u_1 g) + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_2}(\rho h_1 u_2 g) + \frac{\partial}{\partial x_3}(\rho u_3 g) =$$

$$\frac{1}{h_1 h_2}\frac{\partial}{\partial x_1}\Big(\gamma_g \frac{h_2}{h_1}\frac{\partial g}{\partial x_1}\Big) + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_2}\Big(\gamma_g \frac{h_1}{h_2}\frac{\partial g}{\partial x_2}\Big) + \frac{\partial}{\partial x_3}\Big(\gamma_g \frac{\partial g}{\partial x_3}\Big) + S_g,$$

with γ_g corresponds to diffusion coefficient and S_g to the source term for g (Table D.1).

These equations have been solved for bend flows using hexahedra. The discrete formulation of the equations is obtained by integration over the cell.

Table D.1. Source terms of transport equation in the finite-volume method for the three velocity components.

g	S_g
u_1	$-\frac{1}{h_1}\frac{\partial p}{\partial x_1} - \frac{\rho u_1 u_2}{R_{c1}} + \frac{\rho u_2^2}{R_{c2}} + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_1}\Big[h_2\mu\Big(\frac{1}{h_1}\frac{\partial u_1}{\partial x_1} + \frac{2u_2}{R_{c1}}\Big)\Big]$ $+\frac{1}{h_1 h_2}\frac{\partial}{\partial x_2}\Big[h_1\mu\Big(\frac{1}{h_1}\frac{\partial u_2}{\partial x_1} - \frac{u_2}{R_{c2}} - \frac{u_1}{R_{c1}}\Big)\Big]$ $+\frac{\mu}{R_{c1}}\Big(\frac{1}{h_1}\frac{\partial u_2}{\partial x_1} + \frac{1}{h_2}\frac{\partial u_1}{\partial x_2} - \frac{u_2}{R_{c2}} - \frac{u_1}{R_{c1}}\Big)$ $-2\frac{\mu}{R_{c2}}\Big(\frac{1}{h_2}\frac{\partial u_2}{\partial x_2} + \frac{u_1}{R_{c2}}\Big) + \frac{\partial}{\partial x_3}\Big(\frac{\mu}{h_1}\frac{\partial u_3}{\partial x_1}\Big)$.
u_2	$-\frac{1}{h_2}\frac{\partial p}{\partial x_2} - \frac{\rho u_1 u_2}{R_{c2}} + \frac{\rho u_1^2}{R_{c1}} + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_2}\Big[h_1\mu\Big(\frac{1}{h_2}\frac{\partial u_2}{\partial x_2} + \frac{2u_1}{R_{c2}}\Big)\Big]$ $+\frac{1}{h_1 h_2}\frac{\partial}{\partial x_1}\Big[h_2\mu\Big(\frac{1}{h_2}\frac{\partial u_1}{\partial x_2} - \frac{u_2}{R_{c2}} - \frac{u_1}{R_{c1}}\Big)\Big]$ $+\frac{\mu}{R_{c2}}\Big(\frac{1}{h_1}\frac{\partial u_2}{\partial x_1} + \frac{1}{h_2}\frac{\partial u_1}{\partial x_2} - \frac{u_2}{R_{c2}} - \frac{u_1}{R_{c1}}\Big)$ $-2\frac{\mu}{R_{c1}}\Big(\frac{1}{h_1}\frac{\partial u_1}{\partial x_1} + \frac{u_2}{R_{c1}}\Big) + \frac{\partial}{\partial x_3}\Big(\frac{\mu}{h_2}\frac{\partial u_3}{\partial x_2}\Big)$.
u_3	$\frac{\partial p}{\partial x_3} - \frac{\partial}{\partial x_3}\Big(\mu\frac{\partial u_3}{\partial x_3}\Big) + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_1}\Big(h_2\mu\frac{\partial u_1}{\partial x_3}\Big) + \frac{1}{h_1 h_2}\frac{\partial}{\partial x_2}\Big(h_1\mu\frac{\partial u_2}{\partial x_3}\Big)$.

The continuity equation is given by (U,u: upstream nodes, D,d: downstream nodes, W,w,E,e,N,n,S,s: lateral nodes; Fig. D.1):

$$\left(\rho u_1 \Delta x_2 \Delta x_3\right)_e - \left(\rho u_1 \Delta x_2 \Delta x_3\right)_w + \left(\rho u_2 \Delta x_1 \Delta x_3\right)_n - \left(\rho u_2 \Delta x_1 \Delta x_3\right)_s + \left(\rho u_3 \Delta x_1 \Delta x_2\right)_d - \left(\rho u_3 \Delta x_1 \Delta x_2\right)_u = 0,$$

and the transport equation by:

$$\begin{aligned}
&\left[\left(\rho u_1 \Delta x_2 \Delta x_3\, g\right)_e - \left(\rho u_1 \Delta x_2 \Delta x_3\, g\right)_w\right] \\
&+\left[\left(\rho u_2 \Delta x_1 \Delta x_3\, g\right)_n - \left(\rho u_2 \Delta x_1 \Delta x_3\, g\right)_s\right] \\
&+\left[\left(\rho u_3 \Delta x_1 \Delta x_2\, g\right)_d - \left(\rho u_3 \Delta x_1 \Delta x_2\, g\right)_u\right] \\
&= \left[\left(\gamma_g \frac{\Delta x_2 \Delta x_3}{\Delta x_1}\right)_e (g_E - g_P) - \left(\gamma_g \frac{\Delta x_2 \Delta x_3}{\Delta x_1}\right)_w (g_P - g_W)\right] \\
&+\left[\left(\gamma_g \frac{\Delta x_1 \Delta x_3}{\Delta x_2}\right)_n (g_N - g_P) - \left(\gamma_g \frac{\Delta x_1 \Delta x_3}{\Delta x_2}\right)_s (g_P - g_S)\right] \\
&+\left[\left(\gamma_g \frac{\Delta x_1 \Delta x_2}{\Delta x_3}\right)_d (g_D - g_P) - \left(\gamma_g \frac{\Delta x_1 \Delta x_2}{\Delta x_3}\right)_u (g_P - g_U)\right] \\
&+\left(S_g\, \Delta x_1 \Delta x_2 \Delta x_3\right)_P .
\end{aligned}$$

