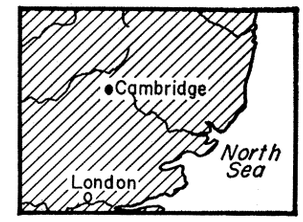
Laboratory of Molecular Biology, Cambridge



Molecular geneticists at Cambridge, England, where the structural model of deoxyribonucleic acid was first proposed 11 years ago, continue to be among the leaders in molecular biology. The group of scientists working with Francis H. C. Crick and Sidney Brenner have been engaged in intensive exploration of the way in which genetic messages govern the operation of living systems.

The molecular genetics story, and the Cambridge group's importance in it, is one of the best-known in the current history of science. But it is perhaps not so well known that Crick and Brenner and their associates are just part of a much larger assemblage, one that includes four winners of the Nobel prize. The molecular geneticists work in the same 2-year-old building with Max Perutz and John Kendrew, crystallographers who have described the structure of the blood proteins hemoglobin and myoglobin; Frederick Sanger, who determined the amino acid sequence of insulin; Hugh E. Huxley, electron microscopist who discovered the sliding mechanism of muscular contraction; and Aaron Klug, who has made important contributions to theory and experiment on the architecture of viruses.

They are all members of the Medical Research Council's Laboratory of Molecular Biology, directed by Perutz. The laboratory has a scientific staff of about 30, assisted by about 80 others—students, technicians, and secretaries. Already crowded in their 1800 square meters of laboratories on four floors, the staff looks forward to almost doubling its space when the British Treasury provides the money for an extension. The new building has been approved already by the MRC, the government body supporting biological and medical research in units closely allied with universities all over Britain and at the National Institute of Medical Research at Mill Hill.

The Cambridge collection of crystallographers, protein chemists, geneticists, and electron microscopists can remember even more cramped days. They are now 3 kilometers away from the crowded room in the Cavendish laboratory building where Crick and Watson worked out the DNA model in the spring of 1953.

It was only after this work that Perutz's unit inherited its well-known "Hut" in the shadow of the Cavendish from the University of Cambridge's metallurgy department. The move to the hut also came soon after Huxley had discovered that muscles contract by the sliding of filaments past one another, and not by contraction in the structure of a protein. Perutz had also just hit on isomorphous replacement by heavy atoms as the way to get a suitable protein crystal target for x-ray diffraction.

"This was the moment, perhaps, when I should have proposed to the MRC the setting up of a proper laboratory of Molecular Biology," Perutz recalled in January 1962 when the building was almost ready, "but was public opinion ready for it, or for that matter, were we?

"At that time, our work stirred up little enthusiasm in this country. When I discussed the implications of Watson's and Crick's discovery with a leading geneticist, he assured me that, as far as his field was concerned, it had none. Most of our crystallographic colleagues continued to be highly sceptical of the prospects of solving protein structures by x-ray analysis, and it was true that Kendrew and I were still facing great difficulties. I thought it wiser to continue on a modest scale until we felt surer of success."

The moment for expansion came in 1957, when Kendrew's work on myoglobin had come close to success, Brenner had started a bacteriophage laboratory, and Sanger had said he would like to join the group. The building was soon approved by the MRC and Treasury, but it took a year's negotiation with a reluctant university to find a site. The one finally chosen was not near the main scientific laboratories of the university but at the edge of town on land intended for a new medical school. When the building was completed, Huxley moved up from University College, London, and Klug moved his virus research group up from Birkbeck College, London, where he had worked under J. D. Bernal (*1*).

Now that all the studies are in the same building, they feed each other, just as crystallography provided the data leading to the Watson-Crick model of DNA.

After Crick and James D. Watson, now a professor of biology at Harvard, had worked out the structure of DNA, theory and experiment raced to explain how the information stored in nucleic acids gets to, and operates in, the regions of cells where proteins are constructed. Scientists in many other laboratories have contributed to the rapidly developing picture. A form of nucleic acid, called "messenger RNA," was postulated by Jacob and Monod as the carrier of instructions from DNA, on which this special form of RNA is supposed to be formed, to the site of protein manufacture in the cytoplasm, the ribosomes. The ribosomes themselves have been found to operate as a kind of assembly line (*2*).

Genetic Mapping

A system of bacteriophage mutants, growing differently on different strains of the colon bacillus, has been exploited by Seymour Benzer of Purdue University and many others for making genetic "maps" of the actual place along a gene where the mutation takes place (*3*).

According to the long-accepted hypothesis of George Beadle and E. L. Tatum, a single gene specifies a single protein (or enzyme). Mutations affecting proteins may involve the change

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of only one amino acid out of hundreds, or they may result in sharply abbreviated sections of protein. Since the DNA molecules are chains, mutations are assumed to be either alterations of material at specific spots along the genetic chain or addition or subtraction of material at those spots. Genetic maps show the apparent relative distances between such changes by depicting the frequency of "recombination" among mutants of the bacteriophage—the probability that mutations, occurring in sequence, will restore the characteristics of the natural, "wild type" phage.

