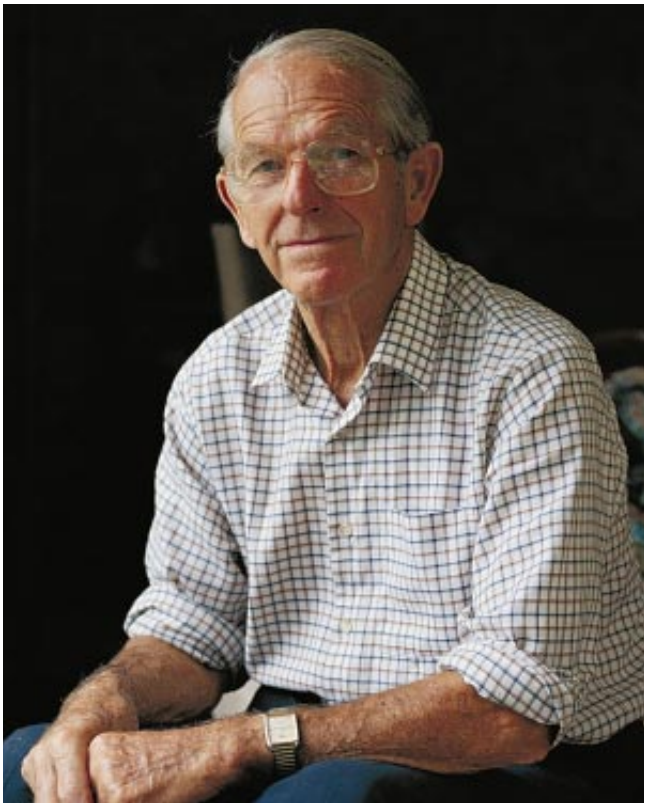
Revealing the Hidden Sequence

One might expect a two-time Nobel Prize winner to spend his days raising funds for a world-class laboratory, giving lectures to adoring colleagues and collecting royalties on his best-selling novel. Yet Frederick Sanger seeks neither fame nor fortune. Instead the man who built the foundations of modern biochemistry lives quietly in Swaffham Bulbeck, England, tending a garden of daffodils, plum trees and herbs. "I think Sanger hasn't been recognized as much as some, partly because he is an undemonstrative person," says Alan R. Coulson, who collaborated with Sanger for 16 years at the MRC Laboratory of Molecular Biology in Cambridge.

Forty years ago, Sanger was the first to reveal the complete structure of a protein. Then during the 1970s he developed one of the first techniques for reading the genetic code. "He deserved two Nobel Prizes," says biochemist G. Nigel Godson of New York University Medical Center. "He single-handedly engineered two revolutions in biology." In addition, Coulson boasts, Sanger deserves much of the credit for laying the groundwork for the Human Genome Project, the multinational effort to determine the entire sequence of nucleotides in human DNA.

Sanger, now 75, chose to meet me at one of his favorite pubs, the Red Lion, near Cambridge. He has mastered the art of understatement in both appearance and action—a no-frills kind of guy. Wearing a red sweater over a plaid shirt, he orders a chicken sandwich and a half-pint of lager. He speaks in a shy voice that is barely audible above the noise of the restaurant. Sanger is uncomfortable talking to journalists, but in the relaxed atmosphere of the pub he reveals some of what made him one of the great scientists of this century.

Sanger was born into a wealthy home. His grandfather made a fortune in the cotton trade and passed it on to his mother, Cicely. As a boy, Frederick was fascinated by nature, collecting everything from rocks to insects. His early ambition was to become a doctor, like his father, Frederick senior. Yet although the younger Sanger enjoyed learning about the science of medicine, he was not interested in the art of diagnosing and treating disease.



DAVID LEVENSON *Black Star*

FREDERICK SANGER transformed biochemistry by developing methods for determining the structure of proteins and DNA.

By all accounts, Sanger was not a brilliant student. In 1936 he was accepted to the University of Cambridge, but he struggled with the basic sciences. Sanger received passing grades in chemistry, but he "bombed" in physics. "I never won scholarships," he notes. "I am not sure I would have been able to attend Cambridge if my parents had not been fairly rich."

Sanger found his calling early in his college career. An enthusiastic professor, Ernest Baldwin, enticed him to study the new science of biochemistry. Sanger enjoyed the subject so thoroughly that he took advanced courses during a fourth, extra year at Cambridge. Two weeks after the final exams, he says, he was "very surprised to learn that he had been awarded a first-class degree."

In 1940, unlike most of his peers, the 22-year-old Sanger did not go off to fight in World War II. "I was brought up as a Quaker, and I felt pretty strongly that people should not go around killing others for any reason," he explains. He successfully defended his position before a military tribunal and spent the war years pursuing a doctorate. Graduate students were obviously in short supply during the war, and the biochemistry department at Cambridge was all too happy to accept a promising student.

After earning his Ph.D. in 1943, Sanger joined the laboratory of Cambridge professor A. C. Chibnall, who was a pioneer in the field of protein chemistry. Chibnall asked Sanger to study insulin, the pancreatic hormone that governs the metabolism of sugar. The suggestion led to a 10-year-long project that established Sanger as the leader of his field. Insulin, like other proteins, is made up of different amino acids. During the 1940s researchers were very aware that the chemistry of proteins depends on the order in which the amino acids are arranged. The problem: no one had found a technique for deducing the sequence.

Chibnall and Sanger chose to work on insulin for several reasons. It was available in a pure form, and it was a small molecule, at least as proteins go. More important, perhaps, investigators realized that if they could work out the structure of insulin, they would understand how it controlled sugar metabolism—insights that had many implications for medicine.

