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# THE MANIPULATION OF GENES

by Stanley N. Cohen

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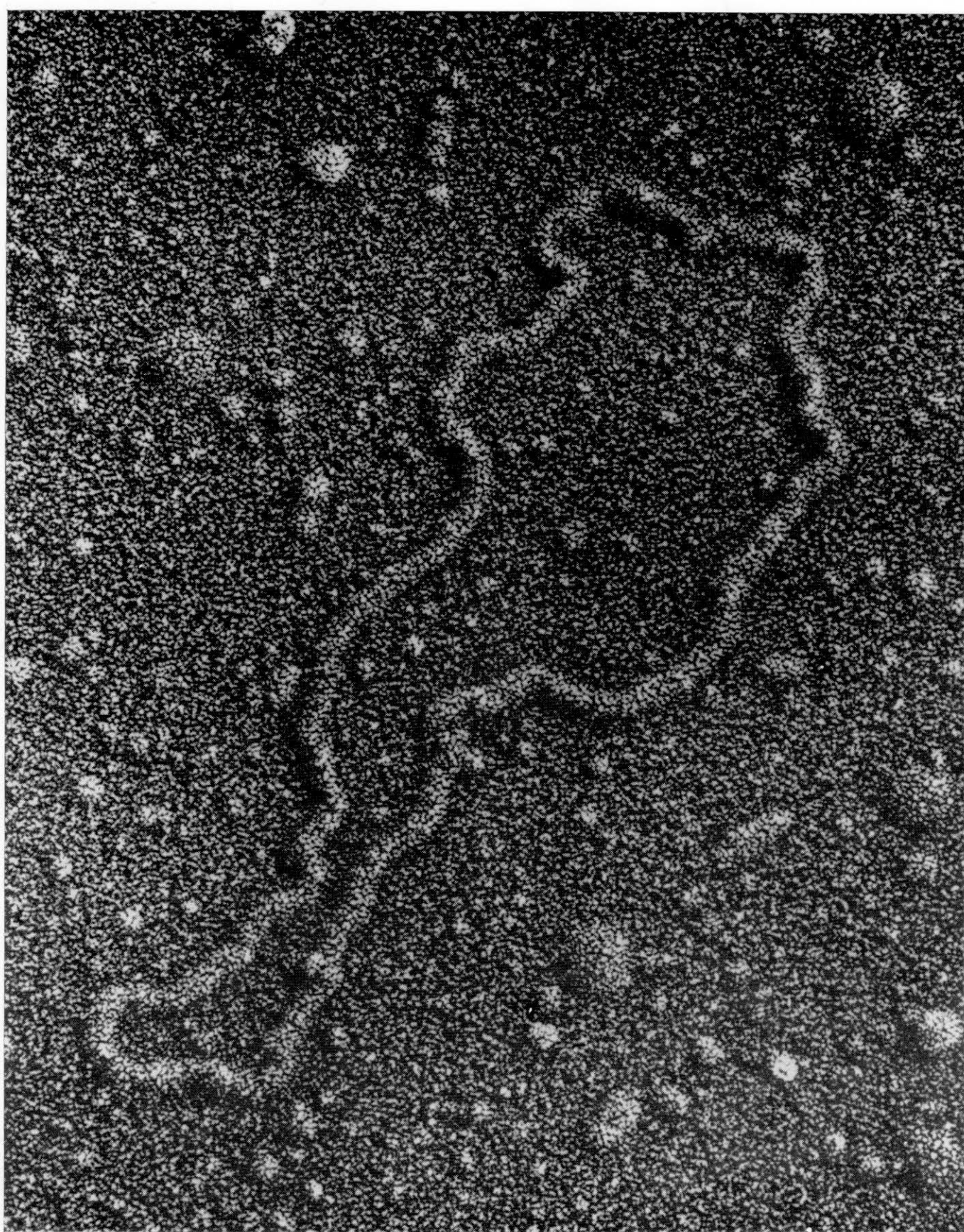
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PLASMID pSC101 is shadowed with platinum-palladium and enlarged 230,000 diameters in an electron micrograph made by the author. A plasmid is a molecule of DNA that exists apart from the chromosome in a bacterium and replicates on its own, often carrying the genes for some supplementary activity such as resistance to antibiotics. This plasmid, a small one made by shearing a larger plasmid native to the bacterium *Escherichia coli*, is a circular, or

closed-loop, molecule of DNA about three micrometers in circumference that carries the genetic information for replicating itself in *E. coli* and for conferring resistance to the antibiotic tetracycline. It was the "vehicle" for the first gene-manipulation experiments by the author and his colleagues. Foreign DNA was spliced to it and the plasmid was introduced into *E. coli*, where it replicated and expressed both its own and the foreign DNA's genetic information.



# THE MANIPULATION OF GENES

Techniques for cleaving DNA and splicing it into a carrier molecule make it possible to transfer genetic information from one organism to an unrelated one. There the DNA replicates and expresses itself

by Stanley N. Cohen

Mythology is full of hybrid creatures such as the Sphinx, the Minotaur and the Chimera, but the real world is not; it is populated by organisms that have been shaped not by the union of characteristics derived from very dissimilar organisms but by evolution within species that retain their basic identity generation after generation. This is because there are natural barriers that normally prevent the exchange of genetic information between unrelated organisms. The barriers are still poorly understood, but they are of fundamental biological importance.

The basic unit of biological relatedness is the species, and in organisms that reproduce sexually species are defined by the ability of their members to breed with one another. Species are determined and defined by the genes they carry, so that in organisms that reproduce asexually the concept of species depends on nature's ability to prevent the biologically significant exchange of genetic material—the nucleic acid DNA—between unrelated groups.

The persistence of genetic uniqueness is perhaps most remarkable in simple organisms such as bacteria. Even when they occupy the same habitat most bacterial species do not exchange genetic information. Even rather similar species of bacteria do not ordinarily exchange the genes on their chromosomes, the structures that carry most of their genetic information. There are exceptions, however. There are bits of DNA, called plasmids, that exist apart from the chromosomes in some bacteria. Sometimes a plasmid can pick up a short segment of DNA from the chromosome of its own cell and transfer it to the cell of a related bacterial species, and sometimes the plasmid and the segment of chromosomal DNA can become integrated into the chromosome of the recipient cell. This

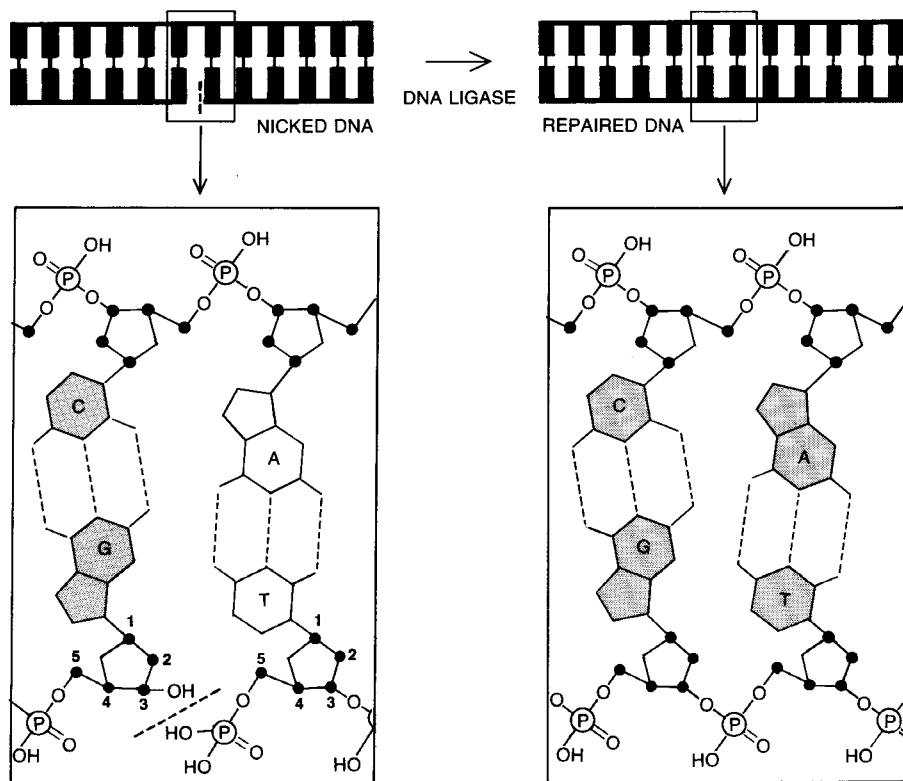
transfer of genes between species by extrachromosomal elements has surely played some role in bacterial evolution, but apparently it has not been widespread in nature. Otherwise the characteristics of the common bacterial species would not have remained so largely intact over the huge number of bacterial generations that have existed during the era of modern bacteriology.

In 1973 Annie C. Y. Chang and I at the Stanford University School of Medicine and Herbert W. Boyer and Robert B. Helling at the University of California School of Medicine at San Francisco reported the construction in a test tube of biologically functional DNA molecules that combined genetic information from two different sources. We made the molecules by splicing together segments of two different plasmids found in the colon bacillus *Escherichia coli* and then inserting the composite DNA into *E. coli* cells, where it replicated itself and expressed the genetic information of both parent plasmids. Soon afterward we introduced plasmid genes from an unrelated bacterial species, *Staphylococcus aureus*, into *E. coli*, where they too expressed the biological properties they had displayed in their original host; then, applying the same procedures with John F. Morrow of Stanford and Howard M. Goodman in San Francisco, we were able to insert into *E. coli* some genes from an animal: the toad *Xenopus laevis*.

We called our composite molecules DNA chimeras because they were conceptually similar to the mythological Chimera (a creature with the head of a lion, the body of a goat and the tail of a serpent) and were the molecular counterparts of hybrid plant chimeras produced by agricultural grafting. The procedure we described has since been used and extended by workers in several laboratories. It has been called plasmid en-

gineering, because it utilizes plasmids to introduce the foreign genes, and molecular cloning, because it provides a way to propagate a clone, or line of genetically alike organisms, all containing identical composite DNA molecules. Because of the method's potential for creating a wide variety of novel genetic combinations in microorganisms it is also known as genetic engineering and genetic manipulation. The procedure actually consists of several distinct biochemical and biological manipulations that were made possible by a series of independent discoveries made in rapid succession in the late 1960's and early 1970's. There are four essential elements: a method of breaking and joining DNA molecules derived from different sources; a suitable gene carrier that can replicate both itself and a foreign DNA segment linked to it; a means of introducing the composite DNA molecule, or chimera, into a functional bacterial cell, and a method of selecting from a large population of cells a clone of recipient cells that has acquired the molecular chimera.

In 1967 DNA ligases—enzymes that can repair breaks in DNA and under certain conditions can join together the loose ends of DNA strands—were discovered almost simultaneously in five laboratories. A DNA strand is a chain of nucleotides, each consisting of a deoxyribose sugar ring, a phosphate group and one of four organic bases: adenine, thymine, guanine and cytosine. The sugars and phosphates form the backbone of the strand, from which the bases project. The individual nucleotide building blocks are connected by phosphodiester bonds between the carbon atom at position No. 3 on one sugar and the carbon atom at position No. 5 on the adjacent sugar. Double-strand DNA, the form found in most organisms, consists of two



DNA LIGASE is an enzyme that repairs "nicks," or breaks in one strand of a double-strand molecule of DNA (*top*). A strand of DNA is a chain of nucleotides (*bottom*), each consisting of a deoxyribose sugar and a phosphate group and one of four organic bases: adenine (A), thymine (T), guanine (G) and cytosine (C). The sugars and phosphates constitute the backbone of the strand, and paired bases, linked by hydrogen bonds (*broken black lines*), connect two strands. The ligase catalyzes synthesis of a bond at the site of the break (*broken colored line*) between the phosphate of one nucleotide and the sugar of the next nucleotide.

chains of nucleotides linked by hydrogen bonds between their projecting bases. The bases are complementary: adenine (A) is always opposite thymine (T), and guanine (G) is always opposite cytosine (C). The function of the ligase is to repair "nicks," or breaks in single DNA strands, by synthesizing a phosphodiester bond between adjoining nucleotides [*see illustration above*].

In 1970 a group working in the laboratory of H. Gobind Khorana, who was then at the University of Wisconsin, found that the ligase produced by the bacterial virus T4 could sometimes catalyze the end-to-end linkage of completely separated double-strand DNA segments. The reaction required that the ends of two segments be able to find each other; such positioning of two DNA molecules was a matter of chance, and so the reaction was inefficient. It was clear that efficient joining of DNA molecules required a mechanism for holding the two DNA ends together so that the ligase could act.

An ingenious way of accomplishing this was developed and tested independently in two laboratories at Stanford: by Peter Lobban and A. Dale Kaiser and

by David Jackson, Robert Symons and Paul Berg. Earlier work by others had shown that the ends of the DNA molecules of certain bacterial viruses can be joined by base-pairing between complementary sequences of nucleotides that are naturally present on single-strand segments projecting from the ends of those molecules: A's pair with T's, G's pair with C's and the molecules are held together by hydrogen bonds that form between the pairs. The principle of linking DNA molecules by means of the single-strand projections had been exploited in Khorana's laboratory for joining short synthetic sequences of nucleotides into longer segments of DNA.

The Stanford groups knew too that an enzyme, terminal transferase, would catalyze the stepwise addition, specifically at what are called the 3' ends of single strands of DNA, of a series of identical nucleotides. If the enzyme worked also with double-strand DNA, then a block of identical nucleotides could be added to one population of DNA molecules and a block of the complementary nucleotides could be added to another population from another source. Molecules of the two populations could then be annealed

by hydrogen bonding and sealed together by DNA ligase. The method was potentially capable of joining any two species of DNA. While Lobban and Kaiser tested the terminal-transferase procedure with the DNA of the bacterial virus P22, Jackson, Symons and Berg applied the procedure to link the DNA of the animal virus SV40 to bacterial-virus DNA.

The SV40 and bacterial-virus DNA molecules Berg's group worked with are closed loops, and the loops had first to be cleaved to provide linear molecules with free ends for further processing and linkage [*see illustration on opposite page*]. (As it happened, the particular enzyme chosen to cleave the loops was the *Eco RI* endonuclease, which was later to be used in a different procedure for making the first biologically functional gene combinations. At the time, however, the enzyme's special property of producing complementary single-strand ends all by itself had not yet been discovered.)

