SPRINGER BRIEFS IN MOLECULAR SCIENCE
HISTORY OF CHEMISTRY

Joe S. Jeffers

Frederick Sanger
Two-Time Nobel Laureate in Chemistry

Springer

SpringerBriefs in Molecular Science

History of Chemistry

Series editor

Seth C. Rasmussen, Fargo, ND, USA

More information about this series at http://www.springer.com/series/10127

Joe S. Jeffers

Frederick Sanger

Two-Time Nobel Laureate in Chemistry

Springer

Joe S. Jeffers
Department of Chemistry
Ouachita Baptist University
Arkadelphia, AR
USA

ISSN 2191-5407 ISSN 2191-5415 (electronic)
SpringerBriefs in Molecular Science
ISSN 2212-991X
SpringerBriefs in History of Chemistry
ISBN 978-3-319-54707-7 ISBN 978-3-319-54709-1 (eBook)
DOI 10.1007/978-3-319-54709-1

Library of Congress Control Number: 2017932639

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface and Acknowledgements

Fred Sanger was a very private person. When I first approached him in 1999 about writing his biography, he made it clear that he was not interested in talking to newspaper types who did not understand biochemistry. After I assured him I was a biochemist, he agreed to see me. At our first session, we talked mainly about biochemistry. When asked if I could return in a week and talk about his early life, he reluctantly agreed. I was clearly invading his privacy. At the same time, his brother Theo and longtime assistant Bart Barrell were encouraging him to meet with me. Dr. Sanger did not volunteer information. He would, however, respond to specific questions or comments. The pattern that characterized our meetings, then, was that I would interview family and colleagues; they would give me stories; and I would ask Dr. Sanger to respond to those stories. While some areas were off limits, he responded honestly. We met a dozen times over the next nine years. By the fourth or fifth meeting, he had decided that I was okay, and from that point on, he seemed to genuinely enjoy our sessions. We always strolled through the garden so he could show me whatever was in bloom at the time.

I gratefully acknowledge the following persons who agreed to interviews. **Family members**: Theodore Sanger (brother), Mary Sanger (sister), Robin Sanger (son), Peter Sanger (son), Sally Sanger (daughter), and Melody Wright (niece). **Colleagues and former students**: Gillian Air, Richard Ambler, Richard Baer, Bart Barrell, Sydney Brenner, George Brownlee, Alan Coulson, Nick Cowan, Francis Crick, Hal Dixon, John Donelson, Margaret Dowding, Allen Edmundson, John Finch, Blas Frangione, Theodore Freidman, Michael Fuller, Carolyn Geczy, P.T. Gilham, John Glegg, Geoff Grigg, Herbert Gutfreund, Brian Hartley, Richard Henderson, Peter Jeppesen, Aaron Klug, Kjeld Marcker, Celia Milstein, César Milstein, Kenneth Murray, Michael Neuberger, Steve Nicklen, Richard Perham, Sam Perry, Max Perutz, Julia Porter, Andrew Ryle, John Sedat, Denis Shaw, John Smith, Les Smith, John Sulston, Jordan Tang, Ted Thompson, Hans Tuppy, Lee Weith, Greg Winter, John Walker, Paul Whitfeld, Ian Young, and Ed Ziff. **Archivists**: Donald Boyd (The Downs, Colwall, Herefordshire), Annette Faux (MRC Laboratory of Molecular Biology, Cambridge), Bill Hetherington (Peace Pledge Union, London), and Alan Shrimpton (Bryanston, Blandford Forum,

Dorset). **Childhood friends**: Merrick Emrys-Roberts and John Jones. **Gloucestershire local historian**: Malcolm Whitaker. **Other archivists** who supplied materials are Laura Baldwin (University of New South Wales, Sydney), Clare Clark (Cold Spring Harbor Laboratory Archives, New York), Fiona Colbert (St. John's College, Cambridge), Christopher Hilton (Wellcome Library, London), John Lagnado (The Biochemical Society, London), and Hazel Zheng (Cambridge Biochemistry). I am deeply indebted to my **Reviewers**: Daniel Casciano, Christine Debouck, Ray Granade, and Randall Wight; and to Series Editor Seth Rasmussen.

I also gratefully acknowledge Ouachita Baptist University, Arkadelphia, Arkansas, USA, and the J.D. Patterson Summer Research Program for financial support.

Arkadelphia, USA Joe S. Jeffers

Contents

About the Author

Joe S. Jeffers is the Charles S. and Elma Grey Goodwin Holt Professor of Chemistry and Pre-Medical Studies at Ouachita Baptist University in Arkadelphia, Arkansas, USA. A native of Warren, Arkansas, he earned a BS in Professional Chemistry from Ouachita Baptist University in 1966. In 1972 he earned a Ph.D. in Biochemistry and Molecular Biology from Purdue University, Lafayette, Indiana, in the College of Science interdisciplinary program in biochemistry. He joined the faculty of Ouachita Baptist University in 1972. He did summer postdoctoral studies at the Open University, UK, in 1972 and at the University of Texas, Austin, in 1974. He had Visiting Researcher status at the Department of Molecular Genetics of SmithKline and French Laboratories, King of Prussia, Pennsylvania, summer 1983; at the Department of Biochemistry and Molecular Biology of the University of Arkansas for Medical Sciences, Little Rock, 1984–1998; and at Arkansas Children's Hospital Research Institute, Little Rock, 1999–2001. He became dean of the J.D. Patterson School of Natural Sciences at Ouachita Baptist University in 2002. He served a 10-year stint as dean before assuming his current position. Jeffers is active in the Division of the History of Chemistry (HIST) of the American Chemical Society. He served four years as HIST program chair and serves on the HIST Executive Committee.

Abstract

Frederick Sanger, British biochemist, won two Nobel Prizes in Chemistry. The first, in 1958, was for being the first person to sequence a protein molecule, namely insulin. That finding led to the idea that there must be a genetic code. He conducted his protein research at the Biochemistry Department of the University of Cambridge. The second Nobel Prize, in 1980, was shared with Paul Berg and Walter Gilbert. Gilbert and Sanger's half of the prize was for developing DNA sequencing techniques. Sanger conducted his nucleic acid research at the Medical Research Council Laboratory of Molecular Biology in Cambridge. Sanger's technique became the basis for the human genome project, whereby the entire DNA sequence of a human was deduced. DNA sequencing has transformed both biology and medicine. This biography covers the early life, the protein period, the RNA period, and the DNA period of Fred Sanger. It also offers a glimpse of family life and a portrait of the man and his legacy. Fred Sanger was the fourth person to win two Nobel Prizes.

Keywords Protein sequence · RNA sequence · DNA sequence · Dideoxy sequence · Fred Sanger · Laboratory of Molecular Biology · British biochemists · Double Nobel laureates · History of proteins · History of DNA

Chapter 1
Introduction

Biographies are but the clothes and buttons of the man—the biography of the man himself cannot be written
—Mark Twain.

British biochemist Frederick Sanger (1918–2013) won two Nobel Prizes in Chemistry, the first, in 1958, "for his work on the structure of proteins, especially that of insulin" [1]. The second, in 1980, he shared with Paul Berg and Walter Gilbert. Half of the prize went to Sanger and Gilbert "for their contributions concerning the determination of base sequences in nucleic acids" [2]. Fred Sanger dedicated his entire professional life to sequences. He was the first person to sequence a protein molecule, insulin. Prior to that time, many protein chemists thought proteins were hopeless mixtures, not unique molecules. Later he developed sequencing methods for ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). Although Walter Gilbert also developed a method for sequencing DNA, Sanger's method was the basis for the human genome project. Sanger considered the DNA sequencing method his most important contribution to science [3].

Fred Sanger was the second of three children born to Frederick and Cicely Sanger. Frederick the father was a country medical doctor.[1] A devoutly religious man, he became active in the Society of Friends (Quakers). The Quaker emphasis on truth and hard work was a tradition that defined Fred. While as an adult he no longer followed Quaker religious beliefs, the personal qualities remained.

Fred's mother came from a wealthy family. Her independence shielded them from most effects of the Great Depression and allowed Dr. Sanger to indulge his strong feelings for the underprivileged and charge little for his services. Dr. Sanger's strong sense of equality is another quality he passed to his children.

Fred had a governess during his early years. At age nine, he went to a Quaker boarding school, The Downs, for boys ages nine to fourteen. Then he went to a public[2] high school, Bryanston, where he passed the School Certificate exams in seven subjects, more than enough to qualify for entry to Cambridge University. He

[1]To distinguish between father and son, Dr. Sanger is used for the father and Fred for the son.
[2]A public school in England is a private school, not a free school operated by the state.

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_1

was expected to follow his father's footsteps by studying medicine at St. John's College, Cambridge. Before entry, however, he decided the life of a country doctor was not for him and chose science instead. School Certificate-level physics at Bryanston did not provide the background needed for university physics, so he dropped physics and changed to physiology his second year, keeping his studies in chemistry, maths, and biochemistry. As a result, the normal three-year course of university study became four.

In 1936, as a conscientious objector, Fred joined the Scientists' Anti-War Group at Cambridge. Here, he met Joan Howe, who would become his wife. While Fred had always been a good, but not a great, student, earning a First on his biochemistry exam surprised him. That result qualified him for graduate studies.

With the outbreak of World War II, a tribunal granted Fred conscientious objector status. After training at a Quaker center for conscientious objectors, Fred became an orderly at a military hospital near Bristol. He did not believe that mopping floors and cleaning toilets was the best use of his skills. During a lull in hospital activity, he went to Cambridge to see if anyone needed a research student. Because he had money and did not need University support, he quickly gained admission. His Ph.D. studies focused on the metabolism of the amino acid lysine, which became his dissertation topic, and war work on nitrogen in the potato. Working under the supervision of Albert Neuberger, Fred received his Ph.D. in 1943.

With Ph.D. in hand, Fred joined the labs of the new head of Biochemistry at Cambridge, Albert Chibnall. Chibnall suggested that Fred study methods of identifying the terminal amino acid of insulin, a protein available in pure form. Fred successfully developed a method for chemically labeling the terminal amino acid that resulted in a bright yellow color for any terminal amino acid. He found two such labeled amino acids, which led to the idea that two chains of amino acids comprised insulin.

Even though it was common practice for a laboratory supervisor to include his name on a protégé's publication, Chibnall declined, saying Sanger did the work and should get the credit. Fred continued this practice throughout his career. If he did not contribute directly to the work, he did not put his name on a paper.

Using partial acid and enzyme hydrolysis to break insulin into smaller fragments, Fred and his coworkers gradually determined the amino acid sequence of both insulin chains. In further work, they established how the two chains were joined, and, in 1954, solved the complete insulin sequence. For the first time, they showed a protein had a definite sequence of amino acids, putting to rest the notion that proteins were hopeless mixtures. For this work, Fred Sanger received the 1958 Nobel Prize in Chemistry.

While affiliated with the Biochemistry Department of Cambridge University during these studies, Fred was never a faculty member, thus not a professor. He did, however, supervise graduate students. Supported first by a Beit Memorial Fellowship, then by the Medical Research Council (MRC), he had no teaching or administrative duties. When Frank Young assumed the chair of Biochemistry in 1949, Fred found himself pushed out of the biochemistry building. Young did not

like the fact that Fred had no teaching duties. Young relocated Ted Thompson, Fred's graduate student, to the Biochemistry Hut, a prefabricated building nearby. Fred soon followed.

In 1953, at the Cavendish Laboratory of Physics, Max Perutz and his colleagues, including Francis Crick, were similarly relocated to a prefabricated building, also called the Hut. The new Cavendish chair, Nevill Mott, did not consider biophysics a proper branch of physics. Max engaged Fred and spearheaded an effort to have the MRC build a new facility for molecular biology, the term coined for the areas of biochemistry and biophysics that involved large biomolecules. The efforts paid off with the 1961 construction of the MRC Laboratory of Molecular Biology (LMB) at the Addenbrooke's Hospital site about two miles from Cambridge University. Max chaired the lab with its three divisions—Protein Chemistry, headed by Fred Sanger; Structural Studies, headed jointly by Max and John Kendrew; and Molecular Genetics, headed by Francis Crick. In October 1962, Crick and James Watson (along with Maurice Wilkins) shared the Nobel Prize in Physiology or Medicine "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material" [4], and Perutz and Kendrew received the Nobel Prize in Chemistry "for their studies of the structures of globular proteins" [5]. With Fred's 1958 Nobel, the LMB now had five Nobel Laureates in its ranks, even though Watson had since moved back to New York.

Fred had begun using radioactive amino acids to label the active centers of enzymes. He continued that idea as he began work on the sequence of RNA molecules. While DNA sequence was the big prize, enzymes necessary to cleave DNA into manageable pieces had not yet been discovered. Such enzymes did exist for RNA, so Fred and his coworkers developed methods to sequence RNA. They had hoped to ferret out the genetic code by comparing messenger RNA (mRNA)[3] sequence to protein sequence. Other labs used binding experiments with small RNA molecules to deduce the genetic code before Fred and his coworkers were successful. Yet Fred's group was the first to sequence a mRNA, thus confirming the genetic code.

Discovery of restriction enzymes that cleaved DNA into manageable pieces led Fred to pursue DNA sequencing methods. He and his coworkers developed early methods that led to some DNA sequencing before he developed the dideoxy method that became coin of the realm in DNA sequencing. For this work, Fred shared the 1980 Nobel Prize in Chemistry, making him one of only four two-time Nobel Laureates.[4]

Hans Krebs, 1953 Nobel Laureate in Physiology or Medicine for his discovery of the citric acid cycle, once said, "Scientific distinction develops if nurtured by distinction" [6]. Fred Sanger fit this description. Three graduate students and one

[3]mRNA has the same coding information as a gene in DNA. mRNA codes for the assembly of amino acids into proteins.

[4]The other three are Marie Curie (Physics 1903, then Chemistry 1911); Linus Pauling (Chemistry 1954, then Peace 1962); and John Bardeen (Physics 1956, then Physics 1972).

post-doctoral fellow who worked with Fred later won Nobel Prizes for work independent of Sanger.[5]

After his retirement, Fred was honored by having the British genomics and genetics research institute, a DNA sequencing center that participated in the Human Genome Project, named The Sanger Centre.

This biography details the life and work of Fred Sanger. Several other sources give additional insights [7–11].

References

1. Nobel Media AB (2014) The Nobel prize in Chemistry 1958. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1958/index.html. Accessed 16 June 2015
2. Nobel Media AB (2014) The Nobel prize in Chemistry 1980. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/index.html. Accessed 16 June 2015
3. Sanger F (1999) Personal communication
4. Nobel Media AB (2014) The Nobel prize in Physiology or Medicine 1962. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1962/. Accessed 17 June 2015
5. Nobel Media AB (2014) The Nobel prize in Chemistry 1962. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1962/. Accessed 17 June 2015
6. Krebs H (1967) The making of a scientist. Nature 215:1441–1445
7. Silverstein A, Silverstein V (1969) Frederick Sanger: the man who mapped out a chemical of life. John Day, New York
8. Sanger F (1988) Sequences, sequences, and sequences. Ann Rev Biochem 57:1–28
9. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Scientific, Singapore
10. Brownlee G (1992) Oral history video interview. A life of research on the sequences of proteins and nucleic acids: Dr. Frederick Sanger in conversation with Prof. George Brownlee. The Biochemical Society, London
11. Brownlee G (2014) Fred Sanger double Nobel Laureate: a biography. Cambridge University Press, Cambridge (includes transcript of interview in reference 10)

[5]Rodney Porter (Physiology or Medicine 1972); César Milstein (Physiology or Medicine 1984); John Walker (Chemistry 1997); and Elizabeth Blackburn (Physiology or Medicine 2009).

Chapter 2
Early Life 1918–1943

Fred Sanger was born on August 13, 1918, in Rendcomb, Gloucestershire, England, one year after his brother Theodore (Theo). They lived in The Old House down the hill from Rendcomb village (Fig. 2.1). The River Churn, little more than a large creek at this point, flowed by the house. Fred's father, Frederick, was a country medical doctor. He saw patients in an outbuilding behind the house, but mostly he made house calls, traveling around by car, bicycle, two-wheeled horse cart, or on foot. He was also the doctor for the little college at Rendcomb.

Earlier Dr. Sanger had been a medical missionary to China. Never one for high church, he went to China with support of the Church Missionary Society. While there, he also founded a school for the lower classes. Illness forced his return to England. He and his mother moved to Rendcomb, where, unfortunately, she died a few months later. In February 1915, his third year at Rendcomb, he was called to attend Daisy Crewdson at Syde, a village some ten miles from Rendcomb. Daisy had broken her arm.[1] Her husband Dilworth was recovering from an illness, so his sister, Cicely, came to Syde to look after Daisy. The good doctor fell in love. He proposed two months later, on his fortieth birthday. They married in September [1].

Cicely Crewdson was the daughter of a wealthy cotton manufacturer from Styal, Cheshire. Squire Theodore Crewdson had at one time been a Quaker, but he had fallen out with them over biblical interpretations. Nevertheless, he had many Quaker books. Dr. Sanger read through them, found his fit, and became a Quaker. Cicely, however, remained Church of England.

Dr. Sanger kept a diary. He either began or ended each year's diary with a letter to his children. He wrote to the boys in 1921, "I am more than ever a Quaker, the social side of our country seems very terrible. There is a false economy about which takes from the poor before it does from the rich, and cannot see where generosity is needed" [2]. The tone was being set that would influence the boys' development.

[1]Both Fred and Theo related the story of their parents' meeting when Cicely had a septic finger during a visit to Syde. No doubt that was the family story later told to the boys. Dr. Sanger's diaries, however, told the story related here. The septic finger episode happened over a long period in 1919, after Fred was born.

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_2

Fig. 2.1 The Old House at Rendcomb (Courtesy Fred Sanger, with permission)

Dr. Sanger stressed to his children that he hopes they grow up to treat all persons as equals [3].

Dr. Sanger loved his wife and doted on his children (Fig. 2.2). As a young child, Theodore could not quite say his own name. It came out Ojo, so Ojo became the pet name for Theo. Fred's nickname was Derrick, Der for short. They had these names

Fig. 2.2 Theo, Fred, and Dr. Sanger with Sammie the donkey (Courtesy Fred Sanger, with permission)

Fig. 2.3 Far Leys in Tanworth-in-Arden (Courtesy Fred Sanger, with permission)

until they went to boarding school. Dr. Sanger was a keen naturalist and Cicely was a lover of wild flowers, so the boys spent much time outside collecting. Dr. Sanger's relentless schedule took a toll on his health. That, combined with his growing enthusiasm for involvement with the Quakers, led him to sell his practice at Rendcomb.

In late 1922, the family moved to Caudle Green, a village across the valley from Syde. Squire Crewdson owned the house, so they paid only peppercorn rent. They lived there while Dr. Sanger found a new practice to buy. During this time, sister Mary (May) was born. Fred slept in the room with his parents. He was in the room asleep when May arrived. Her crying awoke him [4, 5], an incident that became one of Fred's earliest memories.

Dr. Sanger bought a practice in Tanworth-in-Arden, near Birmingham, ideal for him because Birmingham was a Quaker center. The house, Far Leys (Fig. 2.3), lay at the edge of town. They had a large garden and a pond, a site of many activities for the boys. Theo, an avid collector of birds' nests, was the leader of the two. Theo was quite the extrovert; Fred was more introverted. Theo's enthusiasm for nature and collecting became, as Fred put it, the major influence on Fred's early interest in science [6].

The Sangers engaged a governess, Miss Potter. She was stern and the boys disliked her. According to Dr. Sanger, Miss Potter "told Der that she would put a dunce's cap on him and send him through the village with it on." Der went to lessons weeping, which led to Miss Potter's release [7]. Miss Shewell was the new governess. She used the PNEU system,[2] making lessons fun. The boys loved Miss

[2]Parents National Education Union, founded by Charlotte Mason, stressed short fun lessons with afternoon walks outside and habit training—attention, truthfulness, neatness, kindness, punctuality, etc. [8].

Shewell. Several other village children came to Far Leys for lessons with Miss Shewell.

In 1926 at age nine, Theo went to The Downs,[3] a Quaker boarding school at Colwall, Herefordshire. Fred followed a year later. Fred did not like The Downs. It was too regimented with much bullying and little privacy. He started fine, but then plummeted and was placed in the lower form. He lived for holidays back at Far Leys [6] but gradually acclimated to The Downs. They had carpentry, metal working, and art, and Fred liked to work with his hands. He performed better than Theo academically, but neither distinguished himself. As headmaster Geoffrey Hoyland wrote in a Christmas note to Dr. Sanger [10],

> My Dear Sanger, Here are the boys' reports. They are typically British documents—not too good and not too bad with a strong element of compromises. Neither of the boys finds his chief delight and interest in book work, and you are to blame for that by giving them such a vivid sense of the interest of nature, so it's on your head if we can't make scholars of them.

The Downs did not teach science, but Hoyland regularly addressed the students, highlighting new scientific findings. Hoyland saw his job to create a society where "a normal boy could develop naturally and happily under his own steam" [9].

Back at Far Leys, Dr. Sanger had a hut built for the children (Fig. 2.4). Theo had one corner, where he boiled up heads of dead animals and assembled a skull collection. Fred had another corner. May had a third corner near Theo. Fred liked painting, carpentry, and metal working (Fig. 2.5)—the *doing* of things. As May said, she had two brothers who were absolutely different. If she wanted to go out and collect things, she went with the ebullient Theo; if she wanted to do quieter things, she stayed with Fred [11].

Fred built a mouse house where he kept and bred mice. Dr. Sanger regularly reported selling Fred's mice for him. Dr. Sanger wrote [12],

> Fred is coming along with his pictures & carpentry which consists of little original ideas. He has perpetrated a Magnum Opus in the shape of a block of wood carved as a head with open mouth from which is extracted lavatory paper!

The family played many games—Happy Families, Whisk, Charades, Snakes and Ladders, Jacks, and Spillikins.[4] They also donned costumes and did theatricals. Dr. Sanger flew pigeons, so he and the children would drive to Wales, for example, and let the birds fly. They took many trips pulling a caravan. Dr. Sanger and the children rode in the car. Cicely took the train and met them at the destinations [13]. Family life was busy and fun.

Britain had a vast investment in U.S. stocks, so the crash of 1929 led to hard times. More than 7,000,000 were unemployed [14]. The Sangers were well off because of Cicely's family money, so they did not suffer. Dr. Sanger was too

[3] Alan Hodgkin, 1963 Nobel laureate in Physiology or Medicine attended The Downs, leaving just as Fred Sanger was beginning. The poet W. H. Auden taught English at The Downs 1932–1935 [9].

