RECOMBINANT DNA RESEARCH

What are those molecular biologists so excited about?

BY JULIE ANN MILLER

The exhilaration of many molecular geneticists when they consider the technique of recombinant DNA research does not come primarily from dreams of gene therapy, nitrogen-fixing crops or drug-producing bacteria. "The only certain benefit is increased knowledge of basic biologic processes," Maxine Singer of the National Cancer Institute and Paul Berg of Stanford University wrote last summer. The new techniques promise to make a range of challenging research problems manageable for the first time.

"It is no longer a pious dream to expect that the structure of the mammalian genome [hereditary material] will be known to the same resolution as the *E. coli* [bacterium] chromosome," Berg told a recent forum at the National Academy of Sciences. With that knowledge, Berg believes, questions about cell growth, development and differentiation can be more confidently attacked. Then diagnosis, prevention and cure of disease can become more rational and effective.

Years of research on bacteria in many laboratories have resulted in detailed knowledge about their heredity. About 650 genes have been precisely mapped on the single, circular DNA molecule that contains *E. coli*'s genetic information. The map reveals that genes of proteins that function together in a cell are often grouped together. Attached to some of the genes are signals that regulate their action (SN: 11/27/76, p.348). This information helps explain how production of proteins is modulated in response to the bacterium's environment.

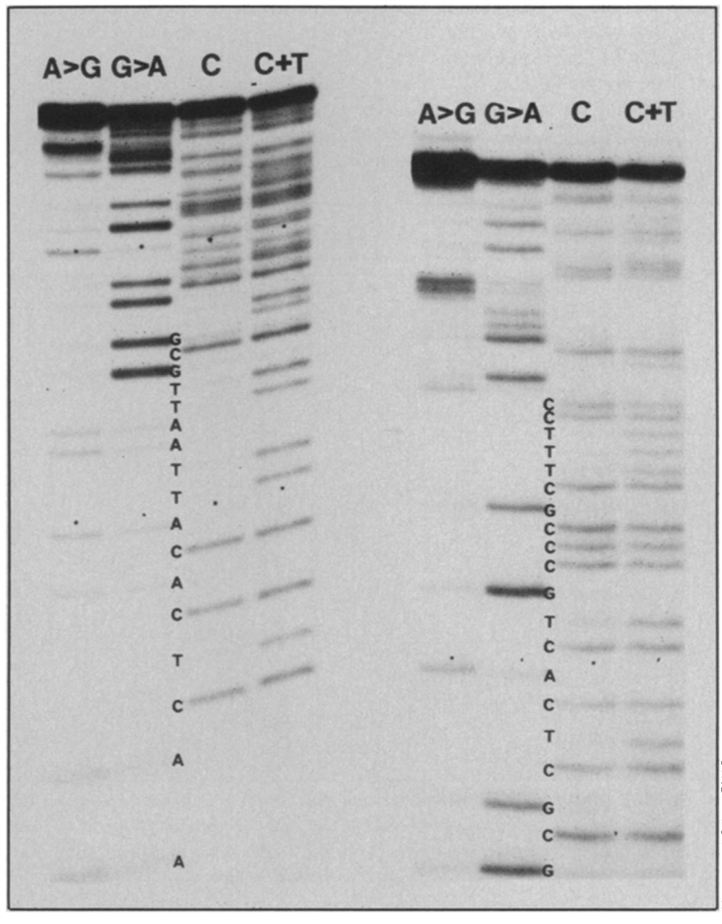
In contrast to that bacterial chromosome, few mammalian genes have been precisely mapped. Little is known about how the genes are organized in the chromosomes, what regulatory signals control them and how they program the development of a single cell into an adult.

The technique of recombinant DNA may be the key to the complex chromosome. It is the first general method for isolating unique segments of DNA directly from the total genetic material of higher organisms. This method is being applied in several ways. The isolated DNA segments can be reproduced in bacteria to provide researchers with enough DNA to determine the exact sequence of nucleotides. The isolated DNA segments can also be used to locate genes on the original chromosomes, providing a physical map. Finally, recombinant DNA techniques are valuable in testing hypotheses about how genes control development.

