RETROSPECTIVE

Fred Sanger: A memorial tribute

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Of the three main activities involved in scientific research, thinking, talking, and doing, I much prefer the last and am probably best at it. I am all right at the thinking, but not much good at the talking.

—Frederick Sanger, 1988

Frederick (Fred) Sanger, twice winner of the Nobel Prize in Chemistry—the first in 1958, for revealing that proteins have a unique molecular structure, and the second in 1980, for developing the knowhow for sequencing DNA—died at age 95 in Cambridge, England on November 19, 2013. It is simply impossible to overestimate the impact he has had on modern genetics and molecular biology, with profound consequences to all of the life sciences. His modest manner and quiet determination to carry out experiments himself, right to the end of his career, was an inspiration for his colleagues and young scientists everywhere.

Frederick Sanger was born on August 13, 1918 into a Quaker family in the ancient village of Rendcomb in Gloucestershire, where his father was the local doctor. Responding to his father's influence, Sanger became interested in biology and aimed for a career in medicine; however, while still at the Bryanston School in Dorset he decided he would be best suited to a scientific career, one which possibly might have an impact in medicine. Sanger won a place at St John's College, Cambridge, where he became interested in the emerging field of biochemistry, convinced that it offered a way to develop a more scientific basis to understand many medical problems.

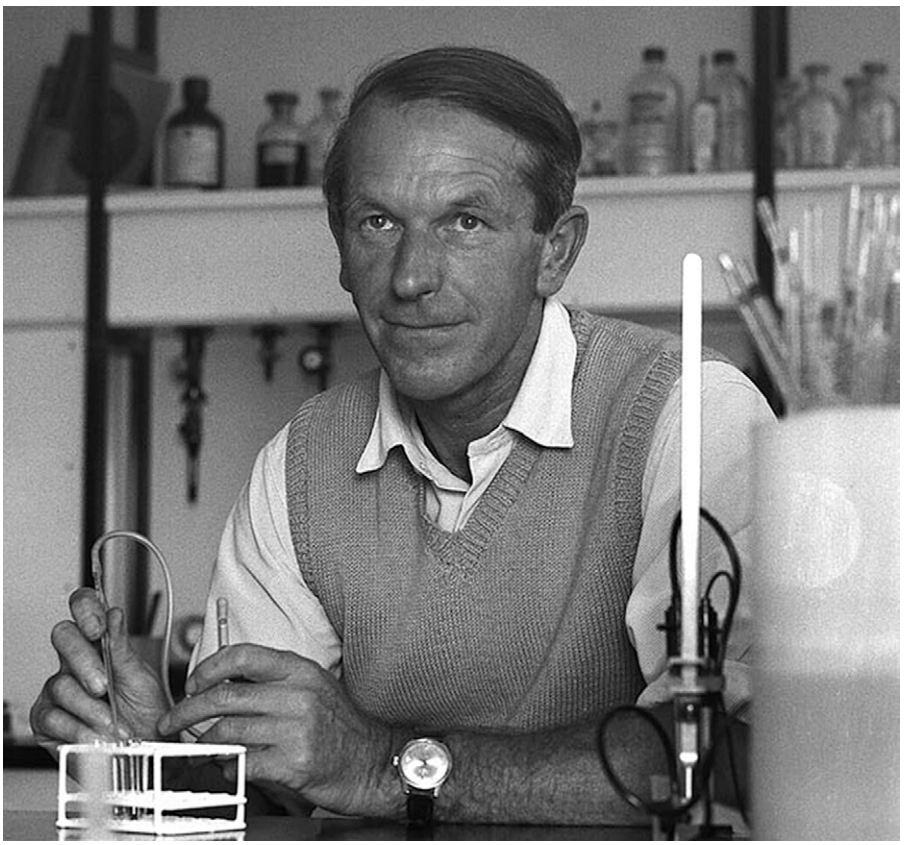

Fig. 1. Frederick Sanger 1918–2013. Image courtesy of MRC Laboratory of Molecular Biology.