[4] Spillikins is pick-up sticks or jackstraws.

Fig. 2.4 Fred in the hut 1931 (Courtesy Fred Sanger, with permission)

Fig. 2.5 Fred and May at the forge 1933 (Courtesy Fred Sanger, with permission)

charitable because his patients had so little [5]. Dr. Sanger wrote, "Sad year to most people feeling the economic strain. Country has become so nationalistic with buy British and tariffs. It all seems so selfish and unChrist-like" [15].

Geoffrey Hoyland suggested Bryanston for education after The Downs. Bryanston was a new school in Dorset that followed a modified Dalton Plan,[5] allowing students to take responsibility for their own education, with increasing freedom and responsibility. Classes decreased progressively at each level [17]. Although not a Quaker school, Bryanston focused on promoting peace. Theo went in 1931; Fred followed the next year. The school was perfect for Fred. He had more freedom than at The Downs and much more freedom than other public secondary schools. He liked projects and stuck to them, unlike many of his classmates, who did not do well with all that freedom.

Dr. Sanger liked Bryanston because it had no Officer Training Corps (OTC). In place of OTC, it had Pioneers. One-half day per week, Pioneers worked on building projects around the school—gardening, laying concrete, and building sheds. At holidays, Pioneers worked with the unemployed, helping them dig their allotments[6] and paying them rent to stay with them. Bryanston offered other physical outlets, especially sports. Fred thought The Downs required participation in too many sports. At Bryanston, he was free to choose. He especially liked squash and Fives.[7]

At the end of one summer holiday, Dr. Sanger wrote two mottos on a slip of paper and gave it to Fred: "Success is naught; endeavor's all." and "If you have built castles in the air, that is where they should be; now put foundations under them."[8] Those mottos resonated with Fred because he enjoyed working hard. He also remembered his father saying on many occasions that there are two worthwhile things in life, science and art—science because it means progress; art because it means creating beauty.

Fred liked the biology master, Bill Hoyland, brother of Geoffrey Hoyland from The Downs. One had to pass only five exams to qualify for Cambridge. Fred took School Certificate exams in seven subjects and passed all seven, earning him the moniker of 'Seven-Credit Fred.'

Fred's major mentor was chemistry master, H. G. Ordish. Mr. Ordish did research with dyes. Because he had already passed the exams, Fred spent much time with Mr. Ordish in the chemistry lab. Fred loved the beautiful crystals that resulted from their work [19].

---

[5]Helen Parkhurst pioneered the Dalton Plan in Dalton, Massachusetts, USA. She believed "The true business of school is not to chain the pupil to preconceived ideas, but to set him free to discover his own ideas and to help him to bring all his powers to bear upon the problem of learning" [16].

[6]A small piece of ground in or near town where one can grow vegetables and flowers.

[7]Fives is a derivative of Eton Fives, a hand ball game played as doubles on a three-sided court, with one wall having a buttress. The game originated outside Eton Chapel, thus the buttress [18].

[8]The first motto is from Robert Browning's *Red Cotton Night-Cap Country*; the second is from Henry David Thoreau's *Walden*.

Bryanston had an exchange program with Schule Schloss Salem in Germany. In 1935, Fred and David Forbes were accepted for a half-term at Salem. Salem put so much emphasis on athletics that Fred and David were ahead of their German peers in coursework. They were stunned when the headmaster started each day with readings from either the Bible or Hitler's *Mein Kampf*. The German boys all stood and saluted 'Heil Hitler' after the readings. Being foreigners exempted David and Fred from the requirement. At end of term, David and Fred went on a walking holiday through parts of Germany, Austria, and Italy. They slept in barns or out in the open. They miscalculated the money they needed and ran short, eating only bread and jam the last few days of the journey [6].

Fred was expected to follow in his father's footsteps, attend St. John's College, Cambridge, and study medicine. In fact, Fred was accepted to St. John's to study Medicine Honours Natural Science Tripos[9] Part 1 [20]. Fred decided a country doctor's life was not for him, so he changed to natural science, much to his parents' disappointment. Fred said,

> I started to think about what I was going to do. I had seen my father's work, you see, which was very scrappy really. You'd drive around to see one patient, try to cure them, and see another patient, and so forth. It occurred to me I really wouldn't enjoy that very much [6].

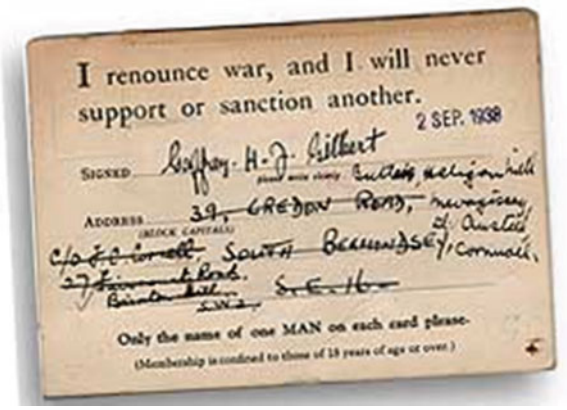
Fred planned to study physics, chemistry, maths, and biochemistry. When he approached the physics master at Bryanston about applying for a physics scholarship to Cambridge, the master told Fred he was not up to it. He was going to St. John's a year early and lacked the background. Fortunately, his family had money to pay his way. Theo went to Cambridge the same year, but he entered Trinity Hall with its focus on law.

Fred learned that he was not ready to compete in physics. Most of his peers had three years of secondary school physics; he had only two. After the first year, he changed from physics to physiology. The change cost him a year, so he took three years for Part 1. Biochemistry was a new subject, so Fred was not behind the other students in biochemistry. Theo stayed 'in college' his first year; that is, he had a room in campus housing. Each student at Cambridge was required to stay in college at least one year. Fred could not get in college first year, so he shared lodgings with Geoffrey Udall, a good friend of Theo's from Bryanston. They lived off campus near the Round Church less than a block from St. John's. Fred did not have a room in college until his third year.

When he came to Cambridge, Fred was political. As a Quaker, he was a pacifist and strongly anti-war. He signed the Peace Pledge Union[10] (Fig. 2.6) and joined the Scientists' Anti-War Group (SAG), which assembled several reports. Fred led a report on the political and economic effects of rearmament. He assembled the

[9]Tripos is the exam pattern necessary for a B.S. honors degree at Cambridge. Part 1 study normally takes two years, Part 2 a third.

[10]Dick Shepherd, a popular Church of England priest, started the PPU. He asked mostly men to "renounce war and never again to support another" by signing a pledge. In a few months of 1934–35, more than 30,000 signed [21].


I renounce war, and I will never support or sanction another.

2 SEP. 1938

SIGNED Geoffrey H. J. Gilbert

ADDRESS (BLOCK CAPITALS) 39, GREDAN ROAD, SOUTH BERMONDSEY

Only the name of one MAN on each card please.

(Membership is confined to those of 18 years of age or over.)

Fig. 2.6 Peace Pledge Union pledge card (Courtesy Peace Pledge Union, with permission)

political parts, but needed help with economics. A friend recruited Joan Howe, an economics student, and they completed the report. The other leaders were not so diligent, so nothing much came of the entire effort. Fred decided he could not make much difference in the peace movement, so he concentrated on his science. Also, his blossoming romance with Joan hastened his drift away from SAG. Joan's father was a Leicester shoe manufacturer. Joan was intelligent and the first in her family to go to college. Her father disapproved of Fred because he did not want her "wasting her time on some good-for-nothing undergraduate" [6].

While Fred was at college, both parents died of cancer. His father opted for surgery and did not survive. His mother moved to Caudle Green and died a year later. Their Uncle Dilworth and Aunt Daisy became de facto parents for Theo, Fred, and May. As a result their holiday home was the house in Caudle Green.

Fred made several trips to Austria, Czechoslovakia, and Germany, usually working in camps sponsored by Quakers to help underprivileged workers. In 1939, he was in Germany for a combined holiday with German students to encourage peace and understanding. Germany invaded Poland, igniting World War II. Fred and the other English students scrambled to catch trains and leave Germany. It was a hectic, fearful time.

Fred took biochemistry for Part 2, requiring a fourth year. He moved out of college to lodgings on Park Parade overlooking Jesus Green. As he began his studies, war broke out. He had already initiated conscientious objector (CO) status following the Military Training Act of 1939, whereby all men aged 20–21 were required to undergo a six-month military training. With the British declaration of war, Parliament passed the National Service Act, imposing conscription [22]. Fred pursued his CO application, going before a Cambridge tribunal, which granted him CO status.

Fred and Joan courted during this trying time. Cambridge did not allow undergraduates to have a car, but Fred bought an old car for £8 and kept it in a

garage out of town. He and Joan bicycled out to the car and then drove around the countryside.

Fred took the exam for Biochemistry Part 2, not expecting to do very well. After all, he did not earn a First on Part 1. Much to his surprise, Fred received a First-Class Honours, the top grade on the exam. He found out from a cousin who read in *The Times* that Fred and one other person had received a First. That result qualified him for graduate school. Fred later reflected that the essay on Comparative Biochemistry at the end of the Part 2 exam probably made the difference. He had some extra time, and he developed the idea of the survival of the fittest molecules [23, 24].

After completing his degree, Fred went to Spiceland [25], a Quaker training center in Devon for war-relief workers. COs had to contribute to the war effort. The three-month training program taught three courses: agriculture, building, and cooking and cleaning. But mostly it was about service. Fred was one of eighteen Spicelanders sent to Winford Hospital near Bristol. As orderlies, they did the drudgework necessary to make the hospital function. Fred wound up cleaning floors and lavatories. He thought this work a waste of his talents, so he wrote to Professor Frederick Gowland Hopkins,[11] chair of biochemistry at Cambridge, about graduate work. His letter went unanswered. During a lull at Winford, Fred went to Cambridge to inquire if anyone needed research help. Discovering that he needed no monetary assistance caught the interest of several researchers. The Ministry of Labour and National Service approved his return to Cambridge to work on his Ph.D.

Fred decided to work with Bill Pirie, who was trying to make edible protein from grass. He gave Fred a bucket of frozen grass and said, "You want to work on this" [23, 24]. Before Fred really got started, Bill left Cambridge for another position. Albert Neuberger, who had recently joined the biochemistry faculty, became Fred's supervisor. Fred indicated that Neuberger taught him how to research, most importantly not to fear trying something new, not to worry when an experiment did not work, but to get on with it and try something else.

In December 1940, Fred and Joan were married in the old family church at Syde (Fig. 2.7). Gasoline was in such short supply that Joan's father gave them several gallons as a wedding present. They left Syde for their wedding trip in Wales, but almost had to spend their wedding night in the car. Fred had not booked a hotel, thinking there would be no problem finding a room. Evacuees from London and other parts of Britain occupied hotel rooms all along their route. Rather late, they finally found a room [27].

Fred's dissertation work was on metabolism of the amino acid lysine. He made acetyl lysines and fed them to rats. In addition to his studies on lysine metabolism, he had war work analyzing nitrogen in potatoes. Fred had fire duty one night a week, where he and a co-worker spent all night watching for fires that might result

[11]Christiaan Eijkman "for his discovery of the antineuritic vitamin" and Sir Frederick Gowland Hopkins "for his discovery of the growth-stimulating vitamins" shared the 1929 Nobel Prize in Physiology or Medicine [26].

Fig. 2.7 Fred and Joan Sanger wedding 1940 (Courtesy Fred Sanger, with permission)

from German bombardments. Fortunately, Cambridge escaped major bombing because it had little industry—only 30 persons were killed and 70 injured in all of the war. Most bombing resulted from German's dropping excess bombs on their way home after an attack elsewhere [28].

Cambridge was awash with London evacuees and with Yanks. The Royal Air Force built airfields all over East Anglia in the mid-1930s; Cambridge was the obvious city for military leave. Rationing allowed the typical resident 12 ounces of sugar, four ounces of bacon, four ounces of butter, and six ounces of meat per day in 1940, and even those items became scarce. Yanks came in with all their money and engendered no small amount of resentment [29]. Joan was a master at finding scarce commodities, so the Sangers fared okay. She also worked two jobs. And in 1943, she bore their first son, Robin. He was a difficult baby the first year, crying all the time. Despite this backdrop, Fred managed to submit his Ph.D. dissertation in late 1943 (Fig. 2.8).

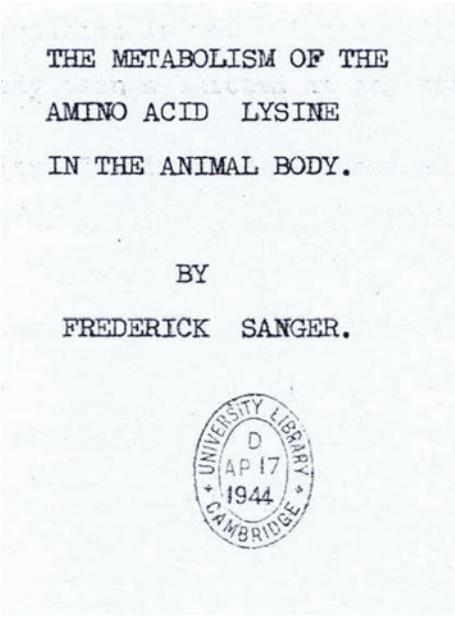


Fig. 2.8 Fred Sanger's dissertation (Courtesy Fred Sanger, with permission)

References

1. Sanger F (1919) Personal diary
2. Sanger F (1921) Personal diary
3. Sanger F (1924) Personal diary
4. Sanger F (1922) Personal diary
5. Sanger F (1923) Personal diary
6. Sanger F (1999) Personal communication
7. Sanger F (1926) Personal diary
8. Charlotte Mason. https://en.wikipedia.org/w/index.php?title=Charlotte_Mason&direction=next&oldid=665003345. Accessed 19 June 2015
9. Brown EJ (1987) The first five: story of a school. EJ Brown, Colwall, UK
10. Sanger F (1928) Personal diary
11. Wilford M (1999) Personal communication
12. Sanger F (1930) Personal diary
13. Sanger T (1999) Personal communication
14. Mauger P, Smith L (1969) British people 1902–1968. Heinemann, London
15. Sanger F (1931) Personal diary
16. Parkhurst H (1922) Education on the Dalton plan. EP Dutton, New York
17. Morgan MC (1978) Bryanston 1928–1978. Bryanston School, Blandford Forum, UK
18. A Brief Introduction to Eton Fives. http://www.fivesonline.net/oldefasite/about.fives/brief.introduction.html. Accessed 24 June 2015
19. Sanger F (2000) Personal communication
20. Fred Sanger Student File, St. John's College Archives
21. Peace Pledge Union. http://www.ppu.org.uk/learn/infodocs/st_ppu.html. Accessed 30 June 2015

22. Hetherington B (2015) Honorary archivist. Peace Pledge Union, Personal communication
23. Brownlee G (1992) Oral history video interview. A life of research on the sequences of proteins and nucleic acids: Dr. Frederick Sanger in conversation with Prof. George Brownlee. The Biochemical Society, London
24. Brownlee G (2014) Fred Sanger double Nobel laureate: a biography. Cambridge University Press, Cambridge, p 74
25. Smith S (1990) Spiceland: Quaker training centre. William Sessions Limited, York, UK
26. Nobel Media AB (2014) The Nobel prize in Physiology or Medicine 1929. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1929/. Accessed 30 June 2015
27. Sanger F (2003) Personal communication
28. Barham J (1977) Cambridgeshire at war. Bird's Farm Publications, Cambridge, UK
29. Brown RD (1980) East Anglia 1939. Terence Dalton Limited, Lavenham, UK

Chapter 3
Protein Period 1943–1962

As of 1943, Fred Sanger needed a job. He had his Ph.D., a wife, and a son. To this point, he had lived on family money. His Ph.D. supervisor, Albert Neuberger, left Cambridge for a position at the National Institute for Medical Research. A.C. Chibnall had just joined the Biochemistry department as chair, replacing the retiring Professor Hopkins. Chibnall, known as Chibs, offered Fred a position supported by a Beit Memorial Fellowship[1] [1]. Fred had his first job!

Insulin, available in pure form, interested Chibs. He and his coworkers determined the amino acid composition of bovine insulin, but knew almost nothing of its structure. They found more free amino groups than could be accounted for by lysine, the only amino acid with a side-chain amino group, suggesting that amino groups were at the end of the structure. Jensen and Evans [2] had shown that phenylalanine was an amino acid at the end of insulin. Chibs and many other protein chemists thought of proteins as pure compounds; others thought of them as heterogeneous mixtures of molecules. Researchers knew that the peptide bond connects one amino acid to another. Bergmann and Niemann [3] had postulated that proteins were composed of repeating units of certain amino acid assemblages. But not much work was happening with protein structure because it was thought to be such an impossible task.

Chibs suggested that Fred investigate the end groups of insulin. Martin and Synge [4] had recently developed a column chromatography method to separate acetyl derivatives of amino acids. Fred tried methanesulfonyl chloride derivatives. Then he tried dinitrochlorobenzene [5]. The heat required to make it reactive enough hydrolyzed some of the protein, but Fred managed to separate the dinitrobenzene (DNB) amino acids on the column. Chibs had a colleague who made dinitrofluorobenzene (DNFB) for the war effort, so Fred tried it. Bingo. It reacted at room temperature. He labeled insulin with DNFB, hydrolyzed the labeled insulin with acid, and three DNB amino acids appeared in the hydrolysate—one for lysine, with its side-chain amino group, and one each for phenylalanine and glycine

[1]Beit Memorial Fellowships for Scientific Research were typically awarded for one year, renewable for a second. Fred Sanger was supported by Beit Fellowships for seven years.

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_3

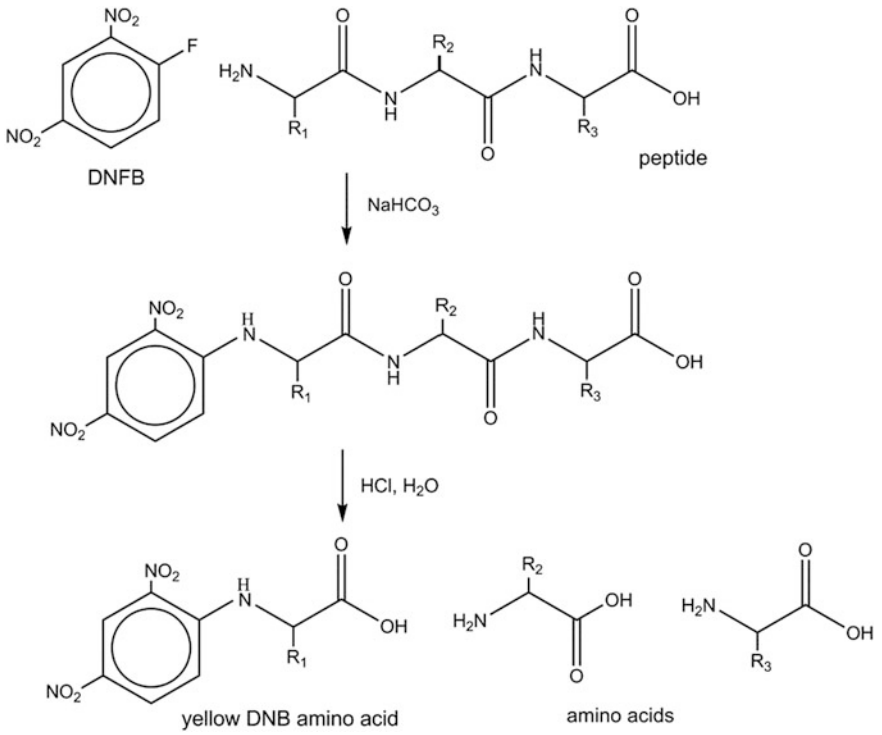


Fig. 3.1 Reaction of DNFB with peptide followed by acid hydrolysis

(Fig. 3.1). The DNB-lysine did not extract into ether, allowing only the end group derivatives to separate on the column [6].

Chibs could have been a coauthor on the *Biochemical Journal* paper describing the free amino groups of insulin. He suggested the project and gave Fred guidance. Because Chibs did not contribute at the bench, he chose not to. Fred published as the sole author and used that same practice throughout his career. By now, Fred was an independent researcher and could supervise graduate students, even though he did not have faculty status. One had to have a teaching appointment to be on faculty, and Fred did not teach.

As WWII ended, graduate students who had postponed studies to serve in the war returned ready to make up for lost time. Fred's first graduate student was Rodney Porter (Fig. 3.2). Rod and his good friend Sam Perry had begun graduate school at Liverpool University just before the war's outbreak. Sam spent most of the war as a POW. He escaped three times, but each escape resulted in recapture. Because he had much time to think, he decided to apply for a scholarship to Cambridge when the war was over. He convinced Rod, a war analyst during combat, to do the same. Rod was assigned to Fred; Sam was assigned to Kenneth Bailey. The four of them shared a lab in the building basement [7]. Both Rod and

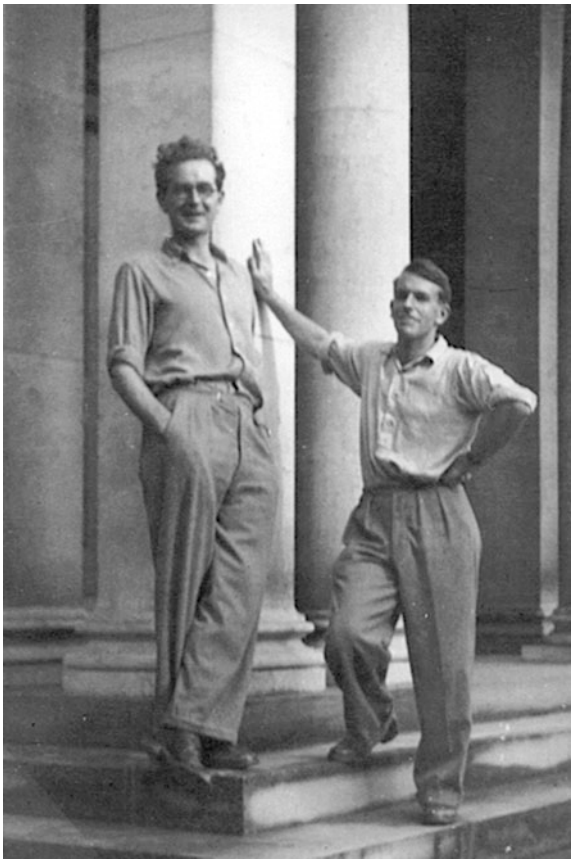


Fig. 3.2 Fred and Rod Porter on steps of biochemistry building 1947 (Courtesy Fred Sanger, with permission)

Sam were slightly older than Fred. The duo bought much energy and exuberance to the lab.