$\Delta x_{i(m)}$ being the distance between node P and adjacent cell center (m) in the direction x_i. When $g \equiv u_i$, S^g contains a pressure gradient term:

$$\left(\Delta x_i \Delta x_j\right)_P \left(p_{in(k)} - p_{out(k)}\right),$$

where subscripts $in(k)$ et $out(k)$ correspond to values at mesh nodes in the entry and exit cross sections associated with velocity component u_k.

A linear interpolation assesses the physical quantity at the required position using a spatial weighting factor, which, for node (m) on coordinate axis x_i between nodes P et M, is given by:

$$f_{(m)} = \left|\frac{x_{i(m)} - x_{iP}}{x_{i(M)} - x_{iP}}\right|.$$

D.3.3 Finite Element Method

The finite element method is an approximation procedure suitable for complicated domain geometries, such as the vasculature (Table D.2). It solves a system of partial differential equations in appropriate functional spaces. The functional spaces V defined for the continuum are approximated by finite-dimension functional spaces V_h:

$$V_h \equiv V_h^n[k,r] \ : \phi(\mathbf{x}) \in C^r(\overline{\Omega}),$$

V_h being the space of polynomials of degree $\leq k$ in $\mathbb{R}^n$, defined in domain Ω_h, with continuous derivatives of order r.[8] The finite element method is based on a representation by interpolation functions, the coefficients of which are obtained from the set of nodal values.

The field of a given variable $\mathbf{u}(\mathbf{x})$ of the physical problem is represented by approximate values at Nn mesh nodes such that the differences $e(\mathbf{x}) = \mathbf{u}_h(\mathbf{x}) - \mathbf{u}(\mathbf{x})$ are small. In each element K, the field of the unknown $\mathbf{u}$ is approximated ($\mathbf{u}_h$) by interpolation function $\{\varphi_i\}$ defined by nodal values $\{\mathbf{u}^{(i)}\}$ which are specified with the type of finite element adequat to the problem, at nodes either uniquely at the element boundary, or both at the element boundary and inside it. Assembling then leads to the solution on the entire domain.[9]

The equations are written using the integral formulation[10] in the volume of element K generated by discretization $\mathcal{T}_h$ of the continuum domain. The approximate value of the unknown $\mathbf{u}_h$ is expressed by a linear combination of

Table D.2. Some features of the finite-difference (FDM) and finite-element (FEM) methods

	FDM	FEM
Formulation	Differential	Integral
Domain geometry	Simple	Complicated
Discret domain	Node network	Element set
Approximation	Pointwise	Piecewise

[8] The space V_h is such that for any sampled element v_h, v_h is continuous over the finite element K, v_h is piecewise continuous over the discrete domain Ω_h, and v_h satisfies the boundary conditions of the problem. In mathematical notation, $\forall\, v_h \in V_h$, $v_h \in C^r(\Omega)$, $v_h\big|_K \in P^k$, $\forall\, K \in \mathcal{T}_h$, $v_h\big|_{\Gamma_h} = v_\Gamma$.

[9] The finite element is characterized by several features: (1) its geometrical type, (2) the node number, (3) the types of nodal variables, and (4) the type of interpolation functions.

[10] The multiplication by test functions and integration of the equations over the domain, followed by by-part integration leads to a weak formulation, the derivatization of the unknown u being reduced and the one of the test function v being augmented.

(sum of products of) interpolation functions $\{\varphi_i(\mathbf{x})\}_{i=1}^{Nn}$ of the basis of space V_h, and nodal values $\mathbf{u}_h^{(i)} = \mathbf{u}_h(\mathbf{x}^{(i)})$ at a given instant:

$$\mathbf{u}_h(\mathbf{x},t) = \sum_{i=1}^{Nn} \varphi_i(\mathbf{x})\mathbf{u}^{(i)}\Big|_t .$$

The interpolation functions satisfy the following conditions: (1) $\varphi_i(\mathbf{x}^{(j)}) = \delta_{ij}$, (2) its degree depends on the node number, (3) its nature depends on the number and on the type of nodal unknowns, and (4) continuity on the element boundary.

The variable $\mathbf{u}_h$ is then defined on the whole domain Ω_h. The simplest interpolation uses piecewise continuous affine basis functions. The continuity is ensured because, for a neighboring element, $\mathbf{u}^{k'}$ is defined by the same value on the shared nodes, or edges or faces: $\mathbf{u}^k = \mathbf{u}^{k'}$.