At Cambridge, Crick, Brenner, L. Barnett, and R. J. Watts-Tobin used this system to provide strong evidence that the genetic "code" in DNA—the one which presumably is read off when messenger RNA is manufactured—is indeed read in sequential groups of three bases along the DNA molecule, as had long been suspected. The code has four different "letters"—the four bases found linked to the sugar phosphate backbones of the nucleic acid chains, and to each other in complementary pairs.

The Crick group announced their results in 1961 (*4*). They had examined a culture of a mutated strain of the T4 bacteriophage, which had lost its ability to grow on strain K of the bacillus *Escherichia coli.* The mutation had been produced by the chemical proflavin, which apparently acts on the nucleic acid by removing or adding a base.

In the culture, a rare mutant phage would revert to "wild type" and grow fairly well on strain K. Other mutants would not grow, however. In their work, the Cambridge group eventually found 80 distinct second mutations from the original mutant. The changes were mapped genetically in order to locate them in the gene.

The mapping showed that in the successful double mutants—revertants which would grow on strain K—the original change had been left intact but a complementary change had taken place, one which, by itself, would also have stopped the phage from growing on strain K. In the unsuccessful double mutants the original site of change was likewise unaltered. A second change had taken place, but without restoring the ability to grow.

What had happened?

In theory, the gene may be likened to a ticker tape, read from one end to some point where there are instructions to stop, or where a mutation may have inserted something which doesn't "make sense."

Crick's group assumed that the successful pair of changes involved events that were opposite. For example, the first mutation may have added a base to the nucleic acid sequence, throwing the rest of the sequence out by one base. Then, a second base could have been subtracted a little way down the gene, leaving a section changed, but restoring the rest of the gene to its original sequence.

The same thing could have happened, of course, if the original change had subtracted a base and the second change had corrected the error by adding a base.

According to this idea, the unsuccessful pairs of mutations were changes "of the same sign," the first mutation, say, adding a base, and the second mutation adding still another base. Through either a second addition or a second subtraction, the "same-sign" changes would throw the sequence even further off.

Lending strength to this view was an examination of triple mutations. Here, the successful trios of changes were changes of the same sign. At the end of three mutations, three bases had been added or three subtracted. Such a sequence of mutations would restore the original sequence if the code were "read" in "words" of three or multiples of three. Other evidence suggested simple triplets.

In all of this work it had never been proved that the sequence of bases in nucleic acid forming the code words of a gene actually corresponded to the sequence of amino acids assembled into proteins on the "instructions" of that code.

In 1963, A. S. Sarabhai, A. O. W. Stretton, and Brenner, of the Cambridge group, and A. Bolle of the Institute of Molecular Biology of the University of Geneva succeeded in proving that the gene for the head protein of the bacteriophage T4D is co-linear with the polypeptide chain (*5*). At about the same time, C. Yanofsky of Stanford University found colinearity in an examination of tryptophan synthetase in *Escherichia coli* (*6*).

The work at Cambridge followed a long search for suitable experimental material. Attention focused on the various structural proteins of bacteriophage. Brenner had begun looking at the head protein in 1957. It was an obvious choice, for the head protein constitutes about 70 percent of the protein made for a new bacteriophage in the host bacterium. But Brenner and his Cambridge colleagues, and Benzer and his co-workers at Purdue, ran into many obstacles.

The material which ultimately proved suitable was discovered by R. H. Epstein, of neither Cambridge nor Purdue but formerly of the California Institute of Technology and now at the Institute of Molecular Biology in Geneva. Epstein was seeking a system in which he could map a large number of genes. He found a class of mutants whose mutations distributed themselves freely all over the genetic map for the phage head protein gene and for other genes as well. The mutants Epstein found are called "amber," and can be produced by such base-analog compounds as 5-bromouracil, 2-aminopurine, and hydroxylamine.

The "amber" mutations are "suppressible"—that is, they will grow on some "permissive" strains of colon bacillus. Benzer had also worked on "suppressible" mutations. He and S. P. Champe, also of Purdue, suggested that the bacteria which would not let the mutants grow could not "read" the genetic message in the mutants' nucleic acid because of a "nonsense codon." The permissive bacteria, however, seemed to be able to read the nonsense codon as a symbol for an amino acid.

By now the goal of the search was clearer: a class of mutations which would produce fragments of protein of varying sizes so that a "protein fragment map" could be prepared and matched against a genetic map.

The "amber" mutants looked like promising material. But there was a question: Did the messenger RNA of the mutants serve only once in the assembly of the truncated head proteins, or would it work again and again? This was another way of asking whether the mutants would produce sufficient quantities of protein fragment to allow the experiments to be performed quickly. Sarabhai demonstrated that the yield was large.

A group of ten mutants was chosen, and their "recombination frequencies" were plotted on a genetic map. The mutants were also set to growing head protein in host cells. Sulfur-35 was used to label the protein.