Rod used the DNFB method to identify end groups of various hemoglobins. During a workup from one of the reactions, the ether-containing flask exploded, sending a piece of glass into Rod's eye. In his two-week recuperation, he started reading Landsteiner's *The Specificity of Serological Reactions*, which summarized known antibody specificity [8]. Sam Perry said, "It became Rod's Bible. He was keen to apply the DNFB method to investigate antibody specificity despite Sanger's misgivings as to whether worthwhile results would be obtained." Rod was "not easy to supervise. With a less tolerant supervisor than Sanger one could have imagined the friction that might have arisen, but it did not" [9]. Rodney Porter would later share the 1972 Nobel Prize in Physiology or Medicine with Gerald Edelman "for their discoveries concerning the chemical structure of antibodies" [10].

Fred and Joan rented a flat on Hills Road, close enough that Fred could bicycle to the biochemistry building. Over time they expanded their Hills Road home to include the entire building and lived there until Fred retired in 1983. Fred would

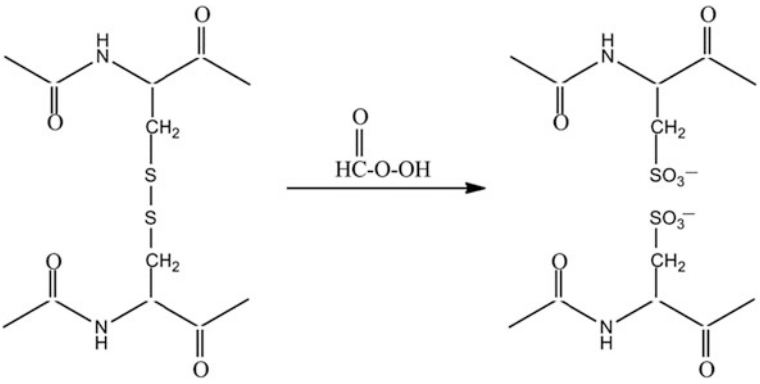


Fig. 3.3 Performic acid oxidation of disulfide cross-link

often take Joan to the cinema in the evening and then return to the lab afterward when it was much quieter. Fred and Joan's second son, Peter, was born in 1945. Peter was a much easier baby than Robin. Rationing continued after the war. Little Peter had teeth problems, probably due to food shortages.

Studies estimated the molecular weight of insulin at 12,000. Given that value, the supposed composition of insulin was four chains, two with glycine termini and two with phenylalanine termini. Fred assumed disulfide linkages between cysteines held the chains together. Toennies and Homiller [11] showed that performic acid oxidizes only the amino acids tryptophan, methionine, and cystine. Insulin contains no tryptophan or methionine, so Fred used performic acid to separate the chains (Fig. 3.3) [12].

In 1947, Fred spent a few months in the lab of Arne Tiselius[2] at Uppsala, Sweden. Sweden seemed a land of plenty without the post-war shortages common in Britain. Fred was surprised that Tselius did not work in the lab but had technicians do the work. Fred and a technician oxidized insulin to separate the chains, then loaded the mixture onto charcoal columns. As material moved down the column, fronts appeared but then disappeared. The technician saw four fronts, so she assumed four chains. That analysis made perfectionist Fred uncomfortable. Tselius had not even been in the lab, yet he wanted to publish the results [14]. Fred later said that it was the only paper he published about which he was ashamed.

Back in Cambridge, Fred oxidized insulin and separated two chains, which he labeled A and B. The A chain had glycine as its amino terminus; the B chain had phenylalanine [15]. Lengthy acid hydrolysis completely degraded insulin to its constituent amino acids. Partial acid hydrolysis, on the other hand, produced a series of random peptides. Alkaline hydrolysis had limited utility because it

[2]Arne Tselius won the 1948 Nobel Prize in Chemistry "for his research on electrophoresis and adsorption analysis, especially for his discoveries concerning the complex nature of the serum proteins" [13].

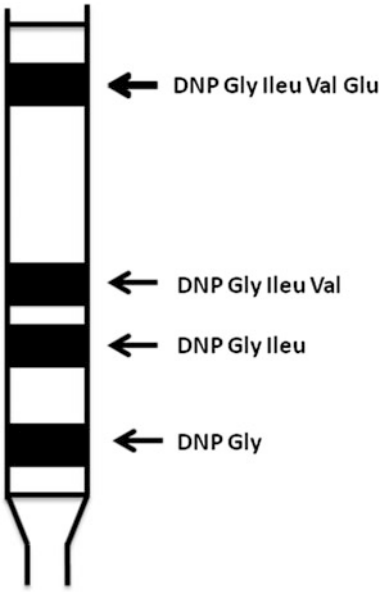


Fig. 3.4 Silica gel partition chromatography of partial acid hydrolysis of DNP–insulin [once a dinitrobenzene group is added to an amino acid, it can be called a dinitrophenyl group (DNP)] (George G. Brownlee, *Fred Sanger-Double Nobel Laureate: A Biography*, (2014), Cambridge University Press, reproduced, with permission, from The Biochemical Society)

degraded many amino acids. Fred labeled the A chain with DNFB and subjected it to partial acid hydrolysis. He then separated the peptides on a column, where the bright yellow bands were easy to follow (Fig. 3.4) [16]. Fred considered this paper his most important of the period because, as he said, "It was the first time that a peptide sequence had been determined at a specific site in a protein.… It also established that proteins could be regarded as pure chemicals rather than heterogeneous mixtures" [17].

Austrian post-doctoral fellow Hans Tuppy joined Fred in 1949 as they tackled the problem of the insulin sequence. Hans worked on the B chain, while Fred worked on the A chain. The B chain had a greater variety of amino acids, making it less difficult than the A chain. War-torn Austria was in shambles; Hans was thankful to have a place to work, and work he did. In the year he was in Cambridge, he mostly completed the B chain sequence. As Hans put it, he came from a German-style lab where the emphasis was more on working than thinking; Fred's was the opposite. Fred thought everything through and planned carefully before beginning any experiment [18].

Acid hydrolysis was not sufficient to complete the entire sequence, so they also used enzyme hydrolysis with pepsin, chymotrypsin, and trypsin. The advent of paper chromatography by A.P.J. Martin's group [19] made separating the peptides much easier. Even so, the initial insulin peptide mixtures were too complex for paper alone, so they conducted preliminary separations by electrophoresis, ion exchange chromatography, and charcoal adsorption chromatography. They then subjected each peptide separated on two-dimensional paper chromatography to further analysis. One fraction was completely hydrolyzed to determine the amino

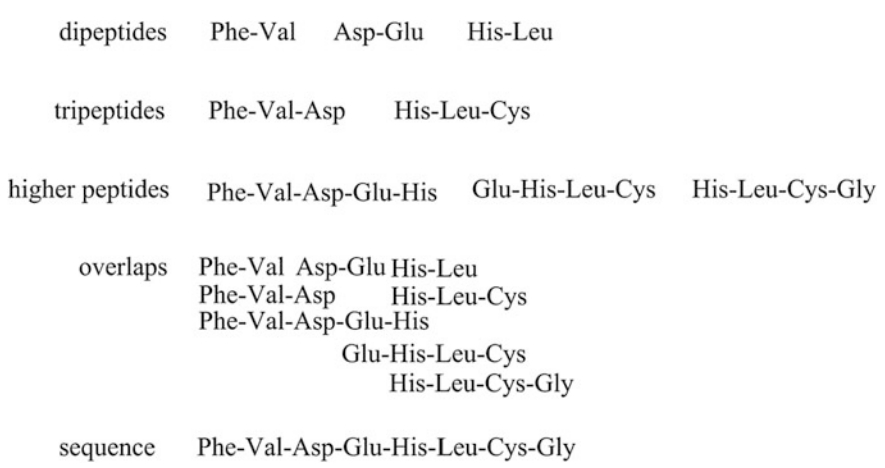


Fig. 3.5 Some peptides obtained from acid hydrolysis of insulin B chain and their overlaps

acid composition; another was labeled with DNFB to discover the terminal amino acid for each peptide. Because they sequenced many peptides that contained overlapping sequences, they were gradually able to piece together the sequence (Figs. 3.5 and 3.6) [20, 21].

As Hans Tuppy finished his year in Cambridge, E.O.P. "Ted" Thompson arrived from Australia. Ted and his wife Adrienne had master's degrees in chemistry from Sydney University. Ted received an Australian two-year scholarship to study for his

Pepsin peptides

Phe-Val-Asp-Glu-His-Leu-Cys-Gly-Ser-His-Leu

His-Leu-Cys- Gly-Ser-His-Leu

Tyr-Thr-Pro-Lys-Ala

Val-Glu-Ala-Leu

Chymotrypsin peptides

Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe

Val-Glu-Ala-Leu-Tyr

Tyr-Thr-Pro- Lys-Ala

Trypsin peptides

Gly-Phe-Phe- Tyr-Thr-Pro- Lys

B chain of insulin

Phe-Val-Asp-Glu-His-Leu-Cys-Gly-Ser-His-Leu- Val-Glu-Ala-Leu-
Tyr- Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala

Fig. 3.6 Some peptides obtained from enzyme hydrolysis of insulin B chain and sequence of B chain

Ph.D. in Cambridge. Because wool was Australia's chief export product, it seemed logical that he should study proteins. The timing was tricky, so Ted and Adrienne set sail for the six-week trip to England before knowing for certain of Ted's acceptance [22]. Frank Young replaced Chibnall when Chibs stepped aside as chair of Biochemistry. Young was not happy that Ted had forced his hand into accepting him for graduate studies. Fred became Ted's supervisor. This big boisterous Aussie was quite a contrast to the serious Tuppy. As Fred put it, "Not only were the subdued strains of Viennese waltzes replaced by bawdy Australian songs, but the whole atmosphere became considerably lighter and more fun" [23].

Even in 1950, the UK was still experiencing great post-war shortages. Laboratory equipment was meager, so they fashioned equipment from whatever was available. Fred's lab was still in the basement. Standard procedure for detecting amino acids was spraying chromatograms with ninhydrin, which reacts with amino acid breakdown products, among them ammonia, producing a purple color at an amino acid's location. The rat room was just across the hall from the lab. Because ammonia from rat urine caused chromatograms to become a sea of purple, the chromatography room had to be located at the other end of the building. Rust was also a problem because they made the chromatography tanks from galvanized sheet iron. They dried paper chromatograms by hanging them in a wooden box with a row of 250-watt light bulbs at its base. If drops hit the bulbs, the bulbs broke, so the hanging was tricky [22]. Clamps were too big to hold capillary reaction vessels, so they used plasticine to hold them. Ted's technique involved kneading the plasticine to a proper consistency. Then he threw it hard against the wall. If it stuck, it was ready; if not, he kneaded it more [23].

The A chain presented many challenges. Gradually Fred and Ted ferreted out the sequence, again using partial acid and enzymatic hydrolysis (Fig. 3.7) [24, 25].

Fred gave many popular lectures about deducing the insulin sequence. About this he said [26],

> I used these cards with the amino acids written on them, and I would lay these out on the bench in front of the lecture and gradually build up the sequence. Maybe we had a dipeptide and then various other dipeptides and I would gradually join them up, and describe how the sequence was built up.

Examples of these referenced cards are illustrated in Fig. 3.8.

Fred Sanger was not a "Eureka!"[3] kind of guy. He had an idea, designed an experiment to test that idea, had another idea, and so forth. Only at this stage did he consider determining the entire primary structure of insulin. Two problems remained: Which of the aspartic acid and glutamic acid side chains were in amide

[3]Archimedes is credited with exclaiming "Eureka! Eureka!" while running naked down the street after being in the bath and deducing that a body displaces an amount of water equal to its volume. He could then tell the local ruler whether or not the ruler's crown was pure gold. Archimedes probably never uttered the words. The story first appeared 200 years later in the writings of Vitruvius in the first century BC [27]. By tradition, a "Eureka!" scientist is one who has a sudden insight of genius.

Gly-Ileu-Val-Glu-Glu-Cys-Cys-Ala-Ser-Val-Cys-Ser-
Leu-Tyr-Glu-Leu-Glu-Asp-Tyr-Cys-Asp

Fig. 3.7 Sequence of insulin A chain

Fig. 3.8 Fred using cards with amino acids glutamic acid, valine, and tyrosine in illustrating how the insulin sequence was determined (George G. Brownlee, *Fred Sanger-Double Nobel Laureate: A Biography*, (2014), Cambridge University Press, reproduced, with permission, from The Biochemical Society)

form—asparagine and glutamine? Where were the disulfide bonds that joined the A and B chains? According to Fred, "Both proved particularly difficult and we would probably not have attempted them but for the challenge of achieving the first determination of a protein structure" [28].

Each afternoon at 4:00, Professor Young hosted a tea for researchers. They sat around a large table and Young controlled the discussion. Fred told Ted, "Professor Young has said you should attend afternoon tea and that I should attend too." Neither did [22]. They did, however, frequent the tearoom at other times. Ruth Kitai, a South African technician, joined Fred and Ted. Fred took Ruth to the faculty tearoom, much to the chagrin of the class-conscious faculty. After all, staff took tea in a separate room [1]. Young wanted more space, so he sent Ted to the Biochemistry Hut, a nearby prefabricated building. Fred remained in the main building, but soon he too moved into the Hut, where they continued work on insulin.

When Fred's seven-year Beit Fellowship ended, Young rallied support from the Medical Research Council. He wanted the MRC to provide Fred's salary. Young "would consider it a 'national tragedy' if Sanger were forced to leave Britain, but insisted it was against his policy to keep on research staff who were not involved in teaching on a permanent basis," including Fred [29].

Acid hydrolysis converted asparagine and glutamine into aspartic acid and glutamic acid, respectively, so they used partial enzyme hydrolysates to identify the amide side chains. Contiguous glutamic acids proved particularly difficult, leading Fred to say, “Few amino acids can have generated so much strong language” [17]. But their persistence paid off [30].

The disulfide question was also difficult. Ted had moved on, so Andrew Ryle and Les Smith joined Fred and Ruth in tackling this issue. They first found conditions to prevent a disulfide exchange reaction from occurring [31], and then, using the new technique of paper electrophoresis [32], located the disulfide linkages, completing the structure of insulin [33]. Because counter-current distribution studies showed insulin to have a molecular weight near 6000 [34], they knew that they had two chains rather than four (Fig. 3.9).

With the sequencing techniques worked out, Fred and his coworkers turned their attention to insulins from other species. They wanted to study insulin function in relation to its structure. They sequenced insulin from cattle, horse, pig, sheep, and whale [35, 36]. The differences were small and revealed little about function.

It was not all work and no play. Fred played on the 1957 Biochemistry cricket team (Fig. 3.10).

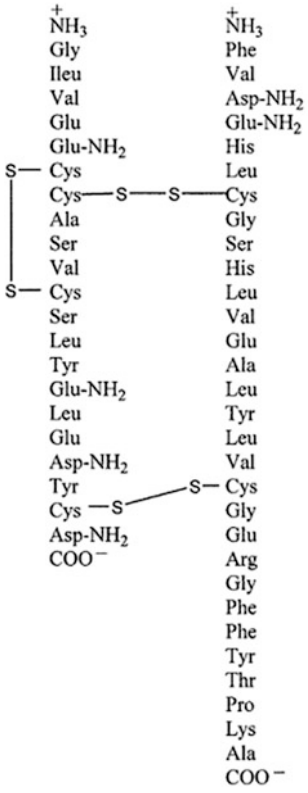


Fig. 3.9 Structure of insulin. NH_2 groups denote amide side chains

Fig. 3.10 Biochemistry Department Cricket Team 1957. Fred is *third* from *left* on row 1. (Courtesy Biochemistry Department, Cambridge University, with permission)

Fred received a telephone call from a Swedish reporter seeking a comment about Fred's winning the 1958 Nobel Prize in Chemistry. Fred had no idea. He had not heard from the Nobel people, and he was hesitant to get excited. On several occasions, Swedish media had contacted persons ahead of the official announcement only to find later that they were wrong. That evening, he took the family to a Jerry Lewis movie. He had trouble concentrating on the movie, wondering if there was any truth to the reporter's call. A few days later, he received the official call. He was indeed the recipient of the 1958 Chemistry Prize [1]. Six people had nominated Fred for the prize [37]. Celebration ensued (Fig. 3.11).

December festivities in Stockholm followed the early fall announcement. Besides Fred, Joan, Robin, and Peter, Fred's siblings Theo and May and Joan's sister attended. They were wined and dined. Professor Igor Tamm, one of three Russians sharing the physics prize, took a special interest in the boys. As the only adolescents attending, Robin and Peter received much attention (Fig. 3.12). The Nobel banquet and ball occurred in Stockholm City Hall, the presentation ceremony in the Stockholm Opera House (Fig. 3.13). Fred delivered his Nobel Lecture on December 1, 1958 [38]. Unfortunately, Joan suffered an appendicitis attack while in Stockholm and had surgery. The Sangers spent the Christmas holidays in a country hotel near Stockholm. Delightful for the boys; not so much for Joan [39].

Fig. 3.11 Fred signing a champagne bottle at Nobel reception in Biochemistry building (Courtesy Biochemistry Department, Cambridge University, with permission)

Fig. 3.12 Peter and Robin Sanger at the Nobel celebrations (George G. Brownlee, *Fred Sanger-Double Nobel Laureate: A Biography*, (2014), Cambridge University Press, reproduced, with permission, from The Biochemical Society and Peter Sanger)

Fig. 3.13 Fred Sanger receiving the 1958 Nobel Prize in Chemistry (Courtesy Fred Sanger, with permission)

Upon his return to Cambridge, Fred and several friends went out to celebrate with a dinner. Someone called, "Let's have a look at that medal, Fred" (Fig. 3.14). As Sam Perry related, "Sanger pulled the case from his pocket, removed the medal and rolled it along the pavement. I am glad to say that as it rolled towards the drain in the gutter, I was able to get my foot in the way. There cannot have been many Nobel medals in such danger" [7].

Fred faced the question of what to do next. Everything to this point had followed from Chib's initial suggestion to look at the insulin structure. He could have sequenced additional proteins, but that did not interest him. Methods excited him now. He liked the idea of attaching a radioactive label to an amino acid and

Fig. 3.14 Fred Sanger's 1958 Nobel Medal (Photo by author. Courtesy Sally Sanger, with permission)

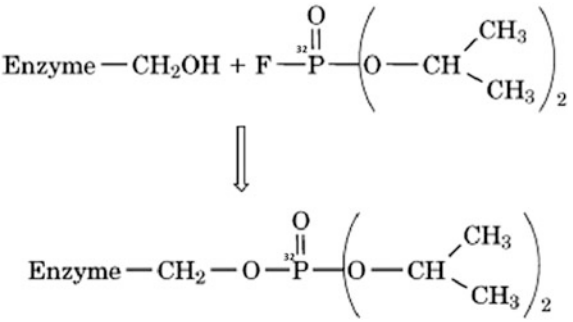


Fig. 3.15 Reaction of serine with ^{32}P-labeled DIFP

sequencing the amino acids around it. An autoradiograph made from exposure of a radioactive spot on a paper to X-ray film would make isolating such a peptide easy to track. Upon development, the film would show a black spot where radioactive material contacted the film. Trypsin and chymotrypsin were known to have serine in the active center. Serine reacts with diisopropyl fluorophosphate (DIFP) to inhibit the enzymes (Fig. 3.15). If DIFP contains radioactive phosphorus (^{32}P), the active center is labeled.[4]

Working with Mike Naughton, Brian Hartley, and Denis Shaw, Fred sequenced the peptides of the active centers of trypsin, chymotrypsin, and elastase [40]. Fred also developed a collaboration with César Milstein, who had earned a Doctor en Quimica (Ph.D.) at the University of Buenos Aires. He came to Cambridge to study and enrolled for a second Ph.D. with Malcolm Dixon as his supervisor. Fred was not César's direct supervisor, but the two worked closely studying the active center of phosphoglucomutase, which contained a serine phosphate group. This work became part of César's Ph.D. dissertation. César stayed on at Cambridge for an extra year, working in Fred's lab [41]. These experiments set the stage for Fred's move into nucleic acid sequencing.

References

1. Sanger F (1999) Personal communication
2. Jensen EH, Evans EA (1935) Studies on crystalline insulin: XVIII. The nature of the free amino groups in insulin and the isolation of phenylalanine and proline from crystalline insulin. J Biol Chem 108:1–9
3. Bergmann M, Niemann C (1938) On the structure of silk fibroin. J Biol Chem 122:577–596
4. Gordon AH, Martin AJP, Synge RLM (1943) Partition chromatography in the study of protein constituents. Biochem J 37:79–86

[4]Isotopes of elements are listed with the chemical symbol for the element preceded by a superscript denoting its atomic mass. ^{32}P is a radioactive form of phosphorus. ^{35}S, a radioactive form of sulfur, appears in Chap. 5.