The finite element is an affine set with interpolation operator: $\{K, P_T, \Sigma_T\}$, where $K \in \mathcal{T}_h$ ($\mathcal{T}_h$: domain mesh) can be the image of the reference finite element $\widehat{K}$, P_T the approximation polynomial space of dimension N_T ($P_T = \{v_h\Big|_T, v_h \in V_h\}$, $\dim(P_T) = n + k$), Σ_T a set of N_T degrees of freedom (N_T linear forms $\{\varphi_i\} \in P_T$, such that $\forall u \in P_T$, $u(\mathbf{x}) = \sum_{i=1}^{N_T} u^{(i)}\varphi_i(\mathbf{x})$. The degrees of freedom are either the variables at the mesh nodes (Lagrange finite element) or the variables and their derivatives (Hermite finite element). The test functions (interpolation functions) can be derived from barycentric coordinates (Fig. D.2).

The domain discretization leads to a set of connected polyhedra with the following rules: (1) the element interior is not empty, and (2) the intersection of two elements is either void, or a common node, edge or face. Different estimators assess the mesh quality.

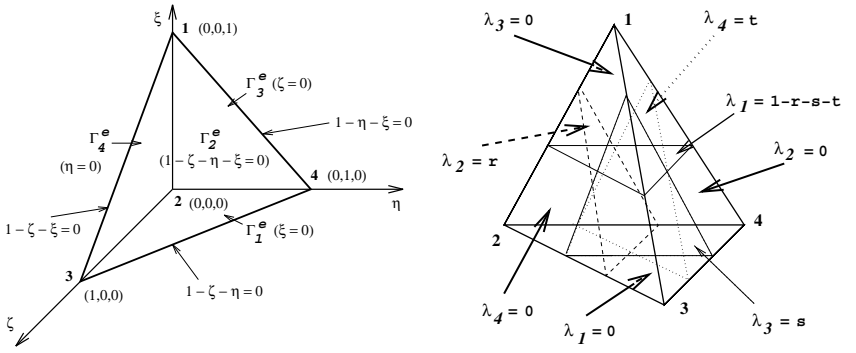

Figure D.2. Barycentric coordinates in the tetrahadron.

The equation formulation is based on projection (with the scalar product meaning) the unknown $\mathbf{u}$ on a functional space, i.e., to multiply by a test function $\mathbf{v}$ and integrate. The integral formulation of the problem can be obtained either by the weighted residual method,[11] or the variational formulation.[12] The integration by parts leads to a weak formulation of the equations because of the reduction in conditions on $\mathbf{u}_h$ in V_h, but the definition criteria of the functional space are strong. Such a method deals with natural boundary conditions[13] and discontinuities.

[11] Let $\mathbf{u}_h$ be the approximate solution of $\mathcal{L}\mathbf{u} = \mathbf{f}$. The test functions $\mathbf{v}_h$, or weighting functions, are interpolation functions. $\mathcal{L}\mathbf{u}_h - \mathbf{f}$ defines the residual. The equation terms are residual-weighted integrals by test functions $\mathbf{v}_h$.

[12] Certain physical problems can be defined by a functional $\pi = \int F\Big(\mathbf{u}(\mathbf{x},t), \nabla\mathbf{u}(\mathbf{x},t), \mathbf{x}, t\Big)$, to be minimized. Because equations are equivalent, the integral formulation is called variational in the absence of a minimization problem. Consider a simple case, the Stokes problem, i.e., the steady flow of an incompressible Newtonian fluid in a domain subjected to body forces $\mathbf{f}$, inertia forces being negligible. The test function $\mathbf{v}$ satisfies the incompressibility condition $\nabla \cdot \mathbf{v} = 0$ in Ω and the boundary condition $\mathbf{v}\big|_{\Gamma} = 0$. The integration in Ω, followed by processing using Green formulas, leads to the following variational formulation:

$$\mathtt{a}(\mathbf{u}, \mathbf{v}) = \mathtt{L}(\mathbf{v}), \ \forall \mathbf{v} \in \mathcal{V},$$

with $\mathtt{a}(\mathbf{u}, \mathbf{v}) = \mu \sum_{1}^{3} \int_{\Omega} u_{,i} v_{,i} \, d\Omega$ and $\mathtt{L}(\mathbf{v}) = \int_{\Omega} \mathbf{f} \cdot \mathbf{v} \, d\Omega$.

[13] There are two kinds of boundary conditions: the essential BCs, which must be explicitly satisfied and the natural BCs implicitly imposed. The latter appear in the equation and are automatically verified.

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Chap. 1. The Cell

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Chap. 2. Cell Membrane and Environment

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Chap. 3. Ion Carriers and Receptors

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Chap. 4. Signaling Pathways

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Chap. 5. Transport and Cell Motion

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Chap. 8. Vessel Wall

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Chap. 9. Endothelium

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Chap. 10. Vascular Wall Growth, Repair and Remodeling

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Concluding Remarks

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App. A. Endocrine System and Hormones

