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Elected FRS 1954

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Frederick Sanger—always known as Fred—was one of the most influential scientists of the twentieth century. A committed molecular biologist, he spent all his academic life in Cambridge devising methods for sequencing proteins and nucleic acids. He twice won the Nobel Prize in Chemistry—once in 1958 for protein sequencing and then again in 1980 for sequencing nucleic acids. He is the only scientist to have achieved this distinction. The impact of his work was enormous. He opened up the field of protein chemistry in the 1950s, stimulating studies of the sequence, structure and function of many proteins and enzymes. In 1977 he devised an ingenious DNA sequencing method that has revolutionized molecular biology and made it possible to completely sequence the $3 \times 10^9$ nucleotides of the human genome. Moreover, he confirmed the genetic code, showed that the genetic code differed in mitochondria, and discovered overlapping genes. Fred Sanger was a modest, reserved man but to his colleagues and friends he always had vision. He was a pioneer and a leader.

EARLY LIFE

Fred’s grandfather, William Albert Sanger (1840–83)—a pharmaceutical chemist—and grandmother, Ann Mary (née Hoff, 1837–1913) had five sons (John, William, Frederick, Edward and Hubert) and one daughter (Mary). Frederick, Fred’s father (1876–1937), the third of the five boys, after graduating from St John’s College, Cambridge (BA, 1897), in Natural Sciences, did postgraduate research in Cambridge (MD, 1905) under Professor George Nuttall FRS on ‘Biological test for blood considered from its medico-legal aspect’. He was a very religious man and went to China as a medical missionary, sponsored by the Church Missionary Society. His most noteworthy contribution was the founding of a school in China open to all and not just the mandarin class. His brother, Revd Hubert Sanger, visited him in China on his way to Australia. Hubert’s daughter, Fred’s cousin, was Ruth Sanger FRS (1918–2001)
of blood group fame. On returning to the UK after more than 10 years in China, Frederick practised as the local General Practitioner in Rendcomb in rural Gloucestershire, marrying Cicely, née Crewdson (1880–1938), in 1916.

Fred’s mother, Cicely, was the daughter of a wealthy cotton manufacturer, Theodore Crewdson (1835–1923), and Rachel Elizabeth, née Jowitt (1841–80). Cicely was the youngest of six children, Theodore, John Wright, Lilian Dora, Helen Mary, Joseph Dilworth and Cicely. The Crewdsons were mainly involved in business. Cicely was a ‘quiet rather shy person with few interests outside the family—in contrast to my father’, Fred recorded (36)*.

Frederick (Fred) Sanger was born in 1918, the second son of Frederick and Cicely Sanger. Fred’s elder brother, Theodore (Theo) (1917–2003), was born a year earlier, and his younger sister, Mary (May) five years later. Fred and Theo—close in age—played together a lot as young children, enjoying the large garden and the river Churn at the bottom of the garden of their substantial ‘Old House’ in Rendcomb, in which the family lived and father had his small adjoining surgery.

Frederick Sanger, senior, had two main influences over his family. First, his medical work stimulated an interest in science and, second, he had converted from the Church of England to become a staunch Quaker after his marriage to Cicely, influenced by the Quaker tradition in the Crewdson family. Ironically, the Crewdson family themselves had rejected Quakerism, and Cicely, who never became a Quaker, was of Church of England persuasion. Thus the three Sanger children, including Fred, were brought up on Quaker principles, saying their prayers morning and night and usually attending the Quaker meetings with their father, although mother went to church instead.

Fred’s family moved to ‘Far Leys’ in Tanworth-in-Arden, near Birmingham, when he was five years old. He and his elder brother’s education started there with the daily visit of a governess, Helen Mary Shewall—a vivacious, Quaker woman, who taught both children and two or three other local boys in the ‘school room’ at home. Fred enjoyed this period because Miss Shewall was a kindly person who gave plenty of individual attention. Theo had a big influence in interesting Fred in science, especially natural history. Fred recorded (36): ‘Being close together in age, Theo and I spent much of our early childhood together. He was always the leader and was much more outgoing and dynamic than me. I have always been somewhat shy and quiet, and early on he was, of the two, more of the scientist.’ The two boys collected skulls, looked for birds’ nests—but did not steal the eggs, simply recorded them—and in spring looked for newts and toads in a nearby pond in a field owned by their parents.

In 1927 Fred, at just nine years old, boarded at the Downs preparatory school (an independent Quaker school) near Malvern, Worcestershire. Fred, a small and rather shy boy, found being a boarder traumatic at first, but gradually adapted to being away from home and the inevitable minor bullying that went on at school. He worked hard, was near the top of his class and also ended up in the school team for both rugby and cricket, but showed no ability in music. He could never sing in tune. The music mistress agreed that it would be better if Fred just mouthed the words when ‘singing’ in class. He was introduced to painting in oils by Maurice Feild, the art master. For his mother’s 50th birthday he produced a book of 50 paintings, which she treasured. However, Fred enjoyed the holidays more than school (figure 1). When Fred was 10 years old he took up carpentry and carved a church on a small piece of wood and then coloured it, which his mother thought was quite original. He had

* Numbers in this form refer to the bibliography at the end of the text.
Frederick Sanger

Frederick Sanger learnt carpentry from Louis G. Byrde, a frequent visitor to ‘Far Leys’, who was the son of a colleague of his father when he was in China. His parents had a shed, called ‘Pansymouse’, built in their garden for the children. (‘Pansy’ was Theo’s nickname, ‘mouse’ was Fred’s.) There Theo had his skull collection and Fred his workbench for his carpentry tools and later on tools for forging and welding. He made a gate, which hung in the garden for a while.

From 1932 to 1936 Fred attended Bryanston, an independent, newly founded public school in Dorset. Bryanston was a progressive school with connections to the Downs preparatory school. The headmaster, T. F. Coad, permitted considerable freedom, and school work was often done as ‘assignments’, in which pupils were given a project and encouraged to obtain help from the masters and older boys to complete the work. This ‘Dalton’ system suited Fred because it ‘meant you had to find out things for yourself rather than having information forced into you’ (36). Science was well taught. Two masters, Frazer Hoyland (Biology) and Geoffrey Ordish (Chemistry), had a particular influence on Fred. Frazer Hoyland had a ‘Biological Society’ in which pupils, including Fred, could mess about in the laboratory after school, making slides and looking at them under the light microscope. Mr Ordish had done research at university on dyestuffs and gave Fred a small project to make some coloured crystals. Fred has recorded (36): ‘working in the lab was a marvellous change for me from just sitting and studying books. I decided this was something I really enjoyed’.

Fred obtained seven credits in his School Certificate Examination (in English, German, elementary mathematics, additional mathematics, physics and chemistry, Latin and biology)—an exceptional achievement, and enough to ensure his admission to St John’s College, Cambridge—the college that his father and two uncles, John and Hubert Sanger, had attended.
Fred’s parents hoped he would study medicine at Cambridge but Fred changed his mind while still at school at Bryanston (Sanger archive, St John’s College, Cambridge; Cicely Sanger, diary; Brownlee 2014). Fred recorded later (36):

Initially I assumed I would follow in my father’s footsteps and become a doctor, though I only began to think about it seriously during my last years at Bryanston, when I had to decide what to go in for at the University. I had seen what the life of a doctor was like, and the more I thought about it the less I liked it. Although I would have liked to be able to relieve people’s suffering it seemed to me to be a very scrappy sort of job, continually running from one problem to another, whereas I preferred the idea of having a single problem I could really get my teeth into.