5. Abderhalden E, Stix W (1923) Weiterer Beitrag zur Frage der Konstitution der Proteine. Untersuchungen über den Aufbau des Seidenfibroins. Hoppe Seylers Z Physiol Chem 129:143–156
6. Sanger F (1945) The free amino groups of insulin. Biochem J 39:507–515
7. Perry SV (1997) Fate has smiled kindly. In: Semenza G, Jaenicke R (eds) Comprehensive biochemistry. Selected topics in the history of biochemistry: personal recollections, vol 40. Elsevier, London
8. Landsteiner K (1946) The specificity of serological reactions. Harvard University Press, Cambridge
9. Perry SV (1987) Rodney Robert Porter 1917–1985. Biographical memoirs of fellows of the Royal Society, vol 33. Cambridge University Press, Cambridge
10. Nobel Media AB (2014) The Nobel prize in Physiology or Medicine 1972. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1972/. Accessed 10 July 2015
11. Toennies G, Homiller RP (1942) The oxidation of amino acids by hydrogen peroxide in formic acid. J Am Chem Soc 64:3054–3056
12. Sanger F (1947) Oxidation of insulin by performic acid. Nature 160:295–296
13. Nobel Media AB (2014) The Nobel prize in Chemistry 1948. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1948/. Accessed 7 July 2015
14. Tselius A, Sanger F (1947) Adsorption analysis of oxidized insulin. Nature 160:433–434
15. Sanger F (1949) Fractionation of oxidized insulin. Biochem J 44:126–128
16. Sanger F (1949) The terminal peptides of insulin. Biochem J 45:563–574
17. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Scientific, Singapore, p 7
18. Tuppy H (2009) Personal communication
19. Consden R, Gordon AH, Martin AJP (1944) Qualitative analysis of proteins: a partition chromatographic method using paper. Biochem J 38:224–232
20. Sanger F, Tuppy H (1951) The amino-acid sequence in the phenylalanyl chain of insulin. 1. The identification of lowerpeptides from partial hydrolysates. Biochem J 49:463–481
21. Sanger F, Tuppy H (1951) The amino-acid sequence in the phenylalanyl chain of insulin. 2. The investigation of peptides from enzymic hydrolysates. Biochem J 49:481–490
22. Thompson EOP (2000) Personal communication
23. Sanger F (1990) Letter to Ted Thompson on the occasion of Ted's retirement
24. Sanger F, Thompson EOP (1953) The amino-acid sequence in the glycyl chain of insulin. 2. The identification of lowerpeptides from partial hydrolysates. Biochem J 53:353–366
25. Sanger F, Thompson EOP (1953) The amino-acid sequence in the glycyl chain of insulin. 2. The investigation of peptides from enzymic hydrolysates. Biochem J 53:366–374
26. Brownlee G (2014) Fred Sanger double Nobel laureate: a biography. Cambridge University Press, Cambridge
27. Biello D (2006) Fact or fiction?: Archimedes coined the term "Eureka!" in the bath. Sci Am Dec 8 http://www.scientificamerican.com/article/fact-or-fiction-archimede/. Accessed July 16 2015
28. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Sci, Singapore, p 9
29. de Chadarevian S (2002) Designs for life: molecular biology after world war II. Cambridge University Press, Cambridge, p 222
30. Sanger F, Thompson EOP, Kitai R (1955) The amide groups of insulin. Biochem J 59:509–518
31. Ryle AP, Sanger F (1955) Disulphide interchange reactions. Biochem J 60:535–540
32. Michl H (1951) Paper electrophoresis at potential differences of 50 volts per centimeter. Monatshefte Chem 82:489–493
33. Ryle AP, Sanger F, Smith LF, Kitai R (1955) The disulphide bonds of insulin. Biochem J 60:541–556
34. Harfenist EJ, Craig LC (1952) Countercurrent distribution studies with insulin. J Am Chem Soc 74:3083–3087

35. Brown H, Sanger F, Kitai R (1955) The structure of pig and sheep insulins. Biochem J 60:556–565
36. Harris JI, Sanger F, Naughton MA (1956) Species differences in insulin. Arch Biochem Biophys 65:427–438
37. Nobel Media AB (2014) Nomination database. Nobelprize.org. http://www.nobelprize.org/nomination/archive/list.php. Accessed 23 July 2015
38. Nobel Media AB (2014) Frederick Sanger—Nobel lecture: the chemistry of insulin. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1958/sanger-lecture.html. Accessed 3 July 2015
39. Sanger P (2000) Personal communication
40. Naughton MA, Sanger F, Hartley BS, Shaw DC (1960) The amino acid sequence around the reactive serine residue of some proteolytic enzymes. Biochem J 77:149–163
41. Milstein C (1999) Personal communication

Chapter 4
Origins of the Medical Research Council Laboratory of Molecular Biology

Austrian Max Perutz came to Cambridge in 1936 to study X-ray diffraction with John Bernal, one of the first to apply X-ray studies to proteins. In 1937, Max began his studies of hemoglobin. That same year Ernest Rutherford,[1] Cavendish Chair of Physics, died following surgery, and Bernal left for London. Lawrence Bragg[2] became Cavendish Chair in 1938. Bragg took an interest in Max. The next year, Hitler invaded Austria, so Max became a refugee, and, as such, ineligible to earn money in Britain. Max's X-ray photos of hemoglobin excited Bragg, who obtained Rockefeller Foundation funding to support Max. American money was okay. Max received his Ph.D. in 1940 [3].

With the war raging, the British government considered Austrians, like Max, threats to national security. Max was interred near Liverpool and on the Isle of Mann and then shipped to Quebec, Canada. After much lobbying on his behalf, the Home Office agreed to release him from detention. Max became a British citizen in 1943 [4]. Still, Bragg could not get a post for Max in this no man's land of biophysics, an area unpopular with traditional physicists. In 1947, Bragg got support from the Medical Research Council (MRC) for the MRC Unit for Study of Molecular Structure of Biological Systems, with Max as Director. The Rockefeller Foundation also supported the Unit, as it was called.

The Unit focused on bringing the physical sciences to bear on biological problems. During this time like-minded researchers started the Hardy Club to discuss biophysics, bringing together biologists and physicists. They often met over lunch at The Eagle (Fig. 4.1), a local pub near the Cavendish Lab [5]. In this milieu, Watson, a postdoctoral fellow, and Crick, a graduate student, proposed the double-helical model of DNA. In 1953, the same year that Watson and Crick proposed the DNA structure, Bragg left for London, and Nevill Mott became Cavendish Chair. Mott,

[1]Ernest Rutherford won the 1908 Nobel Prize in Chemistry "for his investigations into the disintegration of the elements, and the chemistry of radioactive substances" [1].
[2]Sir William Henry Bragg and William Lawrence Bragg shared the 1915 Nobel Prize in Physics "for their services in the analysis of crystal structure by means of X-rays" [2].

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_4

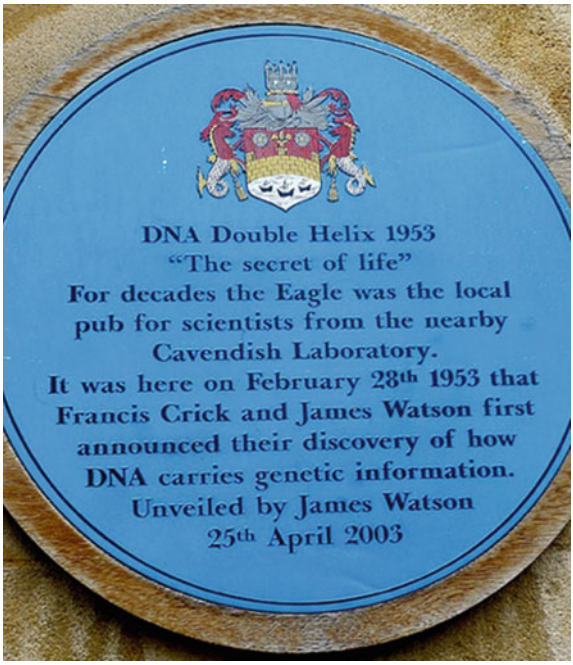


Fig. 4.1 The Eagle Pub plaque commemorating DNA structure (Courtesy Cambridge Ultrasonics and Greene King Brewery, with permission)

a traditional physicist, wanted more space. He wanted the Unit to move because it took up too much space and the researchers did not teach. The General Board of Faculties, however, backed the Unit. Also, the Rockefeller Foundation wanted assurances for the Unit to continue its funding, so in 1957 the Unit moved to the Hut, a prefabricated building similar to the Biochemistry Hut into which Sanger's group had moved (Fig. 4.2).

Both Fred and Max were in a similar situation. Neither held a regular faculty appointment and both were MRC supported. They had chairs who wanted space for faculty who taught. While they had no extensive collaborations, their groups did interact. The Unit had wanted Fred's group to sequence proteins they had under study, but Fred had no interest in being a service provider. Nevertheless, Unit members did visit Fred's lab to learn techniques. Fred, in a chance conversation with Francis Crick on a train trip, said that he wanted to learn something about genetics [4]. These subsequent interactions led to talks about a lab to accommodate both groups. Harold Himsworth, Secretary for the MRC, got wind of the conversations and invited Fred "to discuss some proposals he had heard Sanger was considering" [6]. And he wanted it outlined in writing before the meeting. Fred made his case and a case for including Max's group. Max followed with a letter of his own. They were invited to submit a scientific case for uniting the two groups, and they needed a new name.

Fig. 4.2 The Cavendish Hut (Copyright MRC Laboratory of Molecular Biology, with permission)

Molecular biology was not a new name. It had appeared in a Rockefeller report as early as the late 1930s. In 1957, Max's colleague John Kendrew was in negotiations with Academic Press about editing for a new journal provisionally entitled *Journal of Molecular Biophysics*. That term produced much confusion. Kendrew suggested *Journal of Molecular Biology* and it stuck [7]. The term was picked up in discussions about an institute of molecular biology and gradually the Laboratory of Molecular Biology (LMB) took form.

Politically, it was far from a done deal. Both Fred and Max wanted to stay on central campus with access to Cavendish stores and workshops, pubs, computer facilities, and interaction with teaching faculty. But there was no space. Turf battles made it difficult for an interdisciplinary unit like the LMB. Alexander Todd, chair of chemistry, and Mott favored the lab; Young adamantly opposed it. Young saw the new lab competing for resources, the best researchers, and especially technicians. The relationship with the University was important because Max's and Fred's groups wanted access to graduate students. This debate led to more than two years of negotiations.

Addenbrook's Hospital was moving from campus to a new site on Hills Road, two miles from campus. Initially, Fred and Max thought that too far from the center of activities. As the negotiations dragged on, it became more attractive. Peter Mitchell, Regis Professor of Physic, provided the breakthrough. His radiotherapeutics facility was already approved for the Addenbrook's site, and he suggested that the LMB be built next to it [8].

Fig. 4.3 MRC Laboratory of molecular biology 1962 (Copyright MRC Laboratory of Molecular Biology, with permission)

Three sticking points remained: staff size, employment of technicians, and lab staff's relation to the University. The University wanted to control the staff, i.e., the total number of employees at the new facility. It also wanted to hire the technicians and pay them at university scale. The MRC overrode the University on points one and two and left the third to "sort itself out in practice" [9]. Finally, the Medical Research Council Laboratory of Molecular Biology was to be built on Hills Road (Fig. 4.3).

They occupied the building in 1962. The LMB had three divisions: Max Perutz and John Kendrew headed Structural Studies; Francis Crick Molecular Genetics; and Fred Sanger Protein Chemistry. These men formed the Governing Board, with Max as Chairman. In May 1962, Queen Elizabeth dedicated the building (Fig. 4.4).

The lab was further honored in fall 1962 when Max Perutz and John Kendrew shared the Nobel Prize in Chemistry "for their studies of the structures of globular proteins" [10] and Francis Crick and James Watson (along with Maurice Wilkins) shared the Nobel Prize in Medicine "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material" [11]. By this time, Watson had returned to the United States. Francis Crick, in an interview with Soraya de Chadarevian, chuckled, "The laboratory was run by a committee formed exclusively of Nobel laureates"[3] [12]. Going forward, any researcher who was named a Fellow of the Royal Society came onto the Board (Fig. 4.5).

[3]By 2013, the LMB had 14 Nobel Laureates sharing in ten Nobel Prizes for work conducted at the LMB and 11 LMB alumni who became Nobel Laureates after their time at the LMB [13].

Fig. 4.4 Queen Elizabeth at LMB dedication 1962 (Copyright MRC Laboratory of Molecular Biology, with permission)

Fig. 4.5 LMB Governing Board 1967. From *left*, sitting Hugh Huxley, Max Perutz, Fred Sanger, Sydney Brenner; standing John Kendrew, Francis Crick (Copyright MRC Laboratory of Molecular Biology, with permission)

References

1. Nobel Media AB (2014) The Nobel prize in chemistry 1908. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1908/. Accessed 23 July 2015
2. Nobel Media AB (2014) The Nobel prize in physics 1915. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/physics/laureates/1915/. Accessed 23 July 2015
3. Finch J (2008) A Nobel fellow on every floor. Medical Research Council Laboratory of Molecular Biology, Cambridge, UK
4. Ferry G (2007) Max Perutz and the secret of life. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA
5. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, UK, p 90
6. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, UK, p 199
7. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, UK, p 208
8. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, UK, p 226
9. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, UK, p 228
10. Nobel Media AB (2014) The Nobel prize in chemistry 1962. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1962/. Accessed 23 July 2015
11. Nobel Media AB (2014) The Nobel prize in physiology or medicine. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1962/. Accessed 23 July 2015
12. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, UK, p 261
13. Medical Research Council Laboratory of Molecular Biology http://www2.mrc-lmb.cam.ac.uk/achievements/lmb-nobel-prizes/nobel-facts/. Accessed 28 July 2015

Chapter 5
RNA Period 1962–1971

Fred was still working with active centers of enzymes and with proteins in general. He liked the visual methods of paper chromatography and paper electrophoresis, and he held a certain disdain for work on columns. Protein sequencing, however, had largely gone to column methods because they were more quantitative. Nevertheless, Fred tried to develop a radiolabel method for sequencing proteins that would be faster than columns. To that end he made highly radioactive ovalbumin. His idea was to generate twenty samples of ovalbumin, each with a different radiolabeled amino acid. According to Fred [1],

> The samples were then digested with a proteolytic enzyme and the digests fractionated in parallel by paper electrophoresis and an autoradiograph prepared. Assuming that a peptide was pure, its amino acid composition could be deduced from the darkness (radioactivity) of its band in the separate channels. The whole paper was then to be subjected to the Edman procedure[1] and washed to remove the phenylthiohydantoin, and another autoradiograph was to be prepared. The N-terminal residue of each peptide should then be identified as the band missing or weaker in the second autoradiograph.

Additional Edman treatments should allow the sequences to be deduced. The method did not work out, but it laid the groundwork for the nucleic acid methods to follow.

To this point, Fred had shown no interest in nucleic acids. When he attended Gordon Conferences[2] on Proteins and Nucleic Acids, he passively sat through the nucleic acid talks eagerly awaiting the protein talks. The LMB, however, changed the mix of people for daily contact. As Fred put it, with people like Francis Crick around, one could not ignore nucleic acids.

The problem with nucleic acids was the lack of small molecules on which to develop methods and the monotony of their structures, because they contain only

[1]The Edman procedure is an N-terminal amino acid identification technique that, unlike Fred's DNFB method, does not require the destruction of the peptide under investigation.

[2]Dr. Neil E. Gordon of Johns Hopkins University initiated Gordon Research Conferences to allow scientists with a common interest to share current activity in their area. The 4–5 day meetings had talks in the mornings and evenings with afternoons open for discussion and recreation. A retreat setting lessened distractions [2].

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_5

four types of nucleotides.[3] Deoxyribonucleic acid (DNA) molecules were huge, with no known way to break them into smaller pieces. Even the simplest ribonucleic acid (RNA) viruses contained a few thousand nucleotides. Then transfer RNAs[4] (tRNAs) were discovered [3], and they contained fewer than 100 nucleotides. The appeal to Fred was obvious. He wanted to work out sequence methods for RNA and be the first to sequence one. Also, messenger RNA (mRNA) was known to reflect the DNA structure, so sequencing a mRNA corresponding to a protein of known sequence would reveal the genetic code.

Lipmann et al. [4] showed that leucine-tRNA reacted with ribonuclease to liberate an adenosyl ester of leucine. In bridging the gap between proteins and nucleic acids, Fred and Kjeld Marcker labeled a tRNA from *Escherichia coli* with a radioactive amino acid, using ^{35}S-methionine. They digested it with ribonuclease and separated the products by paper electrophoresis. Their autoradiograph showed a second spot. At first Fred thought it was an artifact. Further study showed it to be formylmethionine [5]. Marcker later extended that work to show the role of formylmethionine in protein synthesis [6].

In 1963, two people joined Fred's group. George Brownlee joined as a Ph.D. student. Fred also hired a new technician, 19-year-old Bart Barrell.[5] Bart had finished school but had not gone to university. He and another applicant showed up at the same time for two available positions. The other position was running an amino acid analyzer for Brian Hartley. Bart met with Fred first while the other fellow met with Hartley. The other applicant was better qualified and probably would have been offered the position with Fred. But he talked himself into a corner with Hartley and had no way out. Fred, reluctantly, offered the job to Bart. Bart still has the letter Fred gave him that said, "As your qualifications are somewhat lower than my previous assistants, it may be necessary to transfer you to the amino acid analyzer" [7]. Bart always felt the threat of the amino acid analyzer hanging over his head.

Fred gave George the choice of working on proteins or RNA. George chose RNA. His first task was to isolate phenylalanine-tRNA. Meanwhile, Fred and Bart used ribosomal RNA (rRNA) to work out methods. rRNA was too large to obtain meaningful sequence results, but fragments of it were good substrates for experiments. Fred liked working with ^{32}P-labeled proteins because he could easily track them after separations on paper by making autoradiographs. Nucleic acids were a suitable extension of this methodology because each nucleotide contains a phosphorus atom. By growing yeast in a ^{32}P-labeled medium, ^{32}P-rRNA was easy to isolate.

George had difficulties in purifying phenylalanine-tRNA. Robert Holly and his coworkers had better fortune and published the sequence of alanine-tRNA [8].

[3]RNA contains A, G, C, and U. DNA contains A, G, C, and T.

[4]tRNAS were first called S-RNAs because they were soluble in water.

[5]Bart Barrell earned a Cambridge Ph.D. based on his years of working with Fred. He never earned an undergraduate degree.

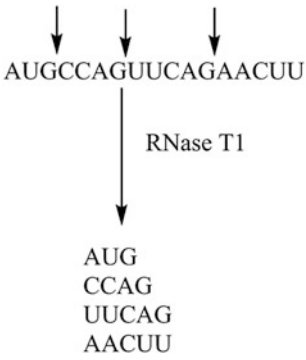


Fig. 5.1 Cleavage pattern for ribonuclease T1

Holly used partial enzymatic digestion methodology similar to what Fred had used for insulin. tRNAs also had some minor nucleotides that made tRNA sequencing easier than other RNAs. The tRNA work lost some of its luster once Holly sequenced a tRNA, but the genetic code was still out there. George joined Fred and Bart in the pursuit. Alas, it was too late. Nirenberg and Khorana[6] used other methods to gradually deduce the code, finishing it by 1966.

Holly used ribonuclease T1 to degrade tRNA. T1 cuts RNA after G nucleotides (Fig. 5.1).

Fred and the boys used T1 on rRNA to produce manageable-sized fragments. They worked out a radioactive method to sequence small oligonucleotides, resolving di-, tri-, and tetra-nucleotide digests. They subjected T1 fragments to partial digestion with the exonuclease spleen phosphodiesterase, which removes one nucleotide at a time from the 5' end of a oligonucleotide fragment. As Fred describes it [10],

> If we consider an oligonucleotide obtained from a ribonuclease T1 digest and suppose its structure is ABCG, where A, B, and C are unknown residues, on partial digestion with spleen phosphodiesterase the following degradation products will be found: BCG, CG, and mononucleotides.[On] the DEAE paper system used for the two-dimensional procedure, any nucleotide will move faster than a corresponding nucleotide having the same structure but with one extra residue added to its 5'-terminal end; i.e. BCG will move faster than ABCG, etc. Consequently, the various degradation products of an oligonucleotide will be arranged in order of their size on fractionation in this system, the larger ones moving slower than the smaller ones. The distance between any two products that differ by only one residue will depend on the nature of that residue. […] hence it is frequently possible to determine the complete sequence from a single degradation and fractionation.

In hindsight, Fred considered this paper his most important RNA work because it made sequencing nucleic acids feasible. They dubbed the spot migration pattern a graticule (Fig. 5.2). The location of each spot revealed its structure (Fig. 5.3).

---

[6]Robert Holley, Gobind Khorana, and Marshall Nirenberg shared the 1968 Nobel Prize in Physiology or Medicine "for their interpretation of the genetic code and its function in protein synthesis" [9].

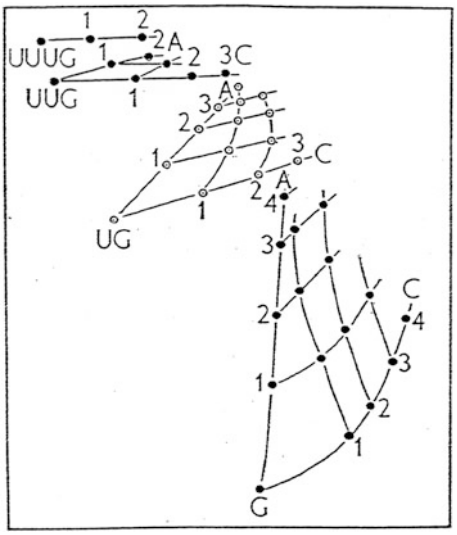


Fig. 5.2 Graticule pattern. First line *bottom right* is G, CG (1), CCG (2), CCCG (3), etc. (J Mol Biol (1967) 23:337–353, with permission)

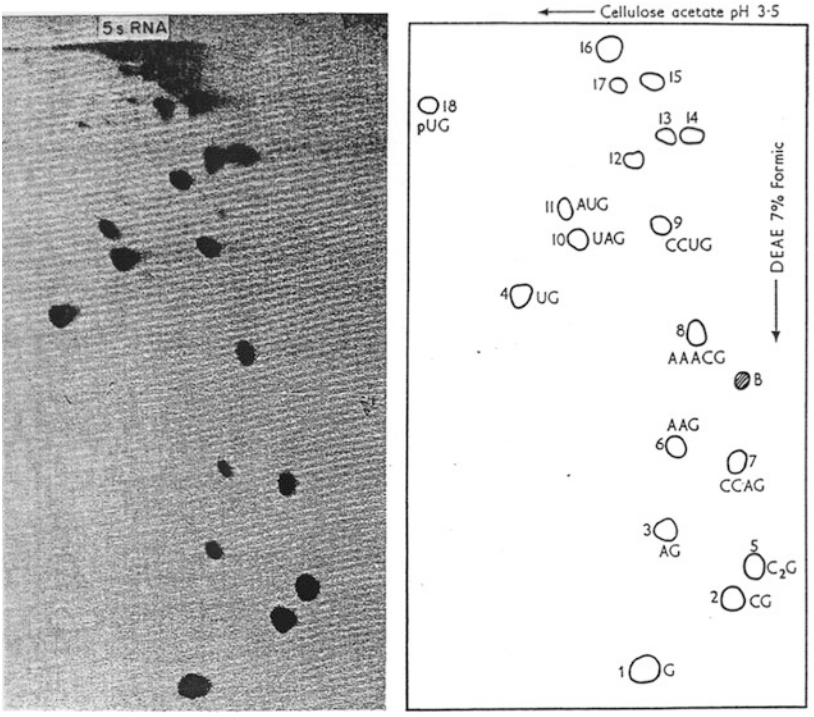


Fig. 5.3 Autoradiograph (*left*) and map showing composition based on graticule (J Mol Biol (1967) 23: 337–353, with permission)

Fig. 5.4 Fred and Bart Barrell about 1980 (George G. Brownlee, *Fred Sanger—Double Nobel Laureate: A Biography*, (2014), Cambridge University Press, reproduced, with permission, from The Biochemical Society and Peter Sanger)

Fred was not given to "Eureka!" moments. He saw small advances with enjoyment, but rarely exhilaration. During the development of RNA autoradiographs, morning after morning they saw streaks of black, indicating unclean separations. The morning Bart brought in the first autoradiograph with clean round spots, Fred was exhilarated [1]. When Fred prepared the manuscript for the first graticule paper, Bart happened by Fred's office and saw 'Bart Barrell' listed as one of the authors. Bart could not believe it. A technician as an author? It was unheard of. He asked Fred, "What's that!" Fred replied, "We're all in this together" (Fig. 5.4) [7].