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Notations

A

$\mathcal{A}(p)$: area-pressure relation
$\mathbf{A}$: Almansi strain tensor
A: cross sectional area
`A`: actin binding site
$\mathbf{a}$: acceleration
a: major semi-axis
AA: arachidonic acid
AAA: abdominal aortic aneurism
Aaa: ATPase associated with diverse cellular activities
AAAP: aneurism-associated antigenic protein
AAK: adaptin-associated kinase
ABC: ATP binding cassette transporter
ABP: actin binding protein
AC: atrial contraction
ACase: adenyl cyclase
ACAT: acyl CoA-cholesterol acyltransferase
ACC: acetyl coenzyme-A carboxylase
ACE: angiotensin converting enzyme
ACh: acetylcholine
ACTH: adrenocorticotropic hormone
Ad: adrenaline
ADAM: a disintegrin and metalloprotease
ADAMTS: a disintegrin and metalloprotease with thrombosspondin
ADP: adenosine diphosphate
AF: atrial fibrillation
AGF: autocrine growth factor
Aip: actin interacting protein
AKAP: A-kinase anchoring protein
ALE: arbitrary Eulerian Lagrangian
AMPK: AMP-activated protein kinase
AmyR: amylin receptor
Ang: angiopoietin
Ank: ankyrin
ANP: atrial natriuretic peptide
ANS: autonomic nervous system
ANT: adenine nucleotide transporter
AOC: amine oxidase copper-containing protein
AoV: aortic valve
AP: activator protein
AP: activating enhancer-binding protein
APC: adenomatous polyposis coli protein
APl: action potential
Apn: adiponectin
Apo: apolipoprotein
Aqp: aquaporin
AR: adrenergic receptor
AR: area ratio
Arf: ADP-ribosylation factor
ARNO: Arf nucleotide site opener
ARP: absolute refractory period
ARP: actin-related protein
Artn: artemin
ARVD: arrythmogenic right ventricular dystrophy
ASAP: artery-specific antigenic protein
ASP: actin-severing protein
ASK: apoptosis signal-regulating kinase
AT: antithrombin

ATF: activating transcription factor
ATG: autophagy-related gene
ATMK: ataxia telangiectasia mutated kinase
ATn: angiotensine
ATng: angiotensinogen
ATP: adenosine triphosphate
ATRK: ATM and Rad3-related kinase
AVN: atrioventricular node
AVV: atrioventricular valves
AW: analysis window

B

B: Biot-Finger strain tensor
B: bulk modulus
$\mathcal{B}$: bilinear form
b: minor semi-axis
b: body force
$\hat{\mathbf{b}}$: unit binormal
BBB: blood-brain barrier
BC: boundary condition
BCL: B-cell leukemia/lymphoma
Bdk: bradykinin
BEM: boundary element method
bFGF: basic fibroblast growth factor
BFU-E: burst forming unit-erythroid
BM: basement membrane
BNP: B-type natriuretic peptide
BOC: brother of CDO
Bφ: basophil

C

C: stress tensor
$\mathtt{C}$: compliance
C: chronotropy
C_{D}: drag coefficient
C_{f}: friction coefficient
C_{L}: lift coefficient
c: stress vector
c_τ: shear
c_{w}: wall shear stress
c: concentration
c_p: wave speed
CA: computed angiography
Ca: calcium
CABG: coronary artery bypass grafting
Cam: calmodulin
CamK: calmodulin-dependent kinase
cAMP: cyclic adenosine monophosphate
CaR: calcium-sensing receptor
CAS: Crk-associated substrate
Cav: caveolin
CBF: coronary blood flow
CBF: core binding factor
CBP: CREB binding protein
CD: cluster determinant protein
Cdc42: cell-division cycle-42
CdK: cyclin-dependent kinase
Cdm: caldesmon
CDO: cell adhesion molecule-related/downregulated by oncogenes
CETP: cholesterol ester transfer protein
CFD: computational fluid dynamics
cFos: cellular Finkel Biskis Jinkins murine osteosarcoma virus sarcoma oncogene
CFU: colony-forming unit
cGMP: cyclic guanosine monophosphate
CGRP: calcitonin gene-related peptide
CI: cardiac index
Cin: chronophin
cJun: avian sarcoma virus-17 oncogene
CK: creatine kinase
CK: casein kinase
CRLR: calcitonin receptor-like receptor
CMC: cardiomyocyte
cMyb: myeloblastosis oncogene
cMyc: myelocytomatosis oncogene
Cn: collagen
CnF: collagen fiber
CNGC: cyclic nucleotide-gated channel
CNS: central nervous system
CO: cardiac output
COx: cyclooxygenase
CoP: coat protein
Cr: creatine
CRAC: Ca^{++}-release–activated Ca^{++} current
CREB: cAMP responsive element-binding protein
cRel: reticuloendotheliosis oncogene
CRH: corticotropin-releasing hormone
Crk: chicken tumor virus regulator of kinase
CRP: C-reactive protein

Cs: cholesterol
CsE: cholesteryl esters
CSF: cerebrospinal fluid
CSK: C-terminal Src kinase
Csk: cytoskeleton
Csq: calsequestrin
CT: computed tomography
CTL: cytotoxic T lymphocyte
CtR: calcitonin receptor
CVI: chronic venous insufficiency
CVLM: caudal ventrolateral medulla
CVP: central venous pressure
CVS: cardiovascular system
cyCK: cytosolic creatine kinase

D

D: dromotropy
D: vessel distensibility
$\mathcal{D}$: diffusion coefficient
$\mathbf{D}$: deformation rate tensor
$\mathbf{d}$: displacement vector
$\mathtt{D}$: flexural rigidity
d: duration
DAG: diacylglycerol
DCA: directional coronary atherectomy
DCT: distal convoluted tubule
De: Dean number
DH: Dbl homology
Dhh: desert hedgehog
DICOM: digital imaging and communication for medicine
DISC: death-inducing signaling complex
DNA: deoxyribonucleic acid
DUS: Doppler ultrasound

