ous, Maynard Smith's postmeeting comment to *Science* would certainly meet with broad agreement: "I thought the meeting was very positive. This was the first time for more than 25 years that there has been serious discussion between paleontologists, geneticists, and the like. This can't be anything but good."

Many people suggested that the meeting was a turning point in the history of evolutionary theory. "I know it sounds a little pompous," Hallam told *Science*, "but I think this conference will eventually be acknowledged as an historic event." Will it prove to be the current equivalent to the 1946 Princeton meeting at which the capstone of the Modern Synthesis was laid? Will a new synthesis emerge, signaling a true paradigm shift in the Kuhnian sense?

Perhaps. Gould expressed his expectations in more modest terms: "I hope that this meeting will lead to a rapprochement. I hope it will set the basis for a reconstruction of ideas."

—ROGER LEWIN

The 1980 Nobel Prize in Chemistry

Three molecular biologists win the prize for discoveries that can be used to study gene structure and control

The current Nobel Prize in Chemistry spotlights contributions to the methodological revolution that is allowing researchers to examine the structure and control of genes of higher organisms in a detail previously unimagined. Half of the prize was awarded to Paul Berg of Stanford University; the other half was awarded jointly to Frederick Sanger of Cambridge University and Walter Gilbert of Harvard. This is Sanger's second Nobel Prize.

Berg is cited for "his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant DNA." According to a press release from the Swedish Royal Academy, "Berg was the first investigator to construct a recombinant DNA molecule, i.e., a molecule containing parts of DNA from different species. His pioneering experiment has resulted in the development of a new technology, often called genetic engineering." Berg does not know whether the Nobel committee had a particular experiment in mind but, he says, "I would like to think it [the prize] was for a body of work and not for a single experiment." Arthur Kornberg, also of Stanford, thinks the only way to interpret the Nobel committee's "carefully worded citation" is as recognition for Berg's 20 years of leadership in the molecular biology of nucleic acids.

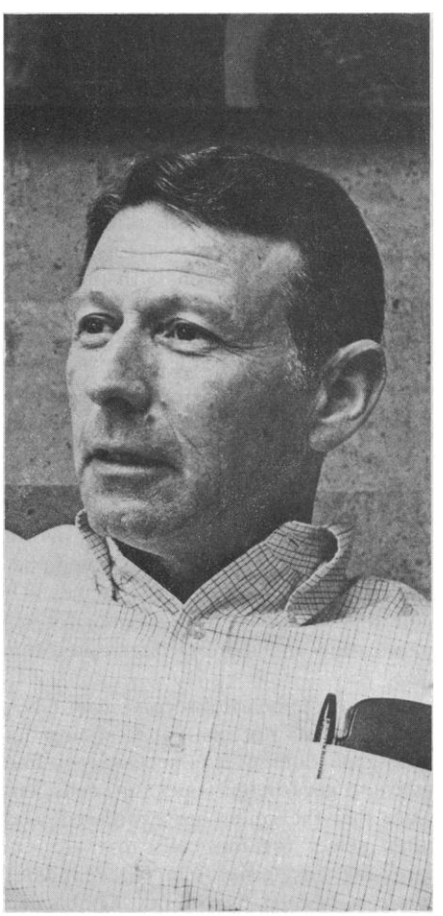
In the 1960's, Berg did a great deal of innovative work on bacterial protein synthesis, particularly the interaction of amino acids with transfer RNA's. His work helped explain how these RNA's are used as adapters in decoding. His group and several others also discovered one of the enzymes that copies DNA into RNA.

Then, about 10 years ago, Berg and many other molecular biologists became interested in applying what is known about bacterial gene expression to the study of gene expression in higher organisms. "We began to think of using SV40 [an animal tumor virus] to carry genes into mammalian cells," Berg says. The foreign genes could then be studied and manipulated to see what controls their expression.

In 1971, Berg and his colleagues David Jackson and Robert Symons opened the circular SV40 molecule with a restriction enzyme, Eco R1. This enzyme, which was discovered in Herbert Boyer's laboratory at the University of California at San Francisco, cleaves DNA at specific base sequences. In the case of SV40 DNA, it cleaves it in exactly one spot. Berg's group then spliced the linear SV40 DNA to the DNA of the bacterial virus λ. The λ DNA also is circular and Berg's group cleaved it too with Eco R1.