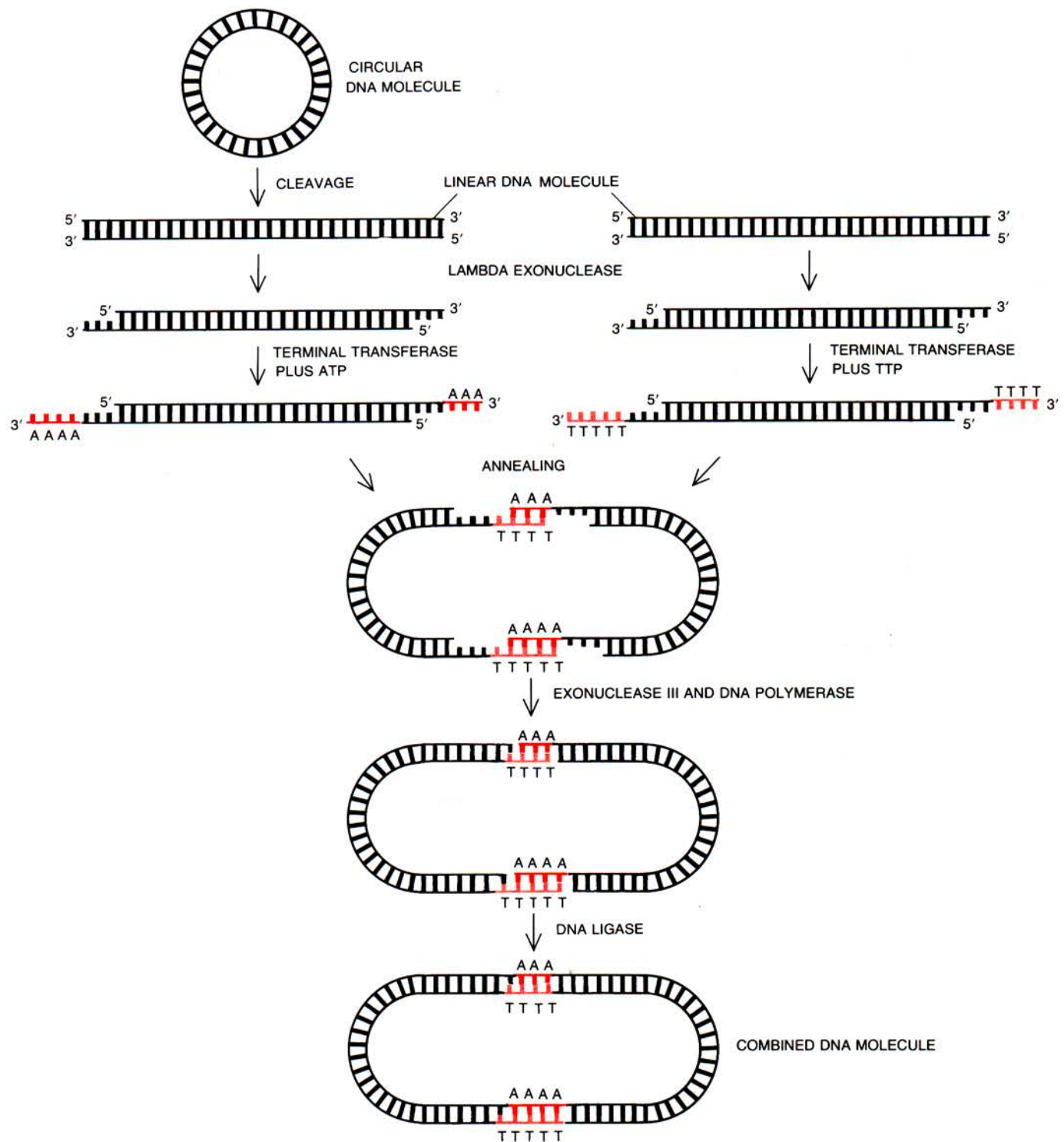
The cleaved linear molecules were treated with an enzyme, produced by the bacterial virus lambda, called an exonuclease because it operates by cutting off nucleotides at the end of a DNA molecule. The lambda exonuclease chewed back the 5' ends of DNA molecules and thus left projecting single-strand ends that had 3' termini to which the blocks of complementary nucleotides could be added. The next step was to add, with the help of terminal transferase, a block of A's at the 3' end of one of the two DNA species to be linked and a block of T's at the 3' ends of the other species. The species were mixed together. Fragments having complementary blocks at their ends could find each other, line up and become annealed by hydrogen bonding, thus forming combined molecules. To fill the gaps at the 5' ends of the original segments the investigators supplied nucleotides and two more enzymes: exonuclease III and DNA polymerase. Finally the nicks in the molecules were sealed with DNA ligase.

The method of making cohesive termini for joining DNA molecules in the first successful genetic-manipulation experiments was conceptually and operationally different from the terminal-transferase procedure. It was also much simpler. It depended on the ability of one of a group of enzymes called restriction endonucleases to make complementary-ended fragments during the cleavage of DNA at a site within the molecule, instead of requiring the addition of new blocks of complementary nucleotides to DNA termini.

Viruses grown on certain strains of *E. coli* were known to be restricted in their ability to grow subsequently on other strains. Investigations had shown that this restriction was due to bacterial enzymes that recognize specific sites on a "foreign" viral DNA and cleave that

DNA. (To protect its own DNA the bacterial cell makes a modification enzyme that adds methyl groups to nucleotides constituting the recognition sites for the restriction endonuclease, making them resistant to cleavage.) Restriction endonucleases (and modification methylases)

are widespread in microorganisms; genes for making them were found on viral chromosomes and extrachromosomal plasmid DNA as well as on many bacterial chromosomes. During the early 1970's the nucleotide sequences at the cleavage sites recognized by several re-



**TERMINAL-TRANSFERASE** procedure for joining DNA molecules involves a number of steps, each dependent on a different enzyme. If one of the molecules to be joined is a closed loop, it must first be cleaved. The linear molecules are treated with lambda exonuclease, an enzyme that cuts nucleotides off the 5' end of DNA strands (the end with a phosphate group on the No. 5 carbon). Then specific nucleotides are added to the 3' end (the end with an OH group on the No. 3 carbon) by the action of the enzyme termi-

nal transferase. One DNA species is supplied with adenosine triphosphate (ATP), the other with thymidine triphosphate (TTP), so that *A* nucleotides are added to one species and complementary *T* nucleotides to the other. When the two species are mixed, the complementary bases pair up, annealing the molecules. Nucleotides and the enzymes DNA polymerase and exonuclease III are added to fill gaps and DNA ligase is added to seal the DNA backbones. The result is a double molecule composed of two separate DNA segments.



striction endonucleases were identified. In every instance, it developed, the cleavage was at or near an axis of rotational symmetry: a palindrome where the nucleotide base sequences read the same on both strands in the 5'-to-3' direction [see illustration below].

In some instances the breaks in the DNA strands made by restriction enzymes were opposite each other. One particular endonuclease, however, the *Eco* RI enzyme isolated by Robert N. Yoshimori in Boyer's laboratory in San Francisco, had a property that was of special interest. Unlike the other nucleases known at the time, this enzyme introduced breaks in the two DNA strands that were separated by several nucleotides. Because of the symmetrical, palindromic arrangement of the nucleotides in the region of cleavage this separation of the cleavage points on the two strands yielded DNA termini with projecting complementary nucleotide sequences: "sticky" mortise-and-tenon ter-

mini. The *Eco* RI enzyme thus produced in one step DNA molecules that were functionally equivalent to the cohesive-end molecules produced by the complicated terminal-transferase procedure.

The experiments that led to the discovery of the capabilities of *Eco* RI were reported independently and simultaneously in November, 1972, by Janet Mertz and Ronald W. Davis of Stanford and by another Stanford investigator, Vittorio Sgaramella. Sgaramella found that molecules of the bacterial virus P22 could be cleaved with *Eco* RI and would then link up end to end to form DNA segments equal in length to two or more viral-DNA molecules. Mertz and Davis observed that closed-loop SV40-DNA molecules cleaved by *Eco* RI would reform themselves into circular molecules by hydrogen bonding and could be sealed with DNA ligase; the reconstituted molecules were infectious in animal cells growing in tissue culture. Boyer and his colleagues analyzed the nucleotide sequences at the DNA termini produced by *Eco* RI, and their evidence confirmed the complementary nature of the termini, which accounted for their cohesive activity.

In late 1972, then, several methods were available by which one could join double-strand molecules of DNA. That was a major step in the development of a system for manipulating genes. More was necessary, however. Most segments of DNA do not have an inherent capacity for self-replication; in order to reproduce themselves in a biological system they need to be integrated into DNA molecules that can replicate in the particular system. Even a DNA segment that can replicate in its original host was not likely to have the specific genetic signals required for replication in a different environment. If foreign DNA was to be propagated in bacteria, as had long been proposed in speculative scenarios of genetic engineering, a suitable vehicle, or carrier, was required. A composite DNA molecule consisting of the vehicle and the desired foreign DNA would have to be introduced into a population of functional host bacteria. Finally, it would be necessary to select, or identify, those cells in the bacterial population that took up the DNA chimeras. In 1972 it still seemed possible that the genetic information on totally foreign DNA molecules might produce an aberrant situation that would prevent the propagation of hybrid molecules in a new host.

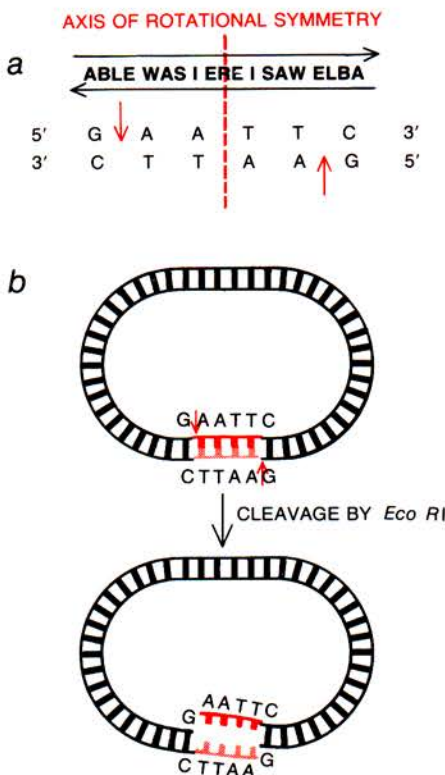
Molecular biologists had focused for many years on viruses and their relations with bacteria, and so it was natu-

ral that bacterial viruses were thought of as the most likely vehicles for genetic manipulation. For some time there had been speculation and discussion about using viruses, such as lambda, that occasionally acquire bits of the *E. coli* chromosome by natural recombination mechanisms for cloning DNA from foreign sources. It was not a virus, however, but a plasmid that first served as a vehicle for introducing foreign genes into a bacterium and that provided a mechanism for the replication and selection of the foreign DNA.

A ubiquitous group of plasmids that confer on their host bacteria the ability to resist a number of antibiotics had been studied intensively for more than a decade. Antibiotic-resistant *E. coli* isolated in many parts of the world, for example, were found to contain plasmids, designated *R* factors (for "resistance"), carrying the genetic information for products that in one way or another could interfere with the action of specific antibiotics [see "Infectious Drug Resistance," by Tsutomu Watanabe; SCIENTIFIC AMERICAN, December, 1967]. Double-strand circular molecules of *R*-factor DNA had been separated from bacterial chromosomal DNA by centrifugation in density gradients and had been characterized by biochemical and physical techniques [see "The Molecule of Infectious Drug Resistance," by Royston C. Clowes; SCIENTIFIC AMERICAN, April, 1973].

In 1970 Morton Mandel and A. Higa of the University of Hawaii School of Medicine had discovered that treatment of *E. coli* with calcium salts enabled the bacteria to take up viral DNA. At Stanford, Chang and I, with Leslie Hsu, found that if we made the cell membranes of *E. coli* permeable by treating them with calcium chloride, purified *R*-factor DNA could be introduced into them [see illustration on opposite page]. The *R*-factor DNA is taken up in this transformation process by only about one bacterial cell in a million, but those few cells can be selected because they live and multiply in the presence of the antibiotics to which the *R* factor confers resistance, whereas other cells die. Each transformed cell gives rise to a clone that contains exact replicas of the parent plasmid DNA molecules, and so we reasoned that plasmids might serve as vehicles for propagating new genetic information in a line of *E. coli* cells.

In an effort to explore the genetic and molecular properties of various regions of the *R*-factor DNA we had begun to take plasmids apart by shearing their DNA mechanically and then transforming *E. coli* with the resulting



**RESTRICTION ENDONUCLEASES** cleave DNA at sites where complementary nucleotides are arranged in rotational symmetry: a palindrome, comparable to a word palindrome (a). The endonuclease *Eco* RI has the additional property of cleaving complementary strands of DNA at sites (colored arrows) four nucleotides apart. Such cleavage (b) yields DNA fragments with complementary, overlapping single-strand ends. As a result the end of any DNA fragment produced by *Eco* RI cleavage can anneal with any other fragment produced by the enzyme.