E

$\mathbf{E}$: strain tensor
E: elastic modulus
$\mathtt{E}$: elastance
$\mathcal{E}$: energy
$\{\hat{\mathbf{e}}_i\}_{i=1}^3$: basis
EBCT: electron beam CT
EC: endothelial cell
ECA: external carotide artery
ECF: extracellular fluid
ECG: electrocardiogram
ECM: extracellular matrix
EDGR: endothelial differentiation gene receptor
EDV: end-diastolic volume
EEL: external elastic lamina
EGF: epidermal growth factor
eIF: eukaryotic translation initiation factor
ELAM: endothelial–leukocyte adhesion molecules
ELCA: excimer laser coronary angioplasty
En: elastin
EnF: elastin fiber
EPDC: epicardial derived cell
Epo: erythropoietin
ER: endoplasmic reticulum
ERGIC: ER Golgi intermediate compartment
ERK: extracellular signal-regulated protein kinase
ERM: ezrin–radixin–moesin
ERP: effective refractory period
ESCRT: endosomal sorting complexes required for transport
ESV: end-systolic volume
ET: endothelin
ETR: endothelin receptor
EVAR: endovascular aneurism repair
Eφ: eosinophil

F

$\mathbf{F}$: transformation gradient tensor
$\mathbf{f}$: surface force
$\hat{\mathbf{f}}$: fiber direction unit vector
f: binding frequency
f_{c}: cardiac frequency
f: friction shape factor
f_{v}: head loss per unit length
FAD: flavine adenine dinucleotide
FADD: Fas receptor associated death domain
FAK: focal adhesion kinase
FB: fibroblast
FC: fibrocyte
FDM: finite difference method
FEM: finite element method
FGF: fibroblast growth factor
FHL: four and a half LIM-only protein

FlIP: flice-inhibitory protein
FN: fibronectin
Fn: fibrin
Fng: fibrinogen
FoxO: forkhead transcription factor
FR: flow ratio
FSH: follicle-stimulating hormone
FSI: fluid-structure interaction
FVM: finite volume method

G

$\mathbf{G}$: Green-Lagrange strain tensor
G: shear modulus
G': storage modulus
G'': loss modulus
$\mathtt{G}$: conductance
G_{p}: pressure gradient
G_{h}: hydraulic conductivity
$\mathbf{g}$: gravity acceleration
$\mathtt{g}$: physical quantity
g: detachement frequency
G protein: guanine nucleotide-binding protein
Gab: Grb2-associated binder
GAG: glycosaminoglycan
GAK: cyclin G-associated kinase
Gal: galanin
GAP: GTPase-activating protein
GCAP: guanylyl cyclase-activating protein
GDP: guanosine diphosphate
GDI: guanine nucleotide-dissociation inhibitor
GEF: guanine nucleotide-exchange factor
GF: growth factor
GFP: geodesic front propagation
GH: growth hormone
GHRH: growth hormone-releasing hormone
GIT: GPCR kinase-interacting protein
GKAP: guanylate kinase-associated protein
GluT: glucose transporter
GnRH: gonadotropin-releasing hormone
GP: glycoprotein
GPI: glycosyl phosphatidylinositol protein
GPCR: G-protein–coupled receptor
GPx: glutathione peroxidase
Grb: growth factor receptor-bound protein
GRK: GPCR kinase
GSK: glycogen synthase kinase
GTP: guanosine triphosphate

H

H: height
$\mathcal{H}$: history function
h: thickness
HAT: histone acetyltransferase
Hb: hemoglobin
HCT: helical CT
HDAC: histone deacetylase
HDL: high density lipoprotein
hERG: human ether-a-go-go related gene
HES: hairy enhancer of split
hFABP: heart fatty acid binding protein
HGF: hepatocyte growth factor
HIF: hypoxia-inducible factor
His: histamine
HMWK: high molecular weight kininogen
Hrt: Hairy-related transcription factor
HS: heparan sulfate
HSC: hematopoietic stem cell
Hsp: heat shock protein
HSPG: heparan sulfate proteoglycan
Ht: hematocrit

I

$\mathbf{I}$: identity tensor
i: current
I: inotropy
IC: isovolumetric contraction
ICA: internal carotide artery
ICAM: intercellular adhesion molecule
ICF: intracellular fluid
ICliP: intramembrane-cleaving protease
IEL: internal elastic lamina
Ifn: interferon
Ig: immunoglobulin
IGF: insulin-like growth factor
IH: intimal hyperplasia

Ihh: indian hedgehog
IL: interleukin
IP: inositol phosphate
IP3: inositol triphosphate
IR: isovolumetric relaxation
ISA: intracranial saccular aneurism
ISG: interferon stimulated gene product
IVC: inferior vena cava
IVUS: intravascular US

J

J: flux
J_m: cell surface current density
JAM: junctional adhesion molecule
JaK: Janus kinase
JNK: c-Jun N-terminal kinase

K

$\mathbf{K}$: conductivity tensor
K: bending stiffness
$\mathtt{K}$: reflection coefficient
$\mathtt{K}_m$: compressibility
k: cross section ellipticity
k_c: spring stiffness
k_{ATP}: myosin ATPasic rate
KHC: kinesin heavy chain
Kk: kallikrein
KLC: kinesin light chain
KlF: Kruppel-like factor

L

$\mathbf{L}$: velocity gradient tensor
$\mathtt{L}$: inertance
L: length
LA: left atrium
LCA: left coronary artery
LCAT: lysolecithin cholesterol acyltransferase
LCC: left coronary cusp
LDL: low density lipoproteins
LDV: laser Doppler velocimetry
Le: entry length
LH: luteinizing hormone
LKlF: lung Kruppel-like factor
Lkt: leukotriene
Ln: laminin
LMR: laser myocardial revascularization
LP: lipoprotein
LPase: lipoprotein lipase
LPS: lipopolysaccharide
LRP: LDL receptor-related protein
LSK: Lin-SCA1+ KIT+ cell
LSV: long saphenous vein
LTBP: latent TGFβ-binding protein
LV: left ventricle
LVAD: left ventricular assist device
LXR: liver X receptor
Lφ: lymphocyte