Rosset and Monier described a small rRNA,[7] designated 5S, 120 nucleotides long and lacking any minor bases [11]. Fred finally had a target molecule of a suitable size—perfect for further methods development. They applied the graticule method to the 5S RNA [12]. Even with ribonuclease T1 and ribonuclease A (cuts after C and U nucleotides), separation of the resulting fragments remained a problem. Good migration of larger fragments occurred with electrophoresis on cellulose acetate, but the large fragments did not move on ion exchange DEAE paper. To that end they developed homochromatography. They used large amounts of nonradioactive RNA fragments to displace the radioactive ones from sites on DEAE paper (Fig. 5.5). Smaller fragments displaced more easily than larger ones, fractionating by size. The presence of large amounts of nonradioactive RNA did not

[7]The various rRNAs are designated by their ultracentrifugation sedimentation coefficients. *E. coli* rRNAs are 5S, 16S, and 23S, with larger numbers corresponding to larger size.

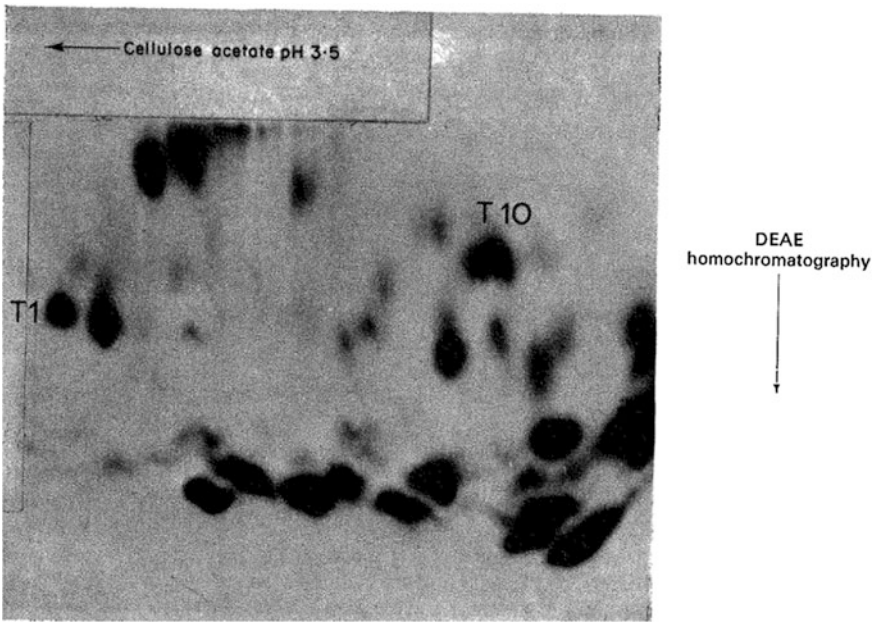


Fig. 5.5 Autoradiograph of partial T1 digest of 5S RNA, cellulose acetate in first dimension and homochromatography mixture in the second (J Mol Biol (1968) 34: 379–412, with permission)

matter because the autoradiographs detected only the radioactively labeled RNA. Their 5S RNA sequence (Fig. 5.6) was the largest at that time and the only one without minor nucleotides [13]. George did most of the sequence work for his Ph.D. dissertation [14].

They grew *E. coli* with radiolabeled nucleotides. The series of separations and reactions took time. To ensure that enough radioactivity persisted to the final steps, they used high doses of ^{32}P. Horace Judson[8] described a day with Fred as Fred made ^{32}P-nucleotides. Fred told him, "This is the hot room. You'll be alright. Don't touch anything. I'm supposed to be the radiation officer, and you're not even wearing a badge." Neither was Fred. Fred was following the elution of ^{32}P-ATP with a radiation counter and it was crackling so much Horace was ill-at-ease. Fred was wearing gloves and working behind Persplex[9] [15]. Bart Barrell noted that when a pair of Fred's shoes got too 'hot,' Fred put them in a bottom desk drawer, awaiting the several half-lives[10] necessary for them to be wearable again [7].

[8]Horace Freeland Judson was a historian of molecular biology and the author of *The Eighth Day of Creation*, a history of molecular biology.

[9]Persplex, Plexiglas, and Lucite are trade names for poly(methyl methacrylate). It provides a barrier against the β-emitting ^{32}P.

[10] ^{32}P has a half-life of 14 days.

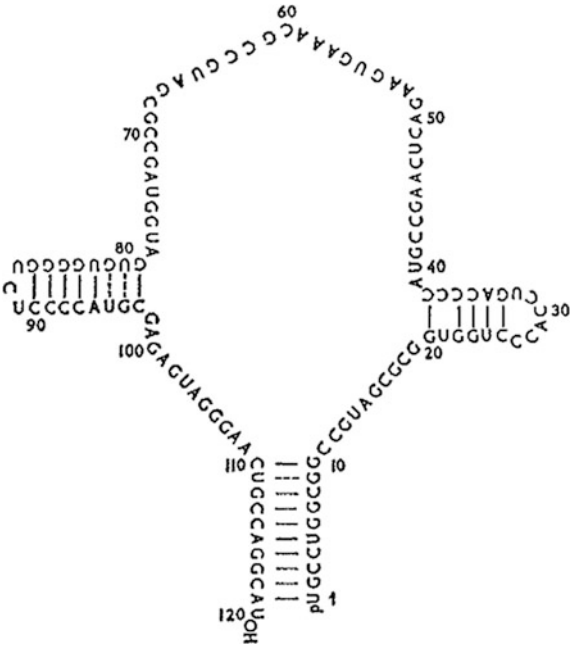


Fig. 5.6 Sequence of 5S RNA (Nature (1969) 215: 735–736)

Fred's group isolated and sequenced phenylalanine-tRNA [16], but his real target was larger RNA molecules. His current separation method for RNA fragments of electrophoresis on cellulose acetate in one dimension followed by homochromatography on DEAE paper in a second dimension had an upper limit of 25-nucleotide fragments. He needed to resolve larger fragments. Fred and George Brownlee extended the method to fragments of up to 50 nucleotides using thin-layer chromatography on mixed DEAE-cellulose and cellulose for the homochromatography step [17].

Binding studies established the genetic code, but no one had compared a mRNA sequence to a known protein sequence. Fred wanted to do so. To that end, he tried to make radioactive mRNA from hen oviducts, but all he managed to do was to make the lab very radioactive. He failed to account for the large amounts of calcium phosphate in egg shells.

Bacteriophages, viruses that infect bacteria, were well known. Bacteriophage R17, a RNA virus, could be prepared in radioactive form. Its coat protein sequence was known. Still, to go from 5S RNA with 120 nucleotides to R17 with more than 3000 was quite a jump. While a T1 digest of 5S RNA yielded 30 RNA fragments, a similar digest of R17 would yield more than 700 fragments [14]. The homochromatography system did provide some suitable products. Fred's graduate student Peter Jeppesen isolated and sequenced one of these fragments. Comparing the possible amino acid sequences that could be coded by this oligonucleotide matched

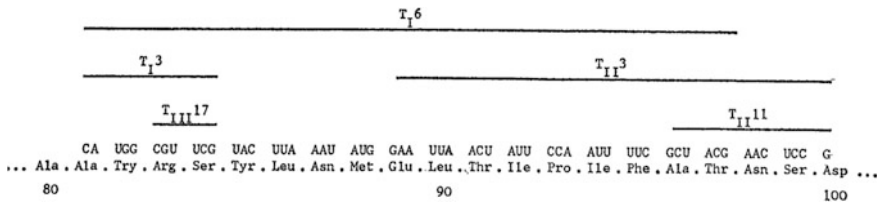


Fig. 5.7 Nucleotide sequence of the fragment from the coat protein cistron of R17 RNA, indicating partial digestion products and the corresponding amino acid sequence of the coat protein (Nature (1969) 223: 1009–1014)

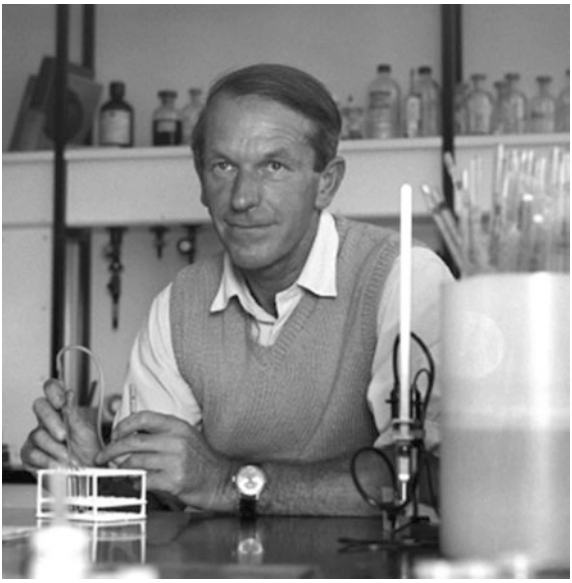


Fig. 5.8 Fred at LMB about 1969 (Copyright MRC Laboratory of Molecular Biology, with permission)

amino acids 89–95 of the R17 coat protein. Jerry Adams, a Harvard post-doctoral fellow working in conjunction with Fred, isolated fragments from partial T1 digests of R17 using polyacrylamide gel electrophoresis. Further analysis and enzyme digests of these fragments led to a 57-nucleotide sequence that corresponded to amino acids 81–99 of the coat protein (Fig. 5.7) [18]. This work was the first chemical confirmation of the genetic code from a messenger RNA. Fred's group did additional RNA sequencing, but by this time his interest had turned to DNA (Fig. 5.8).

References

1. Sanger F (1988) Sequences, sequences, and sequences. Ann Rev Biochem 57:1–28
2. Gordon Research Conferences. http://www.grc.org/about.aspx. Accessed 4 Aug 2015
3. Hoagland MB, Stephenson ML, Scott JF, Hecht LI, Zamecnik PC (1958) A soluble ribonucleic acid intermediate in protein synthesis. J Biol Chem 231:241–257
4. Zachau HG, Acs G, Lipmann F (1958) Isolation of adenosine amino acid esters from a ribonuclease digest of soluble, liver ribonucleic acid. Proc Natl Acad Sci USA 44:885–889
5. Marker K, Sanger F (1964) *N*-Formyl-methionyl-S-RNA. J Mol Biol 8:835–840
6. Clark BFC, Marcker KA (1966) The role of *N*-formyl-methionyl-S-RNA in protein biosynthesis. J Mol Biol 17:394–406
7. Barrell B (1999) Personal communication
8. Holley RW, Apgar J, Everett GA, Madison JT, Marquisee M, Merrill SH, Penswick JR, Zamir A (1965) Structure of a ribonucleic acid. Science 147:1462–1465
9. Nobel Media AB (2014) The Nobel prize in physiology or medicine 1968. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1968/. Accessed 7 Aug 2015
10. Sanger F, Brownlee GG, Barrell BG (1965) A two-dimensional fractionation procedure for radioactive nucleotides. J Mol Biol 14:373–398
11. Rosset R, Monier R (1963) A propos de la présence d'acide ribonucléique de faible poids moléculaire dans les ribosomes d'*Escherichia coli*. Biochim Biophys Acta 68:653–656
12. Sanger F, Brownlee GG (1967) Nucleotide sequences from the low molecular weight ribosomal RNA of *Escherichia coli*. J Mol Biol 23:337–353
13. Brownlee GG, Sanger F, Barrell BG (1968) The sequence of 5S ribosomal ribonucleic acid. J Mol Biol 34:379–412
14. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Scientific, Singapore, p 204
15. Judson HF (1971) Fred Sanger: lab session, 22 January. In Horace Freeland Judson collection, American Philosophical Society, Philadelphia, PA, USA
16. Barrell BG, Sanger F (1969) The sequence of Phenylalanine tRNA from *E. coli*. FEBS Lett 3:275–278
17. Brownlee GG, Sanger F (1969) Chromatography of ^{32}P-labelled oligonucleotides on thin layers of DEAE-cellulose. Eur J Biochem 11:395–399
18. Adams JM, Jeppesen PGN, Sanger F, Barrell BG (1969) Nucleotide sequence from the coat protein cistron of R17 bacteriophage RNA. Nature 223:1009–1014

Chapter 6
DNA Period 1969–1983

DNA sequence was Fred's ultimate goal. Up to this time, DNA sequencing seemed out of reach. No small DNAs were available to work out methods and there were no suitable enzymes for breaking DNA into more manageable fragments. Wu and Kaiser [1] first published a DNA sequence. They used bacteriophage λ, which, while double-stranded, has short single-stranded ends. In vivo, the complementary single-stranded ends allow λ to form a circle. Using *Escherichia coli* DNA polymerase, they measured the rate of incorporation of nucleotides into λ as the single-stranded ends are used as a template to make λ completely double-stranded. Burton and Petersen [2] had developed a method for depurinating DNA, leaving fragments with oligonucleotides containing only C and T. Working with Fred, Maria Szekely developed an in vitro labeling technique using polynucleotide kinase. She took depurination products from bacteriophage fd DNA and added ^{32}P-phosphate to their 5'ends. She then sequenced some of the fragments using the two-dimensional system worked out for RNA [3].

By the early 1970s, Fred had about 10 people in his group, many American postdoctoral fellows. In keeping with the pattern Chibnall established in the early days at the Cambridge Biochemistry Department, Fred had the postdocs working independently. If Fred were not involved hands-on in a project, he did not put his name on the paper. For example, Vic Ling developed an improved method for sequencing oligonucleotides, called the "wandering spot" method. He used it to sequence a 20-nucleotide fragment from depurinated fd DNA [4].

Fred's technician, Bart Barrell, had become quite an independent researcher, so Fred hired another technician, 20-year-old Alan Coulson.[1] Like Bart, Alan came without a university degree, although he did have a higher national diploma from Leicester Polytechnic. Alan (Fig. 6.1), reserved like Fred, was a change from the extroverted Bart. Fred commented [7],

[1]Alan published more papers with Fred that any other researcher. After Fred's retirement, Alan worked with John Sulston, who shared the 2002 Nobel Prize in Physiology or Medicine with Sydney Brenner and Robert Horvitz "for their discoveries concerning genetic regulation of organ development and programmed cell death" [5]. Based on his work with Sulston, Alan earned a Ph.D. from The Open University in 1994 [6].

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_6

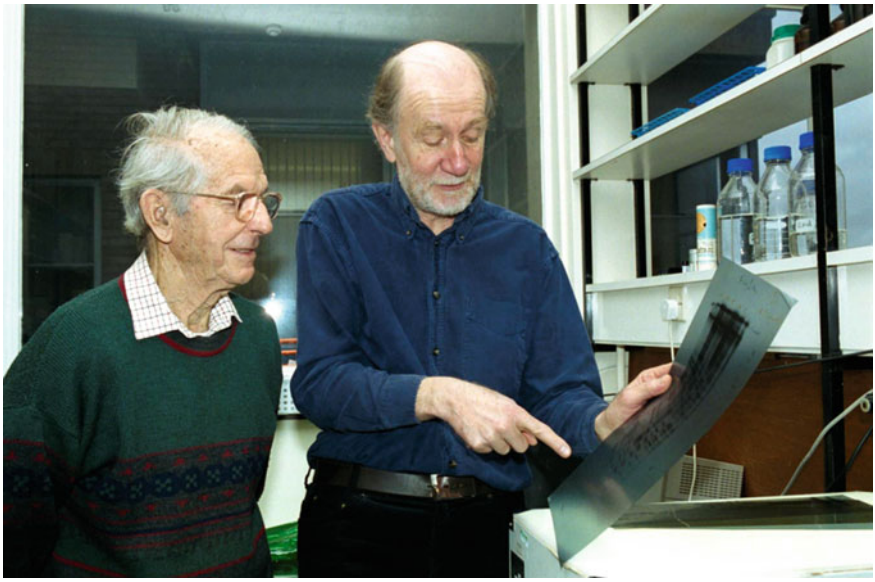


Fig. 6.1 Alan Coulson showing Fred an autoradiograph about 1980 (Copyright MRC Laboratory of Molecular Biology, with permission)

> Hitherto I had preferred working with more outgoing, extroverted types whose personalities were in marked contrast to my own. I suppose I admired them and found them to be stimulating. With Alan it was different; we were both quiet by nature. Perhaps as I became more mature I felt less need for external stimulation, though there were always plenty of noisy people around the lab.

Alan stayed with Fred until Fred's retirement in 1983.

Fred settled on bacteriophage ØX174 DNA because it was single-stranded and because postdoctoral fellow John Sedat knew how to grow the phage. A few DNA endonucleases were available. John Sedat, Francis Galibert, and Ed Ziff used endonuclease IV to produce a fragment of 48 nucleotides from ØX174. At the same time, Hugh Robertson, Bart Barrell, Lee Weith, and John Donelson generated a 50-nucleotide fragment by binding ØX174 DNA with ribosomes. Because the DNA was single-stranded, the ribosomes bound to a sequence corresponding to the mRNA initiation site. The bound ribosomes protected that site while the unprotected DNA was degraded by the nonspecific pancreatic DNAase. The race was on. Both groups were successful. Typical of Fred's humor, he said, "There was a rumor in the lab that there was to be a reward of a three-week holiday for the first person to sequence 50 residues of DNA. As there were ten of us we all took a weekend off" [8].

7Fred remembered Charles Weissman's group, in their studies of the RNA sequence of bacteriophage Qβ, had used a replicase to incorporate radioactive nucleotides in making complementary copies of Qβ RNA [9]. Fred wanted to use DNA polymerase to develop a similar DNA sequencing method. Unlike Qβ replicase, DNA polymerase requires a primer, and such a primer would have to be

synthesized. Khorana was pioneering DNA synthesis methods at MIT. Fred talked with Hans Kössel at a meeting and they had a similar interest in using an oligonucleotide as a primer to study bacteriophage f1 DNA. Kössel had worked with Khorana and knew the techniques. It took Kössel and his colleague Fisher a year to make the octanucleotide primer based on the known coat protein structure [8].

The other problem remained that of breaking DNA into smaller pieces. Berg, Fancher, and Chamberlin developed a ribosubstitution method, incorporating occasional ribonucleotides into a newly synthesized DNA strand [10]. Ribonuclease or alkali degraded those DNA molecules. The only breaks occurred where the ribonucleotides were incorporated (Fig. 6.2).

Fred, with John Donelson and Alan Coulson, used the octanucleotide primer, *E. coli* DNA polymerase, and the ribosubstitution method to synthesize radioactive ribosubstituted DNA strands, complementary to phage f1 DNA. While the octanucleotide primer did not bind to the expected coat protein position, it did bind at a consistent position, suitable for working out the method. Subsequent digestion of the product and separation of the oligonucleotides allowed sequencing using the wandering spot method. They determined a sequence of 81 nucleotides [11, 12].

To this point, Fred based his work on partial enzymatic hydrolysis. He broke large molecules down to smaller ones, separated the smaller ones, and broke the smaller ones down further, gradually giving rise to their sequences. He always looked for a simpler method. Englund [13] described a 3′-exonuclease action of bacteriophage T4 DNA polymerase wherein the presence of an excess of one nucleotide degraded DNA until it reached a residue the same as the nucleotide in excess. For example, if double-stranded DNA is incubated with T4 DNA polymerase in the presence of an excess of TTP (5′ thymidine triphosphate), the 3′ end of the DNA will be degraded until it reaches a T residue. At this point, removing T and putting T back on are in equilibrium, so degradation stops (Fig. 6.3).

This result inspired Fred. From earlier studies, Fred and Alan experimented with using the DNA primer and DNA polymerase to make as complex a mixture of newly synthesized ^{32}P-DNA as possible. The idea was to make every possible length of DNA chain starting at the primer and extending along the template (Fig. 6.4).

They cleaned up the product mixture and used different aliquots to try the T4 DNA polymerase reaction. In one aliquot the mixture was incubated with T4

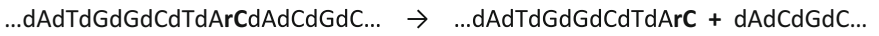


Fig. 6.2 Degradation of ribosubstituted DNA with ribonuclease A or alkali

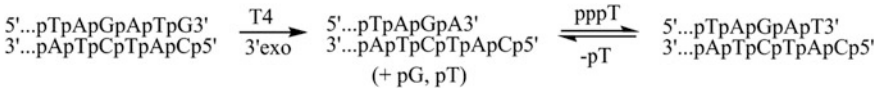


Fig. 6.3 T4 DNA polymerase exonuclease activity in the presence of excess TTP

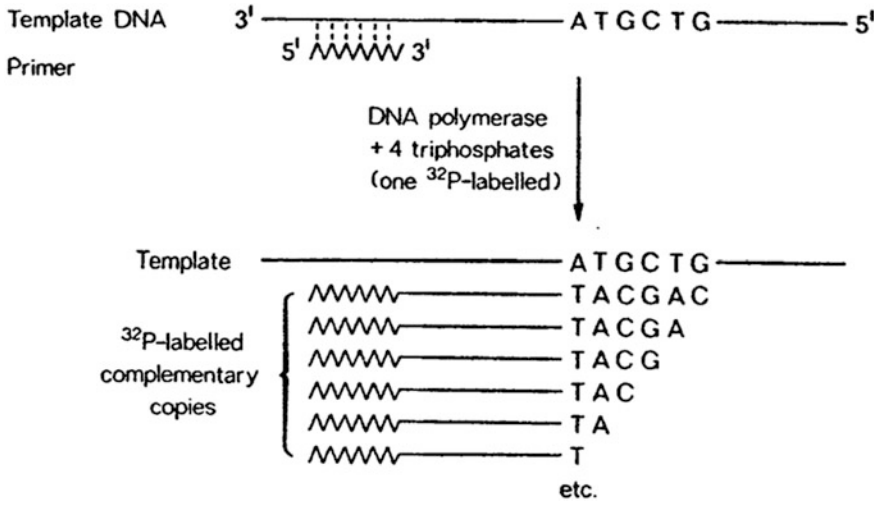


Fig. 6.4 Mixture of newly synthesized DNA products from primed template (J Mol Biol (1975) 94: 441–448, modified with permission)

polymerase and excess ATP, in the next with excess GTP, then with CTP, and finally with TTP. Theoretically, each reaction would produce a population of molecules ending in the residue present in excess. Because they added a nucleotide to the reaction, they called it the "plus" system (Fig. 6.5) [14].