During the second half of his last term at Bryanston (summer 1936), before going up to Cambridge, Fred went on an exchange visit to a German school, Schloss Salem, in southern Germany, founded by Kurt Hahn, who later founded Gordonstoun. On this visit, with another pupil, David Forbes from Bryanston, Fred observed the ‘nazification’ of Germany and its effect in the school he was attending. Sport, particularly hockey and athletics, and outdoor activities were emphasized at the expense of academic work. Fred learned nothing new academically, except German. Sport at Bryanston, however, was not compulsory. Fred initially continued with both rugby and cricket, which he had played at the Downs school, but then gave up these organized games in favour of fives, in which he was captain, and squash.

Fred went up to St John’s College, Cambridge, in October 1936, to read Natural Sciences. Fred’s father had extensive correspondence between 1933 and 1936—more than 20 letters—with the Master of St John’s, Ernest Benians, and Fred’s moral tutor, Robert Howland, regarding Fred’s application, schooling, admission, and living arrangements (Sanger archive, St John’s College, Cambridge). He was clearly very keen that Fred should get into St John’s.

There were no financial problems and neither Fred nor Theo (who went up to Trinity Hall) won scholarships. Fred decided to read chemistry and physics as full subjects and mathematics as a half subject, but needed to find another half subject for the Natural Science Tripos. He wrote later (36):

on looking through the list of possibilities I saw a subject I had not heard of before—biochemistry. This intrigued me; the idea that living matter could be explained in terms of chemistry seemed exciting and to open up all sorts of possibilities. In charge of Biochemistry was Ernest Baldwin [figure 2], and his enthusiasm amply confirmed that this should be my other half-subject. I well remember my first meeting with him. He welcomed me into his room and seemed to be bubbling over with enthusiasm about biochemistry, a marked contrast to other directors of studies whom I had been to see. He was a born teacher and I am grateful to him for introducing me to the subject.

Fred found his first year in Cambridge very demanding academically, and he only gained a third in ‘prelims’ in the first-year exams (Sanger archive, Cambridge University Library). The problem was that Fred had not done ‘Higher Certificate’ at school and did not have sufficient grounding in physics and mathematics. Another factor may have been that Fred’s father died in 1937 from an operation to remove a stomach cancer, and his mother died a year later in 1938 from colon cancer. This may have left the young Fred somewhat vulnerable. Fred liked chemistry but did not enjoy either physics or mathematics. He therefore dropped physics after his first year and took up physiology instead, soldiering on in mathematics. However, this meant he could not complete his Part I in the usual two years. Instead it took him three years to get his BA (a second, in 1939) and normally he would have left Cambridge then. However, as he had inherited £25 000 from his parents’ estate (36) he was not short of
money. He had liked the Part I biochemistry course, so he decided to stay on for a fourth year to do a Part II (advanced course) in biochemistry. Fred particularly liked the Part II practical courses, and the enthusiasm of his lecturers, among whom were Joseph Needham (FRS 1941) on morphogenesis, David Keilin FRS on cytochromes, and Malcolm Dixon (FRS 1942) on enzymes (36).

Outside work, Fred’s main interest centred on Quakerism. He was much influenced by it but never became a member of the Society of Friends. He attended their meetings in Cambridge, took part in various social activities and had many Quakers among his friends. As a confirmed pacifist he had signed the ‘Peace Pledge Union’ and was a member of the scientists’ anti-war group (Brownlee 2014). This was unpopular at that time, because war with Germany had broken out and compulsory conscription had been introduced. Fred had to appear before a tribunal to confirm his status as a conscientious objector—obtaining unconditional exemption from military service. Fred’s interests introduced him to his girlfriend, (Margaret) Joan Howe—a pretty brunette from Leicestershire, who was an undergraduate studying economics at Newnham College, Cambridge. She helped Fred to write a report entitled ‘The political and economic effects of rearmament’ for the scientists’ anti-war group. Fred spent a lot of time with her during the year that he was doing his Part II biochemistry. During that time, Fred obtained permission to drive a car, supported by a letter from his guardian, J. D. Crewdson.
Fred and Joan were married on 28 December 1940 in Syde, Gloucestershire (figure 3). Joan was the eldest daughter of Alfred Howe (1889–1976) and Sarah, née Watson (1890–1956), who had three children, (Margaret) Joan (1918–2012), Edna (1921–99) and Alfred (‘Peter’) (1923–96). Alfred Howe was a successful Leicester shoe manufacturer, owning a shoe factory, J. H. Clarke & Co. Ltd, that he had started with his partners. He was very much a self-made man, although rather strict with his family. He was ambitious for his children, and Joan was probably the first of the Howe family to go to university. He was not quite sure of his future son-in-law and, according to Christopher and Michael Howe, his nephews, probably expected his daughter to marry someone more orthodox than a pacifist scientist who, at least in his early undergraduate career, had not altogether excelled. They recall that Alfred was said to have told Fred that he could not marry his daughter unless he ‘bucked up’ his ideas.

Fred did ‘buck up’ his ideas, obtaining a first-class degree in 1940 for the Part II biochemistry course. He had not planned to read for a PhD after graduating, but only considered he was good enough to do research on being awarded a first (Brownlee 2014). He returned to the Biochemistry Department, Cambridge, in the autumn of 1940, self-funded, initially supervised (for one month only) by Norman Wingate (Bill) Pirie (1907–97; FRS 1949) and then by Albert Neuberger (1908–96; FRS 1951) (figure 4) on the subject ‘The metabolism of the amino acid lysine in the animal body’. Fred, with characteristic modesty, did not rate his PhD highly (Brownlee 2014), but he did get five papers published as a result of his studies, among them a
paper on the topic of ‘Nitrogen of the potato’ as part of the war effort. Fred’s PhD examiners, Albert Chibnall FRS (1894–1988) and Charles Harington FRS (1897–1972), were enthusiastic about his science, although they were not impressed by his presentation and spelling mistakes (Sanger archive, Cambridge University Library). However, after he corrected his thesis he was awarded a PhD (1944).

**SEQUENCE OF INSULIN**

On completion of his PhD under Neuberger’s supervision, the incoming Professor of Biochemistry, Albert Chibnall, persuaded Fred to study insulin. Fred really had no choice. He needed a job and a new scientific problem, because Neuberger had left Cambridge for the National Institute of Medical Research in London. Chibnall had previously worked with insulin and discovered that it had an excess of ‘free’ α-amino acids, indicating that insulin had rather short chains. Nothing was known about the protein sequence of insulin, or indeed of any other protein (other than some short peptides from silk fibroin), except for the amino acid, phenylalanine, at the amino (N) terminus (Jensen & Evans 1935). Fred introduced the Sanger reagent, fluorodinitrobenzene (FDNB) to study the N-terminal amino acids of insulin. This reagent rapidly reacted with insulin at room temperature to modify amino groups and give a dinitrophenyl (DNP-) amino acid (figure 5). The modified amino acid could be liberated from the derivatized insulin by acid hydrolysis, generating yellow dinitro derivatives of the N-terminal amino acid, along with derivatives of the ε-amino group of lysine and the hydroxyl group of tyrosine. Fred used the newly described method of partition chromatography (Gordon
et al. 1943) to identify the DNP-amino acids in insulin. In a classic paper in 1945 (1), he found both phenylalanine and glycine at the N terminus of insulin, indicating that there were at least two polypeptide chains. To do this he had to compare the mobilities of these two DNP-amino acids, in a variety of solvent systems on partition chromatography, with all 18 different marker DNP-amino acids that he had synthesized.