M

Ma: Mach number
MAP: microtubule-associated protein
mAP: mean arterial pressure
MAPK: mitogen-activated protein kinase
MARK: microtubule affinity regulating kinase
MBP: myosin binding protein
MCP: monocyte chemoattractant protein
MEJ: myoendothelial junction
MGP: matrix Gla protein
MHC: major histocompatibility complex
miCK: mitochondrial creatine kinase
MIS: mini-invasive surgery
MIT: mini-invasive therapy
MiV: mitral valve
MKP: mitogen-activated protein kinase phosphatase
MLC: myosin light chain
MLCK: myosin light chain kinase
MLCP: myosin light chain phosphatase
MLP: muscle LIM protein
MMP: matrix metalloproteinase
mtMMP: membrane-type MMP
Mo: monocyte
MPO: median preoptic nucleus
MRI: magnetic resonance imaging
MRTF: myocardin-related transcription factor
MSSCT: multi-slice spiral CT
mTOR: mammalian target of rapamycin
mTORc: mTOR complex

MVO2: myocardial oxygen consumption
MWSS: maximal wall shear stress
mmCK: myofibrillar creatine kinase
MuRF: muscle-specific ring finger
MyHC: myosin heavy chain
Mφ: macrophage

N

N: sarcomere number
$\hat{\mathbf{n}}$: unit normal vector
n: PAM density with elongation x
$\mathtt{n}$: myosin head density
NAD: nicotine adenine dinucleotide
NAd: noradrenaline
NCC: non-coronary cusp
NCS: neuronal calcium sensor
NCX: Na^{+}–Ca^{++} exchanger
NF: nuclear factor
NFAT: nuclear factor of activated T cells
NHE: sodium–hydrogen exchanger
NHERF: NHE regulatory factor
NIK: NFκB-inducing kinase
NIP: neointimal proliferation
NmU: neuromedin-U
NO: nitric oxide
NOS: nitric oxide synthase
NOx: NAD(P)H oxidase
NPC: Niemann-Pick disease C protein
NpY: neuropeptide-Y
NRSTK: non-receptor serine/threonine kinase
NRTK: non-receptor tyrosine kinase
NSF: N-ethylmaleimide-sensitive factor
NST: nucleus of the solitary tract
Nφ: neutrophil

O

OSI: oscillatory shear index
OVLT: organum vasculosum lamina terminalis

P

$\mathcal{P}$: permeability
$\mathtt{P}$: power
p: pressure
PAF: platelet activating factor
PAFAH: platelet-activating factor acetylhydrolase
PAI: plasminogene activator inhibitor
PAK: p21-activated kinase
PAR: partitioning defective protein
Pax: paxillin
PC: protein C
PCMRV: phase-contrast MR velocimetry
PCr: phosphocreatine
PCT: proximal convoluted tubule
PDE: phosphodiesterase
PDE: partial differential equation
PDGF: platelet derived growth factor
PDK: phosphoinositide-dependent kinase
Pe: Péclet number
PE: pulmonary embolism
PECAM: platelet endothelial cell adhesion molecule
PEO: proepicardial organ
PET: positron emission tomography
PF: platelet factor
PG: prostaglandin
pGC: particulate guanylyl cyclase
PGC: PPARγ coactivator
PGI2: prostacyclin
PGF: paracrine growth factor
PH: pleckstrin homology
PI: phosphoinositide
PI3K: phosphatidylinositol 3-kinase
PIP2: phosphatidyl inositol diphosphate
PIV: particle image velocimetry
PIX: p21-interacting exchange factor
PK: protein kinase
PKL: paxillin kinase linker
Pkp: plakophilin
PL: phospholipase
PLb: phospholamban
PLd: phospholipid
PLTP: phospholipid transfer protein
PMCA: plasma membrane Ca-ATPase
PMR: percutaneous (laser) myocardial revascularization
Pn: plasmin
Png: plasminogen
PoG: proteoglycan
Pon: paraoxonase

PPAR: peroxisome proliferator–activated receptor
preKk: prekallikrein
PS: protein S
PSC: pluripotent stem cell
PSEF: pseudo-strain energy function
PSer: phosphatidylserine
PTA: plasma thromboplastin antecedent
PTCA: percutaneous transluminal coronary angioplasty
PTCRA: PTC rotational burr atherectomy
PTEN: phosphatase and tensin homologue deleted on chromosome 10
PTFE: polytetrafluoroethylene
PTH: parathyroid hormone
PTHRP: parathyroid hormone-related protein
PTP: protein tyrosine phosphatase
PTK: protein tyrosine kinase
PuV: pulmonary valve
PVNH: paraventricular nucleus of hypothalamus
PWS: pulse wave speed
PYK: proline-rich tyrosine kinase
P2X: purinergic ligand-gated channel