To determine the sequence of insulin, Sanger began with a simple strate-

gy that chemists often use to analyze large compounds: trying to break the molecule into fragments and then figure out how the pieces fit together. Easier said than done. To snip insulin into pieces of a meaningful size and then sort them, Sanger tried every trick in the chemist's handbook and then came up with some new schemes.

One of Sanger's important innovations was a method for labeling an end of a protein fragment. These tags made it easier to deduce what pieces belonged together. For example, if three amino acids—call them A, B and C—are linked together in some order in a chain, the sequence could be determined in the following way. After labeling the chains, they could be broken down into individual amino acids, indicating, say, that B was at one end of the chain. Then a new sample of the same chains could be cut into pieces consisting of two amino acids. The final step was isolating all those pieces that had a B at one end. If all the B's were linked to A's, then the sequence would have to be BAC.

While pursuing the insulin sequence, Sanger tried hundreds of different techniques. "Most experiments go wrong," Sanger sighs. "I didn't spend too much time trying to figure out what went wrong. I just started thinking about the next experiment. It prevented me from getting depressed."

There was one experiment in particular that Sanger would like to forget. In 1947 he spent a year in Uppsala, Sweden, working in the laboratory of the eminent biochemist Arne W. K. Tiselius. A technique Sanger developed seemed to suggest that insulin was not a single chain but rather four cross-linked chains. When he presented the results to his mentor, Tiselius suggested that they rush to publish a paper together. "I was rather shocked as he had not really contributed anything."

But as a junior member of the laboratory, Sanger gave in. "The paper is the only one of which I am ashamed," he comments. Sanger later discovered that insulin is actually made of two cross-linked chains, one 30 amino acids long and the other 21 long. The larger chain was by far the most complex structure that a protein chemist of that era had ever struggled with. It was not until 1952 that Sanger and his co-workers Hans Tuppy and E.O.P. Thompson figured out the complete sequence of both chains.

Sanger then had to decide how the chains linked together to make an insulin molecule. The problem turned out to be extremely complicated. An initial analysis seemed to show that chains were linked at nearly every point. Then Sanger realized that his techniques for cutting the insulin molecule were introducing new bridges between the strands. By 1955 he found a way to prevent the introduction of cross-links and succeeded in determining the complete structure of insulin.

Four years later, Sanger won the Nobel Prize in Chemistry for the insulin work. He was immediately besieged by professors inviting him to teach and administrators asking him for advice. But Sanger wanted no part of it. "I have actively tried to avoid both teaching and administrative work," he says. "This was partly because I thought I would be no good at them but also out of selfishness."

Sanger's talent for solving the right problem at the right time earned him two Nobel prizes in chemistry.

Ironically, in the year Sanger became a Nobel laureate, his research began to founder. He had taken the insulin study as far as it could go and was looking for new questions to grapple with. "I think these periods occur in most people's research careers and can be depressing and sometimes lead to disillusion," he observes. "I have found that the best antidote is to keep looking ahead."

In 1961 Sanger joined the Laboratory of Molecular Biology at the Medical Research Council in Cambridge and hired Coulson as his research assistant. "He's not the kind of guy you pal around with right away," Coulson says.

Soon after his arrival at the laboratory, Sanger decided his talents might be useful for analyzing DNA, the molecule that stores the genetic code. In the 1950s James Watson and Francis Crick had figured out that DNA was a long, double helix made of four different nucleotides. The arrangement of nucleotides determines what proteins an organism can make, that is, what genes it will express. Yet at the time, scientists could determine the sequence of nucleotides for only a very small section of a DNA strand.

Sanger set an ambitious goal for himself: sequencing the many thousands of nucleotides in the DNA of a virus. Yet he and other biochemists soon found DNA more difficult to analyze than proteins for two reasons. First, they had less experience handling DNA than they did proteins. Second, DNA is made from only four basic building blocks, whereas proteins are constructed from some 20 different amino acids. Just as a puzzle with many similar pieces is more difficult to solve than one with many different pieces is, the sequence of DNA was more difficult to decode than the sequence of insulin was.

For more than 10 years, Sanger investigated techniques for sequencing DNA, competing with several laboratories around the world. Then in 1975, Sanger and Walter Gilbert of Harvard University, working independently of one another, developed methods for rapidly sequencing DNA. Their techniques could determine the arrangement of nucleotides in segments of DNA 200 or more units long in a few days. With earlier methods, the job would have taken years. The new technique enabled Sanger and his collaborators to determine the sequence of 5,375 nucleotides in a virus called ϕX174.

In 1980 Sanger won a second Nobel Prize, which he shared with Gilbert and Paul Berg of Stanford University. Berg had found a way to insert pieces of DNA from one organism into the DNA of another. His work launched the technology of recombinant DNA.

During his career, Sanger published about one major scientific paper every eight years, but his colleagues say each one is a classic in experimental biochemistry. "I don't publish papers unless I have something to write about and something I am sure about," he explains.

These days, although Sanger still gives advice to his colleagues at the Laboratory of Molecular Biology, he has retired from his research. "The aging process was not improving my performance in the laboratory," he complains. "I would have felt guilty about occupying a space that could have been available to a younger person."

Reflecting on his research, Sanger finds it hard to recall any moments of great inspiration. He does not subscribe to the "popular idea that scientific progress depends on sudden breakthroughs." Instead he thinks about many events that "were more often associated with small and gradual advances."

Sanger finishes his lunch and invites me to his home. We drive through the countryside to an unpretentious house surrounded by an elaborate garden. It seems Sanger is as devoted to gardening as he once was to research. He and his wife of 52 years, Joan, grow a dozen varieties of flowers, cultivate several fruit trees and raise chickens. When they are not working in the garden, the Sangers entertain their three children, grandchildren and friends. "I have always had a happy and peaceful life," he remarks. The key, he asserts, "is working on the right thing at the right time." —*Russell Ruthen*