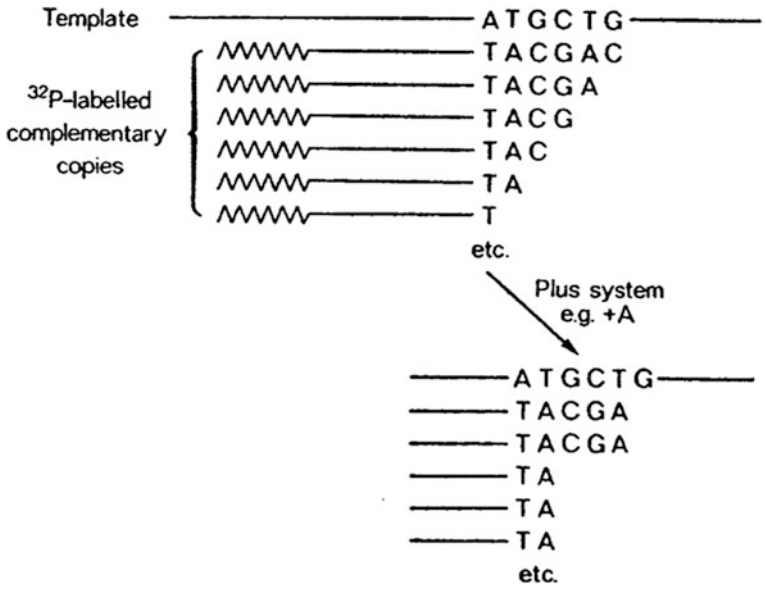


Fig. 6.5 Plus A reaction degrades each DNA molecule to the next A residue (J Mol Biol (1975) 94: 441–448, modified with permission)

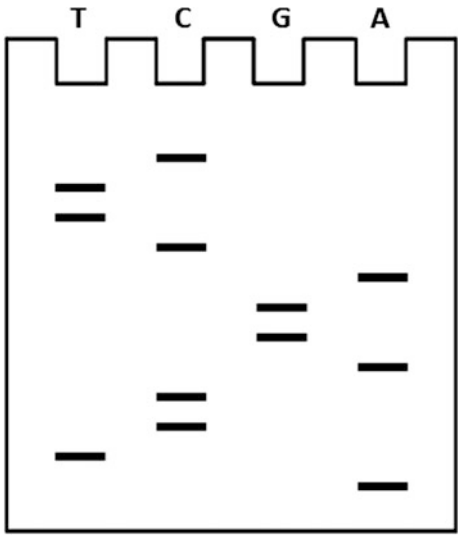


Fig. 6.6 Sketch of all four plus reactions run side by side on PAGE giving sequence ATCCAGGACTTC

They originally analyzed the resulting mixtures with the homochromatography system. John Donelson achieved good results using electrophoresis on polyacrylamide gels (PAGE), so they tried them too. Gradually they found using longer polyacrylamide gels under denaturing conditions (8M urea) and high voltage (heat) allowed them to separate DNA fragments that differed by only one nucleotide in chain length. They loaded the +C, +T, +A, and +G reactions side by side on the same gel. After PAGE, a generated autoradiograph allowed them to read the sequence from the bottom of the gel up because the smallest fragments traveled faster through the gels (Fig. 6.6).

Some bands did not show up where they should; phantom bands also appeared. For a confirmation system, they developed a "minus" system based on the work of Wu and Kaiser [1]. Fred and Alan started with the same product mixture from the DNA polymerase reaction. Again they used four aliquots. When they reinitiated the DNA polymerase reaction without one of the four nucleotides, the DNA molecules would extend until needing the missing nucleotide. The –A reaction did the extension without ATP, so the DNA chain grew until an A was needed. Then it stopped (Fig. 6.7). Similar reactions were done with –C, –T, and –G. The four reactions were run on PAGE and autoradiographed. The minus system also had some faults, so they did all eight fractions, four plus reactions, and four minus reactions, and ran them on the same gel (Fig. 6.8). The minus fragments were, of course, one nucleotide shorter than the plus fragments [14]. Fred said, "I consider this to be my most important paper because it describes an entirely new approach to sequencing" [8].

The lab had already begun sequencing ØX174 DNA using various methods as they developed. A host of researchers in the lab contributed. Restriction enzymes became available, providing tools for cutting DNA at particular sequences. Fred's

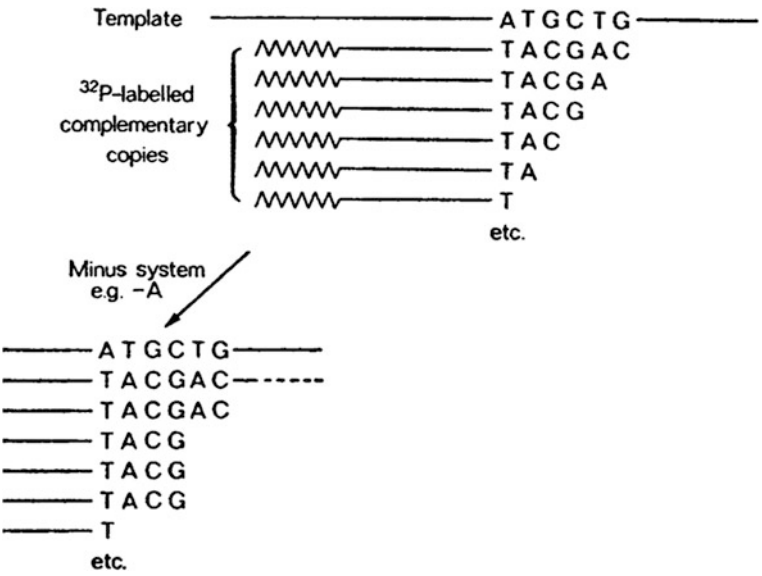


Fig. 6.7 Minus A reaction extends each DNA molecule to where the next A residue is needed (J Mol Biol (1975) 94: 441–448, modified with permission)

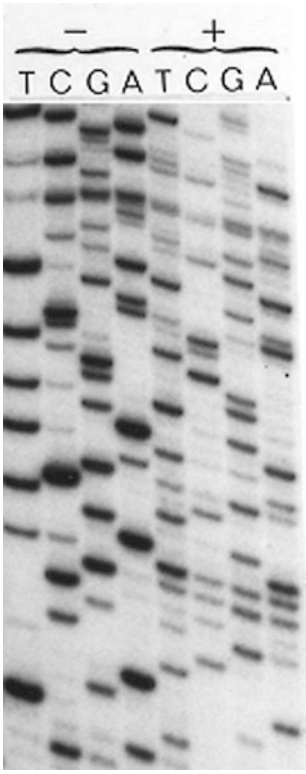


Fig. 6.8 Minus reactions and plus reactions run side by side (Courtesy Andrew J.H. Smith, 1980 Ph.D. dissertation, University of Cambridge, modified with permission)

group used Hind II[2] to cut ØX174 DNA into 13 fragments. A series of papers revealing parts of the ØX174 DNA sequence culminated in the 1977 publication of the entire 5400 base pair sequence [15].

A big surprise came from the sequence of ØX174: it had overlapping genes. The one gene–one enzyme hypothesis of Beadle and Tatum [16] asserted that a region of DNA coded for one protein only. There was not enough DNA, however, to code for the number of proteins produced by ØX174. Fred's group found gene E within the coding region of gene D; it used a different reading frame [17]. Similarly, they found an overlap with genes A and B [18].

The plus and minus system outperformed other techniques, but it still had problems. The band strength varied and some bands did not show up. Fred was inspired by Arthur Kornberg's work showing dideoxythymidine triphosphate (ddTTP) was a substrate for DNA polymerase [19]. Where ddTTP added to a DNA chain, synthesis stopped because there was no hydroxyl group at the end onto which the next nucleotide could add (Fig. 6.9).

Kornberg had no more ddTTP to offer Fred. Fred met Klaus Geider at a meeting in Germany, and Klaus gave Fred some to try. Fred said, "The first experiment we did with it gave a beautiful autoradiograph with sharp bands of equal intensities extending over a long sequence" [7]. Fred and Alan set out to make the ddNTPs. During one work up, Fred dropped the flask containing the product. Disgusted with himself, he went home for the day. Alan swept it up, recovered and purified it [20]. The four syntheses took a year, but they were successful.

At the same time Steve Nicklen was studying the use of arabinonucleotides, which also served as chain terminators. They were better than the plus and minus system, but not as good as the ddNTPs [21]. The use of very thin polyacrylamide gels improved the results markedly [22].

Another advantage the dideoxy method had over plus and minus was that the initial reactions were useful without cleanup and further reaction. They used four aliquots of template and primer, each with all four dNTPs, one ddNTP, some ^{32}P-dCTP, and DNA polymerase. They loaded each completed reaction on a gel (Fig. 6.10) [23].

They selected the loading pattern, so A and T lanes were on the outside, G and C lanes on the inside. When they exposed the photographic film to the radioactive gel, they could read the autoradiograph from either side. If they produced a new copy of the noncoding strand, they merely flipped the film over to read the sequence of the coding strand. TCGA became AGCT.

Maxam and Gilbert [24] developed a chemical method for DNA sequencing, also using polyacrylamide gel electrophoresis. They used double-stranded DNA, replacing the 5′ phosphate with ^{32}P. They used restriction enzymes to cut the DNA and then separated the two parts. Because only the radioactive DNA exposed the film, they had no need to separate the DNA strands. This method of generating DNA fragments differed from Fred's group, but the gel methods were similar.

[2]Hind II cuts DNA at sequence GTPyPuAC, where Py = C or T and Pu = G or A.

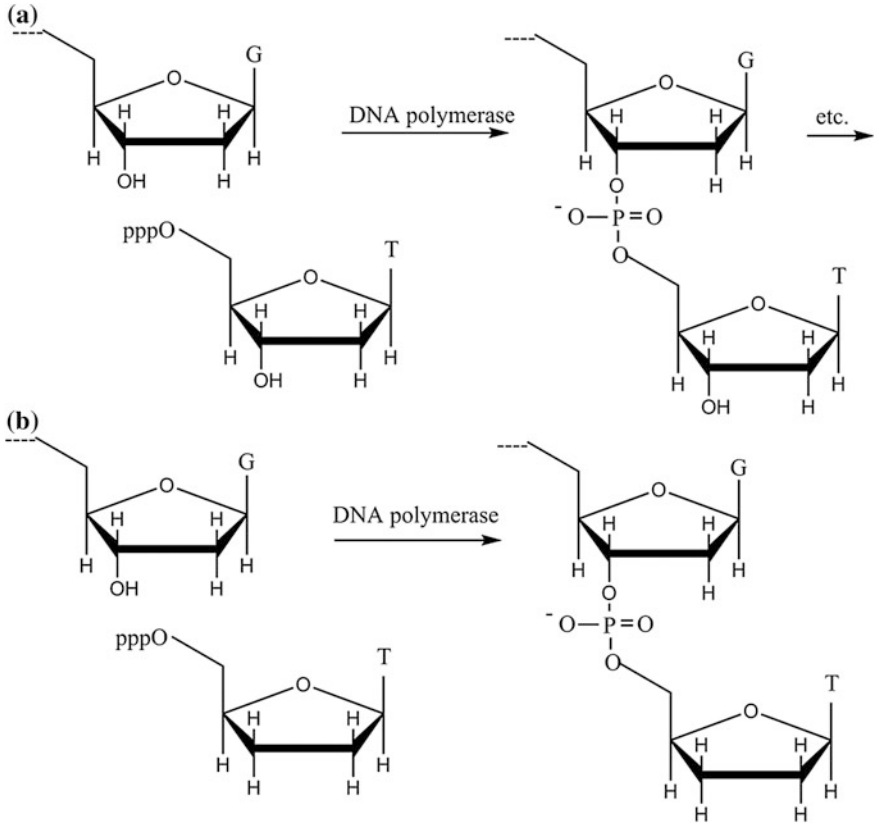


Fig. 6.9 **a** dTTP adds to 3′ end of DNA chain; new end has OH group to accept the next dNTP. **b** ddTTP adds to 3′ end of DNA chain; new end has no OH group to accept another dNTP

Members of Gilbert's lab had visited Fred's lab during the plus and minus method sequencing. Gilbert presented the chemical method at a Gordon Conference before they published the paper. As Fred put it, "It is interesting that although they used many of our techniques they have carefully avoided giving any references to our work" [25]. Fred was not happy about that slight.

Fred's group used the dideoxy method to check the ØX174 sequence because there were several regions of uncertainty. They made several revisions [26].

The need for single-stranded DNA limited the dideoxy method. ØX174 has single-stranded DNA, so it was an easy choice. Most DNAs are double-stranded. Gronenborn and Messing solved the problem [27]. They generated a version of M13 bacteriophage (M13mp2), which has a single-stranded DNA. It has a restriction enzyme site in its double-stranded replicative form. The restriction site is in the middle of a β-galactosidase gene, also added to M13. If no extra DNA is added to the virus at this site, it grows on special agar producing blue plaques. If extra DNA is added, it interferes with the β-galactosidase production producing

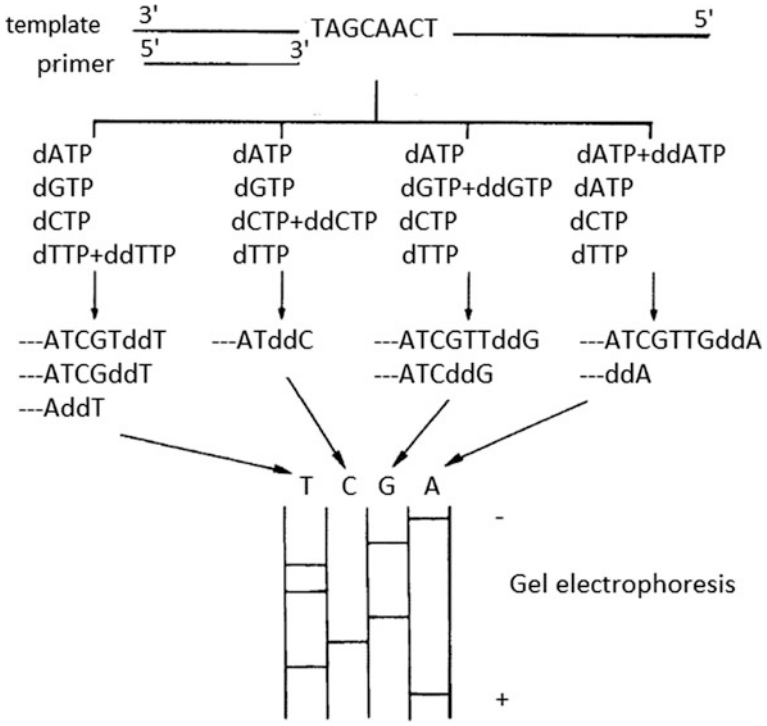


Fig. 6.10 Four reactions of the dideoxy method (Courtesy Andrew J.H. Smith, 1980 Ph.D. dissertation, University of Cambridge, modified with permission)

white plaques. White plaques, then, gave a source of M13 that included the DNA of interest. The mature viral DNA is single-stranded. They made a primer that binds to the M13 sequence just upstream from the inserted DNA, so this cloning procedure was perfect for the dideoxy method (Fig. 6.11).

Fred and his group used the M13 system to begin studies of human mitochondrial DNA and had some surprising finds [28]. First, the genetic code, thought to be universal, differs in human mitochondria. UGA is a tryptophan codon, not a termination codon. AUA codes for methionine rather than isoleucine. Also the tRNA sequences in the D loop and TψC loop differ from all other known sequences [29]. They found only 23 tRNA genes in the mitochondrial genome, suggesting a need for fewer tRNAs to recognize mitochondrial codons than the 31 needed for the universal code wobble hypothesis [30]. They sequenced human mitochondrial DNA [31], a result Fred proudly regarded as the "start of the human genome project" [32].

John Walker had the task of going to a maternity home in Cambridge to collect placentas from which they isolated the human mitochondria. He joined the expectant fathers in the waiting room, distinguished only by his holding an ice

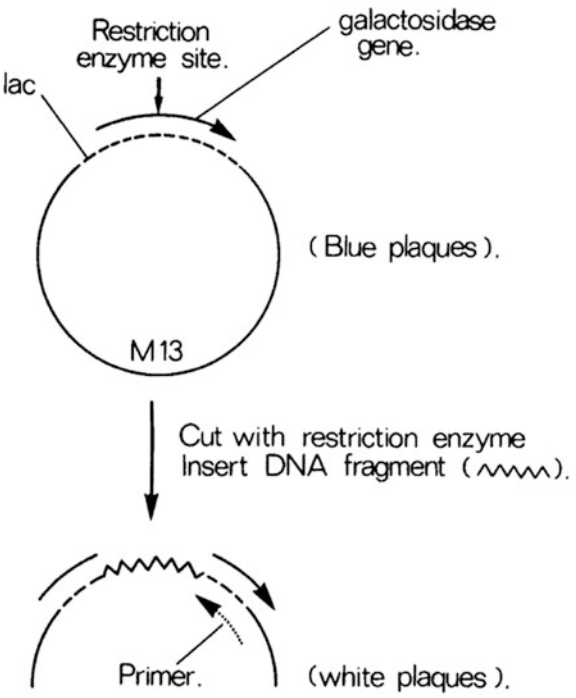


Fig. 6.11 M13mp2 with restriction sites in β-galactosidase gene (Courtesy *Selected Papers of Frederick Sanger* (*with commentaries*), Sanger F, Dowding M (eds). Copyright 1996, World Scientific Publishing, Singapore, with permission)

bucket. Delays in getting the placentas meant that they were often too degraded to be useful. A switch to caesarian placentas yielded much better results [33].

John joined Fred's group as a postdoctoral fellow in 1974. As a protein chemist, his task was to do protein sequences while the others did DNA. John had been a postdoctoral fellow at the Pasteur Institute in Paris. After unethical treatment by his department chair, John happened to attend a protein chemistry workshop at Cambridge. At the end of the meeting a dinner was held at St. John's College. As John tells the story [33],

> I was seated next to this little fellow who I didn't know who the hell he was. I introduced myself, and he said, 'I'm Fred Sanger.' And I said, 'Really, I thought you were dead.' We laughed and he said 'Why?' I said, 'When I was a student at Oxford, I read chemistry. In the final examination there was a question, 'Describe the Grignard reagent, the Perkin reaction, and the Sanger reagent.' Naturally, I thought that all of these people had to be dead if I was being asked a question of this kind. Fred thought this was quite amusing.

They talked science for the rest of the evening. At the end, Fred asked John if he had thought about coming back to England. Within two weeks he was back.

Fred was home for lunch when he received the call telling him that he was a corecipient of the 1980 Nobel Prize in Chemistry. Half of the prize went to Paul Berg "for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA." Fred and Walter Gilbert shared the other half "for their contributions concerning the determination of base sequences in nucleic

Fig. 6.12 Champagne party at the LMB after the announcement of the 1980 Nobel Prize (Copyright MRC Laboratory of Molecular Biology, with permission)

acids" [34]. Fred became only the fourth two-time recipient of a Nobel Prize. Marie Curie won for Physics in 1903 and Chemistry in 1911; Linus Pauling won for Chemistry in 1954 and Peace in 1962; and John Bardeen won for Physics in 1956 and Physics in 1972.

It was party time at the Laboratory of Molecular Biology. Champagne all around (Fig. 6.12). The family, except for Peter, joined Fred in Stockholm for the celebration. Fred received the Nobel Medal from the King of Sweden (Fig. 6.13). The medal is shown in Fig. 6.14.

The last major sequencing project Fred undertook was bacteriophage λ. Other labs determined some 6000 of the 48,502 base pairs. Fred and his coworkers used shotgun cloning with M13mp2 to generate workable fragments. Because the human mitochondria DNA studies required special containment facilities, Fred and Alan Coulson started working on the λ sequence while the containment facilities were being equipped [32]. After finishing the mitochondrial sequences, they returned to the λ project, completing it in 1982 [35].

Fred retired in 1983 at age 65. On the day of his retirement, John Walker collected Fred from his lab at 4:00 for the awaiting party in the LMB canteen. John said [33],

> Fred was still fiddling away with bits of tubing and green plasticine and I said, 'Come on, Fred, it's your party.' He just put it down. And that was the end. He never touched anything after that. Quite dramatic actually.

And that was that. He began his career at the bench; he ended it there.