Fred had not initially decided to use FDNB for his endgroup analysis of insulin. He had previously worked with methane sulphonyl chloride and chlorodinitrobenzene before deciding to use FDNB. Fred decided to use FDNB because the fluoro derivative was more reactive than the chloro derivative. Despite this initial success, Fred had not yet decided to sequence insulin. He had noticed, however, in experiments in which he had varied the conditions of acid hydrolysis of insulin after tagging with DNP (because of the instability of DNP-glycine in acid), that partial acid hydrolysis products were detected on partition chromatography. In the meantime Fred had worked out a method for oxidizing insulin to break the disulphide bonds with performic acid. He then separated the two chains of insulin—the glycol (Gly) and phenylalanyl (Phe) chains—although at the time he thought there were four chains because its molecular mass had been overestimated (2). Using partial acid hydrolysis, Fred deduced the sequence of the first four amino acids in the Phe chain of insulin as Phe-Val-Asp-Glu and five amino acids in the Gly chain as Gly-Ile-Val-Glu-Glu, as well as an internal tetrapeptide sequence of Thr-Pro-Lys-Ala from the Phe chain. He benefited from the newly described method of paper chromatography (Consden et al. 1944) to identify unmodified amino acids.

Fred himself, writing later, thought his 1949 paper (3) was more important than his better-cited 1945 paper (1), because it was the first paper to accurately position a sequence of amino acids adjacent to one end of a protein chain. It was now clear that it was possible to define amino acid sequences in proteins, so it was unlikely that proteins were statistical mixtures of amino acid sequences, as some had previously claimed.

The year 1949 was a watershed moment for Fred because he had now decided to sequence insulin. He stated then (4), ‘It is probable … that the complete amino-acid sequence in a protein will be elucidated in the not too distant future.’ Fred was undoubtedly thinking about
his own work on insulin when he wrote this because he clearly states, in his 1949–50 report to the Beit Trustees (Wellcome/Beit/Sanger archive), who were supporting his research as a Beit Fellow, that he ‘considered that it might be possible to determine the complete peptide sequence of these [insulin] chains.’

Once this decision had been made, there followed four extremely detailed classic papers, published in Biochemical Journal (5–8). He first worked out the sequence of the longer, 30-residue-long B chain (the Phe chain) with Hans Tuppy—a visiting British Council supported scientist from Vienna. Then he sequenced the shorter, 21-residue-long, A chain (the Gly chain) with Ted Thomson, a PhD student from Sydney, Australia. This was the first time that Fred had any help with his work on insulin. All previous insulin papers reported work done entirely by himself. Essentially the approach was identical with both the A and B chains, although the longer B chain was easier to sequence than the shorter A chain.

As an example of the approach that Fred took to sequencing insulin, let us consider Fred’s results with the B chain (5). First he attempted to sequence the chain just from the result of an analysis of partial acid products: 23 dipeptides, 15 tripeptides, 8 tetrapeptides, 2 pentapeptides and 1 hexapeptide were isolated and individually sequenced. When they were ‘overlapped’ with one another, and knowing the overall composition of the amino acids in this chain, Fred was able to deduce five non-overlapping peptides covering 27 residues of the 30-residue long chain. It proved impossible to sequence the B chain completely from the results of partial acid hydrolysis, partly because of purification problems of more polar peptides and partly as a result of the lability of peptide bonds adjacent to serine and threonine residues. The main problem in this work was the purification of the large and complex mixture of peptides obtained by partial acid hydrolysis, so that it was necessary to use purified B chain for this work and a preliminary group separation of peptides by various techniques. Ion-exchange chromatography, adsorption on charcoal and ionophoresis were all used to simplify the complex mixtures before purification by fractionation on two-dimensional paper chromatography.

To complete the B-chain sequence, Fred had to use enzymatic hydrolysis with trypsin, chymotrypsin and pepsin to obtain more specific cleavages. He had been reluctant to use these proteolytic enzymes before, because proteases had been shown to have synthetic as well as proteolytic activity. However, no synthetic activity was detected in Fred’s work. Ironically, he was later to prove that partial acid hydrolysis, not proteolytic enzymes, could lead to artefacts. As expected, much simpler mixtures of peptides were obtained with enzymatic hydrolysis than with partial acid hydrolysates, although Fred now used ionophoresis—separating peptides into acid, neutral and basic peptide mixtures—before separation and purification on two-dimensional paper chromatography. Analysis of these peptides, coupled with a knowledge of the previous results from partial acid hydrolysis, allowed the 30 residues of the B chain to be deduced. The sequence of a protein had been solved—but not quite.

There remained the amide problem—the problem of whether the glutamic (Glu) or aspartic (Asp) residues were actually glutamine (Gln) or asparagine (Asn), because the amide bonds in glutamine or asparagine were hydrolysed by acid. Fred had to use two more enzymes, papain and a mould protease, to isolate more peptides to solve this problem. Peptides derived from enzymatic digestion generally retained the amides, unlike those from partial acid hydrolysis, so their mobility on ionophoresis at different pH values would generally inform one of their amide content. The B chain caused little problem, but sorting out position 4 of the A chain was a particularly frustrating part of the sequence work because of conflicting results with different peptides. Finally, the problem was resolved by Ted Thomson, who made 17 different
estimations of the amide content in one particular 13-residue papain peptide (9) before they were satisfied that the sequence was correct.

The final problem of deciding on the arrangement of the three disulphide bonds was equally frustrating. The idea was to isolate peptides by partial acid hydrolysis of native insulin, then treat with performic acid to break the S–S bond and isolate the two halves, thus establishing which pairs of cysteine were linked. However, this theory was thwarted initially because peptides became scrambled during acid hydrolysis as a result of ‘disulphide interchange reactions’ (10). There were two separate mechanisms but Fred found out how to inhibit this scrambling by adding SH (thiol) reagents to the reaction. Even so it was very difficult to establish one particular disulphide bridge in the A chain because two cysteine residues were adjacent.

The final solution (11) (figure 6) took Fred and his colleagues Hans Tuppy, Ted Thompson, A. P. Ryle, Ruth Kitai and Les Smith 11 years to solve. But it was worth it to describe the first ever sequence of a protein. The scientific community was amazed at this tour de force. In his work Fred gave huge credit to Martin for the development of partition chromatography and then paper chromatography, acknowledging how crucial these techniques had been for his work on insulin. Nevertheless, Martin’s group did not undertake any analysis as ambitious as Fred’s. They had sequenced the cyclic pentapeptide gramicidin S, a much simpler problem than sequencing insulin. It was the persistence, focus and dogged determination to complete the project that characterized Fred’s approach and ultimately led to his success. Fred had to surmount many hurdles and develop and adapt techniques from the literature to solve the insulin sequence, as Stretton (2002) has emphasized.