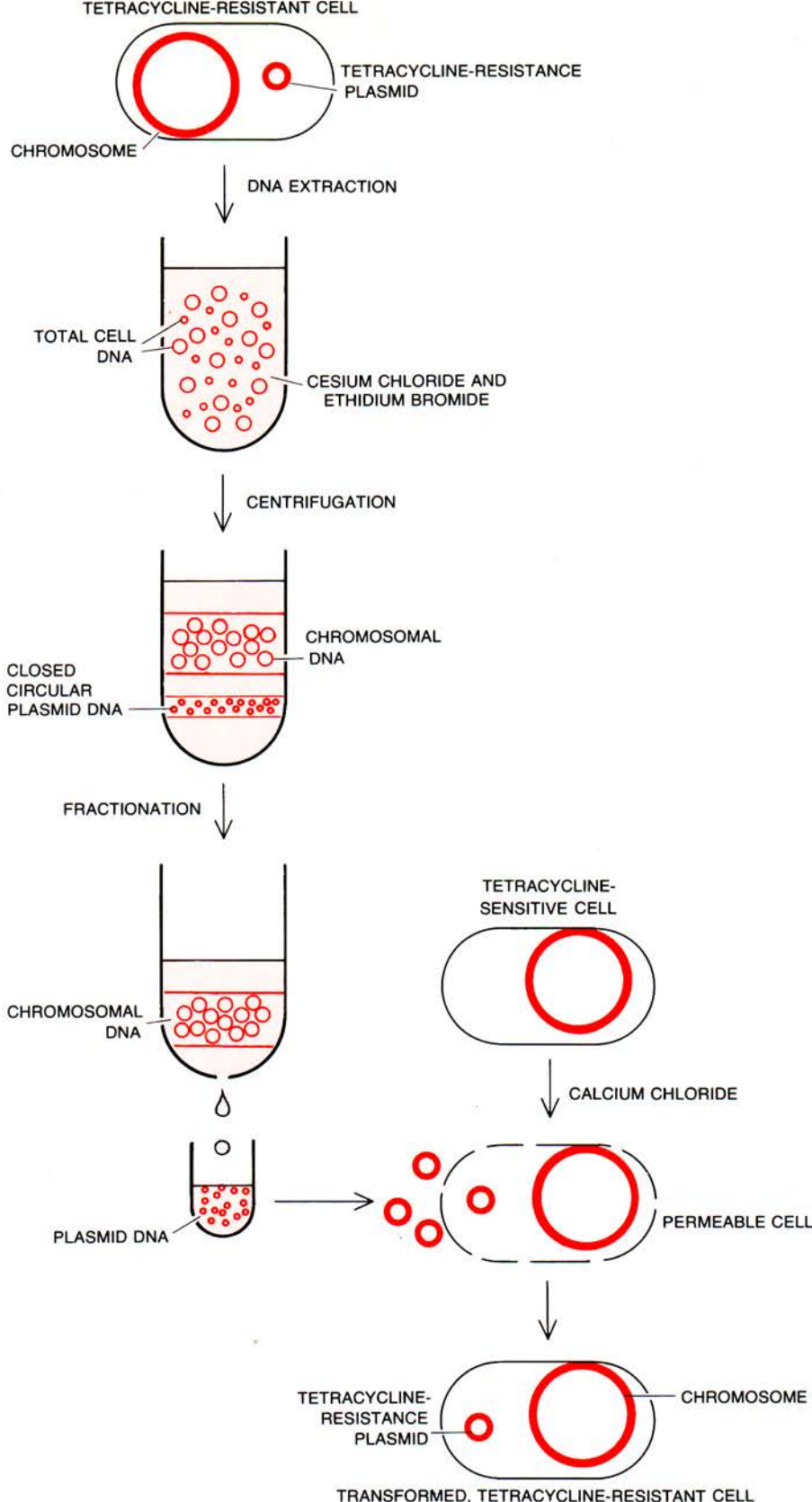


fragments. Soon afterward we began to cleave the plasmids with the *Eco* RI enzyme, which had been shown to produce multiple site-specific breaks in several viruses. It might therefore be counted on to cleave all molecules of a bacterial plasmid in the same way, so that any particular species of DNA would yield a specific set of cleavage fragments, and do so reproducibly. The fragments could then be separated and identified according to the different rates at which they would migrate through a gel under the influence of an electric current.

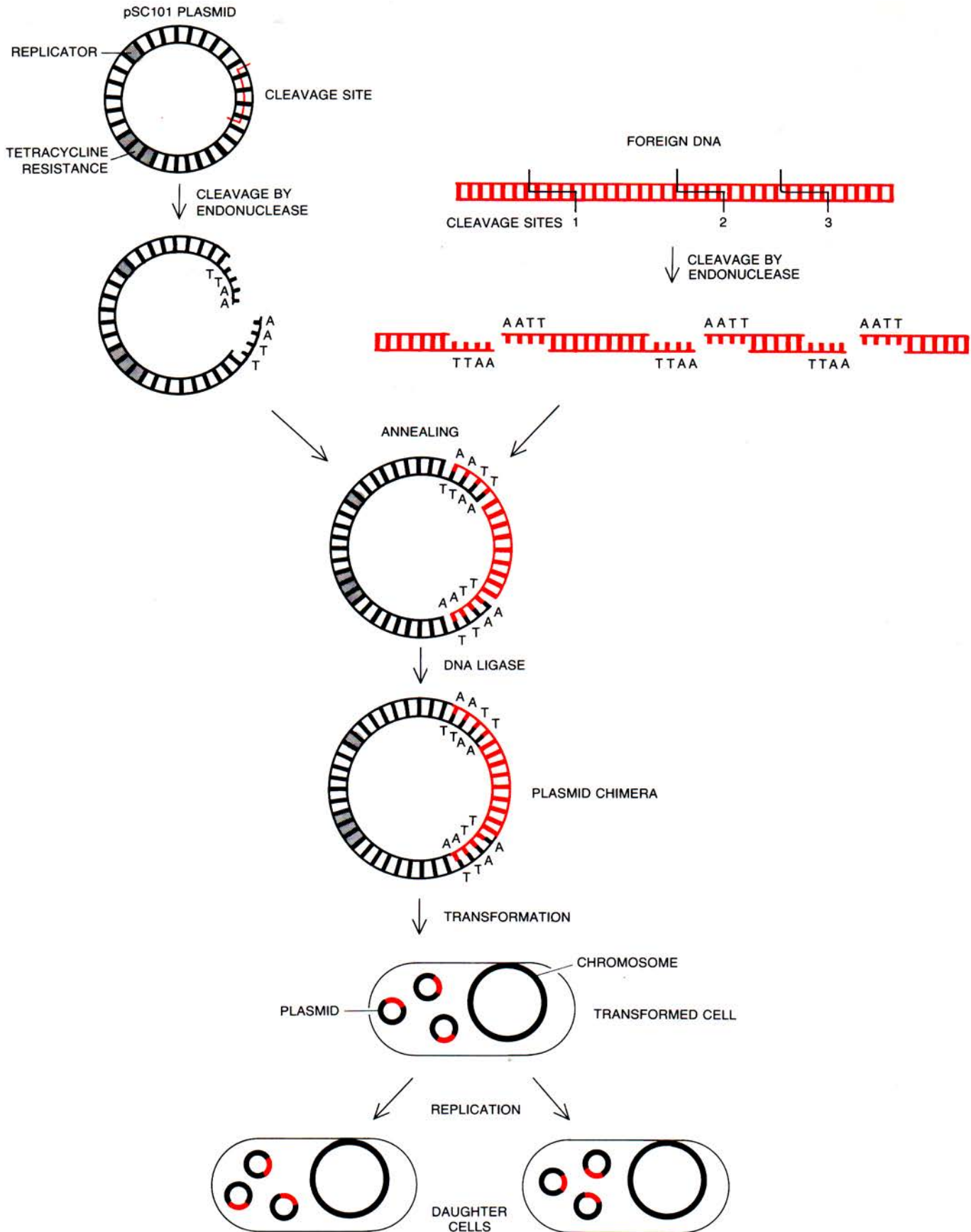
When the DNA termini produced by *Eco* RI endonuclease were found to be cohesive, Chang and I, in collaboration with Boyer and Helling in San Francisco, proceeded to search for a plasmid that the enzyme would cleave without affecting the plasmid's ability to replicate or to confer antibiotic resistance. We hoped that if such a plasmid could be found, we could insert a segment of foreign DNA at the *Eco* RI cleavage site, and that it might be possible to propagate the foreign DNA in *E. coli*.

In our collection at Stanford there was a small plasmid, *pSC101*, that had been isolated following the mechanical shearing of a large plasmid bearing genes for multiple antibiotic resistance. It was less than a twelfth as long as the parent plasmid, but it did retain the genetic information for its replication in *E. coli* and for conferring resistance to one antibiotic, tetracycline. When we subjected *pSC101* DNA to cleavage by *Eco* RI and analyzed the products by gel electrophoresis, we found that the enzyme had cut the plasmid molecule in only one place, producing a single linear fragment. We were able to join the ends of that fragment again by hydrogen bonding and re-seal them with DNA ligase, and when we introduced the reconstituted circular DNA molecules into *E. coli* by transformation, they were biologically functional plasmids: they replicated and conferred tetracycline resistance.

The next step was to see if a fragment of foreign DNA could be inserted at the cleavage site without interfering with replication or expression of tetracycline resistance and thus destroying the plasmid's ability to serve as a cloning vehicle. We mixed the DNA of another *E. coli* plasmid, which carried resistance to the antibiotic kanamycin, with the *pSC101* DNA. We subjected the mixed DNA to cleavage by *Eco* RI and then to ligation, transformed *E. coli* with the resulting DNA and found that some of the transformed bacteria were indeed resist-



**PLASMID DNA** can be introduced into a bacterial cell by the procedure called transformation. Plasmids carrying genes for resistance to the antibiotic tetracycline (*top left*) are separated from bacterial chromosomal DNA. Because differential binding of ethidium bromide by the two DNA species makes the circular plasmid DNA denser than the chromosomal DNA, the plasmids form a distinct band on centrifugation in a cesium chloride gradient and can be separated (*bottom left*). The plasmid DNA is mixed with bacterial cells that are not resistant to tetracycline and that have been made permeable by treatment with a calcium salt. The DNA enters the cells, replicates there and makes the cells resistant to tetracycline.



FOREIGN DNA is spliced into the pSC101 plasmid and introduced with the plasmid into the bacterium *Escherichia coli*. The plasmid is cleaved by the endonuclease *Eco* RI at a single site that does not interfere with the plasmid's genes for replication or for resistance to tetracycline (top left). The nucleotide sequence recognized by *Eco* RI is present also in other DNA, so that a foreign DNA exposed to the endonuclease is cleaved about once in every 4,000 to

16,000 nucleotide pairs on a random basis (top right). Fragments of cleaved foreign DNA are annealed to the plasmid DNA by hydrogen bonding of the complementary base pairs, and the new composite molecules are sealed by DNA ligase. The DNA chimeras, each consisting of the entire plasmid and a foreign DNA fragment, are introduced into *E. coli* by transformation, and the foreign DNA is replicated by virtue of the replication functions of the plasmid.



ant to both tetracycline and kanamycin. The plasmids isolated from such transformants contained the entire *pSC101* DNA segment and also a second DNA fragment that carried the information for kanamycin resistance, although it lacked replication functions of its own. The results meant that the *pSC101* could serve as a cloning vehicle for introducing at least a nonreplicating segment of a related DNA into *E. coli*. And the procedure was extraordinarily simple.

Could genes from other species be introduced into *E. coli* plasmids, however? There might be genetic signals on foreign DNA that would prevent its propagation or expression in *E. coli*. We decided to try to combine DNA from a plasmid of another bacterium, the *pI258* plasmid of *Staphylococcus aureus*, with our original *E. coli* plasmid. The staphylococcal plasmid had already been studied in several laboratories; we had found that it was cleaved into four DNA fragments by *Eco* RI. Since *pI258* was not native to *E. coli* or to related bacteria, it could not on its own propagate in an *E. coli* host. And it was known to carry a gene for resistance to still another antibiotic, penicillin, that would serve as a marker for selecting any transformed clones. (Penicillin resistance, like combined resistance to tetracycline and kanamycin, was already widespread among *E. coli* strains in nature. That was important; if genes from a bacterial species that cannot normally exchange genetic information with the colon bacillus were to be introduced into it, it was essential that they carry only antibiotic-resistance traits that were already prevalent in *E. coli*. Otherwise we would be extending the species' antibiotic-resistance capabilities.)

Chang and I repeated the experiment that had been successful with two kinds of *E. coli* plasmids, but this time we did it with a mixture of the *E. coli*'s *pSC101* and the staphylococcal *pI258*: we cleaved the mixed plasmids with *Eco* RI endonuclease, treated them with ligase and then transformed *E. coli*. Next we isolated transformed bacteria that expressed the penicillin resistance coded for by the *S. aureus* plasmid as well as the tetracycline resistance of the *E. coli* plasmid. These doubly resistant cells were found to contain a new DNA species that had the molecular characteristics of the staphylococcal plasmid DNA as well as the characteristics of *pSC101*.

The replication and expression in *E. coli* of genes derived from an organism ordinarily quite unable to exchange genes with *E. coli* represented a breach in the barriers that normally separate

biological species. The bulk of the genetic information expressed in the transformed bacteria defined it as *E. coli*, but the transformed cells also carried replicating DNA molecules that had molecular and biological characteristics derived from an unrelated species, *S. aureus*. The fact that the foreign genes were on a plasmid meant that they would be easy to isolate and purify in large quantities for further study. Moreover, there was a possibility that one might introduce genes into the easy-to-grow *E. coli* that specify a wide variety of metabolic or synthesizing functions (such as photosynthesis or antibiotic production) and that are indigenous to other biological classes. Potentially the *pSC101* plasmid and the molecular-cloning procedure could serve to introduce DNA molecules from complex higher organisms into bacterial hosts, making it possible to apply relatively simple bacterial genetic and biochemical techniques to the study of animal-cell genes.

Could animal-cell genes in fact be introduced into bacteria, and would they replicate there? Boyer, Chang, Helling and I, together with Morrow and Goodman, immediately undertook to find out. We picked certain genes that had been well studied and characterized and were available, purified, in quantity: the genes that code for a precursor of the ribosomes (the structure on which proteins are synthesized) in the toad *Xenopus laevis*. The genes had properties that would enable us to identify them if we succeeded in getting them to propagate in bacteria. The toad DNA was suitable for another reason: although we would be constructing a novel biological combination containing genes from both animal cells and bacteria, we and others expected that no hazard would result from transplanting the highly purified ribosomal genes of a toad.

Unlike the foreign DNA's of our earlier experiments, the toad genes did not express traits (such as antibiotic resistance) that could help us to select bacteria carrying plasmid chimeras. The tetracycline resistance conferred by *pSC101* would make it possible to select transformed clones, however, and we could then proceed to examine the DNA isolated from such clones to see if any clones contained a foreign DNA having the molecular properties of toad ribosomal DNA. The endonuclease-generated fragments of toad ribosomal DNA have characteristic sizes and base compositions; DNA from the transformed cells could be tested for those characteristics. The genes propagated in bac-

teria could also be tested for nucleotide-sequence homology with DNA isolated directly from the toad.

When we did the experiment and analyzed the resulting transformed cells, we found that the animal-cell genes were indeed reproducing themselves in generation after generation of bacteria by means of the plasmid's replication functions. In addition, the nucleotide sequences of the toad DNA were being transcribed into an RNA product in the bacterial cells.

Within a very few months after the first DNA-cloning experiments the procedure was being used in a number of laboratories to clone bacterial and animal-cell DNA from a variety of sources. Soon two plasmids other than *pSC101* were discovered that have a single *Eco* RI cleavage site at a location that does not interfere with essential genes. One of these plasmids is present in many copies in the bacterial cell, making it possible to "amplify," or multiply many times, any DNA fragments linked to it. Investigators at the University of Edinburgh and at Stanford went on to develop mutants of the virus lambda (which ordinarily infects *E. coli*) that made the virus too an effective cloning vehicle. Other restriction endonucleases were discovered that also make cohesive termini but that cleave DNA at different sites from the *Eco* RI enzymes, so that chromosomes can now be taken apart and put together in various ways.