Fig. 6.13 Fred receiving the 1980 Nobel Prize in chemistry (Courtesy Fred Sanger, with permission)

Fig. 6.14 Fred Sanger's 1980 Nobel Medal (photos by author. Courtesy Sally Sanger, with permission)

References

1. Wu R, Kaiser AD (1968) Structure and base sequence in the cohesive ends of bacteriophage lambda DNA. J Mol Biol 35:523–537
2. Burton K, Petersen GB (1960) The frequencies of certain sequences of nucleotides in deoxyribonucleic acid. Biochem J 75:17–27
3. Székely M, Sanger F (1969) Use of polynucleotide kinase in fingerprinting non-radioactive nucleic acids. J Mol Biol 43:607–617
4. Ling V (1972) Fractionation and sequences of the large pyrimidine oligonucleotides from bacteriophage fd DNA. J Mol Biol 64:87–102
5. Nobel Media AB (2014) The Nobel prize in physiology or medicine 2002. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2002/. Accessed 7 Jan 2016
6. Coulson A (1999) Personal communication
7. Sanger F (1988) Sequences, sequences, and sequences. Ann Rev Biochem 57:1–28
8. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Sci, Singapore, pp 338–339
9. Billeter MA, Dahlberg JE, Goodman HM, Hindley J, Weissmann C (1969) Sequence of the first 175 nucleotides from the 5′ terminus of Qβ RNA synthesized in vitro. Nature 224:1083–1086
10. Berg P, Fancher H, Chamberlin M (1963) In: Vogel HJ, Bryson V, Lampen JO (eds) Symposium on informational macromolecules. Academic Press, New York and London, pp 467–483
11. Sanger F, Donelson JE, Coulson AR, Kösse H, Fischer D (1973) Use of DNA polymerase I primed by a synthetic oligonucleotide to determine a nucleotide sequence in phage f1 DNA. Proc Natl Acad Sci USA 70:1209–1213
12. Sanger F, Donelson JE, Coulson AR, Kössel H, Fischer D (1974) Determination of a nucleotide sequence in bacteriophage f1 DNA by primed synthesis with DNA polymerase. J Mol Biol 90:315–333
13. Englund PT (1971) Analysis of nucleotide sequences at 3′ Termini of duplex deoxyribonucleic acid with the use of the T4 deoxyribonucleic acid polymerase. J Biol Chem 246:3269–3276
14. Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 94:441–448
15. Sanger F, Air GM, Barrell BG, Brown NL, Coulson AR, Fiddes JC, Hutchison CA III, Slocombe PM, Smith M (1977) Nucleotide sequence of bacteriophage φX174 DNA. Nature 265:687–695
16. Beadle GW, Tatum EL (1941) genetic control of biochemical reactions in neurospora. Proc Natl Acad Sci USA 27:499–505
17. Barrell BG, Air GM, Hutchison CA III (1976) Overlapping genes in bacteriophage φX174. Nature 264:34–41
18. Smith M, Brown NL, Air GM, Barrell BG, Coulson AR, Hutchison CA III, Sanger F (1977) DNA sequence at the C termini of the overlapping genes A and B in bacteriophage φX174. Nature 265:702–705
19. Atkinson MR, Deutscher MP, Kornberg A, Russell AF, Moffatt JG (1969) Enzymatic synthesis of deoxyribonucleic acid. XXXIV. Termination of chain growth by a 2′,3′-dideoxyribonucleotide. Biochemistry 8:4897–4904
20. Sanger F (2000) Personal communication
21. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467
22. Sanger F, Coulson AR (1978) The use of thin acrylamide gels for DNA sequencing. FEBS Lett 87:107–110

23. Nobel Media AB (2014) Frederick Sanger—Nobel lecture: determination of nucleotide Sequences in DNA. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/sanger-lecture.html. Accessed 12 Jan 2016
24. Maxam AM, Gilbert W (1977) A new method for sequencing DNA. Proc Natl Acad Sci USA 74:560–564
25. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Sci, Singapore, p 344
26. Sanger F, Coulson AR, Friedmann T, Air GM, Barrell BG, Brown NL, Fiddes JC, Hutchison CA III, Slocombe PM, Smith M (1978) The nucleotide sequence of bacteriophage φX174. J Mol Biol 125:225–246
27. Gronenborn B, Messing J (1978) Methylation of single-stranded DNA in vitro introduces new restriction endonuclease cleavage sites. Nature 272:375–377
28. Sanger F, Coulson AR, Barrell BG, Smith AJH, Roe BA (1980) Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J Mol Biol 143:161–178
29. Barrell BG, Bankier AT, Drouin J (1979) A different genetic code in human mitochondria. Nature 282:189–194
30. Barrell BG, Anderson S, Bankier AT, de Bruijn MHL, Chen E, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1980) Different pattern of codon recognition by mammalian mitochondrial tRNAs. Proc Natl Acad Sci USA 77:3164–3166
31. Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465
32. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Scientific, Singapore, pp 346–347
33. Walker J (2003) Personal communication
34. Nobel Media AB (2014) The Nobel prize in chemistry 1980. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/. Accessed 15 Jan 2016
35. Sanger F, Coulson AR, Hong GF, Hill DF, Petersen GB (1982) Nucleotide sequence of bacteriophage λ DNA. J Mol Biol 162:729–773

Chapter 7
Life Outside the Lab

Fred and Joan rented a flat on the first floor at 252 Hills Road in Cambridge (Fig. 7.1). The war was on, so rationing was in place and certain foods, especially meat, were scarce. Imports were not available. They grew some vegetables in the back garden. Joan was social and enjoyed chatting with people. She developed a special rapport with the butcher, so if meat was available, she could usually get it. They had a few chickens in the garden. Young chickens produced eggs; old chickens produced dinner [1].

Before the children came along, Joan worked part-time at a language school. Fred biked back and forth to the biochemistry building on Tennis Court Road. They still had the car Fred had kept outside Cambridge during his student days. The old Jowlett Fred had bought for £8 finally died, and they had to push it the last mile to the junk yard. They replaced it with a Morris Octavia Fred got from his cousin. Joan had a good friend, Doret Sergeant, whose husband John was a sculptor. Fred traded the Morris 8 to John for a garden sculpture (Fig. 7.2), which Fred kept for the rest of his life.

Robin was born in 1943 in the flat at 252. Robin was a difficult baby, so Joan went to Leicester to stay with her mother for a time [2]. Fred had a punt, so punting on the Cam was common. Robin related his first memory from punting (Fig. 7.3). He fell off the punt as a tyke. He could not swim, but somehow managed to grab a trailing rope. Fred jumped into the river trying to find him. Meanwhile, Joan pulled Robin back into the punt. Fred surfaced in a panic because he could not find Robin, and there Robin sat, wet, but otherwise okay [1, 3].

Peter was born in 1945. He was a much easier baby, but rationing, which lasted well after the end of the war, took its toll, especially with Peter's teeth. They hired Mrs. Patterson as a helper. Over time, Fred and Joan rented the upper floors too and eventually bought the house. At this point, Mrs. Patterson and her daughter moved into the house on the third level, so Fred and Joan had help whenever they needed it. Around 1960, Mrs. Patterson's daughter married and moved to America; Mrs. Patterson followed [4].

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_7

Fig. 7.1 252 Hills Road in Cambridge, on right (photo by author)

Fig. 7.2 Fred's garden sculpture (sculpture is shown here at Far Leys, Swaffam Bulbeck, Fred's home in retirement) (photo by author)

Fred and Joan went to the theater often. They liked the cinema too, particularly comedies—Marx Brothers, Charlie Chaplin, Jacques Tati, and Dean Martin and Jerry Lewis. They also enjoyed hikes in the parks and woods, bird-watching, gathering conkers,[1] camping (sometimes in the back garden), and collecting blue bells. They read to the boys and played games like Monopoly® and canasta. At Christmas, Fred hid the presents and then set the boys on a treasure hunt with clues

[1]Horse Chestnut tree seeds.

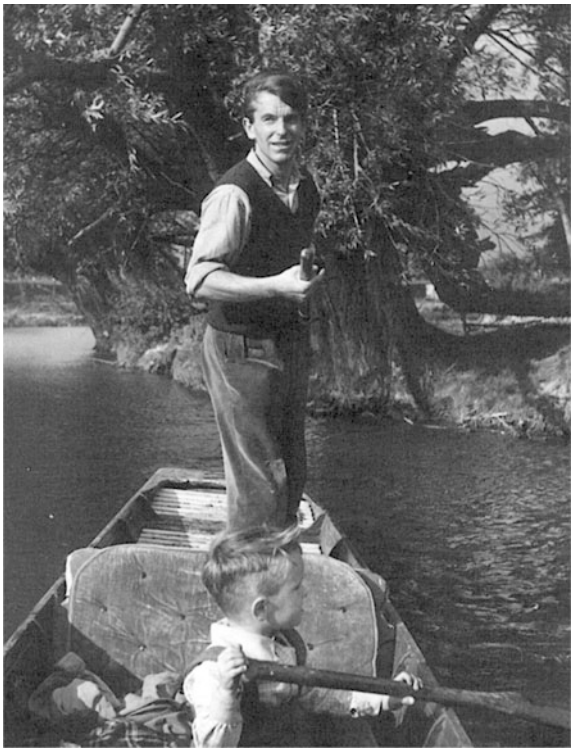


Fig. 7.3 Fred and Robin punting on the Cam 1945 (Courtesy Fred Sanger, with permission)

to find them. Ever working with his hands, Fred built a tree house for the boys. He also built a ping pong table and a layout for electric trains in the attic [3, 5, 6].

They kept a tortoise named Charlie in the garden for more than 10 years. On one of their walks, they found two barn owls. Fred made a frame by the apple tree for them. He brought home post-experimental lab rats for food. Peter once stood out by Hills Road with an owl on each shoulder, watching people's expressions as they drove by.

Family travel at holidays usually meant the beach or the Channel Islands. Fred occasionally took the family with him to professional conferences. The 1958 Nobel Prize opened up many opportunities for travel. In 1959 the family went to America, sailing on the SS Liberte to New York (Fig. 7.4). Seeing the Statue of Liberty was especially exciting. A US Customs official, noticing Robin liked to read, asked him if his reading included *Lady Chatterley's Lover*, an odd question to a 16-year-old. The book was banned in America, and 1959 was the year Grove Press was testing obscenity laws by publishing it, so it was in the news [7]. Then they flew to Chicago, and from there took a train to San Francisco. They came back through Las Vegas, Salt Lake City, the Grand Canyon, Madison, and on to Boston. With the excitement of the Nobel ceremonies the year before and now America, the boys

Fig. 7.4 SS Liberte (Courtesy cruiselinehistory.com, with permission)

were thrilled. The boys flew home from Boston while Fred and Joan continued around the world [3, 6].

Sally was born in 1960. By this time Robin was off to Bryanston, the same secondary school Fred had attended. Peter followed two years later. Mrs. Gifford became the new nanny for Sally. Fred, ever the family man, planned family activities to coincide with school holidays. Fred especially liked to ski, so there were trips to Austria and Switzerland for skiing. Squash and tennis were also fun. As Sally got older, she and Fred went on many bird-watching walks. When finding a No Trespassing sign, Fred went right over the fence—"always flaunting protocol" [8].

Because they were so private, Fred and Joan had limited family friendships with Fred's coworkers from the lab. Rodney Porter, Fred's first graduate student, and his wife Julia were exceptions. They developed a friendship that lasted until Rodney's untimely death in a 1985 car crash. Herbert "Freddie" Gutfreund (Fig. 7.5) worked in the biochemistry building as a physical chemist. He became close friends with the Sangers. As a bachelor, Freddie was like an extra member of the family, often spending time with Fred, Joan, and the boys [9].[2] Fred and Joan were quiet family types and did not go to many social gatherings. As Freddie put it, "I never saw Fred at a biochemistry social function. If he came to a reception, he was dragged there."

Another graduate student, Ted Thompson (Fig. 7.6), and his wife Adrienne were also frequent visitors to 252. The Australians had a way of bringing out the kid in Fred. Ted was the first of these Aussies. Surprisingly, there were a few language

[2]Fred would later be best man in Freddie's wedding and godfather to Freddie's first child.

Fig. 7.5 Freddie Gutfreund 1953 (Courtesy CSHL archives, modified with permission)

barriers. Fred and Joan invited Ted and Adrienne over for "supper." Joan had prepared a large joint of meat. Ted signaled that they might run a bit late because Ted had to have his "tea" first, so they arrived at the Sanger's having already had a large meal [10]. Ted and Adrienne also managed to get Fred and Joan to join them twice for the college's May Ball, all-night affairs ending with a punt trip up the Cam for breakfast [11].

César Milstein and his wife Celia were also close friends. César worked with Fred for part of this Ph.D. dissertation, but then returned to his native Argentina. Political turmoil there led César to return to Cambridge, by this time to the Laboratory of Molecular Biology (Fig. 7.7). Fred and César became great friends, a friendship that lasted until César's death in 2002.

Boats played a major part in Fred's life outside of lab. In addition to the punt, Fred bought a water scooter at the Earl's Court boat show. Peter grew interested in boats, so Fred and Peter made a canoe. Fred rented cabin cruisers at holidays, cruising on the nearby rivers and onto the wash near King's Lynn. Then he bought one. He and Peter built a runabout they could use for water skiing. He also bought a two-mast converted lifeboat for sailing and outfitted it to suit himself. He kept the

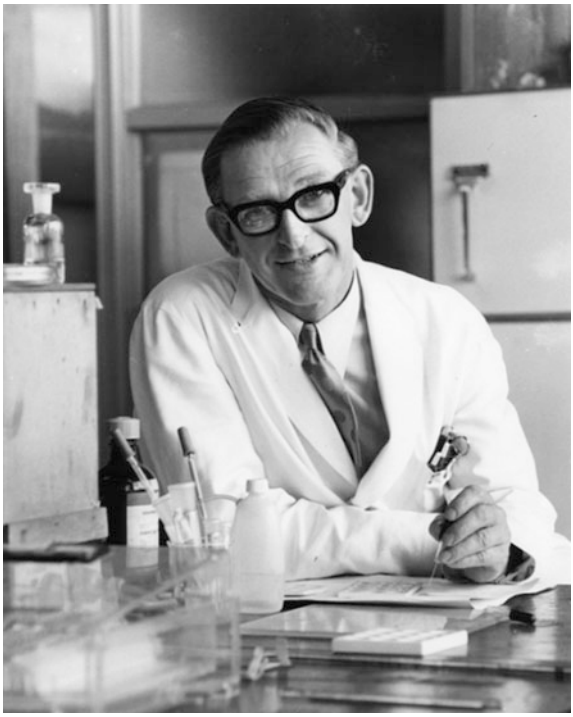


Fig. 7.6 Ted Thompson 1975 (Photo Val Sowada. Courtesy UNSW archives, with permission)

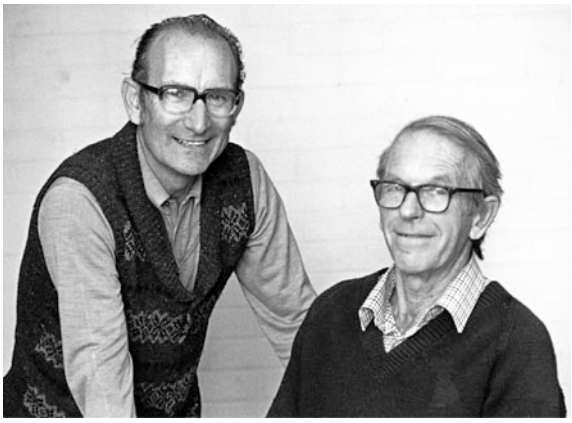


Fig. 7.7 César Milstein with Fred Sanger 1980 (Courtesy Fred Sanger, with permission)

cabin cruiser on the River Ouse near Cambridge; he kept the sail boat on the river at Aldeburgh and, during the summer, at Blakeney. He named his cabin cruisers "Regnas" (Fig. 7.8, Look at the name carefully) [5, 12]. He also named his second sail boat "El Frederico".

Boat stories abound. They would often take a boat on the north shoal mud flats of East Anglia. When the tide went out, the boat would sit on the mud, and they

Fig. 7.8 Fred and grandchildren on Regnas 1981 (Courtesy Peter Sanger, with permission)

would collect cockles.[3] On one occasion Fred and Robin sat the boat on a mud flat and went ashore. The water started rushing in. As they hastened to get back to the boat, the mud was particularly soft and they had to be careful to not bog down. It was quite dangerous. As Robin put it, "We were both light. If we had been heavy, we would have been sucked into the mud and it would have been dodgy." Once, sailing off the coast at Aldeburgh, rip currents caught Fred and Robin where a strong wind coming down from the North Sea and the tide coming out and up from the Thames estuary had them in rough water unable to go anywhere. The lighthouse at Aldeburgh signaled dangerous rocks. They spent the entire night taking turns at the watch. Finally the tide changed, and they pulled into an estuary and had breakfast [3]. Another time, when Sally was small, they put her play pen on top of the cruiser and were negotiating the channels of the mud flats when they hit a rock. Supplies went overboard, but Sally's play pen stayed on [5]. Fortunately, most of the time it was smooth sailing.

Boating was not limited to family. César was Fred's "first mate" on frequent sailing ventures. The Porters occasionally joined them. César teased Fred that he would never cross the English Channel. Fred, César, and Rod Porter set sail one day to make the crossing. A bit of fishing and some unfavorable tides ran them late, so they never made that crossing [13]. Fred, Joan, César, and Celia went sailing in the Greek Islands. Their boat was one of a group with an experienced Greek sailor in the lead boat. Fred was the main sailor; César did the cooking [12].

[3]Salt-water clams.

When Ted Thompson visited Cambridge, he also joined in. Fred, Ted, and César were out in the runabout on an estuary at night. The estuary was loaded with phosphorescent bacteria. When they urinated into the water, they saw flashes of light. They got the bright idea to see what it was like to water ski at night. Ted was driving while Fred and César skied. Fred yelled "port" and Ted went "starboard." César ended up thigh-deep in mud in the shallow estuary. They decided it was a stupid idea and went back and drank some rum [14].

Other than travels associated with lectures, the family often took holidays to Sark, a Channel Island, and to the Canary Islands. Because Sally was so much younger than Robin and Peter, she was much like an only child with two older brothers. Once the boys were grown and gone, invited lectures took Fred, often Joan, and sometimes Sally on many trips. Fred particularly liked the deserts of southern California (Fig. 7.9).

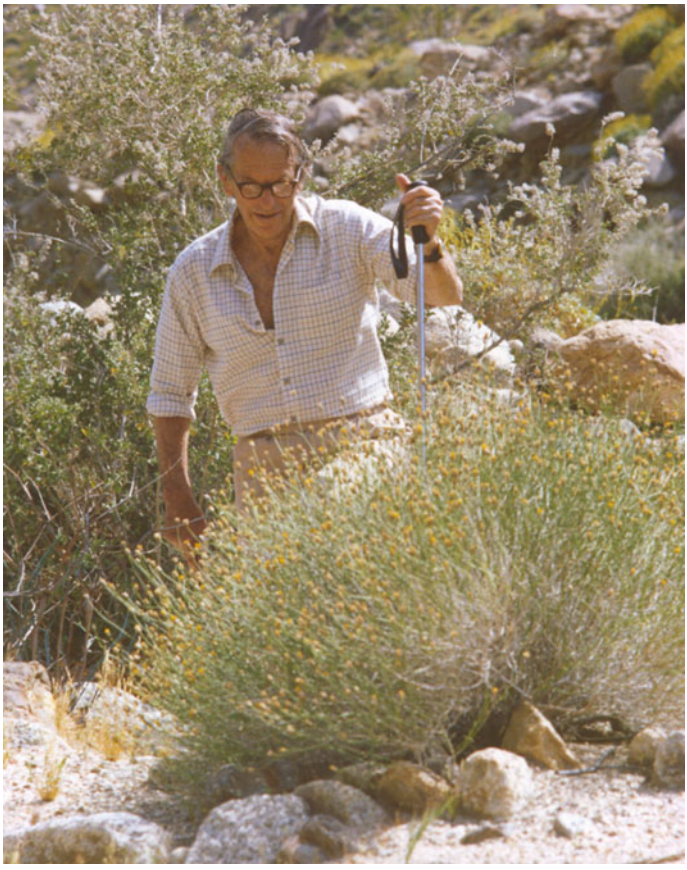


Fig. 7.9 Fred hiking in California desert 1981 (Courtesy of Dr. Theodore Friedmann, with permission)

Fred generally did not take work home. Because he lived close to lab, he often went back after supper or even after the cinema. He liked to work when no one else was around. Only on rare occasions did the children come to the lab. Fred's secretary of long standing, Margaret "Peggy" Dowding, only recalled two times Joan called the lab needing Fred's attention at home. On one of those occasions, an injured kestrel was in the back garden. Fred hurried home to tend to it. On the other, Fred had poured some cement. Joan rang up and said, "Would you tell Fred that a naughty little girl has walked on his cement?" Of course, he had to go and quickly repair that [15].

Fred took only two short sabbaticals, both to Australia. The first was to Sydney, set up by Geoff Grigg and Ted Thompson. The second was to Adelaide, set up by Bob Symons. Sally was still school age when they went to Sydney, so she went to school there. They lived in a house overlooking Sydney Harbor and The Heads (Fig. 7.10). At the end of the sabbatical, folks at the lab asked how he liked the house. He replied that he liked it, that he went for a swim every morning. They were aghast. Sydney Harbor was teeming with sharks [16]. The Sangers toured the world on that trip, making many stops for lectures. Fred and Sally took advantage and did much snorkeling [8]. Fred also attended some conferences in Australia. After a keynote address in Sydney, a dinner-dance followed. Ted had arranged for his assistant, Wendy, to dance with Fred. While dancing, Wendy called him Professor. Fred corrected her and said he was not a professor. Wendy replied, "Never made it, huh?" [11].

Fig. 7.10 View of Sydney Harbor and The Heads from Fred's house (photo by author)

Fig. 7.11 Far Leys in Swaffam Bulbeck (photo by author)

When Fred retired, he sold the house on Hills Road and moved to Swaffam Bulbeck, not far from Cambridge. He named the place Far Leys (Fig. 7.11), just like his boyhood home in Tanworth-in-Arden. There was room for a large garden (Fig. 7.12), his other passion besides boating. Joan died in 2012. Fred had Alzheimer's. Family and live-in care providers looked after Fred until his death from pneumonia November 19, 2013, at Addenbrooke's Hospital in Cambridge, fittingly, immediately adjacent to the MRC Laboratory of Molecular Biology [8, 18].

Robin (Fig. 7.13) studied law at the University of Leeds and worked for a bank and trust company for 26 years. He lives in Yorkshire, where he and his wife Brenda are active in amateur theatricals [3].

Peter (Fig. 7.14) studied sociology at the College at Preston and worked as a civil servant at the Inland Revenue Office (now Her Majesty's Revenue and Customs) in Manchester until his retirement. He now lives in Lancashire. Peter has two sons, Ben and David. He also has a grandson named Fred Sanger, born in 2014 [5, 17].

Sally (Fig. 7.15) studied English at Girton College, Cambridge; earned an M.A. in Medieval English from Bristol University; and began Ph.D. studies in literature at the University of Manchester. She had a change of plans and went to the University of Sheffield, where she earned an M.A. in Information Studies. She worked in the health services industry until 2013. She then returned to academia gaining an M.Sc. in Health Informatics from University College London and is now studying for a Ph.D. in Health Informatics at the University of Sheffield [8, 18].