Although this was the first time that DNA's from two different species were joined, it was not the first time that any DNA's were joined. H. Gobind Khorana, of the Massachusetts Institute of Technology, discovered in the 1960's that an enzyme produced by the bacterial virus T4 can catalyze the linking together of DNA molecules. Berg, Jackson, and Symons enzymatically constructed complementary or "sticky" ends on the two DNA segments to be joined and then used the T4 enzyme to do the joining. The method they used was developed and tested independently by Berg's group and by Peter Lobban and Dale Kaiser of Stanford. Although no one knew it at the time, it was unnecessary to construct sticky ends, since they are automatically produced when Eco R1 cleaves DNA. This fact was discovered in 1972 by Janet Mertz and Ronald Davis and independently by Vittorio Sgaramella, all of Stanford University.

It had been Berg's intention to introduce the SV40-λ hybrid molecule into the bacterium *Escherichia coli*, which λ can infect. In that way, he could get many copies of the molecule to be used for future experiments in gene expression in



Paul Berg

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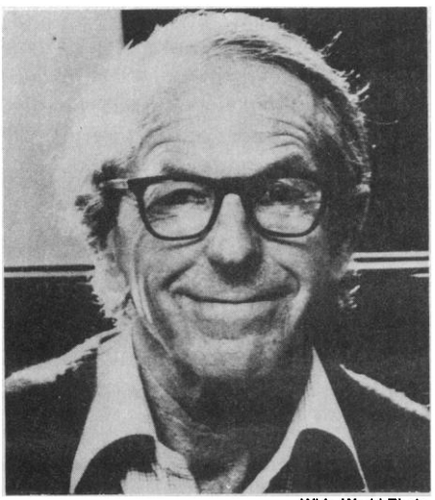


Wide World Photo

Frederick Sanger

mammalian cells. In the summer of 1971, Mertz, who was Berg's graduate student, described the plan at a tumor virus conference held at Cold Spring Harbor, New York. Robert Pollack of Cold Spring Harbor Laboratories reacted immediately with dismay, pointing out that SV40 transforms human cells in culture and that *E. coli* lives in the human gut. If any *E. coli* infected with the SV40-λ DNA escaped from the laboratory, they could be dangerous.

Berg was persuaded by this argument and decided not to do the experiment. He led molecular biologists in calling for a moratorium on recombinant DNA research until the risks could be assessed and the safety of the experiments ensured. It was a period, Berg recalls, "of more controversy than science." In 1975, the moratorium was conditionally lifted and the National Institutes of Health developed guidelines for the conduct of recombinant DNA experiments. The guidelines have since been softened as the experiments turned out to be less risky than anticipated.

Ironically, the experiment that Berg originally wanted to do would not have succeeded, and no one at the time would have known why. The SV40-λ hybrid would not have replicated in bacteria because Berg inserted the SV40 genes at a site now known to be essential for λ's replication and thereby interrupted this site.

In fact, the heart of recombinant DNA technology is not just gene splicing but also gene cloning. It is necessary to find ways to get foreign genes into cells, ensure that the genes are expressed, and then select for the cells that are expressing those genes. Cloning techniques were pioneered by Stanley Cohen and Annie Chang of Stanford University and Herbert Boyer and Robert Helling of the University of California at San Francisco, who, in the early 1970's, developed a plasmid, which is a small piece of extra-chromosomal DNA, that could carry foreign genes into bacterial cells. The plasmid contained genes that made the bacteria resistant to the antibiotic tetracycline, so that the cells which took up the plasmid and expressed its genes could easily be selected.

In the past decade, recombinant DNA techniques have become increasingly sophisticated. Berg has played a major role in these developments. Most recently, he and others, particularly Daniel Nathans of Johns Hopkins University Medical School, who won a Nobel Prize for his work in restriction enzymes, extensively studied the structure, organization, and replication of SV40 genes. After constructing deletion mutants of SV40 that have proved extremely useful in studies of SV40 gene functions, Berg went back to his original idea of using SV40 to introduce genes into mammalian cells. He spliced to SV40 an *E. coli* gene that allows cells to use xanthine as a substrate in nucleotide synthesis. Then, in separate experiments, he spliced animal genes for globin, histone, or the enzyme dihydrofolate reductase to this hybrid SV40 molecule. When the SV40 carrying the added bacterial and animal genes was introduced into cultured cells, Berg could pick out the cells that were transformed by SV40 by selecting for cells that grow on xanthine. In this way, Berg was able to show that the added animal genes are expressed in cultured cells.