The investigative possibilities of DNA cloning are already being explored intensively. Some workers have isolated from complex chromosomes certain regions that are implicated in particular functions such as replication. Others are making plasmids to order with specific properties that should clarify aspects of extrachromosomal-DNA biology that have been hard to study. The organization of complex chromosomes, such as those of the fruit fly *Drosophila*, is being studied by cloning the animal genes in bacteria. Within the past few months methods have been developed for selectively cloning specific genes of higher organisms through the use of radioactively labeled RNA probes: instead of purifying the genes to be studied before introducing them into bacteria, one can transform bacteria with a heterogeneous population of animal-cell DNA and then isolate those genes that produce a particular species of RNA. It is also possible to isolate groups of genes that are expressed concurrently at a particular stage in the animal's development.

The potential seems to be even broader. Gene manipulation opens the pros-

pect of constructing bacterial cells, which can be grown easily and inexpensively, that will synthesize a variety of biologically produced substances such as antibiotics and hormones, or enzymes that can convert sunlight directly into food substances or usable energy. Perhaps it even provides an experimental basis for introducing new genetic information into plant or animal cells.

It has been clear from the beginning of experimentation in molecular cloning that the construction of some kinds of novel gene combinations may have a potential for biological hazard, and the scientific community has moved quickly to make certain that research in genetic manipulation would not endanger the public. For a time after our initial experiments the pSC101 plasmid was the only vehicle known to be suitable for cloning foreign DNA in *E. coli*, and our colleagues asked for supplies with which to pursue studies we knew were of major scientific and medical importance. Investigators normally facilitate the free exchange of bacteria and other experimental strains they have isolated or developed, but Chang and I were concerned that manipulation of certain genes could give rise to novel organisms whose infectious properties and ecological effects could not be predicted. In agreeing to provide the plasmid we therefore asked for assurance that our colleagues would neither introduce tumor viruses into bacteria nor create antibiotic-resistance combinations that were not already present in nature; we also asked the recipients not to send the plasmid on to other laboratories, so that we could keep track of its distribution.

When still other cloning vehicles were

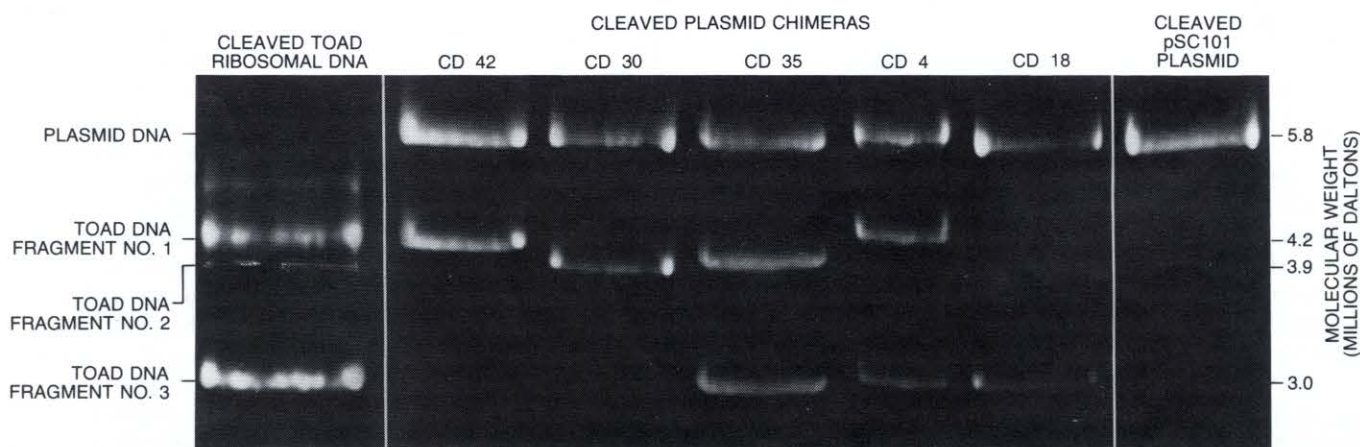
discovered, it became apparent that a more general mechanism for ensuring experimental safety in gene-manipulation research was advisable. The groundwork for such control had been established earlier: the National Academy of Sciences had been urged to consider the "possibility that potentially biohazardous consequences might result from widespread or injudicious use" of these techniques and had asked Paul Berg to form an advisory committee that would consider the issue. Berg too had been concerned about the potential hazards of certain kinds of experimentation for some years, and had himself decided to abandon plans to try to introduce genes from the tumor virus SV40 into bacteria because of the possible danger if the experiment were successful.

Berg brought together a number of investigators, including some who were then directly involved in molecular cloning, in the spring of 1974. In a report released in July and in a letter to leading professional journals the members of the committee expressed their "concern about the possible unfortunate consequences of indiscriminate application" of the techniques and formally asked all investigators to join them in voluntarily deferring two types of experiments (which had, as a matter of fact, been avoided by informal consensus up until that time). Experiments of Type I involved the construction of novel organisms containing combinations of toxin-producing capabilities or of antibiotic-resistance genes not found in nature. Type 2 experiments involved the introduction of DNA from tumor viruses or other animal viruses into bacteria; the committee noted that "such recombinant molecules might be more easily dissemi-

nated to bacterial populations in humans and other species, and might thus increase the incidence of cancer or other diseases."

The Academy committee was concerned largely because of our inability to assess the hazards of certain experiments accurately before the experiments were undertaken. Guidelines for safety had long been available in other areas of potentially hazardous research, such as studies involving known disease-causing bacteria and viruses, radioactive isotopes or toxic chemicals. Because of the newness of the microbial gene-manipulation methods, no such guidelines had yet been developed for work in this area, however; there was the possibility that potentially hazardous experiments might proceed before appropriate guidelines could be considered and implemented. We recognized that most work with the new methods did not and would not involve experiments of a hazardous nature but we recommended the deferral of Type I and Type II experiments until the hazards were more carefully assessed, until it was determined whether or not the work could be undertaken safely and until adequate safety precautions were available. The committee also proposed that an international meeting be held early in 1975 to consider the matter more fully.

Such a meeting was held in February at the Asilomar Conference Center near Pacific Grove, Calif. It brought together 86 American biologists and 53 investigators from 16 other countries, who spent three and a half days reviewing progress in the field of molecular cloning and formulating guidelines that would allow most types of new hereditary characteristics to be introduced into bacteria and



GEL ELECTROPHORESIS demonstrates the presence of toad DNA in chimeric plasmids. Fragments of DNA migrate through a gel at different rates under the influence of an electric current, depending on their size. Linear molecules of plasmid DNA (right) and the cleavage products of toad ribosomal DNA (left) therefore

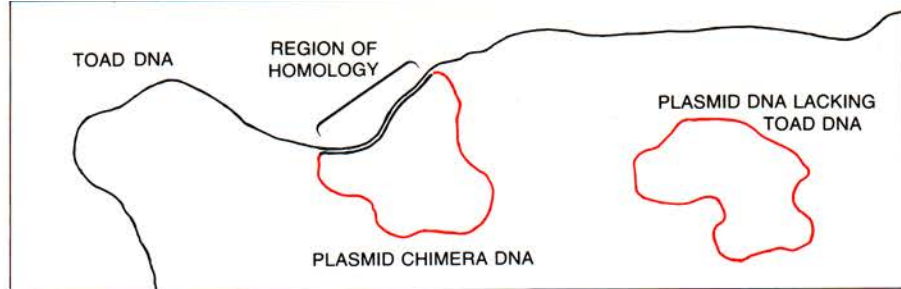
have characteristic sizes and migrate characteristic distances in a given time. The bands of DNA, visualized by a fluorescent dye, are photographed in ultraviolet. All five chimeric plasmids (center) contain a plasmid DNA molecule; in addition each chimera includes one or more fragments characteristic of original toad DNA.



viruses safely. Invited nonscientists from the fields of law and ethics participated in the discussions and decisions at Asilomar, along with representatives of agencies that provide Federal funds for scientific research; the meetings were open to the press and were fully reported. The issues were complex and there were wide differences of opinion on many of them, but there was consensus on three major points. First, the newly developed cloning methods offer the prospect of dealing with a wide variety of important scientific and medical problems as well as other problems that trouble society, such as environmental pollution and food and energy shortages. Second, the accidental dissemination of certain novel biological combinations may present varying degrees of potential risk. The construction of such combinations should proceed only under a graded series of precautions, principally biological and physical barriers, adequate to prevent the escape of any hazardous organisms; the extent of the actual risk should be explored by experiments conducted under strict containment conditions. Third, some experiments are potentially too hazardous to be carried out for the present, even with the most careful containment. Future research and experience may show that many of the potential hazards considered at the meeting are less serious and less probable than we now suspect. Nevertheless, it was agreed that standards of protection should be high at the beginning and that they can be modified later if the assessment of risk changes.

Physical containment barriers have long been used in the U.S. space-exploration program to minimize the possibility of contamination of the earth by extraterrestrial microbes. Containment procedures are also employed routinely to protect laboratory workers and the public from hazards associated with radioactive isotopes and toxic chemicals and in work with disease-causing bacteria and viruses. The Asilomar meeting formulated the additional concept of biological barriers, which involve fastidious cloning vehicles that are able to propagate only in specialized hosts and equally fastidious bacterial strains that are unable to live except under stringent laboratory conditions.

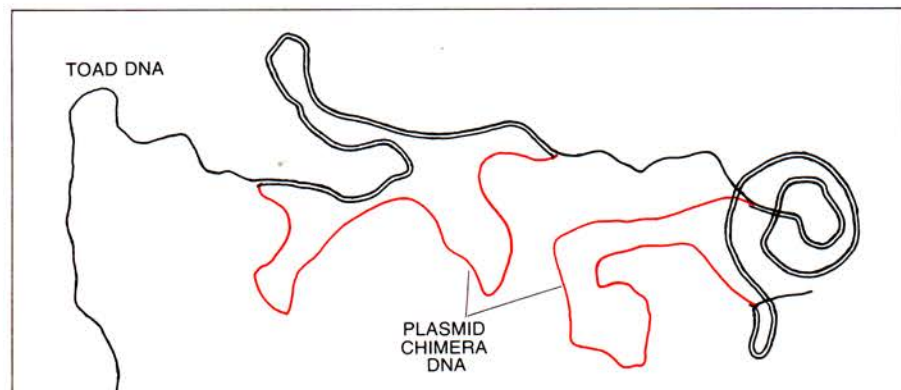
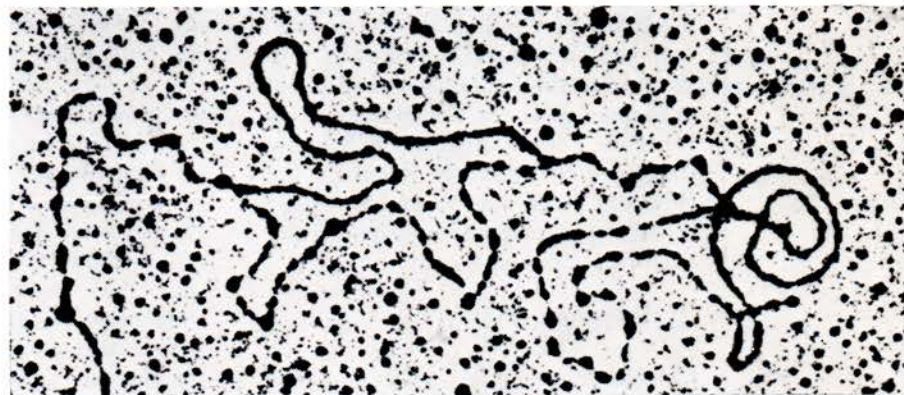
In the past the scientific community has commonly policed its own actions informally, responding to ethical concerns with self-imposed restraint. Usually, but not always, society at large has also considered the public well-being in determining how knowledge obtained by basic scientific research should be applied. Extensive public scrutiny and



**HETERODUPLEX ANALYSIS** identifies regions of a toad DNA (black) that have been incorporated in a chimeric plasmid DNA molecule. DNA isolated from toad eggs and the DNA of the chimera are denatured, that is, each natural double-strand molecule is split into two single strands of DNA, by alkali treatment. The toad and the chimeric DNA's are mixed together, and any complementary sequences are allowed to find each other. The toad DNA incorporated in the chimeras has nucleotide sequences that are complementary to sequences in the DNA taken directly from the animal source. Those homologous sequences anneal to form heteroduplex double-strand DNA that can be identified in electron micrographs.

open discussion by scientists and non-scientists of the possible risks and benefits of a particular line of basic research has been rare, however, when (as in this case) the hazards in question are only potential and, for some experiments, even hypothetical. As this article is being written it is still too early to know what the long-range outcome of the pub-

lic discussions initiated by scientists working in genetic manipulation will be. One can hope that the forthright approach and the rigorous standards that have been adopted for research in the cloning of recombinant DNA molecules will promote a sharper focus on other issues relevant to public and environmental safety.



**PRESENCE OF TOAD DNA** in two separate chimeric plasmid molecules is demonstrated by an electron micrograph made by John F. Morrow at the Stanford University School of Medicine. As is indicated in the drawing (bottom), there are DNA strands from two plasmids and a strand of toad DNA. The micrograph shows thickened regions of DNA where nucleotide sequences are homologous and two single strands have been annealed. The toad DNA in the chimeras codes for ribosomes, and the space between the two heteroduplex regions is compatible with the spacing of multiple ribosomal genes in toad DNA.



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## Bibliography

CONSTRUCTION OF BIOLOGICALLY FUNCTIONAL BACTERIAL PLASMIDS IN VITRO. Stanley N. Cohen, Annie C. Y. Chang, Herbert W. Boyer and Robert B. Helling in *Proceedings of the Na-*

*tional Academy of Sciences of the United States of America*, Vol. 70, No. 11, pages 3240–3244; November, 1973.

REPLICATION AND TRANSCRIPTION OF EUKARYOTIC DNA IN *ESCHERICHIA COLI*. John F. Morrow, Stanley N. Cohen, Annie C. Y. Chang, Herbert W. Boyer, Howard M. Goodman and Robert B. Helling in *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 71, No. 5, pages 1743–1747; May, 1974.

POTENTIAL BIOHAZARDS OF RECOMBINANT DNA MOLECULES. P. Berg et al. in *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 71, No. 7, pages 2595–2599; July, 1974.

FIRST ASM CONFERENCE ON EXTRA-CHROMOSOMAL ELEMENTS IN BACTERIA in *Microbiology—1974*. American Society for Microbiology, 1975.