Having been raised as a Quaker with strong antiwar sentiments, Sanger was relieved from serving in the British armed services during World War II. Instead, he engaged in antiwar efforts and participated in social relief work and, briefly, as a hospital orderly. Sanger received his bachelor's degree in 1939 and remained at Cambridge throughout the war years to work on his doctorate. His research advisor, Professor A. Neuberger, encouraged him to focus on the metabolism of lysine as well as a more important problem confronting England at the time, namely the nutritional value of potatoes. Sanger was awarded the doctorate in 1943.

Professor A. C. Chibnall, a protein chemist, having just replaced the very influential F. G. Hopkins as Head of the Biochemistry Department, offered Sanger space and the freedom to do his own research. Within a year, Sanger was awarded a Beit Memorial Fellowship for Medical Research and turned his attention to the chemistry of insulin, an astute choice as it was one of very few proteins that could be obtained in pure form at the time. Sanger made use of a newly available colored reagent, fluorodinitrobenzene (FDNB), to tag and identify the termini of insulin's two chains as glycine and phenylalanine. By using partial acid hydrolysis and enzymatic digestions with different proteases, Sanger generated with each digestion different populations of peptides whose amino-termini could be tagged with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and separated by the then new techniques of paper chromatography and electrophoresis. Painfully but methodically, Sanger deduced the amino acid sequence of the two insulin chains. Determining which cysteins were involved in the single intra-A chain disulfide bond and in the two disulfide bonds linking the A and B chains together proved to be more difficult, and took several years more to solve. By 1955, the entire sequences of the insulin A and B chains were known and the linkages that held the two chains together were established.

This remarkable achievement was rewarded with the 1958 Nobel Prize in Chemistry. At the Prize ceremony, the Swedish Academy of Sciences citation speech noted:

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> You knew when you began to look into the structure of the insulin molecule 15 years ago that the problem was a formidable one. So did the whole scientific world. Those who knew you, were confident, however, that you would ultimately succeed, and each successive publication from your laboratory strengthened our confidence. . . . Now that many years of work have been crowned with success you may look back and rejoice.

However, rejoicing was not Sanger's style, for in his acceptance speech at the banquet he noted:

> If one takes stock at the end of a day or a week or a month and asks oneself what have I actually accomplished during this period, the answer is often 'nothing' or very little and one is apt to be discouraged and wonder if it is really worth all the effort that one devotes to some small detail of science that may in fact never materialize. It is at times like the present that one knows that it is always worth-while and I am extremely grateful to the Academy and the Nobel Foundation for giving me this great encouragement.

Sanger's formidable accomplishment that insulin—and by inference other proteins as well—have precise amino acid sequences, brought to the fore the puzzle of how organisms are able to manufacture the tens of thousands of different proteins, each with a unique arrangement of its constituent amino acids. Remarkably, the clue to that conundrum was the basis for awarding the 1958 Nobel Prize in Physiology or Medicine to George Beadle and Edward Tatum at the very same ceremony at which Sanger received his prize. The contribution of these men, made during the 1940s, established that genes provide the information for ordering the amino acids for each protein: "one gene, one protein." Precisely how genes direct the assembly of proteins became the central question of molecular biology during the succeeding decades. Unsurprisingly, Fred Sanger played a seminal role in that advance, for which he received a second Nobel Prize in Chemistry in 1980.

In 1962, Sanger moved from the university biochemistry laboratories to the newly opened Medical Research Council Laboratory of Molecular Biology on the outskirts of Cambridge. Amid the conjectures of Francis Crick and Sydney Brenner on the existence of a genetic code, Sanger recognized that solving the code and learning how its message is translated into proteins would ultimately require knowing the nucleotide sequences of the relevant RNAs and DNAs.

Focusing first on determining the sequence of RNAs, Sanger adopted the approach that was so successful in sequencing insulin: use a variety of nucleases to partially or completely digest the RNA; use the newly developed electrophoretic and chromatographic methods to separate the resulting fragments; determine the nucleotide sequence of each fragment; and deduce their order in the parent molecule. This basic approach succeeded for determining the sequence of *Escherichia coli* ribosomal 5S RNA, several special tRNAs, and the bacteriophage R17 RNA genome.

