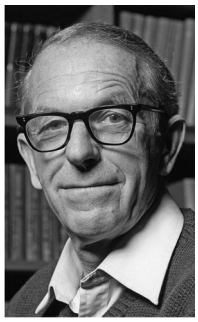

F. Sanger

Frederick Sanger (1918–2013)

Frederick (Fred) Sanger died on 19th November, 2013 aged 95. He is the only person to have been awarded two Nobel Prizes in Chemistry, in 1958 and 1980 (*Angew. Chem.* **1981**, *93*, 127), for his development of methods for the sequencing of proteins and nucleic acids, respectively. He was modest and undemonstrative in the extreme, but he was hugely inspirational and influential. He was a pivotal figure in 20th century science and the medical applications of what he made possible are only now beginning to be realized.

Fred Sanger was born in Rendcomb in Gloucestershire into the family of a doctor. He was educated at Bryanston School, where he was first drawn towards practical chemistry, and went on to St John's College, Cambridge, where he took a first-class BA in biochemistry in 1940. Being a Quaker and conscientious objector, he was able to take up research (on lysine metabolism) supervised by Albert Neuberger in the Department of Biochemistry at Cambridge. This was the beginning of an entire working life spent in Cambridge.

In 1943, he started his independent research stimulated by Albert Chibnall. He began his determination of the amino-terminal groups of insulin, the eventual complete sequencing of which led to the awarding of his first Nobel Prize. The sequencing required the development of the novel reagent fluorodinitrobenzene (Sanger's reagent), and the implementation of a partial hydrolysis strategy to generate separable (by electrophoresis and chromatography) overlapping fragments, a theme apparent in much of his later work.

In 1962, Fred moved to the newly founded Medical Research Council (MRC) Laboratory of Molecular Biology. He began then to develop methods for the sequencing of RNA based on the labeling of nucleotides with radioactive ^{32}P. Enzymatic fragmentation methods and analytical electrophoretic two-dimensional fractionation techniques were developed with his assistant Bart Barrell. The sequence of the 120 nucleotide *E. coli* 5S rRNA was determined in 1967 with Barrell and George Brownlee. The sequencing of longer RNA, parts of the 3300 nucleotide bacteriophage R17 genome, demonstrated directly for the first time the correspondence between the genetic code and an amino acid sequence (of the previously sequenced coat protein).

In the late 1960s, Fred began to develop methods for sequencing DNA. The extension by polymerase of a short oligonucleotide primer on a single-stranded DNA template to produce analyzable fragments, which became ubiquitous in DNA sequencing strategies, was an early development. This was applied to the sequencing of 50 nucleotides of bacteriophage f1 DNA. Analysis was facilitated by the incorporation of ribonucleotides into the extending DNA synthesis, enabling fragmentation and two-dimensional chromatographic or electrophoretic analysis. This process was extremely laborious, but the potential for much more rapid sequencing became apparent with the realization that denaturing polyacrylamide gel electrophoresis had the capability to resolve single-nucleotide differences in the length of even relatively long oligonucleotides. Fred described this as "the best idea I ever had".

Fred's first method to generate fragments for one-dimensional "sequence reading" on polyacrylamide gels was the "plus and minus" method, whereby a set of purified ^{32}P-labeled fragments derived from primer extension were extended a second time in reactions with or without one of the four triphosphates. This advance allowed the Sanger group to publish the sequence of the whole 5386 nucleotide genome of bacteriophage ΦX174 in 1977. However, the generation of the "plus and minus" sequence fragments was laborious, and Fred then developed the method for which he is probably best known—chain terminator sequencing, whereby a proportion of the 2′,3′-dideoxy derivative of one of the nucleoside triphosphates is included in each of the four polymerization reactions. Having shown the potential of the method with the available dideoxyT, the A, C, and G dideoxy derivatives had to be synthesised by Fred and myself. This "Sanger dideoxy sequencing" was used by the Sanger group to resequence the ΦX174 genome, to sequence human mitochondrial DNA (16589 nucleotides) and the bacteriophage λ genome (48502 nucleotides). Fred was awarded his second Nobel Prize for this work (along with Walter Gilbert and Paul Berg). The dideoxy sequencing method was used for the sequencing of many genomes, including the human genome, and modifications of it form the basis of methods used today.

Fred Sanger received many awards in addition to his Nobel Prizes—Order of the Companions of Honour (CH) and the Order of Merit (OM) among them. The genome research center, the Sanger Institute in Cambridge, was named in his honor. Somewhat to the surprise of many, Fred retired in 1984 particularly to spend time in his large garden in the countryside near Cambridge. Fred was married to Joan, who died in 2012, for 72 years. He is survived by their three children and two grandchildren.

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