REPORT OF THE WORKING PARTY ON THE EXPERIMENTAL MANIPULATION OF MICROORGANISMS. Her Majesty's Stationery Office, London, 1975.

# The Recombinant-DNA Debate

by Clifford Grobstein

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# The Recombinant-DNA Debate

*The four-year-old controversy over the potential biohazards presented by the gene-splicing method and the effectiveness of plans for their containment is viewed in a broader context*

by Clifford Grobstein

The guidelines for research involving recombinant-DNA molecules issued a year ago by the National Institutes of Health were the culmination of an extraordinary effort at self-regulation on the part of the scientific community. Yet the policy debate over recombinant-DNA research was clearly not laid to rest by the appearance of the NIH guidelines. Instead the debate has escalated in recent months both in intensity and in the range of public involvement. A watershed of sorts was reached in March at a public forum held by the National Academy of Sciences in Washington. The forum was in part a repeat performance by scientists arguing fixed positions that were established early in the debate. There were, however, new participants on the scene, and they presented a varied and rapidly shifting agenda. They made it clear that research with recombinant DNA had become a political issue. As one speaker remarked, the Academy forum may have been the last major public discussion of recombinant DNA arranged by the scientists involved in the research. Nonscientists at the forum, by word and deed, reiterated the theme that science has become too consequential either to be left to the self-regulation of scientists or to be allowed to wear a veil of political chastity.

Science of course is crucially consequential to society, precisely because it is an intensifying source of both benefits and risks. Research with recombinant DNA may provide major new social benefits of uncertain magnitude: more effective and cheaper pharmaceutical products; better understanding of the causes of cancer; more abundant food crops; even new approaches to the energy problem. These and other possible outcomes are envisioned in "best-case scenarios" for the future application of

recombinant-DNA technology. "Worst-case scenarios" can also be conceived: worldwide epidemics caused by newly created pathogens; the triggering of catastrophic ecological imbalances; new tools for militarists and terrorists; the power to dominate and control the human spirit.

Both the best-case and worst-case scenarios are largely speculative; the gap between them symbolizes the large degree of uncertainty that surrounds this major step forward in molecular genetics. The material basis of biological heredity has been broken into in the past two decades, and it seems as though each of the fragments has acquired a life of its own. In this resulting period of instability fear threatens to override wonder as the implications of the research diffuse more widely. The fear is not so much of any clear and present danger as it is of imagined future hazards. The classic response to such fears is rigid containment: the Great Wall, the Maginot Line, the cold war. All are manifestations of the effort to provide absolute security against unpredictable risks, and yet each generates its own risk. The escalation of the recombinant-DNA de-

bate has a component of this kind of behavior, but there is a more rational component as well.

The first round of the fateful debate began in 1974, when investigators at the leading edge of work in this field declared a voluntary moratorium on several types of experiment judged to be conceivably risky. A set of techniques had been developed that made it possible to cut the long, threadlike molecules of DNA into pieces with the aid of certain enzymes, to recombine the resulting segments of DNA with the DNA of a suitable vector, or carrier, and to reinsert the recombinant into an appropriate host cell to propagate and possibly to function.

The significance of the new developments is rooted in the central biological role of DNA as the transmitter of genetic information between generations. The transmission of the encoded genetic message depends on the ability of a cell to generate exact replicas of the parental DNA and to allocate the replicas among the offspring. In addition the success of genetic transmission depends on the ability of the offspring to "express" the

**GENETIC CODE** of an extremely small bacterial virus, the bacteriophage designated  $\phi$ X174, is given by the sequence of letters on the opposite page. The letters stand for the four nucleotides cytosine, guanine, adenine and thymine, which are linked end to end to make up each strand of the normally double-strand DNA molecule. The genetic message embodied in each strand of DNA is represented by the particular sequence of nucleotides, any one of which may follow any other. In the  $\phi$ X174 virus the DNA molecule, which has only a single circular strand for part of its life cycle, consists of approximately 5,375 nucleotides; the nucleotides are grouped into nine known genes, which are responsible in turn for coding the amino acid sequences of nine different proteins. For example, the dark-color segment of the molecule, called gene J, codes for a small protein that is part of the virus; this segment also happens to be the shortest gene in the  $\phi$ X174 genome. The complete nucleotide sequence for the DNA in  $\phi$ X174 was worked out recently by Frederick Sanger and his colleagues at the British Medical Research Council Laboratory of Molecular Biology in Cambridge. About 2,000 pages of this type would be required to show the nucleotide sequence for the DNA in the chromosome of a typical single-cell bacterium; roughly a million pages would be needed to similarly display the genetic code embodied in DNA molecules that make up chromosomes of a mammalian cell.



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encoded information properly by referring to it to control essential life processes. The mechanism of genetic expression in higher organisms is at present only dimly understood, and the discovery of the new recombinant-DNA techniques seemed immediately to open a broad new avenue to increased knowledge in this field.

The detailed mechanisms of genetic replication and expression are enormously complex. The essence of the matter, however, is found in the famous "double helix" structure of DNA. Both of the two long, interwound and complementary strands of the DNA molecule are made up of four kinds of nucleotides, cytosine, guanine, adenine and thymine (abbreviated *C*, *G*, *A* and *T*), which are linked end to end like a train of boxcars. The genetic message of each strand is embodied in the particular sequence of nucleotides, any one of which may follow any other. For example, the sequence *CATTACTAG* contains five identifiable English words: *CAT*, *AT*, *TACT*, *ACT* and *TAG*. The genetic message, however, is "written" in triplets: *CAT*, *TAC* and *TAG*. In general each triplet "codon" determines, through a series of intermediate steps, the position of a specific amino acid in a protein molecule.

Proteins, like nucleic acids, can be visualized as long trains of boxcars coupled end to end; here, however, the subunits are amino acids rather than nucleotides. The sequence of nucleotides in a given DNA molecule determines the sequence of amino acids in a particular protein, with each triple-nucleotide codon placing one of 20 possible amino acids at each successive position in the protein chain. The sequence of amino acids in turn specifically establishes both the structure and the function of

the protein. Thus the nucleotide sequence of DNA precisely specifies the protein-building properties of the organism. Moreover, virtually every property of the organism, from enzymatic action to eye color, depends on protein structure in one way or another.

The transmission of the essential genetic information between generations depends on the precise replication of the nucleotide sequences of DNA. The mechanism for replication stems from the complementary relation between the two strands of the DNA molecule. A sequence on one strand (for example *CATTACTAG*) lies immediately opposite a complementary sequence (*GTAATGATC*) on the other strand. The strands are complementary because *C* and *G* are always opposite on the intercoiled strands, as are *A* and *T*. Complementarity depends on the special chemical affinity, or binding, between *C* and *G* on the one hand and *A* and *T* on the other. The sum of these bonds, repeating along the length of the strands, is what holds the strands together in the double helix. Under appropriate conditions affinity is reduced and the two strands can unwind and separate. The single strands can again pair and rewind when conditions for high affinity are restored.

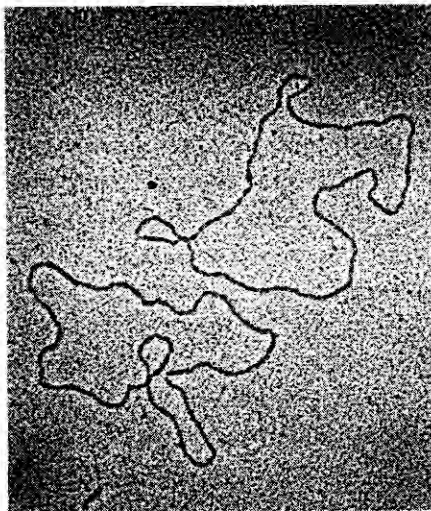
Double-strand DNA replicates by means of an extension of these properties. The unwinding and separation of the strands begins at a localized site along the DNA molecule. In the presence of suitable enzymes and free nucleotides a new chain is formed next to the exposed portion of each unpaired older chain. Each nucleotide lines up next to its opposite number (*C* next to *G*, *A* next to *T*). The complementary sequence thus established is then linked end to end by an enzyme that closes the

nucleotide couplings. When the replication process has traveled along the entire length of the original double helix, two new helices identical with the first one have been formed. The replication of DNA is the most fundamental chemical reaction in the living world. It fully accounts for the classical first principle of heredity: like begets like.

If DNA replication always worked without error, life would be far more homogeneous than it is. Here, however, a second classical principle of heredity intervenes: the principle of mutational variation, or the appearance in the offspring of new hereditary characteristics not present in the progenitors. Mutations arise through error, at least partly in the replication process. For example, the substitution of one nucleotide by another changes the triplet codon and puts a different amino acid in the corresponding position in the resulting protein. Single-nucleotide errors lead to single-amino-acid errors. Thus, a single-nucleotide error is responsible for the human disease sickle-cell anemia. Most mutations are not such simple, single-nucleotide exchanges; nevertheless, they correlate directly with altered, transposed or deleted nucleotide sequences in DNA. When these changes appear in a gene (that is, a segment of DNA that codes the amino acid sequence of a particular protein), a change in the protein and hence in the hereditary properties it controls is the result.

Therein lies the crux of recombinant-DNA technology. It makes possible for the first time the direct manipulation of nucleotide sequences. Changes in nucleotide sequence that are produced by "natural" errors are random, even when their overall frequency is artificially increased. In natural populations Darwinian selection "chooses" among the random errors, increasing the representation in breeding populations of those errors that lead to more offspring in particular environments. Artificial selection, practiced by human beings for millennia, favors errors that meet human needs (agricultural breeding) or whims (exotic-pet breeding). The success of both natural and artificial selection, however, is dependent on the random occurrence of desirable mutations. There was no way to direct genetic change itself until recombinant-DNA techniques came along. The new techniques enable one to deliberately introduce known and successful nucleotide sequences from one strain or species into another, thereby conferring a desired property.

The recombinant-DNA approach involves experimental ingenuity and detailed knowledge of the DNA molecule. It begins with an attack on DNA by the proteins called restriction enzymes, which are isolated from bacteria. The enzyme attack breaks the double chain

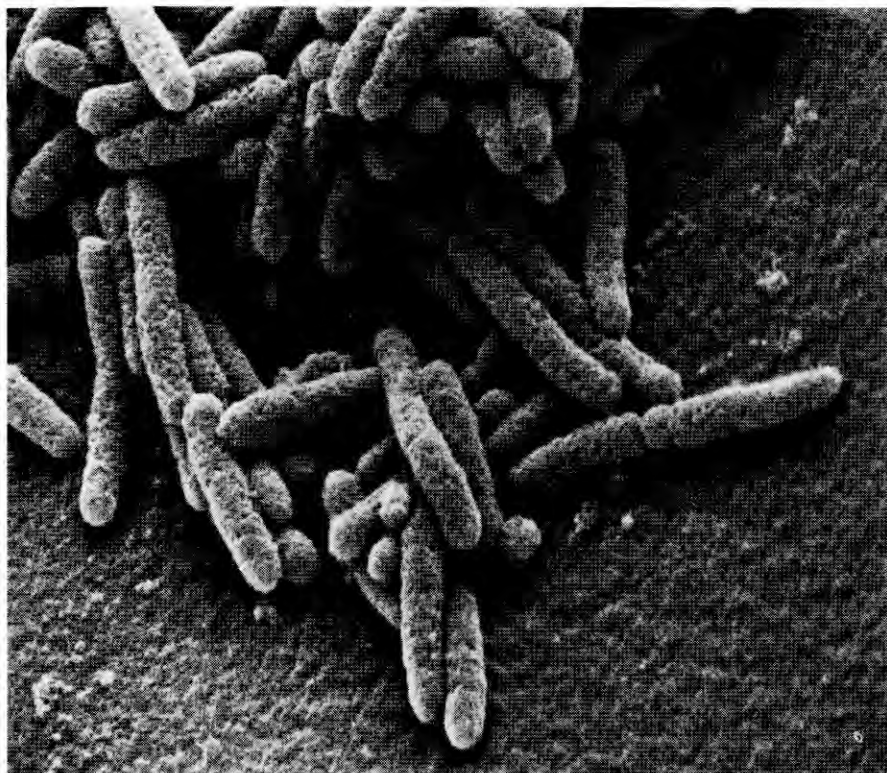


**BACTERIOPHAGE  $\phi$ X174 AND ITS DNA** are portrayed in this pair of electron micrographs. The virus infects the common intestinal bacterium *Escherichia coli*. In the micrograph at left, made by Jack D. Griffith of the Stanford University School of Medicine and Andrew Staehelin of the University of Colorado, two  $\phi$ X174 particles are seen attached to surface of an *E. coli* cell. In micrograph at right, made by Griffith, the DNA molecules of two  $\phi$ X174 viruses are seen in their double-strand form; each molecule is about 18,000 angstroms long.

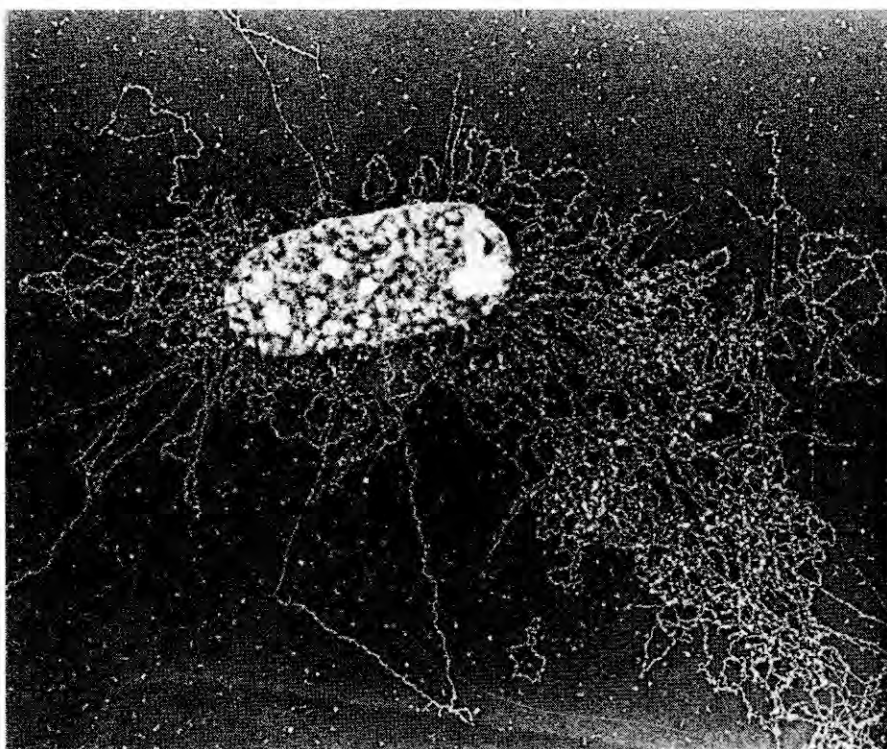
of DNA at particular sequences, say at the sequence *CATTAC*, which is opposite the complementary sequence *GTAATG*. The break does not always occur at the same point on the two strands. It may, for example, be between the two *T*'s in the first strand but just to the right of the *ATG* in the second strand. On separation one piece therefore ends in *TAC*, whereas the other ends in *ATG*. Since the single-strand ends are complementary, they will under suitable conditions stick side by side, and they can then be coupled together end to end. If the same restriction enzyme is used on the DNA from two different sources, both of which have the appropriate target sequence, then sequences with the same "sticky" ends will result. By taking advantage of this stickiness two sequences from any source can be recombined into a single DNA molecule.