Because insulin had a defined and unique amino acid sequence, this suggested that there were no obvious general principles defining the arrangement of amino acids in insulin, or by implication in other proteins. This put paid to earlier hypotheses that proteins, in general, might have regular repeating sequences or were heterogeneous in some undefined way. More importantly, it proved that there must be a specific mechanism of protein synthesis, although mRNA and tRNA were unknown in the early 1950s. By implication, a genetic code must exist to specify this amino acid sequence of proteins. The Nobel committee did not take long to award Fred the Nobel Prize in Chemistry in 1958. Fred’s work stimulated numerous studies by others to sequence other proteins, such as ribonuclease, chymotrypsin, myoglobin and the α and β globin chains of haemoglobin, although Fred’s partial acid hydrolysis procedure was unsuitable for these longer proteins because of the complexity of the mixtures.
SEQUENCE OF RADIOLABELLED PROTEINS

After completing the sequencing of insulin in 1954, Fred wished to relate its structure to function but made only limited progress in this direction. However, he introduced the idea of comparative sequencing of related insulins. He showed, with Ieuan Harris, H. Brown, Ruth Kitai and Mike Naughton, that pig, sheep, horse and whale insulins differed from cattle insulin, thus defining amino acid residues that could vary without obviously affecting function (12, 13). This is the first ever example of comparative sequencing to identify homologous amino acid residues in different species—a method still widely used to assess which amino acid residues in proteins are important for function. But he had another idea: he wished to explore the idea of simplifying the tedious procedures of protein sequencing by the use of radioactive isotopes. For example, he introduced radioactive $^{32}$P-phosphate into chicken ovalbumin. The dipeptide, SerP-Ala, was identified simply from its properties on ionophoresis and chromatography, without purifying it from other non-radioactive peptides. More importantly, with Brian Hartley (FRS 1971), Denis Shaw and Mike Naughton (14), he labelled the active centre of enzymes such as elastase, trypsin and chymotrypsin with $^{32}$P-labelled diisopropylfluorophosphate, showing that all three proteases had an identical tetrapeptide ‘fingerprint’ and an identical sequence, Gly-Asp-SerP-Gly, at their active centre. With César Milstein (FRS 1975) (15) he characterized a short pentapeptide sequence, Thr-Ala-SerP-His-Asp (or Asn), around the active centre of another enzyme, phosphoglucomutase, after radiolabelling its active-centre serine residue. Ingenious as this approach was, there were limits to the radioactive sequencing of proteins, because there were 20 different amino acids to contend with. Nucleic acids were simpler than proteins in this respect. There were, in general, only four different bases to order in a sequence.

RNA SEQUENCING

Fred moved from the Biochemistry Department of Cambridge University to the newly-built Medical Research Council (MRC) Laboratory of Molecular Biology on the outskirts of Cambridge in 1962. There, with more space to expand his group than in the Biochemistry Department, he started work on nucleic acids, introducing the new concept of sequencing $^{32}$P-labelled nucleic acids, influenced by his earlier ideas of sequencing labelled proteins. No short DNA was known, and the only accessible small RNAs known then were the transfer RNAs (tRNAs)—about 80 nucleotides long—but it was non-trivial to purify any one specific aminoaeryl-tRNA from the mixture of naturally occurring tRNAs in the cell. So Fred, with Bart Barrell (his assistant), started by ‘fingerprinting’ high-molecular-mass, $^{32}$P-labelled 16S and 23S ribosomal RNA, which could be purified on sucrose gradients. T$_1$ ribonuclease (RNase) was a guanine-specific ribonuclease and gave high-resolution fingerprints on a two-dimensional modified paper system (cellulose acetate ionophoresis at pH 3.5 in one dimension, and diethylaminoethyl (DEAE)-cellulose paper ionophoresis at pH 1.9 in the second dimension) that Fred, with his typical ingenuity, had devised (16). This was an excellent fractionating system giving sharp spots and was the basis (with modification of the method used in the second dimension) of all the subsequent work on RNA.

The initial fingerprints of ribosomal RNAs showed remarkable resolution, separating for example isomers of trinucleotides such as ACG from CAG, and CUG from UCG. These
trinucleotide sequences were not identified by comparison of their mobility with known, model unlabelled trinucleotides; rather, they were sequenced from first principles. They were eluted from the ion-exchange paper used in the second dimension and their sequences deduced from the isolation of smaller products obtained by degradation with other nucleases or by alkaline hydrolysis to derive their constituent mononucleotides. The $^{32}$P label acted as the tag to identify the constituent mononucleotides and their positions in the sequence. To sequence longer oligonucleotides Fred used partial digestion with exonucleases and analysed the intermediate degradation products by ionophoresis on DEAE-cellulose paper in one dimension. This was a powerful new sequencing method because the mobility shifts (or ‘M values’) indicated which residue was present. Thus Fred was using positional information to sequence RNA (16) as, indeed, he had done previously to sequence radioactive proteins.

The first short RNA to be fully sequenced by radioactive methods was the 120-nucleotide 5S ribosomal RNA from *Escherichia coli* (17). It proved a good model RNA with which to develop the radioactive sequencing methods, especially of the longer T$_1$ RNase products and for finding ways of isolating even longer products of partial digestion with T$_1$ RNase, long enough for the deduction of a complete sequence. The methods used to sequence RNA were essentially the same as had been used to sequence insulin. Starting with the shorter products of degradation, longer and longer fragments were isolated and sequenced, until a unique sequence could be logically deduced by ‘overlapping’ the smaller fragments. For a few years 5S RNA (figure 7), and then the 184-nucleotide 6S RNA—also from *E. coli* (Brownlee

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Figure 7. Sequence of 5S ribosomal RNA of *E. coli*. (From Brownlee, G. G., Sanger, F. & Barrell, B. G. (1968) *J. Mol. Biol.* 34, 379–412; reproduced with permission.)
were the longest known RNA sequences. To solve these sequences it was necessary to devise improved fractionation procedures to separate the longer products of partial digestion with T₁ RNase. Homochromatography—a type of displacement chromatography using high concentrations of degraded RNA on DEAE-cellulose paper (17)—was used instead of ionophoresis in the second dimension. This was subsequently improved by carrying out the homochromatography on mixed thin layers of cellulose and DEAE-cellulose at 60°C in the second dimension (18). Oligonucleotides up to about 50 nucleotides long could now be isolated and sequenced.

Fred did sequence tRNA, somewhat later—once methods for purifying individual aminoacyl-specific tRNAs had been worked out. He was not the first to sequence tRNA, a feat managed by Bob Holley using classical methods (Holley et al. 1965). Fred himself was more interested in using his radioactive sequencing methodology. With Bart Barrell he purified and sequenced the phenylalanine tRNA of *E. coli* (19). tRNA was easier to sequence than 5S RNA, being shorter, and the minor bases helped with the overlapping.