After digestion of the products with the enzymes trypsin and chymotrypsin, the fragments were studied to see

which mutants made which fragments. As it turned out, the mutants produced segments of the natural head protein which could be rank-ordered by length. The rank order of the segments matched the genetic map.

The Architecture of Viruses

The virus group, under Klug, is testing ideas of the required geometry of viruses, refined from some early views of Watson's and Crick's, reached when they were searching for examples of simplicity in the use of genetic information and saw such simplicity in the apparent tendency of viruses to construct protective shells out of many identical protein subunits.

Watson and Crick stated in 1956 that the only real possibilities for generating so-called "spherical" viruses from identically placed and identical protein subunits would be arrangements with the symmetry of a tetrahedron (with 12 possible locations), an octahedron (with 24), or an icosahedron (with 60).

Virus observations in electron microscopes soon followed, made with the technique of negative staining described by Brenner and R. W. Horne. The viruses clearly had more than the maximum of 60 protein subunits suggested by Watson and Crick. The adenovirus was observed to have six units on each of its six edges, indicating a total number of 252 units spread almost evenly over its surface at an apparent spacing of 70 angstroms. The units at the vertices had only five neighbors, not six, like all the other units which could be seen. Nonetheless, the adenovirus exhibited icosahedral symmetry.

By 1962, Klug and an American colleague, Donald L. D. Caspar of the Children's Cancer Research Foundation in Boston, were proposing a slightly modified picture, in which certain multiples of 60 units could generate a spherical surface of icosahedral symmetry with only slight departures from strict equivalence (*7*). The statements by Watson and Crick clearly almost fit the facts, and Caspar and Klug wished to depart from them as little as possible. They called the modified equivalence requirements "quasi-equivalence."

The morphological subunits visible in electron micrographs probably consist of either five or six individual protein molecules, each molecule having a molecular weight of about 20,000, the weight calculated for the protein surface units of tobacco mosaic virus. The units seen in electron micrographs of several kinds of virus studied by Klug's group are several times too large for the molecular weights being determined in Sanger's group by J. I. Harris and John Hindley.

Klug's group of researchers in the structural-studies division under Kendrew is testing these notions by electron microscopy, x-ray, and biochemical methods. With J. T. Finch, electron microscopist, Klug is preparing to publish results of a comparative study of human-wart virus, simian-40 virus, and polyoma virus. All three viruses follow the structural scheme proposed by Caspar and Klug, and all three appear to have the same number of protein subunits on their surface, a number much larger than the hitherto accepted figure of 42. The results are based on new micrographs, but the same numbers are visible in earlier micrographs.

The strong agreement with theory gave pleasure at the laboratory. Perutz commented recently that the results had "surprised all of us. The theory was pretty far-fetched, you know."

Others in the virus group are Kenneth C. Holmes, who is studying tobacco mosaic virus with x-ray diffraction patterns; William Longley, who is studying the "spherical" viruses with x-ray techniques; and Ruben Leberman, biochemist. They are all careful to mention the important role the late Rosalind Franklin played in their work in London.

Other viruses under study are the polio, turnip yellow, turnip crinkle, and tomato bushy stunt viruses.

Support for Molecular Biology

The large number of research successes in the Laboratory of Molecular Biology make Perutz and Kendrew, who is deputy chairman of the laboratory, very optimistic about future results. But they are not so happy when they look at the present levels of European support for molecular biology. Both men have been active in the formation of the European Molecular Biology Organization, whose council held its first meeting in Geneva on 2 February. Perutz is chairman.

The council, set up after more than a year of informal meetings culminating with a meeting at Ravello, Italy, in September 1963, has established two committees. One, with A. A. Buzzati-Traverso of Naples as chairman, will consider a European fund for molecular biology, to make grants for research, travel, fellowships, working groups, and training courses. The other, with Kendrew as chairman, will look into the desirability of establishing a European laboratory for molecular biology on the model of the CERN nuclear center at Geneva.

While the organization is still setting up its legal charter and collecting replies from 150 molecular biologists invited to join, it is already pushing to start a small program of travel grants.

During the council meeting in Geneva, the group issued a manifesto that began this way:

"The new approach to biology which has become known as molecular biology has developed since the war both in Europe and America. Some of the most important advances in the subject were made in European laboratories, but shortages of funds, together with the rigid divisions of science into the classical disciplines in many European universities, and the limited interchanges of workers and of ideas between European countries have retarded further growth: there are very few well-equipped interdisciplinary centres, and most work is done by isolated groups with inadequate means.

"Furthermore, a substantial proportion of the funds made available in many European countries have been provided from American sources, and recent changes in U.S. policy make it likely that this support will be reduced or even completely withdrawn in the next year or two.

"In America, on the other hand, the implications of molecular biology have been quickly grasped, and many excellently equipped institutions for its study have been established. In consequence, many Europeans are being tempted to accept attractive offers to pursue their research under better conditions in American laboratories."

—VICTOR K. MCELHENY

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