Many people view the ability to splice genes of different organisms as a sharp break with past experimental methods. "I believe science has not taken so large a step into the unknown since Rutherford began to split atoms," Robert Sinsheimer, a biologist at the California Institute of Technology, told the Academy forum. "The recombinant DNA technology brings us at one bound into a new domain. . . ."

In strategy, however, the technique is clearly a continuation of past experimental approaches. Work on the genetics of bacteria depended on the researchers' ability to move genes from one cell to another. At first, DNA was identified as the hereditary material in experiments where pieces of chromosomes of one cell passed into another. In the bacterial experiments, biologists were often able to rely on natural methods of gene transfer. Certain viruses will package a small piece of bacterial DNA along with, or even instead of, their own viral DNA, and transfer those genes to the next bacterium they infect. Similarly there are natural small rings of DNA, or plasmids, which can incorporate genes of one bacterium and carry them to another. These techniques differ from the controversial gene-splicing methods in that the genes were shifted among bacteria of the same species, rather than into bacteria from other organisms.

The basic information of a chromosome is contained in the order of its nucleotides, the four subunits that make up the alphabet of the genetic code. In the earlier days of DNA study, the nucleotide sequence was deduced from the protein product of a gene. This method was only an approximation, because most amino acids can be represented by several different three-nucleotide "words." This technique was also limited because it could be used only for genes that produce large enough amounts of protein. Most important, the regions of DNA that control expression of



Reading a DNA molecule: New techniques allow rapid determination of the nucleotide order.

other genes would never be described. Biologists have calculated that in higher organisms there is far more DNA than is necessary just to code for the proteins. Whether all the excess DNA has regulatory functions, or whether it plays some other role, is currently a major question.

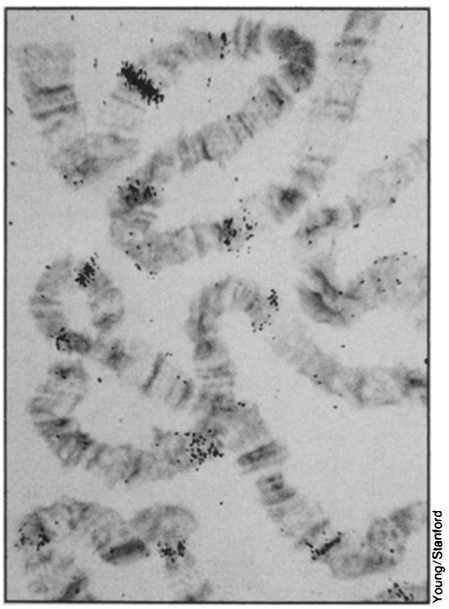
When it comes to sequencing, the tables have now turned. With the isolation and multiplication of segments of DNA, and new methods for determining the nucleotide sequence, proteins may be described by analyzing their genes. "You might as well get the gene out, put it on a plasmid and sequence it," Walter Gilbert, a Harvard biochemist, told scientists at a recent genetics meeting in Park City, Utah. "It's faster and more accurate than doing protein sequence by conventional techniques." Gilbert said one of his students sequenced a bacterial gene and discovered that the researchers who originally analyzed its protein product had made mistakes in the sequence.

There are now several rapid sequencing methods. One, developed by Fred Sanger and colleagues at the Medical Research Council in England, has been used to completely determine the nucleotide order of a virus (SN: 3/5/77, p. 148). Gilbert and Allan M. Maxam have devised a somewhat different technique. These methods are useful both to bacterial geneticists studying natural DNA fragments and to researchers examining segments of recombinant DNA.

Maxam and Gilbert use four chemical treatments to break a radioactively labeled molecule into a set of fragments. The different chemical procedures break DNA selectively after different nucleotides. The resulting fragments can be separated on gels that resolve pieces differing in length by a single base. "It takes the magic out of sequencing," Gilbert says. "You can just read it."