Many other tRNAs were sequenced by his visiting postdoctoral researchers (Kjeld Marcker and Moshe Yaniv), by his PhD students (Shyam Dube and Howard Chadwell) or by others in the wider Laboratory of Molecular Biology in the group of John Smith (FRS 1976), Sydney Brenner FRS or Brian Clark. Early sequenced tRNAs include methionine tRNA (Cory et al. 1968), formylmethionine tRNA (Dube & Marcker 1969), valine tRNA (Yaniv & Barrell 1969) and tyrosine and tyrosine suppressor tRNA (Goodman et al. 1970). Other significant discoveries—using Fred’s fingerprinting method—arose from the isolation of tyrosine tRNA precursors (Altman 1971) and from the study of ribosome-binding sequences in bacteriophage R17 RNA (Steitz 1969).

After 5S RNA and tRNA, Fred took up the challenge of sequencing longer RNAs, present in RNA-containing bacteriophages, such as bacteriophage R17 RNA, which was about 3000 nucleotides long. Initially, Dahlberg—working in Fred’s laboratory—had managed to sequence a 10-nucleotide T₁ RNase end product from the 3’ end of 32P-labelled bacteriophage f2 RNA by using an ingenious diagonal technique (Dahlberg 1968). Surprisingly, partial T₁ RNase digests of radiolabelled phage R17 RNA gave discrete bands on polyacrylamide gel electrophoresis. Several bands were sequenced, mainly by Jerry Adams and Peter Jeppesen. The first one analysed turned out to be a region of RNA coding for a known amino acid sequence of the phage’s coat protein sequence (20). This was 57 nucleotides long and was the first time that any sequence of mRNA had been directly sequenced (figure 8). There were no surprises. The genetic code—worked out by indirect methods in the mid 1960s, was confirmed, but Fred thought that this effort was worthwhile (Brownlee 2014). However, Fred did not complete the sequence of the bacteriophage R17 RNA. This was done by Walter Fiers’s group for the related MS2 bacteriophage RNA (Fiers et al. 1976), because by the late 1960s Fred had turned his attention to DNA.
DNA sequencing

Early approaches

The problem with sequencing DNA in the early 1970s was the obvious limitation that DNA was too long to attempt to sequence. Unlike the situation with RNA, there were no short DNAs on which to test sequencing methods. Moreover, no highly specific DNases were available: restriction enzymes had not yet been discovered. The smallest known DNA bacteriophages (f1, fd and ΦX174) were about 5000 nucleotides long. Despite these limitations, much earlier—before Fred had become interested in DNA—Burton & Petersen (1960) perfected a chemical degradation method, the ‘depurination’ reaction, to isolate and sequence various short pyrimidine-containing oligonucleotides. Somewhat later, Murray & Offord (1966), working in Fred’s laboratory, also purified and characterized short labelled DNA oligonucleotides that had been derived by enzymatic digestion. They used the two-dimensional separation methods developed for sequencing RNA.

In the early 1970s several postdoctoral researchers and PhD students were working in Fred’s laboratory trying to initiate DNA sequencing. John Sedat, Ed Ziff and Francis Galibert collaborated by modifying the sequencing methods previously used with RNA, to sequence regions of ΦX174 phage DNA—despite its size. In an ambitious experiment they uniformly labelled it with $^{32}$P-phosphate and then digested the $^{32}$P-labelled ΦX174 DNA with enzymes. Endonuclease IV, under carefully defined conditions, gave surprisingly good results on polyacrylamide gel fractionation, and a 48-nucleotide sequence of DNA was established (Ziff et al. 1973).

Another important early initiative was by Vic Ling (Ling 1972). Building on earlier work of Maria Székely in Fred’s laboratory (21), Vic Ling sequenced some of the longer depurination products of bacteriophage fd DNA by post-labelling their 5′ ends with $^{32}$P, using T4 polynucleotide kinase. In particular, he defined the sequence of a 20-nucleotide product by using a two-dimensional ‘wandering spot’ method to determine its sequence. In this method the sequence was deduced from the relative positions of the successive degradation products of a partial 3′-exonuclease digest. Thus this paper made extensive use of positional information to establish sequence.

In yet another approach, Elizabeth Blackburn (FRS 1992) (Blackburn 1975) copied the now known 48-nucleotide fragment of ΦX174 DNA with RNA polymerase, introducing the $^{32}$P label as a labelled nucleoside triphosphate in the transcription reaction. Clearly several different approaches to sequencing DNA were going on in Fred’s laboratory in the early 1970s. Yet Fred was not an author on all this early DNA sequence from his laboratory because he felt he had not made a significant contribution to this work.

Instead, Fred himself became interested in a different sequencing approach based on copying DNA with DNA polymerases. This was stimulated by the earlier success of Wu & Kaiser (1968), who had copied the short 12-nucleotide sticky ends of another, much longer DNA, phage λ, with DNA polymerase, as an aid to sequencing. Fred wanted to generalize their approach and had chosen to copy the single-stranded DNA of bacteriophage f1 with DNA polymerase. But he needed a primer (a short DNA oligonucleotide) to hybridize with the single-stranded template f1 DNA to initiate DNA synthesis. On the basis of the supposedly known sequence (Met-Try-Val) in the coat protein of f1, Fred designed a unique octanucleotide primer and collaborated with D. Fischer and H. Kossel to synthesize it for him. A year later (it took that long to synthesize it), Fred tried this octanucleotide, introducing a $^{32}$P label into the
transcript by incorporating one $^{32}$P-labelled nucleoside triphosphate, usually $^{32}$P-dATP, with the other three unlabelled triphosphates and DNA polymerase. Working with John Donelson (a postdoctoral researcher from the USA) and Alan Coulson (his assistant), he analysed the synthesized DNA on the two-dimensional system, using homochromatography in the second dimension on thin layers. To aid in the sequence analysis, Fred substituted CTP (cytidine triphosphate) for dCTP (deoxycytidine triphosphate) by using the known misincorporation of CTP, catalysed by Mn$^{2+}$ (Berg et al. 1963). Despite the fact that the octanucleotide failed to prime synthesis in the predicted region of the coat protein gene (because of an error in the protein sequence), it did prime in a unique position elsewhere. Fred was able to successfully deduce a sequence of 50 residues.

He took advantage of the CTP incorporated into DNA to digest successively longer primed fragments with pancreatic RNase (22).

Now in theory he could start with unlabelled f1 DNA but still use his radioactive approach by introducing the radioactivity into the products of the synthetic reaction. In this way he could study a long DNA by sampling a short section of the DNA template. This was a bold decision at the time and paved the way for one-dimensional sequencing by the plus and minus method.

**The plus and minus method**

In this method radioactively labelled DNA products, synthesized by DNA polymerase in a primed synthesis reaction, were ‘read off’ by examining the positions of bands in adjacent lanes of a polyacrylamide gel fractionation (23). Fred recorded later that ‘this new approach to DNA sequencing was I think the best idea I have ever had, being original and ultimately successful’ (34). In this new method, unlike all previous methods, there was no need to analyse the products further after their fractionation.