Although Sanger had dallied with attempts to sequence short stretches of DNA in the late 1960s, the prospect for having cloned DNAs in the early 1970s made having a robust way to sequence DNA imperative. Again, Sanger rose to the challenge. For that purpose, he chose a different approach. Instead of making fragments of the molecules to be sequenced, he determined the nucleotide sequence of copies of the DNA produced by DNA polymerases. After a more tedious procedure he referred to as "the plus-minus method," Sanger hit on what has been referred to as the dideoxy method. By including a 2′,3′-di-deoxy derivative of the four different nucleoside triphosphates along with the normal deoxy nucleoside triphosphates in separate polymerase reactions, the growing chains were terminated wherever the di-deoxy nucleotide was incorporated. Separating the resulting nested set of fragments with thin layer polyacrylamide gels allows the sequence to be read directly by their positions in the electrophoretic gels. Sanger himself sequenced human mitochondrial DNA (16,589 nucleotides), and the bacteriophage λ genome (48,502 nucleotides). Capping that era of remarkable achievements, the Sanger method was at the core of the multinational effort to sequence the human genome and, subsequently, the genomes of innumerable other organisms, even of ancient humanoid and prehistoric species. Indeed, it is now possible to sequence a human genome or those of human cancers in one or two days.

For this achievement, The Nobel Foundation awarded Sanger a second Nobel Prize in Chemistry in 1980. Sharing in that occasion enabled me to experience the magic of his personality. At every point during the Nobel week's many interviews, panel discussions, and research presentations, Sanger's modesty and retiring demeanor were evident. He did not shrink from the many pranks and tasks the Swedish students put upon the Nobel Prize recipients. Sanger's modesty, sometimes bordering on self-deprecation, is embodied in a widely quoted self-description: "I was just a chap who messed about in his lab."

At the formal banquet, Sanger rose to say:

> When I was here 22 years ago I addressed the students of 1958 as "fellow students" because although I was 40 years old I still felt that I was one of them, and I still feel the same today. I and my colleagues here have been engaged in the pursuit of knowledge. We have been learning, are still learning and I hope will continue to learn. I believe that we have been doing this not primarily to achieve riches or even honour, but rather because we were interested in the work, enjoyed doing it and felt very strongly that it was worthwhile. Scientific research is one of the most exciting and rewarding of occupations. It is like a voyage of discovery into unknown lands, seeking not for new territory but for new knowledge. It should appeal to those with a good sense of adventure. When I was young my Father used to tell me that the two most worthwhile pursuits in life were the pursuit of truth and of beauty and I believe that Alfred Nobel must have felt much the same when he gave these prizes for literature and the sciences. Through art and science in their broadest senses it is possible to make a permanent contribution towards the improvement and enrichment of human life and it is these pursuits that we students are engaged in. So, fellow students, I would like to thank you for your part in these celebrations and for your words. Finally, we would like to salute you young students on whom the future depends [Frederick Sanger—Banquet Speech (2014) Nobelprize.org. Nobel Media AB 2013. www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/sanger-speech.html].

Fred Sanger retired at the age of 66 to spend more time with his family and to enjoy his hobbies of boating and gardening. In 1992 the Wellcome Trust and the Medical Research Council established the Sanger Centre for furthering the knowledge of genomes. Sanger received many honors and prizes, including Fellowship of the Royal Society and Foreign Associate of the US National Academy of Sciences. He was awarded the Royal Medal and Copley Medal of the Royal Society and the Albert Lasker Basic Medical Research Award, and he held honorary degrees at Oxford, Cambridge, and many other universities. Characteristically, not wanting to be called "Sir," Sanger turned down a knighthood. "A knighthood makes you different, doesn't it?" he said, "and I don't want to be different." Sanger did, however, accept the considerably more distinguished Order of Merit from the Queen in 1986.

Frederick Sanger was married in 1940 to Margaret Joan Howe, who died in 2012. She was not a scientist, but Sanger described her as having contributed more to his work than anyone else by providing a peaceful and happy home. Surviving are his three children, Robin, Peter, and Sally, and two grandchildren.