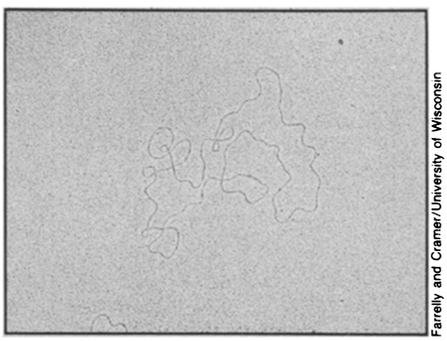
The photograph of gels shows how clearly the nucleotide sequence can be determined. A strong band in the left-hand column arises from adenine (A), in the second column from guanine (G). A band in both the third and fourth columns is from cytosine (C) and in the fourth column alone from thymine (T). Maxam and Gilbert say in the February PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES that now any DNA molecule that can be obtained from a virus or a plasmid can be sequenced.

Besides increasing knowledge of the language of the genetic code and providing the instructions to chemists interested in synthesizing specific genes, nucleotide sequences may also be useful in studying evolution. Theories about mutation and



Young/Stanford

Labeled DNA bound to chromosomes.



Farrelly and Cramer/University of Wisconsin

Recombinant DNA molecule composed of a bacterial plasmid and yeast DNA.

selection can be explored by comparing the chromosomes of closely and distantly related species. There is already evidence of surprising similarities between some sections of the chromosomes of seemingly distant organisms.

Nucleotide sequencing is today experiencing such a boom that Lee Hartwell, a yeast geneticist at the University of Washington, jokingly complained at the genetics meeting that "the geneticists were invited just to suggest to the sequencers what might be interesting to sequence."

In addition to learning the exact sequence of nucleotides in DNA, biologists also hope to discover how genes are organized into chromosomes. David Hogness and collaborators at Stanford University, for example, have used recombinant DNA to study fruit fly (*Drosophila*) chromosomes. Radioactively labeled segments of DNA will, under proper conditions, bind to the region of an intact chromosome that contains the same sequence. The black spots on the photograph show the position of these segments bound to the giant chromosomes of *Drosophila* salivary glands.

The researchers found that some pieces of DNA have only one possible position. Other genes seem to be present in multiple copies. One segment of DNA attaches to 30 to 40 different sites on the chromosomes, Michael Young told the Utah meeting. Young suggested that different copies of a gene might shuttle in and out of special chromosome sites during development.

Although most genes in higher organisms do not seem to be grouped by their functions, research on *Drosophila* and on sea urchins has shown that some genes form tandemly repeated, multigene families. These groups must have sophisticated controls. For example, in one family, some genes are read in one direction and others in the opposite direction. To understand coordination of gene expression, the researchers want to learn much more about the interactions of adjacent genes, the location of signal regions and the meaning of the repeated sequences.

Finally, recombinant DNA is being used to answer specific questions about gene expression, such as, do genes move around in the chromosome during development (SN: 3/12/77, p. 164)? Also genes-splicing techniques provide a chance for scientists to look at cellular activities outside the nucleus. Segments of DNA can be used to identify specific messenger RNA. Researchers can then distinguish control of protein production at the DNA-to-RNA step and at the RNA-to-protein step and ask when in development a gene is first expressed.

"So far recombinant DNA methods have been applied almost exclusively to the chromosomes of lower organisms," Berg explained to the Academy forum. "In fact, until recently, such experiments with mammalian and particularly human chromosomes were forbidden wherever the NIH guidelines are applicable." Now the guidelines allow transfer of mammalian genes to bacteria under specified safety conditions.

Sir Francis Crick of the Medical Research Council summarized the state of research using recombinant DNA when he commented on the Utah meeting. "People have reported their systems are promising, their methods are exciting, they have some results, but no deep results. We are in an era of very rapid progress. But it will be two to three years, maybe even just one year, before we see the sunlight, and even more rapid progress." □