Q

q: flow rate

R

R: resistance
R_{h}: hydraulic radius
R_{g}: gas constant
r: radial coordinate
RA: right atrium
RAAS: renin–angiotensin–aldosterone system
Rab: Ras from brain
RACC: receptor-activated cation channel
RACK: receptor for activated C-kinase
RAMP: receptor activity–modifying protein
Ran: Ras-related nuclear protein
Rap: Ras-related protein
RAR: retinoic acid receptor
Ras: (superfamily of small GTPases/genes)
RBC: red blood cell
RC: ryanodine calcium channel
RCA: right coranary artery
RCC: right coronary cusp
Re: Reynolds number
RFA: radio-frequency ablation
Rheb: Ras homolog enriched in brain
RHS: equation right hand side
Rho: Ras homology
RIAM: Rap1-GTP-interacting adapter
RIP: receptor-interacting protein
RKIP: Raf kinase inhibitor protein
RNA: ribonucleic acid
dsRNA: double-stranded RNA
mRNA: messenger RNA
miRNA: microRNA
rRNA: ribosomal RNA
siRNA: small interfering RNA
tRNA: transfer RNA
RNABP: RNA binding protein
RNP: ribonucleoprotein
snoRNP: small nucleolar ribonucleoprotein
Robo: roundabout
ROI: region of interest
ROK: Rho kinase
ROS: reactive oxygen species
RPTP: receptor protein tyrosine phosphatase
RSE: rapid systolic ejection
RSMCS: robot-supported medical and surgical system
RSK: ribosomal S6 kinase
RSTK: receptor serine/threonine kinase
RTK: receptor tyrosine kinase
Runx: Runt-related transcription factor
RV: right ventricle
RVF: rapid ventricular filling
RVLM: rostral ventrolateral medulla
RVMM: rostral ventromedial medulla
RXR: retinoid X receptor

S

S: Cauchy-Green deformation tensor
s: sarcomere length

SAA: serum amyloid A
SAC: stretch-activated channel
sAC: soluble adenylyl cyclase
SAH: subarachnoid hemorrhage
SAN: sinoatrial node
SAPK: stress-activated MAPK
Sc: Schmidt number
SCA: stem cell antigen
SCF: stem cell factor
SDF: stromal cell-derived factor
SE: systolic ejection
SEF: strain-energy function
SERCA: SR Ca ATPase
SFK: Src-family kinase
SFO: subfornical organ
sGC: soluble guanylyl cyclase
SGK: serum- and glucocorticoid-induced kinase
SH: Src homology
Shc: Src-homologous and collagen-like substrate
Shc: Src homology 2 domain containing transforming protein
Shh: sonic hedgehog
SHIP: SH-containing inositol phosphatase
SHP: SH-containing protein tyrosine phosphatase
SIP: steroid receptor coactivator–interacting protein
SKIP: sphingosine kinase-1 interacting protein
SLAM: signaling lymphocytic activation molecule
SMC: smooth muscle cell
SNAP: soluble N-ethylmaleimide-sensitive factor-attachment protein
SNARE: SNAP receptor
SOD: superoxide dismutase
Sos: Son-of-sevenless
SPECT: single photon emission CT
Sph: sphingosine
SPN: supernormal period
SR: sarcoplasmic reticulum
SR: scavenger receptor
SRF: serum response factor
SSAC: shear stress-activated channel
SSE: slow systolic ejection
Ssh: slingshot protein
SSV: short saphenous vein
St: Strouhal number
STAT: signal transducer and activator of transduction
STIM: stromal interaction molecule
Sto: Stokes number
SUMO: small ubiquitin-related modifier
SV: stroke volume
SVC: superior vena cava
SVF: slow ventricular filling
SVR: systemic vascular resistance
SW: stroke work
S1P: sphingosine 1-phosphate

T

$\mathbf{T}$: extrastress tensor
T: temperature
$\hat{\mathbf{t}}$: unit tangent
t: time
TACE: tumour necrosis factorα-converting enzyme
TACE: transarterial chemoembolization
TAK: TGFβ-activated kinase
TC: thrombocyte
TCF: T-cell factor
TcR: T-cell receptor
TCA: tricarboxylic acid
TEA: transluminal extraction atherectomy
TEM: transendothelial migration
Ten: tenascin
TFPI: tissue factor pathway inhibitor
TG: triglyceride
TGF: transforming growth factor
TGN: trans-Golgi network
TJ: tight junction
TKR: tyrosine kinase receptor
TLR: toll like receptors
TLT: TREM-like transcript
TM: thrombomodulin
TMC: twisting magnetocytometry
TMy: tropomyosin
TN: troponin
Tn: thrombin
TNF: tumor necrosis factor
TNFR: tumor necrosis factor receptor
TORC: transducer of regulated CREB

tPA: tissue thromboplastin activator
Tpo: thrombopoietin
TRADD: tumor necrosis factor-receptor associated death domain
TRAF: tumor necrosis factor-receptor associated factor
TREM: triggering receptor expressed on myeloid cells
TRH: thyrotropin-releasing hormone
TRPC: transient receptor potential channel
TrV: tricuspid valve
Trx: thioredoxin
TSH: thyroid-stimulating hormone
Tsp: thrombospondin
TxnIP: thioredoxin-interacting protein

U

$\mathbf{U}$: right stretch tensor
$\mathbf{u}$: displacement vector
$\mathtt{u}$: electrochemical command
Ub: ubiquitin
UCP: uncoupling protein
UDP: uridine diphosphate-glucose
UK: urokinase
Unc: uncoordinated receptor
US: ultrasound
USC: unipotential stem cell
USI: ultrasound imaging
UTP: uridine triphosphate