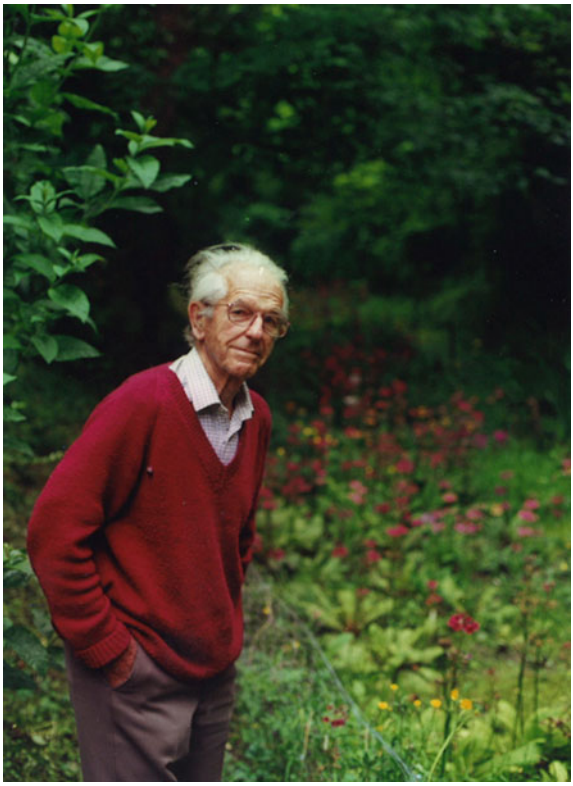


Fig. 7.12 Fred Sanger in garden at Far Leys (photo by author. Courtesy Fred Sanger, with permission)

Fig. 7.13 Robin Sanger about 2000 (Courtesy Robin Sanger, with permission)

Fig. 7.14 Peter Sanger in 2000 (photo by author. Courtesy Peter Sanger, with permission)

Fig. 7.15 Sally Sanger in 2014 (photo by author. Courtesy Sally Sanger, with permission)

References

1. Sanger F (2003) Personal communication
2. Sanger F (2007) Personal communication
3. Sanger R (2003) Personal communication
4. Sanger F (2006) Personal communication
5. Sanger P (2000) Personal communication
6. Sanger R (2016) Personal communication
7. Kaplan F (2009) The day obscenity became art. *The New York Times*. http://www.nytimes.com/2009/07/21/opinion/21kaplan.html?pagewanted=all&_r=0, accessed 8 July 2016
8. Sanger S (2014) Personal communication
9. Gutfreund H (1999) Personal communication
10. Sanger F (1990) Letter to Ted Thompson on the occasion of Ted's retirement
11. Thompson EOP (2000) Personal communication
12. Sanger F (2004) Personal communication
13. Milstein C (1999) Personal communication
14. Thompson EOP (2002) Personal communication
15. Dowding M (1999) Personal communication
16. Air G (2003) Personal communication
17. Sanger P (2014) Personal communication
18. Sanger S (2016) Personal communication

Chapter 8
The Man and His Legacy

How does one paint a picture of Fred Sanger (Fig. 8.1)? After all, his life of techniques was devoted to the visual. He loved painting as a child and maintained that hobby until his twilight years. He shunned column chromatography, when possible, for paper and thin-layer techniques. Fred wanted to *see* the results. His polyacrylamide sequencing gels were visual masterpieces.

Fred's Quaker upbringing with its emphasis on honesty, fairness, and hard work defined him, even though he was an agnostic as an adult. His reserved nature and love of working with his hands defined his work. Fred was not one for idle chatter. He limited his social contact to family and close friends. Even at the lab, he attended only those meetings relevant to his science or his limited administrative duties. In his mind, if one were not in the lab, one wasted time. He worked at the bench his entire career, even after two Nobel Prizes.

Fred once observed, "I'm just a chap who messed about in the laboratory" [1]. In his autobiographical sketch for *Annual Review of Biochemistry*, he said, "Of the three main activities involved in scientific research, thinking, talking, and doing, I much prefer the last and am probably best at it" [2]. And doing he did. Mostly he made decisions based on what would be the most fun to do [3].

In Fred's classic pattern, he worked closely with one or two people. Even when he had many postdoctoral fellows, he left them to do their own work while he did his. He was a great believer in distributing responsibility. His main concern was quality of people, and he knew how to choose people. Once he decided you were independent, you had to solve your own problems [4]. While he was available for help and comments, he often said, "Why don't you try harder. It will come" [5]. When John Walker asked Fred's advice about working with ATPase, Fred said, "Why don't you get on with it?" That was approval—always a man of few words [6]. "Jolly good" and "That's very encouraging" were also classic responses. A half-suggestion from Fred was the equivalent of an imperative from others. Woe be to the student who ignored such a comment [7]. His long-time technician Bart Barrell said, "When Fred said, 'We ought to be doing …', that was a direct order to go out and do it" [5]. Theodore Friedmann added, "When Fred asks a question, he's

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1_8

Fig. 8.1 Fred Sanger at Far Leys 1999 (photo by author. Courtesy Fred Sanger, with permission)

not asking a question, he's giving you advice. He's telling you in his subtle way that's something you should try" [8].

According to César Milstein [4],

> It is really difficult for someone who hadn't worked with him to appreciate the impact he has on other people working close to him. He's really inspirational. He has this knack for hitting at the center of a problem and asking the most absurd questions, of which, you suddenly said, my God, he's right.

For all his seriousness in the lab, the Australians had a way of bringing out the little kid in Fred. They had no inhibitions. They would run around the lab with pipets, blowing things at one another, and Fred was right in the thick of it [5]. Denis Shaw joked that Fred used to practice swearing when he knew an Australian was coming to visit [9].

Fred did not like confrontations. He rarely interfered if there were personality conflicts in lab. He let such situations resolve themselves [3]. He did not even like to say 'Good morning.' His secretary, Peggy Dowding, said Fred would walk the long way around to get to his office so he did not have to pass her desk and say hello [7].

Most people describe Fred as a nice guy—easy going, non-confrontational. But there was steel running through him. Fred could be hard on his protégés. John Walker was close to finishing the sequence of the protein bacteriorhodopsin. Fred went to a meeting in Boston, where he met with Gobind Khorana and found that Khorana's group had just completed the bacteriorhodopsin sequence. When Fred came back, he would not speak to John. Finally Fred said, "You got beat." He made it clear he was not happy about it. It was important to win [6]. Fred was a perfectionist and could get quite angry, but he usually did not express it verbally. If someone offended Fred in any way, that person received a frosty response, leaving no doubt about Fred's feelings. If he was not pleased, he could present "a cold and distant face—quite formidable" [7].

Fred had great patience in the lab. If an experiment did not work, he designed the next one and got on with it. As Francis Crick said, "Fred didn't have great intentions. He just followed his nose. That was the way he worked" [10]. Fred described the years between insulin and RNA sequencing as his "lean years." He did not publish much, but he was laying the groundwork for his nucleic acid work. The 1958 Nobel Prize bought him freedom to work on topics that took more time to develop [2]. When he proposed plans for the sequence determination of a bacteriophage genome involving a large program, little precedence, and the need for much new chemistry, the referees were concerned. One reviewer replied, "Fred Sanger continues to bang his head against brick walls. And the walls keep falling down" [11]. His plans were approved.

Most people did not see Fred as the brilliant star of the lab. Unlike Francis Crick or Sydney Brenner, Fred did not self-promote; he disapproved of flashy people [8]. He was modest, but if someone assumed he was modest, he could snap back quickly [12]. At the lab party when Fred won his second Nobel Prize, Sydney, as lab director, praised Fred's modesty and added that Fred never had to do a calculation in his life, in essence damning Fred with faint praise. When Fred got up, he said, "That's true, but I think I'm bloody good" [13]. Brian Hartley described it as modesty combined with arrogance—arrogance because he thinks he can do "it"; modesty because he does not want to tell everybody he can [14].

That modesty extended to dress and actions. In the early days in biochemistry when everyone else wore a tie, Fred never did [15]. Steve Nicklen reported his first meeting with Fred. "I thought he was the cleaner. I waited in his office. He shuffled in looking pretty shabby and started shuffling in the waste bin. I said, 'Do you mind? I am waiting for Dr. Sanger. I wonder if you can clean up later.' He said, 'Well, I am Fred Sanger.'" [16]. Sidney Altman,[1] during his postdoctoral days, was doing an experiment using 5mCi of ^{32}P in a bubbler tube in a water bath. As he pulled the tube out of the bath, he discovered it had a hole in it and radioactivity scattered all over the lab area. He reported it to Sydney Brenner, who muttered, "I am too busy. Go and see Fred." Sidney explained the situation to Fred. Without saying a word, Fred put on gloves and started cleaning up as much as he could. Sidney said [17],

> I objected to what he was doing because I thought I should be doing that job. Quite soon, he said that I had done enough and decided to clear the area.… I cannot imagine a person of Fred's reputation taking on such a modest and thankless task and refusing help.… There are many ways, I suppose, of exhibiting humility and greatness, and this was one of them.

Fred was a methods guy. He always viewed methods as the door to enter another room. One could not get into that room if one did not know how to open the door. Once he got the door open, he was not interested in using that method for routine follow-up. He left that to others [4]. In the early days in biochemistry, Perutz's Unit wanted Fred to sequence proteins for them. He declined. Sydney Brenner had ideas

[1]Sidney Altman shared the 1989 Noble Prize in Chemistry with Thomas Cech "for their discovery of catalytic properties of RNA" [18].

to bounce off Fred, but Fred knew Sydney had ideas where he wanted Fred to do the work. When Fred knew that Sydney was coming, Fred would always be somewhere else. He once remarked, "That bloody man Brenner is coming again." and Fred disappeared into town [6, 19].

Fred was the master of the throwaway remark. At the celebration for the Nobel Prizes for Max Perutz and John Kendrew, Fred, playing on Max's reputation for miserliness, said, "Now, Max, perhaps you can buy yourself a new bicycle" [20]. When Max lavishly praised Fred at Fred's second Nobel party, Fred said, "The reason Perutz knows so much about me is he is writing my obituary" [6].

Fred Sanger was an understated man. His name is not among the twentieth-century scientists often mentioned by the public or by scientists outside biochemistry–molecular biology. He easily walked the streets of Cambridge in almost complete anonymity. So what is his legacy? Clare Sansom proposed Fred as the Grandfather of Proteomics for his sequencing of insulin from several species [21]. Fred's insulin work laid to rest the view among some protein chemists that proteins were hopeless mixtures and showed insulin to have a definite sequence of amino acids. Avery et al. showed earlier that DNA was the genetic material [22]. Horace Judson reported, "Sequence specificity demanded a specific instruction from a gene." Jacques Monod[2] told Judson [24],

> The first determination of the exact amino acid sequence of a protein by Sanger was absolutely essential; one could not even have begun to think seriously about the genetic code until it had been revealed to begin with that a protein is beyond a shadow of a doubt a polypeptide in which amino acid residues really are arranged in a definite, constant, genetically determined sequence.

Jeremy Farrar, Director of the Wellcome Trust at the time of Fred's death, called Fred the Father of Genomics because his "work laid the foundations of humanity's ability to read and understand the genetic code, which has revolutionized biology and is today contributing to transformative improvement in health care" [25]. His DNA sequencing method was at the base of the human genome project. John Newell asked Fred, "Looking back on your life in science, what accomplishment do you feel is most satisfying?" Fred responded, "Well, I think the work on DNA. It was the climax of my work. DNA is really the ultimate, I think, because it controls all other sequences" [26]. The Wellcome Trust and the Medical Research Council opened the human genome center near Cambridge in 1993. John Sulston,[3] first Director of the center, called Fred and asked if they could honor him by naming it The Sanger Centre. Fred said, "Fine, but it better be good" [28]. The center is now called the Wellcome Trust Sanger Institute.

---

[2]François Jacob, André Lwoff, and Jacques Monod shared the 1965 Nobel Prize in Physiology or Medicine "for their discoveries concerning genetic control of enzyme and virus synthesis" [23].

[3]Sydney Brenner, Robert Horvitz, and John Sulston shared the 2002 Nobel Prize in Physiology or Medicine "for their discoveries concerning genetic regulation of organ development and programmed cell death" [27].

John Walker made the case that Fred's "impact on biology is as dramatic as that of Charles Darwin" [29]. "Darwin completely transformed the way we think about biology. Fred invented DNA sequencing…. [that] has transformed both biology and medicine." Walker further stated that genome sequencing allowed us to follow human and animal migration patterns and the associated spread of diseases and to find evolutionary links between ancient and modern human populations. In medicine, it has led to new genomic diagnostics and therapies [30]. Historians of science are unlikely to elevate Fred to Darwin's status. Nevertheless, the impact of genome sequencing will continue to reap huge rewards for science and society.

Hans Krebs, 1953 Nobel Laureate in Physiology or Medicine for his discovery of the citric acid cycle, once said, "Scientific distinction develops if nurtured by distinction" [31]. Fred Sanger fit this description. Three graduate students and one postdoctoral fellow who worked with Fred later won Nobel Prizes for work independent of Sanger.

Rodney Porter (Fig. 8.2), Fred's first graduate student, shared the 1972 Nobel Prize in Physiology or Medicine with Gerald Edelman "for their discoveries concerning the chemical structure of antibodies" [32]. César Milstein (Fig. 8.3), who worked with Fred for part of his Ph.D. studies, shared the 1984 Nobel Prize in Physiology or Medicine with Niels Jerne and Georges Köhler "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies" [33]. John Walker (Fig. 8.4), who, as a postdoctoral fellow, worked on parallel projects with Fred, shared the 1997 Nobel Prize in Chemistry divided, one half jointly to Paul Boyer and John Walker "for their elucidation of the enzymatic mechanism

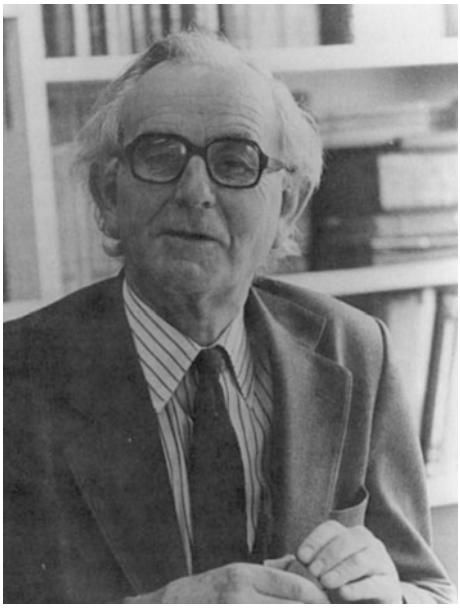


Fig. 8.2 Rodney Porter (Courtesy Julia Porter, with permission)

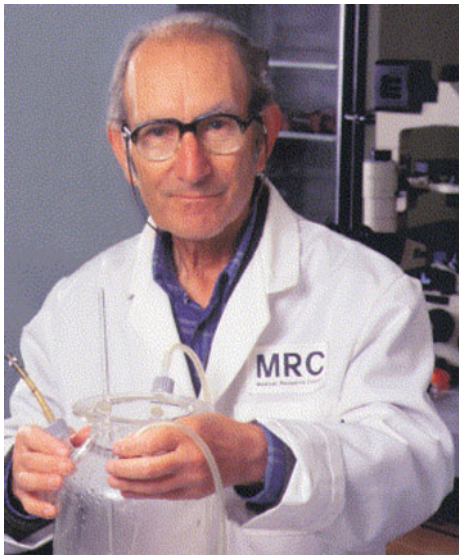


Fig. 8.3 César Milstein (Copyright MRC Laboratory of Molecular Biology, with permission)

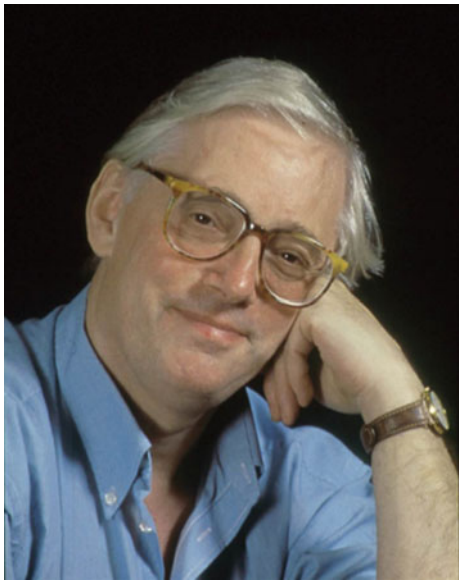


Fig. 8.4 John Walker (Courtesy John Walker, with permission)

underlying the synthesis of adenosine triphosphate (ATP)" and the other half to Jens Skou "for the first discovery of an ion-transporting enzyme, Na+, K+ -ATPase" [34]. Elizabeth Blackburn (Fig. 8.5), a graduate student with Fred at the LMB, shared the 2009 Nobel Prize in Physiology or Medicine with Carol Greider and Jack Szostak

Fig. 8.5 Elizabeth Blackburn (photo Joe Belcovson. Courtesy Elizabeth Blackburn and the Salk Institute, with permission)

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase" [35]. Several other students were named Fellows of the Royal Society.

Fred won many scientific and civilian awards. Of the civilian awards, he was most proud of the Order of Merit, the highest British honorary award given to those who provided especially eminent service in the armed forces or particularly distinguished themselves in science, art, literature, or the promotion of culture. The order is limited to only 24 living members.

Fred turned down a Knighthood. He did not want to be called Sir Fred. His father's teachings and his Quaker upbringing instilled in him an egalitarian view of people. His daughter, Sally, said that her parents had one another on a pedestal. One of the few times she saw them at odds was over the Knighthood. Joan was quite upset; Fred was mulish and stubborn. Joan wanted him to accept it; Fred did not want people to look at him differently. He was not into peerage and ranking. He was a lifetime Labour voter [36]. His actions over the years bore that out. He took his technician into the faculty lounge in the Biochemistry Department during his protein days. He put the names of technicians on research papers. He listed his former secretary as a coeditor of his *Selected Papers of Frederick Sanger* (*with commentaries*) [37].

His turning down the Knighthood had its critics. John Walker said that Joan would have loved to be Lady Sanger. That side of Fred seems selfish. Other people derive pleasure from that recognition—family, coworkers, and friends [6].

Fred was also criticized for not pushing the Medical Research Council and the National Research Development Corporation[4] (NRDC) to patent César Milstein and Georges Köhler's monoclonal antibody work. They did approach the NRDC to

[4]The NRDC was set up after World War II to commercialize British publicly funded research.

inquire about the possibility. When the NRDC did not see any 'immediate practical application' in it, they did not pursue it. It seemed to be a combination of bureaucratic mishandling and bad timing [38]. Each of the founding division heads at the LMB had direct access to the MRC. Max Perutz indicated that if Fred had worked through him, he would have raised hell about it with the MRC [39]. Other people made a fortune from it. The loss of revenue to the LMB for that failure led to policy changes at the MRC and the LMB [40].

While Fred was almost universally lauded by his colleagues and students, he had a dark side. "Sweetness and darkness" was the description Sally gave him. Sweetness because he taught her how to swim and ride a bicycle, because he took her for walks in the woods and on snorkeling adventures, and because he was kind in welcoming Sally's husband Chris into the family. Darkness because he was a perfectionist. He wanted to see perfection all around him, but he only let it out around family, and he pushed it too much at home. Because he had Joan on a pedestal, he took out his emotional dark side on Sally. He told her she was a mistake. He had quite a temper; he would be normal one moment and then suddenly very angry. Nothing physical, just angry words. He had a deep-seated anger [36].

Where did this anger come from? Was it from work? It was well known that he and Sydney Brenner had issues. Was it the perfection that family could never meet? Was it the burden of always being good old Fred? We will never know. As Mark Twain said, "Biographies are but the clothes and buttons of the man—the biography of the man himself cannot be written" [41].

References

1. Marks L (2015) Just a chap who messed about in the lab: Fred Sanger and DNA sequencing. History & Policy. http://www.historyandpolicy.org/opinion-articles/articles/just-a-chap-who-messed-about-in-the-lab. Accessed 22 July 2016
2. Sanger F (1988) Sequences, sequences, and sequences. Ann Rev Biochem 57:1–28
3. Brownlee G (1999) Personal communication
4. Milstein C (1999) Personal communication
5. Barrell B (1999) Personal communication
6. Walker J (2003) Personal communication
7. Dowding M (1999) Personal communication
8. Friedmann T (2004) Personal communication
9. Shaw D (2002) Personal communication
10. Crick F (2001) Personal communication
11. Dodson G (2005) Fred Sanger: sequencing pioneer. The Biochemist 27(6):31–35
12. Air G (2003) Personal communication
13. Klug A (2009) Personal communication
14. Hartley B (1999) Personal communication
15. Fuller M (2007) Personal communication
16. Nicklen S (2007) Personal communication
17. Altman S (2003) RNA processing: a postdoc in a great laboratory. Genetics 165:1633–1639
18. Nobel Media AB (2014) The Nobel prize in chemistry 1989. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1989/. Accessed 3 Aug 2016

19. Young I (2002) Personal communication
20. Ambler R (2000) Personal communication
21. Sansom C (2006) The beginnings of bioinformatics. The Biochemist 28(6):48–49
22. Avery OT, MacLeod CM, McCarty M (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from Pneumococcus Type III. J Expt Med 79:137–1157
23. Nobel Media AB (2014) The Nobel prize in physiology or medicine 1965. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1965/. Accessed 4 Aug 2016
24. Judson HF (1993) Frederick Sanger, Erwin Chargaff, and the metamorphosis of specificity. Gene 135:19–23
25. Gallagher J (2013) Frederick Sanger: double Nobel prize winner dies at 95. BBC News. http://www.bbc.com/news/science-environment-25020112. Accessed 3 Aug 2016
26. Newell J (1992) DNA research was the climax of my work. Helix Amgen's Mag Biotechnol 1 (1):4–9
27. Nobel Media AB (2014) The Nobel prize in physiology or medicine 2002. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2002/. Accessed 4 Aug 2016
28. Sulston J (2007) Personal communication
29. Walker J (2014) Frederick Sanger (1918-2013): double Nobel-prizewinning genomic pioneer. Nature 505:27
30. Walker J (2014) Memorial service for Dr. Frederick Sanger (Honorary Fellow). University of Cambridge Collection. https://sms.cam.ac.uk/collection/1842965. Accessed 3 Aug 2016
31. Krebs H (1967) The making of a scientist. Nature 215:1441–1445
32. Nobel Media AB (2014) The Nobel prize in physiology or medicine 1972. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1972/. Accessed 10 July 2015
33. Nobel Media AB (2014) The Nobel Prize in physiology or medicine 1984. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1984/. Accessed 10 July 2015
34. Nobel Media AB (2014) The Nobel prize in chemistry 1997. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1997/. Accessed 10 July 2015
35. Nobel Media AB (2014) The Nobel prize in physiology or medicine 2009. Nobelprize.org. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2009/. Accessed 10 July 2015
36. Sanger S (2014) Personal communication
37. Sanger F, Dowding M (eds) (1996) Selected papers of Frederick Sanger (with commentaries). World Scientific, Singapore
38. de Chadarevian S (2002) Designs for life: molecular biology after World War II. Cambridge University Press, Cambridge, p 357
39. Perutz M (1999) Personal communication
40. Henderson R (2007) Personal communication
41. Twain M (1922) Unpublished chapters from the autobiography of Mark Twain. Harper's Magazine 144:273–280

Index

J.S. Jeffers, *Frederick Sanger*, SpringerBriefs in History of Chemistry,
DOI 10.1007/978-3-319-54709-1