The only further step necessary is to put the recombinant DNA into a suitable host organism. The recombinant must have the ability to penetrate the host and become part of its genetic system. An effective way to accomplish this has been developed for the common intestinal bacterium *Escherichia coli*. In addition to its single large circular chromosome the *E. coli* bacterium may have one or more independently replicating, smaller loops of DNA known as plasmids. The plasmids can be isolated from the bacteria, broken open by restriction enzymes and used as one component of a recombinant. After linking up the plasmid DNA with the "foreign" DNA the circular form of the plasmid can be restored and the structure returned to a whole cell. There it can resume replication, duplicating not only its own native sequence but also the foreign one. A strain of bacteria is thus obtained that will yield an indefinite number of copies of the inserted nucleotide sequence from the foreign source.

Standing alone, none of this appears to be particularly momentous or threatening; it is only a new and intriguing kind of chemistry applied to living organisms. Given the complexity of living organisms and the still more complex world of social phenomena, however, this new chemistry quickly builds into varied new potentials, both speculative and real. Suppose, for example, one were to isolate the nucleotide sequence necessary to produce a potent toxin and to transfer it to *E. coli*, usually a harmless inhabitant of every human intestinal tract. Would a dangerous new pathogen be created? Would the transformed *E. coli* release a toxin in the human gut? Might such a new pathogen escape from control and induce epidemics? Questions of this kind have answers, but they take time to find. To gain some time for reflection investigators in 1974 called for a partial and temporary moratorium on those experi-



**PILE OF *E. COLI* CELLS** appears in this scanning electron micrograph made by David Scharf. Some of the cells have been caught in the act of asexual reproduction (cell division); a few appear to be transferring their DNA by means of the threadlike connection characteristic of the process known as conjugation. *E. coli* bacteria are considered by most investigators to be most suitable host cells for recombinant-DNA experiments. Magnification is 11,000 diameters.



***E. COLI* SPEWS OUT DNA** through its chemically disrupted cell wall in this electron micrograph by Griffith. Most of the DNA is in the form of a single large molecule of double-strand DNA, which constitutes the chromosome of this simple prokaryotic organism. In addition the *E. coli* bacterium may have one or more of the independently replicating loops of DNA known as plasmids; one of these smaller extrachromosomal DNA molecules can be seen near the bottom. Plasmids derived from *E. coli* cells play an important role in recombinant-DNA research, since they form one class of vectors, or carriers, into which segments of "foreign" DNA can be spliced prior to their being reinserted into an appropriate host cell to propagate, thereby duplicating not only their own native nucleotide sequence but also the foreign sequence.



ments thought to be potentially the riskiest. The separation of the certainly safe experiments from the less certainly safe ones became the chief function of the guidelines released by the NIH in June, 1976. The guidelines, which replaced the temporary moratorium, were derived from worst-case analyses of various kinds of experiments; the object was to evaluate the possible range of hazards and to prescribe appropriate matching safeguards in order to minimize the unknown risks. The guidelines assigned heavy responsibility to individual investigators, and they buttressed this responsibility with special monitoring committees in the sponsoring institutions and in the funding agency.

If such regulations have been adopted, why is debate continuing? Briefly, it is because the matching of estimated risk and prescribed containment adopted by the guidelines is regarded by critics as being inadequate in dealing with potential biohazards and incomplete in failing to address other important issues. The most vocal critics have presented their own worst-case analyses in the scientific and general press. These accounts have led to widespread alarm and to public-policy deliberations at the level of local communities, states and the Federal Government. The expressed concerns of the critics have generated a revised agenda for what is now emerging as a broadened second round of policymaking.

Potential biohazards and estimated degrees of risk continue to dominate the

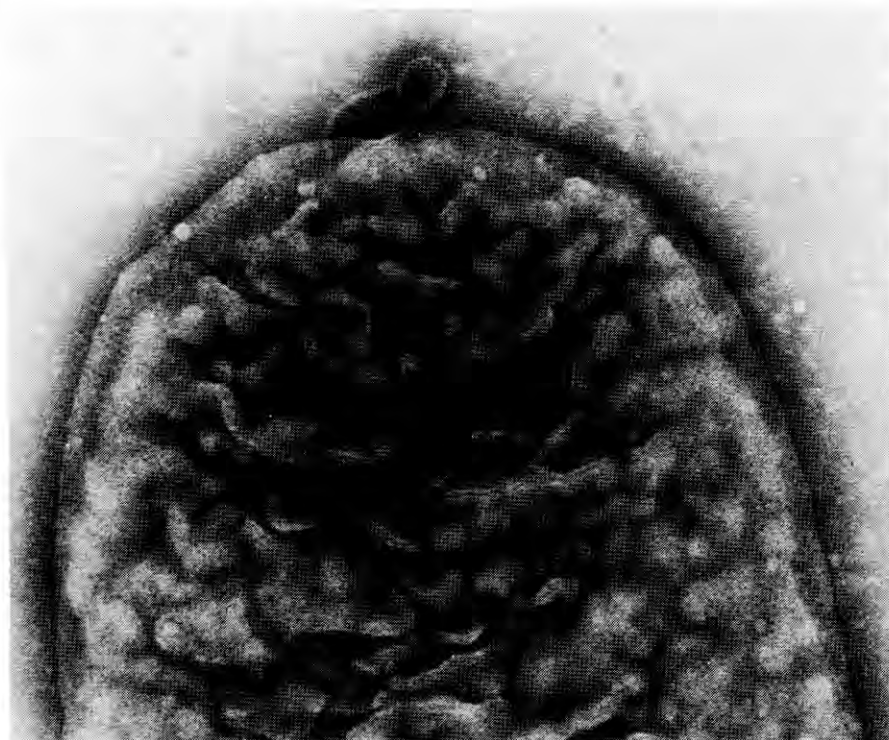
debate. The NIH guidelines balance the estimated risk of a given experiment and recommend specific measures for containing the risks. (Risk, it must be remembered, means possible danger, not demonstrated danger.) Those experiments judged to present an excessive risk are entirely proscribed. At the other end of the spectrum experiments judged to present an insignificant risk require only the safeguards of good laboratory practice. Between these extremes the guidelines establish various levels of estimated risk and prescribe combinations of suitably increasing physical and biological containment. The release into the environment of any recombinant organisms is forbidden.

Unfortunately, given the growing but still limited state of knowledge, wide disagreement is possible, both as to estimated degrees of risk and as to the efficacy of the proposed containment. Some critics project fragmentary information into the inevitable spread of dangerous, newly created organisms, threatening both the public health and the environment. Some defenders project the same fragmentary information to the conclusion that the NIH guidelines are already overly cautious. They believe the actual hazard under existing precautions will turn out to be no greater than that routinely faced in the use of automobiles, jet aircraft and other accepted technologies. The wide range of estimates is possible because of the multiplicity of conceivable experiments and because experience and critical data

are inadequate for certainty on many points. One fact that is certain is that no known untoward event has yet resulted from recombinant-DNA research.

What emerges on the new policy agenda, then, is the need for effective policy-oriented research to reduce the current uncertainty as to the risk of particular kinds of experiments. For example, there is dispute over the use of *E. coli* as a host for recombinant DNA. One side argues that scientists must be mad to pick a normal human inhabitant (and a sometime human pathogen) to serve as a host for recombinant DNA. This view, in extreme form, demands the suspension of all recombinant-DNA research until an organism safer than *E. coli* can be found. The other side argues (1) that the vast amount of information available on *E. coli* makes it invaluable, (2) that the K-12 strain of *E. coli* actually used in laboratory research has been so modified genetically in adapting to laboratory conditions that it survives only with difficulty in the human intestine and (3) that new strains of K-12 have been developed with additional genetic deficiencies that will make survival outside of laboratory conditions essentially impossible. The use of such genetically deficient strains is what is meant by the term "biological containment." The concept is supported by proponents of the research as an efficacious new approach to safety and derided by critics as likely to be circumvented by natural recombination.

Such differences of opinion are normally reduced by scientists to experimental questions. For example, the suitability of the K-12 strain of *E. coli* as an experimental organism can be judged only from the effect of recombinant genes on the ecological relations of *E. coli* within the human intestine, including the degree of success of recombinant strains in competing with other strains of *E. coli* and with other organisms. Information on these matters is growing. Such questions, however, are not normally subjects of profound scientific interest. They have recently become matters of priority only because they may provide information that would be useful in arriving at a policy decision. Research on policy-oriented questions has never had a very high status among scientists engaged in basic research or even among those engaged in applied research. Therefore policy-oriented research must be encouraged through special funding mechanisms and through suitable new institutional arrangements. A regulatory agency for recombinant-DNA research and other conceivably hazardous kinds of research is urgently needed outside the NIH, and it should include a research component. The Center for Disease Control and its National Institute for Occupational Safety and Health come



**BACTERIAL VIRUS IS ATTACHED** to the wall of an *E. coli* cell in this electron micrograph made by Maria Schnoss of the Stanford School of Medicine. This particular virus, named bacteriophage lambda, normally infects the bacterium by injecting its DNA into the host cell through a long taillike appendage. The magnification is approximately 140,000 diameters.

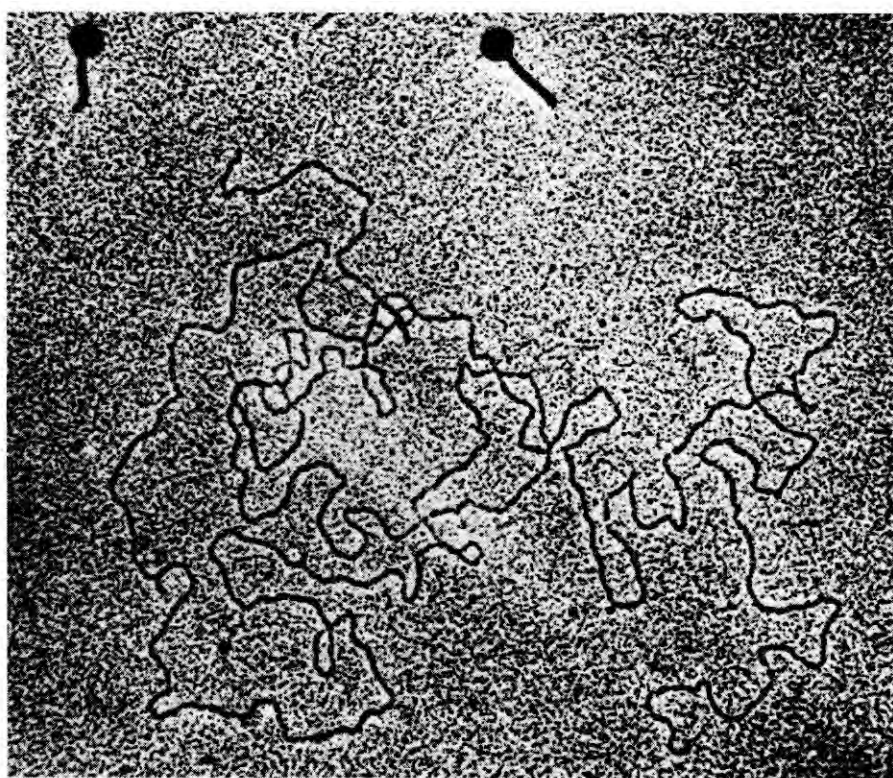


to mind as possible models for such a dual-purpose agency.

Also related to the question of biohazards is a controversy over the desirability of centralizing recombinant-DNA research facilities. Some of those who fear severe dangers from recombinant organisms have urged that the potentially more hazardous research be concentrated in remote places with extremely stringent containment procedures. Those who minimize the hazard are opposed to the concentration concept because it would tend to separate the research from the intellectual mainstream and would be unnecessarily expensive in facilities. The argument has been particularly strenuous with respect to experiments requiring *P3* facilities, which are defined as those necessary to contain "moderate risk" experiments. *P4* facilities for "high risk" experimentation are expected to be fewer in number because of their high cost; generally speaking they are likely also to be comparatively isolated. The current NIH guidelines provide little direction in these matters. A decision on a firmer policy belongs on the discussion agenda. Particularly urgent is careful consideration of such intermediate possibilities as the use of centralized, high-risk facilities for making particular recombinations for the first time. These activities, together with preliminary testing of new recombinants for possible hazards, might also be carried out by the proposed new regulatory agency.

A special case that emphasizes the advantages of initial testing in a central facility is provided by what are called "shotgun" experiments. These experiments, which offer special advantages to the investigator, may also present special hazards. Shotgun experiments involve exposing the total DNA of a given organism to restriction enzymes in order to obtain many DNA fragments. The fragments are then each recombined with DNA from a suitable vector and the recombinants are randomly reinserted into *E. coli* host cells. The next step is to spread the *E. coli* cells on a nutrient substrate so that each recipient cell, containing a particular inserted foreign sequence, grows into a colony. If the experiment is successful, the yield is a "library" of all the nucleotide sequences of a particular organism, each sequence growing in a separate strain and accessible to manipulation and cross-combination at will.

This experimental approach is laborious but far less so than anything else available for the exploration of the complex genetic systems of higher organisms. There is, however, a risk of unknown magnitude that portions of the DNA with unknown or repressed functions might duplicate and create unanticipated hazards. The result might be particularly unfortunate if the original



**BACTERIOPHAGE LAMBDA AND ITS DNA** are both represented in this electron micrograph provided by Griffith. Two complete lambda viruses are at the top; the long double-strand DNA molecule of a disrupted lambda is below them. DNA from bacteriophage lambda can also serve as a vector for recombinant-DNA experiments involving *E. coli* host cells.