It is of some interest to know when, and how, Fred developed this first one-dimensional sequencing method. One difficulty in establishing this is that Fred has admitted that his laboratory records were incomplete at the time that he was developing the plus and minus method (31). However, it seems that the first attempt was in about April 1973 (Welcome/Sanger archives) in a series of experiments using primed extension with the octanucleotide, an f1 DNA template and DNA polymerase. Fred first randomized the size range of products of radioactive synthesis—labelled with either $^{32}$P-dATP or $^{32}$P-dGTP (synthesized in-house). He purified the labelled, synthetic DNA from unincorporated triphosphates on a size-exclusion column. Then, and this is the crucial part, he incubated four aliquots of the column sample with one triphosphate, either dCTP, dATP, dGTP or dTTP, and T4 DNA polymerase—the plus system. T4 DNA polymerase degrades the labelled DNA by using its 3′-exonuclease activity until the required triphosphate, either C, A, G or T, is required for synthesis, so all fragments end in a known residue (Englund 1971). With another four aliquots he incubated with only three triphosphates and the Klenow fragment of *E. coli* DNA polymerase, omitting in turn either dCTP, dATP, dGTP or dTTP—the minus system. In this case the DNA polymerase would synthesize a short section of DNA until the absent triphosphate, either dCTP, dATP, dGTP or dTTP, was needed. Such products would end before the absent dCTP residue. These eight aliquots were fractionated by one-dimensional homochromatography. Initial results were unclear, but they did ‘show that some things have happened’ (37). In a subsequent experiment, Fred repeated the primed extension reaction and gave a sample to his postdoctoral researcher, John Donelson, who fractionated the samples side by side on a polyacrylamide gel. This looked slightly better than the separation by homochromatography and paved the way for optimizing polyacrylamide gel conditions.
Polyacrylamide gels were fairly standard for separating proteins and nucleic acids from one another at that time but they had never been used to sequence DNA before. It had not been realized that quite long nucleic acids differing by only a single residue could be resolved from one another. Fred subsequently optimized conditions, finding eventually that long, very thin, highly denaturing 7 M urea gels, run at about 50 °C, were best, and resolved up to about 450 residues (27). Although there were some limitations to the plus and minus method—in that the intensities of bands on the gels could be somewhat uneven—Fred and his colleagues Gillian Air, Bart Barrell, Nigel Brown, Alan Coulson, John Fiddes, Clyde Hutchinson III, Pat Slocombe and Mike Smith used this method to describe a provisional sequence of approximately 5375 nucleotides of bacteriophage ΦX174 DNA (24). Fortunately, restriction fragments of ΦX174 DNA could now be used as primers: there was no need to synthesize primers chemically.

The dideoxy method

Despite the huge advance of the plus and minus method for sequencing DNA, Fred was not satisfied with its accuracy and wished to devise another method to define the nucleotide at the end of a sequence. He was stimulated to consider the use of chain terminators by the group under Arthur Kornberg (ForMemRS 1970), who had shown that 2′,3′-dideoxy-TTP (figure 9) acted as a chain terminator for *E. coli* DNA polymerase (Atkinson *et al*. 1969). The 2′,3′-dideoxy residue, once incorporated into the DNA, could not be extended further because it lacked the 3′-hydroxyl group necessary for chain extension. The idea then was to mix the 2′,3′-dideoxy-TTP with the normal dTTP, in the presence of the other three usual triphosphates (dCTP, dATP and dGTP, one of which was radioactively labelled), so that partial termination occurred in a primed synthesis reaction. In that way one should obtain a series of bands covering a region of the DNA template in which individual dideoxy-terminated bands would represent a known residue. Only 2′,3′-dideoxy-TTP had been synthesized before; dideoxy-CTP, dideoxy-ATP and dideoxy-GTP were not available. To establish this method, Fred and Alan Coulson, therefore, had to synthesize these themselves, with advice from Mike Gait and Bob Shepherd. About a year later, now armed with the four dideoxy triphosphates, Fred, Alan Coulson and Steve Nicklen described their method in their classic 1977 paper—arguably Fred’s most important paper (25). This dideoxy method (figures 10 and 11) proved to be simpler and more accurate than the plus and minus method. He and his colleagues, now
including Ted Friedmann, then repeated the sequence of ΦX174 DNA, revising about 30 residues in the now 5386-nucleotide sequence (28). In the same year Maxam & Gilbert (1977) described an alternative, direct, sequencing method that depended on the partial chemical degradation of 5′ or 3′ end-labelled DNA strands. This alternative method was quite widely used initially because it was equally applicable to double-stranded and single-stranded DNA.

Fred now wanted the challenge of sequencing even longer DNAs, such as human mitochondrial DNA (about 16.5 × 10^3 nucleotides), and phage λ DNA (about 48.5 × 10^3 nucleotides). These longer DNAs, being double-stranded, had to be converted to single-
stranded DNA for his dideoxy sequencing method. There was also the need for more efficient and faster sequencing to speed up the data acquisition, significantly helped by Rodger Staden’s computer programs (Staden 1980). Fred, with Alan Coulson, Bart Barrell, Andrew Smith and Bruce Roe, realized both aims by introducing a random, ‘shotgun’ cloning of DNA into a modified M13 phage DNA (Gronenborn & Messing 1978) from which individual, random recombinant clones could be isolated (29). This speeded up sequencing immeasurably because cloning in M13 effectively purified the individual DNAs before sequencing. Dideoxy sequencing was also standardized, because ‘universal primers’, which hybridized to the M13 DNA vector sequences flanking the cloned insert, could now be used. Fred was always keen on shotgun sequencing, which he used extensively in the phage λ work with Coulson, in which about 90% of the DNA was obtained by a shotgun approach. Sonication was the preferred method of obtaining randomly cut DNA. However, directed approaches, which were both slow and frustrating, were needed to complete the sequence of the 48,502 nucleotides with the help of Guo-Fong Hong, Diana Hill and George Petersen (33).

Fred confirmed his preference for shotgun sequencing in later correspondence with Craig Venter—congratulating Venter on his shotgun cloning of the genome of Mycoplasma genitalium in 1995. Writing to Venter (Brownlee archives, ex Sir Ken Murray FRS) Fred said:

I was interested to see how far it is possible to go with the shotgun approach. I was always keen on it but one of the main problems was that it was not popular with my collaborators. Each person likes to have a bit of sequence they can call their own. I suppose this is not a problem now with all the automation. It has certainly come a long way since the λ work.

Many scientists who worked with Fred were amazed at the techniques that Fred Sanger used and developed in his experimental approach. Perhaps the most obvious was the use of micro-methods. Undoubtedly he developed them because of the need to apply very small volumes of peptides or nucleotides onto paper, and later onto gels—methods that were required for sequencing insulin, RNA and DNA. Learning how to manipulate very small reaction volumes by evaporating solutions onto a polythene sheet in a desiccator and setting up 1 μl reactions in glass capillary tubes were essential if you were to succeed in Fred’s laboratory. Equally ingenious were his techniques for eluting peptides or oligonucleotides from paper, from ion-exchange paper, from thin layers or from gels. Bits of wood and Plasticine—a commercial, coloured, malleable modelling clay—were essential items of laboratory equipment for holding capillary tubes or small glass test tubes in place.