V

$\mathbf{V}$: left stretch tensor
V: volume
V_q: cross-sectional average velocity
$\mathbf{v}$: velocity vector
$\mathtt{v}$: recovery variable
VAMP: vesicle-associated membrane protein
VAV: ventriculoarterial valve
VCAM: vascular cell adhesion molecule
VCt: vasoconstriction
VDC: voltage-dependent channel
VDt: vasodilation
VEGF: vascular endothelial growth factor
VF: ventricular filling
VGC: voltage-gated channel
VIP: vasoactive intestinal peptide
VLDL: very low density lipoprotein
VN: vitronectin
VR: venous return
VVO: vesiculo–vacuolar organelle
vWF: von Willenbrand factor

W

$\mathbf{W}$: vorticity tensor
$\mathcal{W}$: strain energy density
W: work, deformation energy
$\mathbf{w}$: grid velocity
WBC: white blood cell
WSS: wall shear stress
WSSTG: WSS transverse gradient

X

$\mathtt{X}$: reactance
$\mathbf{x}$: position vector
$\{x, y, z\}$: Cartesian coordinates

Y

$\mathtt{Y}$: admittance coefficient

Z

$\mathtt{Z}$: impedance
ZO: zonula occludens

Miscellaneous

3DR: three-dimensional reconstruction
5HT: serotonin

Σc: sympathetic
pΣc: parasympathetic

Greek Letters

α: volumic fraction
α: convergence/divergence angle
α: attenuation coefficient
α_{k}: kinetic energy coefficient
α_{m}: momentum coefficient
β: inclination angle
$\{\beta_i\}_1^2$: myocyte parameters
Γ: domain boundary
Γ_{L}: local reflection coefficient
Γ_{G}: global reflection coefficient

γ: activation factor
$\gamma_{\mathtt{g}}$: amplitude ratio of $\mathtt{g}$
$\dot{\gamma}$: shear rate
δ: boundary layer thickness
ϵ: strain
ε: small quantity
ζ: singular head loss coefficient
ζ: transmural coordinate
$\{\zeta_j\}_1^3$: local coordinate
η: azimuthal spheroidal coordinate
θ: circonferential polar coordinate
θ: $(\hat{\mathbf{e}}_x, \hat{\mathbf{t}})$ angle
κ: wall curvature
κ_{c}: curvature ratio
κ_{d}: drag coefficient
κ_{h}: hindrance coefficient
κ_{o}: osmotic coefficient
κ_{s}: size ratio
$\{\kappa_k\}_{k=1}^9$: tube law coefficients
κ_{e}: correction factor
Λ: head loss coefficient
λ_{L}: Lamé coefficient
λ: stretch ratio
λ: wavelength
μ: dynamic viscosity
μ_{L}: Lamé coefficient
ν: kinematic viscosity
ν_{c}: cardiac frequency
ν_{P}: Poisson ratio
Π: osmotic pressure
ρ: mass density
τ: time constant
Φ: potential
$\phi(t)$: creep function
φ: phase
γ_i: wetted perimeter
$\psi(t)$: relaxation function
Ψ: porosity
ω: angular frequency
Ω: computational domain

Subscripts

A: atrial (alveolar)
Ao: aortic
a: arterial
app: apparent
c: contractile
c: center
$\cdot_{\mathrm{c}}$: point-contact
D: Darcy (filtration)
$\mathtt{d}$: diastolic
dyn: dynamic
e: external
$\mathtt{e}$: extremum
eff: effective
f: fluid
g: grid
i: internal
inc: incremental
l: limit
$\cdot_\ell$: line-contact
max: maximum
m: muscular
P: pulmonary
p: parallel
p: particule
$\cdot_{\mathrm{q}}$: quasi-ovalisation
r: radial
rel: relative
S: systemic
s: solute
$\mathtt{s}$: serial
$\mathtt{s}$: systolic
$\cdot_{\mathrm{t}}$: stream division
T: total
t: time derivative of order 1
tt: time derivative of order 2
V: ventricular
v: veinous
w: wall
$\mathtt{w}$: water (solvent)
$\cdot_\Gamma$: boundary
θ: azimuthal
+: positive command
−: negative command
$\bullet_*$: at interface
0: reference state ($\cdot_0$: unstressed or low shear rate)
$\cdot_\infty$: high shear rate

Superscripts

$^{\mathrm{a}}$: active state
$^{\mathrm{e}}$: elastic
$^{\mathrm{f}}$: fluid
$^{\mathrm{h}}$: hypertensive
$^{\mathrm{n}}$: normotensive

$^{\mathrm{p}}$: passive state
p: power
$^{\mathrm{s}}$: solid
$^{\mathrm{T}}$: transpose
$^{\mathrm{v}}$: viscoelastic
*: scale
*: complex variable

Mathematical Notations

$\Delta\bullet$: difference
$\delta\bullet$: incriment
$d\bullet/dt$: time gradient
$\boldsymbol{\nabla}$: gradient operator
$\boldsymbol{\nabla}\cdot$: divergence operator
∇^2: Laplace operator
$|\ |_+$: positive part
$|\ |_-$: negative part
$\dot{\bullet}$: time derivative
$\overline{\bullet}$: time mean
$\breve{\bullet}$: space averaged
$\langle\bullet\rangle$: ensemble averaged
$\tilde{\bullet}$: dimensionless
$\bullet^+$: normalized ($\in [0,1]$)
$\hat{\bullet}$: peak value
$\bullet_\sim$: modulation amplitude
$\det(\bullet)$: determinant
$\mathrm{cof}(\bullet)$: cofactor
$\mathrm{tr}(\bullet)$: trace

Chemical Notations

$[\bullet]$: concentration

Index

D

E

F

G

H

S

T

U

V

W

Z