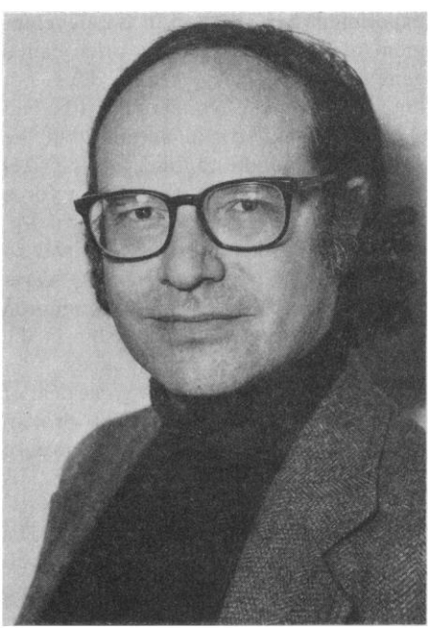
Dean Hamer and Philip Leder of the National Institute of Child Health and Human Development have also used SV40 as a cloning vector in cultured cells. But, says Nathans, "Clearly the notion that you could construct a vector with animal viruses was Berg's idea."

An important aspect of Berg's work has been his extraordinary ability to develop methodologies. For example, he was the first to use nitrocellulose binding assays to study interactions between proteins and nucleic acids. He also developed the nick translation method, which is used to make isotopically labeled DNA probes and is central to current studies of gene functions. "His style of biochemistry helped set the standards in the nucleic acid field," says Nathans.

The second half of the chemistry prize was also given to developers of methodologies. Sanger and Gilbert were honored for their discoveries of ways to sequence DNA. In the past few years, these techniques have become widely used to determine amino acid sequences of proteins because with these methods it is easier and more accurate to sequence the DNA coding for proteins than to sequence the proteins directly. The techniques are also used to determine the intervening sequences that occur in eukaryotic genes and the sequences that occur in control regions of bacterial DNA. By using these methods, molecular biologists hope to learn which sequences control gene expression in higher organisms and how they do so. "DNA sequences are the basic, underlying structures [of molecular biology]. There is nothing more primitive. Your questions are ultimately posed there," says Gilbert.

Sanger and Gilbert are about as different as two scientists can be, and they came upon their sequencing methods by entirely different paths. Sanger is quiet, modest, self-effacing; Gilbert is much more flamboyant. Ted Friedman of the University of California at San Diego, who spent a sabbatical year with Sanger, says, "If you talk to Sanger and do not know who he is, you would think he is the lab caretaker. If you allow him to, he will melt into the woodwork." George Brownlee of Oxford University, who until recently was at Cambridge with Sanger, adds, "Sanger certainly doesn't give himself airs. But in my view, he ranks among the great scientists of our time."

According to Friedman, Sanger's outstanding feature is his "uncanny belief and knowledge that sequencing can be determined by very simple methods." In the 1950's, Sanger studied protein sequencing at a time when no one knew whether all proteins of a particular type



Walter Gilbert

have the same sequences. His first Nobel Prize was awarded for this work. Then he attacked the problem of RNA sequencing, developing the widely used fingerprinting method. About 10 years ago, he set out to sequence DNA, even though this problem, too, was considered intractable.

Sanger's method evolved gradually from more than one line of attack on the problem. In the early 1970's, he discovered the plus-minus sequencing method, a direct precursor of the method he uses today. In the plus-minus method the object is to obtain a set of nested segments of the DNA to be sequenced. The first segment consists of the first nucleotide, the second consists of the first two nucleotides, the third of the first three nucleotides, and so on. These segments are constructed in such a way that the identity of the last nucleotide of each nested segment is known. Once obtained, the nested segments can be separated according to size by electrophoresis on an ultrathin polyacrylamide gel. The separated fragments can be detected because each is isotopically labeled.

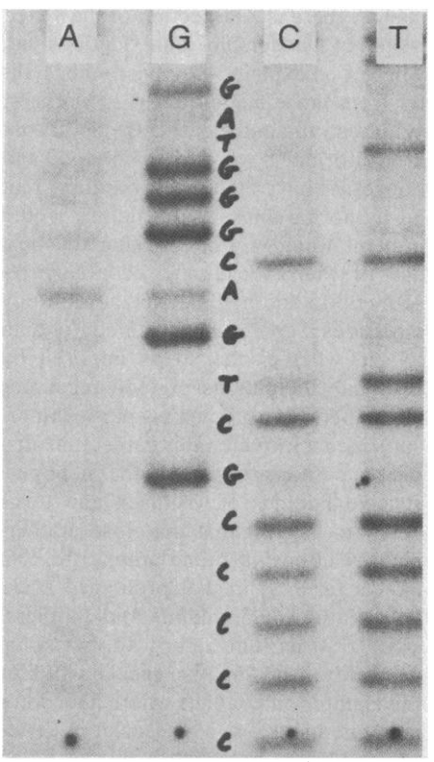
The key to the plus-minus method is obtaining the nested segments. Sanger constructs them by synthesizing them. He separates the two strands of the DNA to be sequenced and then makes partial copies of one of those strands. To ensure that the partial copies include all nested segments and that the terminal nucleotide of each segment is known, Sanger makes the copies under conditions in which one nucleotide is limited in quantity. For example, he provides limited quantities of adenine so that the DNA copying will eventually stop because of lack of adenine. Then all of the copies made will end just before an adenine, and the next nucleotide of each of the resulting segments is adenine. In a similar way, Sanger synthesizes segments ending before each of the other three DNA nucleotides.