**OVERLAPPING GENES IN ΦX174 DNA; DIFFERENT CODE IN MITOCHONDRIA**

Fred’s approach was always methodological. How long a DNA could he sequence? How could he improve his methods? His motivation for sequencing was not primarily to discover the biological significance of the sequence he described. Nevertheless, by sequencing phage ΦX174 DNA and human mitochondrial DNA he and his co-workers discovered two quite unexpected and novel properties in gene organization.

The first surprise was the presence of ‘overlapping genes’ in ΦX174 DNA, described in a classic paper by Bart Barrell, Gillian Air and Clyde Hutchinson III, but not including Fred’s name as an author (Barrell et al. 1976). This was another occasion on which Fred felt he had not done enough to warrant authorship. Unambiguous evidence for an overlap of genes D and E was obtained by studying the sequence of wild-type ΦX174 DNA and amber (UAG-
producing) stop codon mutations in both genes. Another paper, now including Fred Sanger’s name along with Mike Smith and Nigel Brown, showed that genes A and B also overlapped (26). It was suggested that in the evolution of small genomes such as ΦX174 DNA there may have been selective pressure for new proteins overlapping with existing proteins, because there was insufficient non-coding DNA elsewhere.

The second surprise was that human mitochondrial DNA (16 569 nucleotides) and bovine mitochondrial DNA (16 338 nucleotides) have a different genetic code from the standard genetic code (Barrell et al. 1979) (31, 32). (The human mitochondrial DNA (Cambridge reference sequence) was re-sequenced and slightly revised by Andrews et al. (1999).) In mammalian mitochondria, UGA (normally a chain terminator codon) now codes for tryptophan, and AUA (normally coding for isoleucine) for methionine. This unusual mitochondrial genetic code was a complete surprise because previously the genetic code, worked out in the early 1960s, was considered to be universal. Moreover it was proposed (31, 32) that AGA and AGG (normally coding for arginine) could now be chain terminators in addition to the usual terminators UAA and UAG. However, more recent work by Temperley et al. (2010) has suggested that AGA and AGG may not be terminators, because backwards slippage of the mRNA on the ribosome (ribosome frameshifting) may occur at a U residue one nucleotide upstream of these proposed terminators, converting them to classical UAG terminators.

Along with the unusual genetic code in mitochondria, there emerged another unexpected feature of mitochondrial-encoded tRNAs. Only 22 different mitochondrial tRNAs were predicted from the DNA sequence, in contrast with the 32 or more normal cytoplasmic number of tRNAs. Many of the mitochondrial tRNAs were predicted to have an unusual ‘cloverleaf’ structure lacking the invariant features of tRNA, such as the TψCG sequence (where ψ represents pseudouridine) and the dihydro-U arm. The most bizarre was the serine tRNA coding for AGC or AGU. The small number of mitochondrial tRNAs strongly suggested that some mitochondrial tRNAs must recognize the redundant third position of the codon with a modified anticodon:codon base pairing. In such mitochondrial tRNAs a U residue in the first position of the anticodon may ‘wobble’ with either A, C, G or U in the third position of the codon (30). Overall, the different genetic code of mitochondria and the different nature of the tRNAs was consistent with the endosymbiotic origins of mitochondria, presumed to derive from some primitive prokaryote (31).

Sequencing mitochondrial DNA involved many of Fred’s co-workers, including Steve Anderson, Alan Bankier, Maarten de Bruijn, Bart Barrell, Ellson Chen, Alan Coulson, Jacques Drouin, Ian Eperon, Don Nierlich, Bruce Roe, Peter Schreier, Andrew Smith, Rodger Staden and Ian Young. According to Fred, Bart Barrell was particularly involved in analysing the mitochondrial DNA data and predicting where tRNA sequences were present (34, 36).

Fred was awarded his second Nobel Prize in Chemistry in 1980 (35), jointly with Paul Berg and Walter Gilbert. Fred is one of only four scientists worldwide to have been awarded the Nobel Prize twice, the others being Marie Curie, Linus Pauling ForMemRS and John Bardeen ForMemRS. He is the only British scientist to have achieved this distinction.

INFLUENCE ON OTHERS

Fred rarely tried to influence others directly, although he always encouraged his scientific colleagues if they asked his advice. He said his own experiments often failed and one should just keep trying again. His main influence was through example. He was an experimentalist par excellence, who liked to ‘mess about’ in the laboratory (figure 12). His experimental notebooks
(archived in the Wellcome Library, London) are mainly in his own handwriting. They cover his whole career from 1940 to 1983, indicating the importance that he attached to an experimental approach with his own hands. At the MRC Laboratory of Molecular Biology I observed that he rarely spent time in his small office other than to dictate letters to his secretary, Peggy Dowding, or to plan his experiments for the day, or to write up his experimental results. He had no great liking for committee meetings. Only very rarely was there a meeting with his group leaders, Ieuan Harris, Brian Hartley and César Milstein. Normally he was to be found working in his small laboratory, which he shared with his assistant, adjoining his office. Many of his critical contributions were work done by himself with either Bart Barrell or, later, Alan Coulson. By establishing the sequence of insulin—the first protein ever to be sequenced—he opened up the field of protein sequencing in general. By finding a quick method of sequencing DNA he opened up the possibility of whole-genome sequencing—for example, the possibility of sequencing the human genome. This goal was achieved in 2001 with his dideoxy sequencing method, albeit modified with fluorescent tags replacing radioactive tags. Since then, DNA sequencing has been automated, so that many billions of DNA fragments are sequenced in massively parallel approaches (Brownlee 2014). However, most currently used sequencing methods still rely on the principles that Sanger developed in his 1977 paper (25). DNA sequencing now impacts on the whole of biology in many varied fields, including medicine, evolutionary biology, virology, bacteriology, cell biology, archaeology, botany and forensic science.
Early on in his career Fred was constrained by the limited space allocated to him in the Biochemistry Department. In the late 1950s he collaborated with Max Perutz FRS, head of an MRC Unit at the Cavendish Department of Physics (who was also short of space), to lobby the MRC for a new joint research institute, which opened in 1962 as the MRC Laboratory of Molecular Biology, Cambridge. New archive material (Wellcome/Sanger archive) suggests that it was Fred who took the initiative to join forces with Perutz’s group.

Later, in 1993, Fred officially opened the Sanger Centre, renamed the Wellcome Trust Sanger Institute, at Hinxton Hall, Hinxton, Cambridgeshire, under John (now Sir John) Sulston FRS as director. This became a world-leading laboratory, spearheading the considerable UK contribution to the human and nematode (*Caenorhabditis elegans*) genome sequencing projects. Fred is reported to have agreed that the building be named after him on condition that ‘It had better be good’ (Sulston & Ferry 2002). The Department of Biochemistry building in Cambridge—the Sanger Building—was also opened by Fred in 1997. Later, in 2007, he opened the Sanger Centre for Science and Mathematics at his old school, Bryanston.