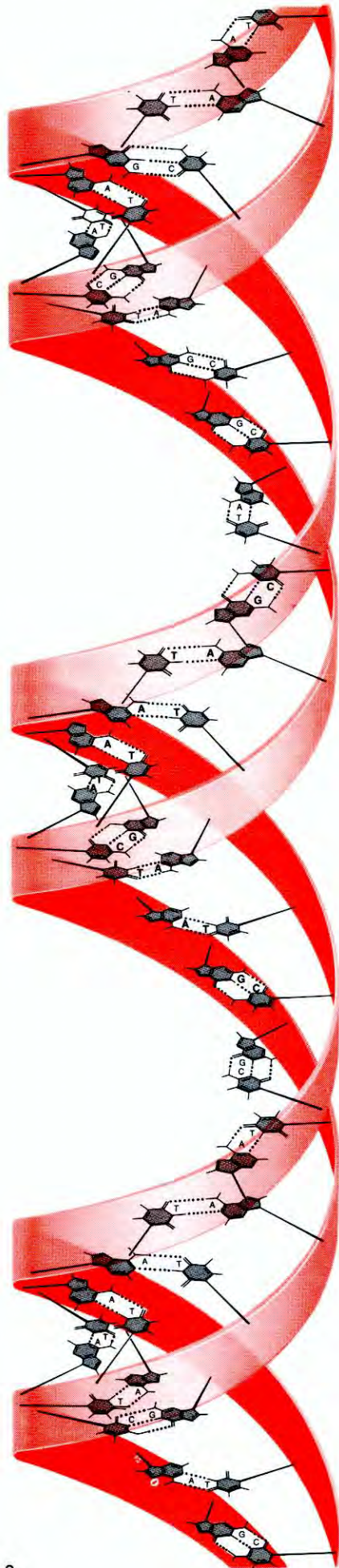
DNA preparation were to contain genetic material from parasites or from viruses associated with the species under study. Under the NIH guidelines, therefore, shotgun experiments are regarded as being more dangerous than those involving purified and characterized DNA. Experiments in this category are treated as being increasingly more dangerous as the test organism under study is biologically more like the human organism. Thus experiments with primate DNA are considered to be more dangerous than experiments with mouse DNA. This approach appears to represent a reasonable precaution with respect to human health hazards, but it is less reasonable with respect to potential ecological effects. For example, shotgun recombinants involving DNA from plant sources could conceivably lead to ecologically dangerous effects if they were to escape into the environment. Shotgun procedures might therefore be best conducted first in special centralized facilities that could also act as storage and distribution centers for the recombinant products once they had been tested for safety.

These examples suggest several advantages for the creation of a Center for Genetic Resources. The center might not only carry out DNA recombinations suspected to be hazardous but also function to preserve genetic information contained in threatened natural species

and in special strains of cells or organisms developed for research and other purposes. Stored genetic information can be expected to be increasingly important in the future. For example, new genetic infusions into domesticated stocks of plants and animals from their wild progenitors have long been used to strengthen the response of the domesticated stocks to changing conditions of husbandry. The sources of wild progenitors are threatened by the reduction of wild habitats all over the world.

The possibility of a biohazard need not arise only as a by-product of basic research. The practical applications of recombinant-DNA techniques, together with the applied research and development leading to them, are at least equally likely sources. For example, recombinant techniques may enormously expand the use of bacteria (and other microorganisms) for the production of certain proteins and other pharmacological products. Microorganisms have long played an essential role in the food, beverage, pharmaceutical and chemical industries, and more precise genetic control of their characteristics has already yielded large benefits. The recombinant-DNA techniques not only offer advances on current practice but also suggest a new realm of "bacterifactory" in which the rapid, controlled growth of microorganisms is coupled to the pro-





duction of specific products normally made only by higher organisms. Included among the possibilities are the production of insulin, blood-clotting factors and immunological agents. The probability of those possibilities ever being realized is no more easily assessed than the risks, but success in realizing them clearly could provide substantial economic and social benefits. Accordingly entrepreneurial interests have been aroused.

The NIH guidelines are silent on the matter of commercial applications other than stipulating that large-scale experiments (beyond production batches of 10 liters) with recombinants "known to make harmful products" be prohibited unless specially sanctioned. The guidelines also require detailed reporting of proposed recombinant-DNA experiments, a provision that runs counter to the protection of proprietary interest. There have been discussions of these matters between the NIH and representatives of industry. In addition industry spokesmen have testified at Congressional hearings. It is known that some industrial research already is under way and that representatives of industry generally endorse the precautionary approach of the NIH guidelines, but they are resistant to limitations on proprietary rights and on the size of batch production. Moreover, patent policy has come up as an issue and there has been some uncertainty in the Department of Commerce as to how it should be handled. Indeed, the possible commercial applications of recombinant-DNA techniques have yet to be publicly evaluated as a serious policy question, and they must be high on the agenda of the next round of discussions.

The problems of commercial applications lead from immediate issues to broader ones and to a larger time frame. Recombinant-DNA techniques have revived the debate over "genetic engineering" and have once again raised questions about the applications of fundamental biomedical research to technology, to the quality of life and to the future of society. Recombinant DNA has now joined nuclear fission, overpopulation, famine and resource shortages in the

doomsday scenarios of "creative pessimism." These issues are even more difficult to deal with objectively than those related to potential biohazards, but they are plainly apparent in the general public discussion and in the public statements of respected scientists.

For example, Robert L. Sinsheimer of the California Institute of Technology has persistently raised issues that are in part practical and in part philosophical. Along with George Wald of Harvard University and Erwin H. Chargaff of the Columbia University College of Physicians and Surgeons, he suggests that the entire recombinant-DNA approach to gaining an understanding of the complexities of higher genetic systems is misbegotten. The argument is not that the approach may not work but that its alleged huge risks are unnecessary because less risky, although slower, means are available. Sinsheimer emphasizes the fundamental difference between simple prokaryotic organisms such as bacteria and complex eukaryotic organisms, including human beings. Prokaryotes, typically one-cell organisms, have a single, comparatively simple chromosome floating freely within the cell body, whereas eukaryotic cells have a nucleus that is bounded by a membrane and contains a number of far more complex chromosomes. The paleontological record suggests that prokaryotes existed on the earth for a billion or more years before the more complex eukaryotes arrived on the scene. Sinsheimer proposes that throughout the evolution of the eukaryotes there has been a genetic barrier between them and the prokaryotes, behind which eukaryotes have developed their more complex mechanisms of genetic control. To transfer these mechanisms, which are possibly the key to the evolutionary success and enormous diversity of eukaryotes, to prokaryotes may introduce, he says, incalculable evolutionary dangers. The prokaryotes may be made far more effective, both as competitors and as parasites, negating an ancient evolutionary strategy.

Sinsheimer's argument has won only a few vocal adherents among biologists, and he himself concedes that it is speculative. Nevertheless, his argument

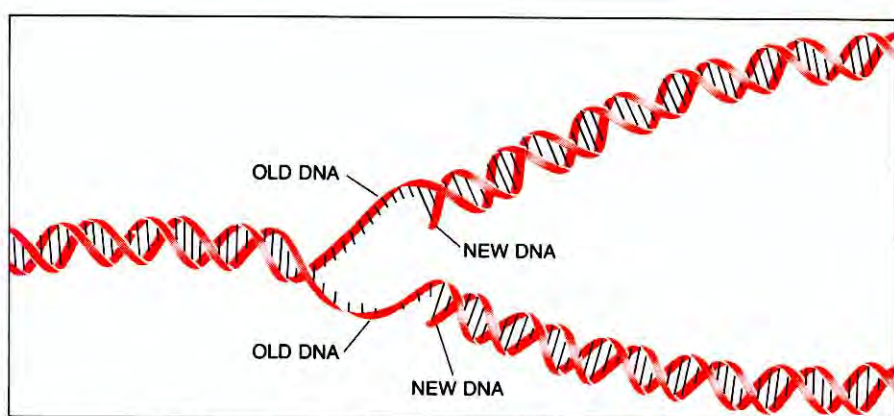
**DOUBLE-HELIX STRUCTURE OF DNA** is evident in this simplified diagram of a short segment of the deoxyribonucleic acid (DNA) molecule. The sugar and phosphate groups that are linked end to end to form the outer structural "backbones" of the double-strand molecule are represented schematically here by the two helical colored bands. The inner portion of each polynucleotide chain, drawn in somewhat greater detail, consists of a variable sequence of four kinds of bases: two purines (adenine and guanine, or A and G) and two pyrimidines (thymine and cytosine, or T and C). The two chains, which run in opposite directions, are held together by hydrogen bonds (dotted black lines) between pairs of bases. Adenine is always paired with thymine, and guanine is always paired with cytosine. The planes of the bases are perpendicular to the common axis of the two helices. The diameter of the double helix is 20 angstroms. Adjacent bases are separated by 3.4 angstroms along the axis and are displaced successively around the axis by an angle of 36 degrees. The structure therefore repeats after 10 bases on each chain (360 degrees), or at intervals of 34 angstroms. The genetic information is stored in the sequence of bases along each chain. In this case the sequence **CATTACTAG** on one strand is identified in boldface type opposite complementary sequence **GTAATGATC** on other strand.



has attracted significant public attention, and it is widely cited to support opposition to continued recombinant-DNA research. Bernard D. Davis, a Harvard Medical School microbiologist, has provided a rebuttal, particularly with respect to the concept of a genetic barrier between prokaryotes and eukaryotes. He believes there has been an ample and continuous opportunity for the exchange of DNA between the two groups. He points out that bacteria can take up naked DNA from their immediate environment and that *E. coli* would be exposed to such DNA arising from dead human cells in the human intestine. Microorganisms might similarly take up DNA in the process of decomposing dead animals. Therefore, Davis argues, most recombinants probably have already been tried in the natural evolutionary arena and have been found wanting. Reasoning on analogy with extensive information on pathogenic bacteria, Davis concludes that under the existing NIH guidelines the probability for survival in nature of laboratory-produced prokaryote-eukaryote recombinants is vanishingly small.

This clash of opinion on a major biological issue illustrates the difficulty of assessment of even comparatively value-free questions when critical information is fragmentary. The controversy over the risk-benefit ratio becomes even more intense when issues involve substantial value judgments as well. Here again a concern of skeptics and opponents of recombinant techniques is sharply articulated by Sinsheimer. He asks: "Do we want to assume the basic responsibility for life on this planet? To develop new living forms for our own purposes? Shall we take into our own hands our own future evolution?" Since the questions include such concepts as responsibility, purpose and control of the future, they clearly involve considerations beyond science alone.

The human species has, of course, been altering life on this planet from the beginnings of human culture. When hunting and gathering gave rise to animal husbandry and agriculture, human choice and purpose began to influence the evolution of selected species. Unconscious human selection was replaced by deliberate plant and animal breeding, and the further development of human culture is now clearly altering the entire ecosystem. Moreover, the biocultural progression of the human species, based partly on human purpose, is undoubtedly altering the human gene pool and will slowly modify the species in unpredictable ways. Nevertheless, the advent of recombinant-DNA techniques has obviously enhanced the prospects for genetic engineering and has restressed the need to assess its implications. Can it be assumed that success in introducing recombinant DNA's into *E. coli* means

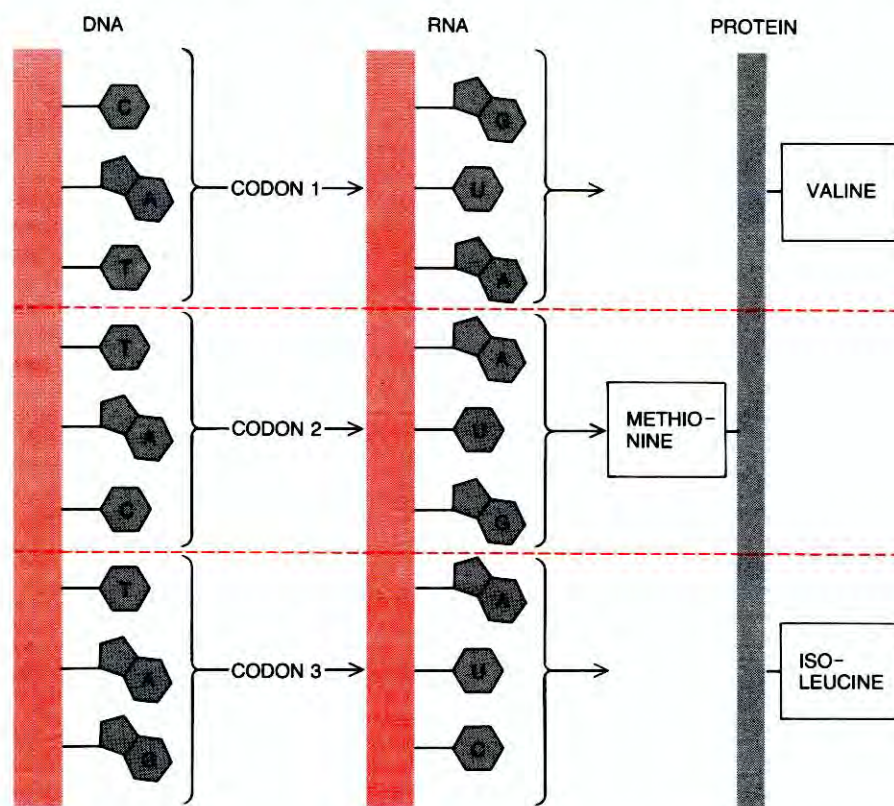


**REPLICATION OF DNA depends on the complementary relation between the nucleotide sequences on the two strands of the DNA molecule. Under appropriate chemical conditions the hydrogen bonds between the bases are weakened and the two strands can unwind and separate. In the presence of suitable enzymes and free nucleotides a new chain can be formed next to the exposed portion of each unpaired older chain. The complementary sequence that is formed by each nucleotide lining up next to its opposite is then linked end to end by an enzyme that "zips up" the nucleotide couplings. In this way two new helixes identical with the first can be formed.**

that there will be similar success in introducing them into the human species? If it can, what is the probable time frame for applying the technique to the human species? Is it accurate and responsible to suggest that we have almost in hand control of "our own future evolution"?