Sanger has since improved the plus-minus method to make it more efficient. Instead of supplying limited quantities of each nucleotide, he supplies derivatives of the nucleotides that cause DNA synthesis to stop.

In contrast to Sanger, Gilbert did not deliberately set out to sequence DNA. A highly visible, active scientist who runs a large laboratory, Gilbert has worked on a wide variety of problems in the past 20 years, ranging from how bacterial genes are organized and expressed to gene control in higher organisms and genetic engineering. He is also chairman of the board and cochairman of the board of directors of the gene splicing firm Biogen.

Gilbert, working with Allan Maxam, who is now at Harvard Medical School's Sidney Farber Cancer Institute, came upon a DNA sequencing technique almost by chance. Gilbert recalls that one day in early 1975, Andrei Mirzabekov, of the USSR Academy of Sciences, appeared in his office and urged him to try a new approach to studying how proteins recognize specific sequences of DNA. Gilbert had long been interested in the lac repressor protein of *E. coli*, which binds to the lac operon segment of DNA, and, in fact, it was Gilbert who isolated the lac repressor and operator. Mirzabekov and his colleagues had been probing protein-DNA interactions with dimethyl sulfate, a reagent that methylates the DNA nucleotides adenine and guanine. After reacting with dimethyl sulfate, DNA breaks easily at these bases.

Gilbert decided to expose lac operon DNA to dimethyl sulfate and then break the DNA at adenines and guanines. For comparison, he would bind lac repressor to the operon and repeat the experiment. The adenines and guanines that reacted with the repressor should be protected from the dimethyl sulfate, and so the DNA should not break there. Since the sequence of lac operon DNA was known (it had been copied into RNA and the RNA sequenced), it would be possible to learn where the repressor binds on this DNA.



Part of the sequencing pattern obtained from a piece of DNA about 130 base pairs in length. The letters at the top of the columns, A, G, C, and T (adenine, guanine, cytosine, and thymine) indicate which base was preferentially cleaved by chemicals. The darkest band in each column represents the base missing from the end of the initial segments, with the exception of cytosine (C). All dark bands in the C column represent cytosines even if bands also appear in the T column at that position. Bands that appear in the T column but not in the C column represent thymines (T). To read the sequence of the DNA, read off the base represented by each band, starting from the bottom of the columns. [Source: Walter Gilbert and Allan Maxam]

After these experiments, Gilbert and several of his associates discovered a second lac operon of unknown sequence. Maxam repeated the dimethyl sulfate experiments with this new lac operon and the lac repressor. When he and Gilbert saw the results, they realized that they had the beginning of a DNA sequencing method. By using dimethyl sulfate and adjusting the reaction conditions, they could break DNA at either adenines or guanines. Now if they could find a way to break DNA preferentially at thymines or cytosines, they could generate nested segments whose terminal nucleotides were known. With this idea, Maxam set to work to develop ways of breaking DNA at thymines or cytosines. He recalled that under appropriate chemical conditions, hydrazine preferentially weakens DNA at one or the other of these nucleotides. After a summer of work, Maxam succeeded in perfecting the chemical method of sequencing DNA.

The difference between the Sanger method and the Maxam-Gilbert method is that Sanger generates nested segments by synthesizing them and Maxam and Gilbert generate the segments by breaking the DNA at specific bases. Both methods are currently used, and researchers experienced with both say that the choice between them depends in part on the length of DNA to to be sequenced and in part on the personal preferences of the investigator. Tom Maniatis of the California Institute of Technology, for example, uses Sanger's method for very long sequences of DNA because it is faster. For shorter sequences, one or a few genes long, the two methods are comparable in speed, but Maniatis prefers the Maxam-Gilbert method because "Allan has established the protocol so completely that anyone who tries the method is successful."

The full ramifications of recombinant DNA technology and DNA sequencing methods are not yet known. But these techniques are changing molecular biologists' perceptions of what can be learned about the genes of higher organisms.

—GINA BARI KOLATA