Fred supervised more than 10 PhD students, including me, throughout his career. His most renowned students were Rodney Porter (FRS 1964) and Elizabeth Blackburn. Rodney Porter (1917–85) was his first student—joining him in 1947, and was actually a year older than Fred (figure 13). He was, according to Fred, more a colleague than a student. Rodney was always
interested in antibodies and did not participate in the insulin sequence work that Fred was doing at that time. Porter was awarded the 1972 Nobel Prize in Physiology or Medicine for his work on antibody structure. Elizabeth Blackburn—a student with Fred much later, from 1971 to 1974—from Melbourne, Australia, became a Nobel laureate in 2009 for her work on telomerase and the sequence at the ends of chromosomes.

CONFERENCES, RECREATION, HOBBIES

Fred was brought up as a strict Quaker, but after he retired he recorded (36), ‘Although I never became a Quaker and eventually found an absolute religious belief difficult to reconcile with a scientific life, I feel the Quaker upbringing was a major influence on me, especially in regard to the importance of telling the truth and [acting according to] one’s own conscience.’ Moreover, he always valued his family life and took regular family holidays, sometimes combining them with invitations to scientific conferences to which he was invited as a speaker. He and Joan particularly enjoyed their trip to Australia as part of an International Wool Festival in 1955, after which they joined Ted Thompson and his wife for a holiday at Heron Island on the Great Barrier Reef. Another memorable trip with Joan, after his first Nobel prize in 1958, was to South America, including Peru, Argentina, Brazil and Chile, a mixture of work—giving lectures—and pleasure. Later, Fred attended scientific conferences somewhat less frequently, although he was happy to travel when he felt that he had made a significant scientific advance, especially if Joan could accompany him. He visited Australia several more times, including a three-month mini-sabbatical to Bill Elliott’s department in Adelaide in 1974. Over his career he arranged several extensive, multi-centre lecture tours in the USA.
and Canada. Fred had many friends, scientific colleagues and admirers throughout the world. He accepted an invitation sponsored by the British Council to visit Russia in 1963 and a visit to China sponsored by the Royal Society in 1980—jointly with his first PhD student, Rodney Porter (figure 14).

One of Fred’s main hobbies was boating. He owned a punt in the 1940s, when his children were quite small, but then he built several boats himself from kits, helped by his son Peter, and he also owned a cabin cruiser for river use. Subsequently, in about 1960, Fred bought a four-berth, estuary and seagoing sailing vessel with two masts and inbuilt engine. It was moored at Orfordness, Suffolk, a short way in from the North Sea on the river near Aldeburgh—of Benjamin Britten fame. Fred entertained family and friends on this quite impressive boat. In about 1980 Fred bought a river cruiser, which he and his family used on the Nene and Ouse on the Norfolk Broads. He had fitted this out himself using his carpentry skills. Earlier in his career Fred was an accomplished squash player and as an undergraduate played for the St John’s College second team. Later, when he was a member of the Biochemistry Department, he was at times top of the departmental squash ladder. Richard Perham FRS (1937–2015) said he had a knack of dominating the centre of the court and, even at the age of 45 years, was difficult to beat (personal communication). Fred also occasionally played cricket for the Laboratory of Molecular Biology laboratory team. He was quite a keen skier, later managing to combine skiing holidays with conferences in Banff in the Canadian Rockies, at Lake Tahoe in the USA and in Queenstown in New Zealand.

RETIRED

Fred retired in 1983, aged 65 years, saying he now wanted to devote his remaining time to his hobbies of boating, gardening and spending more time with his family, his elder son, Robin, his younger son, Peter, his two grandchildren, Ben and David, and his daughter, Sally. Fred did not pursue any academic activity after retirement, other than compiling a collection of his more important papers with Peggy Dowding, who had been his secretary (37), and writing his reminiscences (34). But he did keep in touch with his trusted assistants, Bart Barrell and Alan Coulson, both of whom had now become PhDs (figure 15). Fred and Joan moved from 252 Hills Road, Cambridge, to ‘Far Leys’ (named after his parents’ house in Tanworth-in-Arden) in Swaffham Bulbeck in the Fens, with a large enough garden (1.25 acres) to keep...
Fred occupied in retirement. In fact, the house and garden took up most of Fred’s time, because Joan was becoming less active then and needed his help. Nevertheless, besides his gardening interests—especially in cultivating roses and candelabra primulas—Fred managed some painting and carpentry, skills he had learned during his boyhood. After some decline in physical and mental health latterly, Fred died in 2013, aged 95 years. A moving memorial service was held at his old college, St John’s College, Cambridge, on 8 November 2014 to commemorate his immense contribution to molecular biology. On that occasion, Sir John Walker FRS likened the impact of Fred’s work to that of Charles Darwin. Fred Sanger was awarded many honours besides his two Nobel prizes. He was awarded the Order of Merit in 1986, although had earlier refused a knighthood, not wanting to be addressed as ‘Sir’. His full honours are listed below. He wore them lightly and without pretence. Fred’s wife, Joan, predeceased Fred, who is survived by his sister, Mary (May) Willford, and his three children, Robin, Peter and Sally.

Honours

Civic

1963 Commander of the Order of the British Empire (CBE)
1981 Companion of Honour (CH)
1986 Order of Merit (OM)

Scientific

1951 Corday–Morgan Medal and Prize, Chemical Society
1954 Fellow of King’s College, Cambridge
Fellow of the Royal Society
1958 Foreign Honorary Member of the American Academy of Arts and Sciences
Nobel Prize in Chemistry
1961 Honorary Member of the American Society of Biological Chemists
Member of the Academy of Science of Argentina
Member of the Academy of Science of Brazil
Honorary Member of the Japanese Biochemical Society
Corresponding Member of the Asociación Química of Argentina
1962 Member of the World Academy of Art and Science
1966 Alfred Benzon Prize, Denmark
Honorary Fellow, National Institute of Sciences of India
1967 Foreign Associate of the US National Academy of Sciences
1968 Honorary DSc, Leicester University
1969 Royal Medal, Royal Society
1970 Honorary DSc, Oxford University
Honorary DSc, Strasbourg University
1971 Sir Frederick Gowland Hopkins Memorial Medal, Biochemical Society
Gairdner Foundation Annual Award, Canada
1976 William Bate Hardy Prize, Cambridge Philosophical Society
Hanbury Memorial Medal, Pharmaceutical Society of Great Britain
Fellow of the Royal Society of Edinburgh
Frederick Sanger

1977 Copley Medal, Royal Society
1978 G. W. Wheland Medal, Chicago University
1979 Louis Gross Horwitz Prize, Columbia University, New York
Alpert Lasker Award, New York
Gairdner Foundation Annual Award, Canada
1980 Biochemical Analysis Prize, German Society of Clinical Chemists
Nobel Prize in Chemistry
1981 Foreign Associate, French Academy of Sciences
1982 Corresponding Member, Australian Academy of Sciences
Dale Medal, Society for Endocrinology
1983 Honorary Fellow of King’s College, Cambridge
Gold Medal, Royal Society of Medicine
Honorary ScD, University of Cambridge
1984 Honorary Member, Biochemical Society
1994 Association of Biomolecular Resource Facilities Award
2010 Honorary Fellow, St John’s College, Cambridge
2013 Fellow, American Association for Cancer Research Academy

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The frontispiece photograph was taken in 1980 at the Laboratory of Molecular Biology, Cambridge. Copyright © MRC Laboratory of Molecular Biology; reproduced with permission.

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