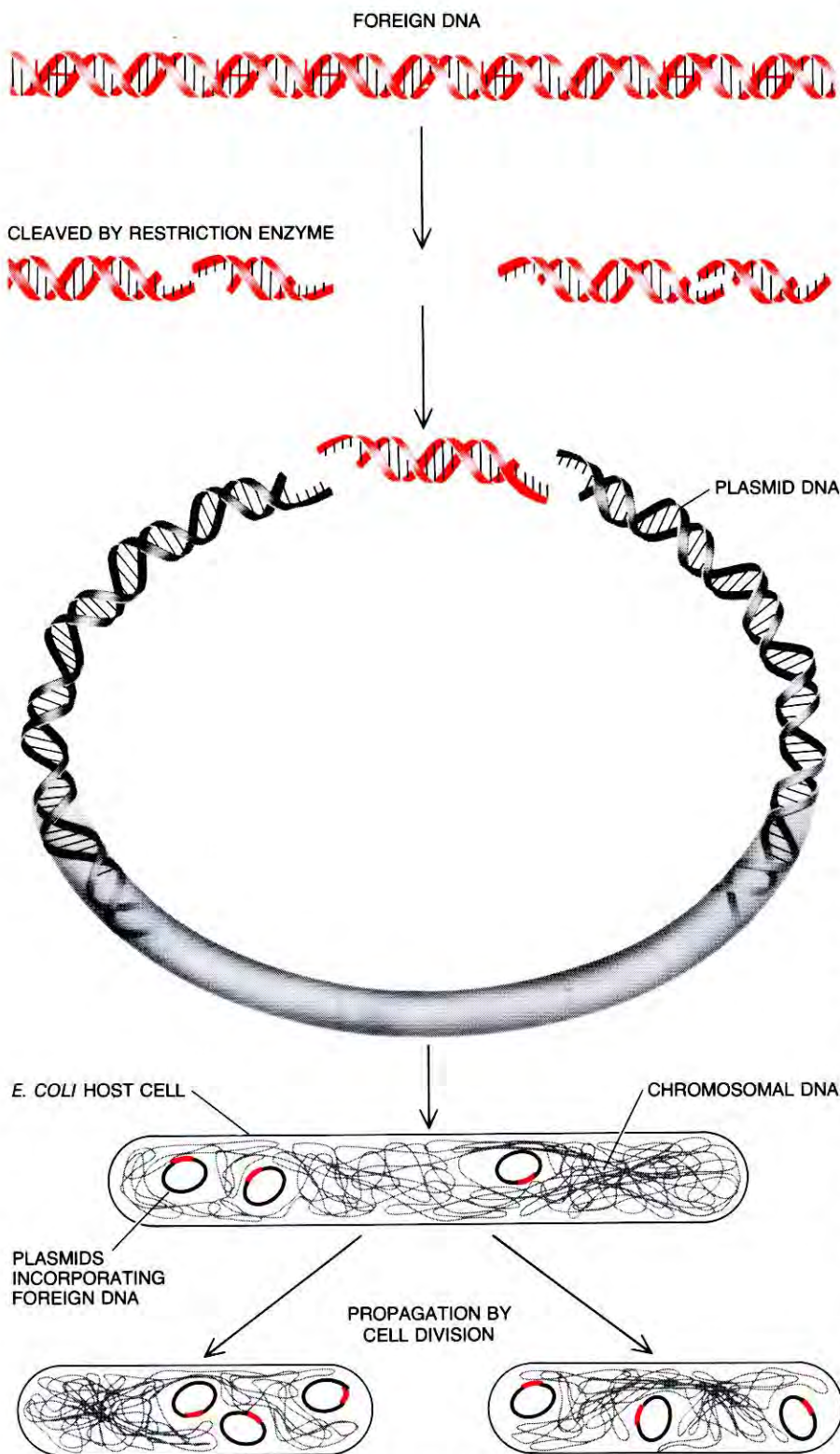
These certainly are questions for scientific assessment, and they should have a prominent place on the new policy agenda.

Sinsheimer has gone into still another controversial area, not only for the scientific community but also for the entire



**ROLE OF DNA IN PROTEIN SYNTHESIS is suggested by this highly schematic diagram. The genetic message contained in the nucleotide sequence CATTACTAG, for example, is "written" in the form of the triplet "codons" CAT, TAC and TAG. Each codon determines, through a series of intermediate steps involving a molecule of ribonucleic acid (RNA), the position of a specific amino acid in a protein molecule. Thus the sequence of nucleotides in a given DNA molecule specifies the corresponding sequence of amino acids in a particular protein, with each triple-nucleotide codon placing one of 20 possible amino acids at each successive position in the protein chain. Since the sequence of amino acids in turn establishes both the structure and the function of the protein, the nucleotide sequence of DNA determines virtually every property of organism. Letter U stands for the pyrimidine uracil, a constituent of RNA.**





**RECOMBINANT-DNA TECHNIQUE** makes it possible for the first time to deliberately introduce nucleotide sequences from the DNA of one strain or species of organism into the DNA of another. The DNA of the "foreign" organism is first treated with restriction enzymes, which cleave the double-strand molecule at particular nucleotide sequences (typically thousands of base pairs apart) on a random basis. The same enzyme is then used to cleave the DNA of a suitable vector, in this case a plasmid isolated from *E. coli* bacteria. Since the break caused by the enzyme does not occur at the same point on both strands, the chemical treatment results in a mixture of DNA segments that have complementary single-strand ends. Under suitable conditions the "sticky" ends of two different sequences can be coupled to form a single DNA molecule. For example, after recombining the foreign DNA with the plasmid DNA the circular form of the plasmid can be restored and the structure can be inserted into a suitable host cell (in this case *E. coli*), where the plasmid can resume replication, thereby propagating an indefinite number of "cloned" copies of the inserted nucleotide sequence from the foreign source.

society. Arguing that time may be needed to "pace" new genetic knowledge to human capacities for putting nature to intelligent use, he wonders whether "there are certain matters best left unknown, at least for a time." This is high heresy in the scientific community, whose fundamental premise is that the growth of knowledge is the driver and not the captive of other values. The rejection of the concept of "forbidden knowledge" was part of the heroic period at the beginning of modern science, when it included willingness to face the Inquisition and the stake. Having been seared by the nuclear flame and now confronting the more subtle implications of the innermost language of life, 20th-century scientists fear not the stake but the judgment of history. Chargaff, a pioneer in the investigations that led to the decipherment of the genetic language, says: "My generation, or perhaps the one preceding mine, has been the first to engage, under the leadership of the exact sciences, in a destructive colonial warfare against nature. The future will curse us for it."

Sinsheimer and Chargaff, along with a number of philosophers, historians and sociologists of science, are clearly suggesting that the possible consequences of knowing must be consciously included in decisions about the directions of the search for knowledge itself. No issue cuts more deeply to the core of modern science. The self-doubt expressed by some scientists reflects a general questioning in the U.S. of the net benefits of science and technology. Cost-benefit analysis is a current preoccupation, and it is being increasingly applied to the generation of knowledge itself. It is hard enough to assess what we may gain or lose from particular new knowledge; it is even harder to assess the costs of not having it. This problem is epitomized by the recombinant-DNA controversy. The rise of molecular genetics in the U.S. is the direct product of a series of decisions made after World War II that provided funds for biomedical research. The objective was the conquest of the "killer" diseases: cancer, heart disease and stroke. Those diseases are still much with us, although they are better understood and cared for. Meanwhile, out of Federally supported research also came the impetus that led to the discovery of the double helix, the genetic code, the structure of proteins and recombinant DNA. In a classic "double take" the public is now asking whether it has been buying health and well-being or chimeric monsters. Is molecular genetics and all biomedical technology a sorcerer's apprentice? Are we increasing rather than lessening our burden of pain and anxiety?

The last question leads to yet another issue. Biohazard and ecohazard may arise inadvertently, but "sociohazard" may be the product of deliberate malev-



olence. The U.S. is a signatory to an international legal convention that has renounced biological warfare, including research to produce the necessary agents. Not all countries have taken this step, and public renunciation without adequate inspection cannot ensure that covert activities do not exist. Opponents of recombinant-DNA research see its techniques as being ideally suited to serve malevolent purposes, either as agents of organized warfare or of sabotage and terrorism. The techniques do not require large installations or highly sophisticated instrumentation. Contrary views have not denied this but have noted that recombinant-DNA techniques would not be the first technology to have potential malevolent applications. Explosives have such applications, but society does not completely ban them; it takes prudent precautions against their misuse.

Nevertheless, the issue of the possible misuse of recombinant-DNA technology deserves a place on the policy agenda, because it emphasizes the need for international discussion of the implications and management of recombinant-DNA research and recombinant-DNA applications. It can be argued that the U.S. is not ready for such discussion until its own policies are in better order. It is not too early, however, to begin the internal consideration of how best to approach the international arena.

These are the chief issues that have emerged from the policy debate so far. It is a not inconsiderable list. The debate has not been raging on every street corner, but it became strenuous enough in Cambridge last summer to have repercussions across the continent. For example, an evaluation presented by a panel of nonscientists to the Cambridge City Council was not too different in content from one produced by a task force of the Quality of Life Board of the City of San Diego, where I live. Both groups accepted within their community the continuance of recombinant-DNA research requiring P3 facilities but sought somewhat greater assurances of safety than those provided by the NIH guidelines. Meanwhile the Attorney General of the State of New York held a public hearing, and a joint hearing was conducted by two committees of the Assembly of the State of California Legislature. Legislation regulating DNA research was later introduced in the California Assembly, and it is still under consideration. Congress has also held several hearings and various items of regulatory legislation have been introduced in both the Senate and the House of Representatives. These local, state and Federal initiatives emphasize the necessity to get on with the policy agenda.

The agenda should be viewed in at least two time frames: immediate and

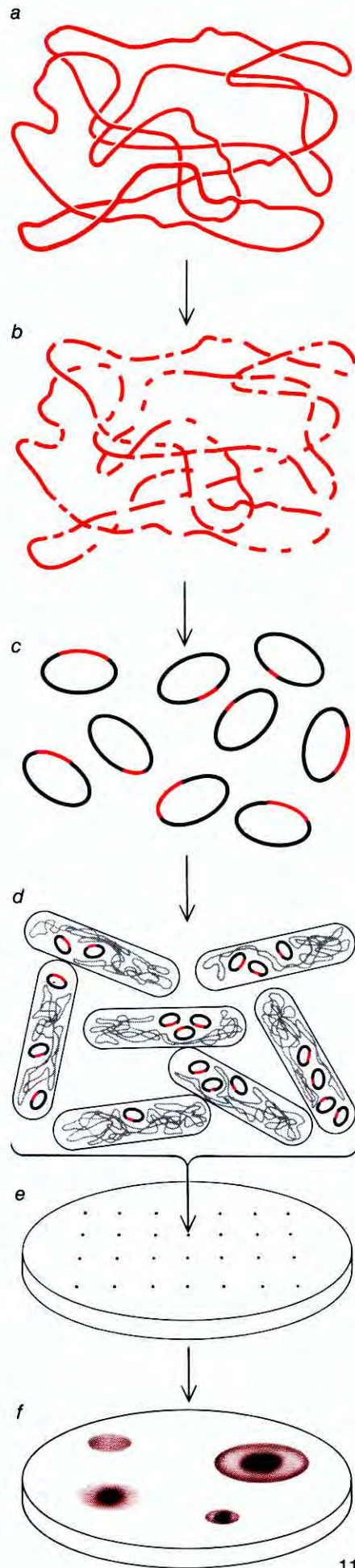
longer range. A consensus has been growing that there is an immediate need to give the quasi regulation represented by the NIH guidelines a statutory base. In particular, regulation must be extended to activities not supported by Federal agencies, especially in the industrial sector. However this is to be done, it is important to maintain flexibility, since the problems to be dealt with will change as greater knowledge and experience are acquired.

Moreover, given the complexity of the longer-term issues, immediate legislation probably should be provisional and limited. A mechanism should be included, however, that actively leads toward a more definitive future policy. This requires provision for a new, comprehensive assessment of all the issues raised by recombinant-DNA research, including the probable effectiveness of the regulatory devices put in place under the NIH guidelines.

The need for such a new national assessment is demonstrated by the nature of the critical challenge to the product of the earlier assessment. First, it has been alleged that the 1975 Asilomar conference establishing the pattern for the NIH guidelines was dominated by scientists involved in the research, and therefore it could not yield a broad enough perspective. Second, it is argued that the earlier assessment was devoted primarily to the question of potential biohazards and did not address in any depth other gravely important questions. The passage of time has added several more points: that circumstances already have changed as research has progressed, that experience has grown and that a wider range of opinion has come to bear on the issue. Whatever format is adopted for the reappraisal of the recombinant-DNA issue, the public must be assured that the process is a comprehensive and objective one.

Whoever undertakes this new national review should first carefully examine the current situation, including the actual effectiveness of the regulatory mecha-

**"SHOTGUN" EXPERIMENT** is a type of recombinant-DNA experiment in which the total DNA of an organism (a) is exposed to restriction enzymes in order to yield many fragments (b), which are then recombined with the DNA from a suitable vector (c) and randomly reinserted with the vector into the host cells (d). The *E. coli* hosts are next spread on a nutrient substrate (e) so that each recipient cell, containing a particular inserted foreign nucleotide sequence, can grow into a colony (f). The result, if the experiment is successful, is a "library" of all the nucleotide sequences of the organism. Under the guidelines issued by the National Institutes of Health last year shotgun experiments are regarded as being potentially more hazardous than those involving purified and characterized DNA, since it is not known whether portions of the DNA with unknown or repressed functions might cause unexpected problems.





nisms provided by the NIH guidelines. Particular attention needs to be paid to the local institutional biohazards committees mandated by the NIH guidelines. Beyond the responsibility assigned to the principal investigator these committees are the only source of local surveillance and standard-setting. Their composition and charge are unique, yet their authority and procedures are stipulated only generally in the NIH guidelines. They may well need the stimulus and support of external interests to carry out their important task. Moreover, no provision has been made for budgeting what may turn out to be their considerable cost for technical surveillance, personnel training and medical monitoring. Like all insurance, security against biohazard must be bought. The cost should be borne as an additional expense of the research, not as a competitor for existing funds.

Similarly, the actual performance of the NIH study sections, which are mandated by the guidelines to be independent evaluators of biohazards and containment, needs to be examined. Study sections are already heavily overloaded with the job of evaluating scientific quality. Yet these part-time peer groups are asked to assume another difficult function. If the responsibility is to be taken seriously, it too will entail additional costs.

Of special importance for early attention is an effective monitoring system for following the actual directions of recombinant-DNA research. The techniques involved are so rich in possibilities, whether for fundamental research or applications, for benefit or risk, that "early warning" is essential. Systematic following of the directions of investigators' interests, from applications for support through informal communication to formal publication, is essential to the early detection and assessment of either risks or opportunities. Needless to say, monitoring is particularly difficult in industrial research. It might therefore be desirable to limit or postpone certain development efforts pending closer study and greater knowledge of the underlying problems.

Equally urgent is a determined effort toward a more effective assessment of risks and their limitation. The specific assignment of responsibility for this kind of policy-oriented research should be an early recommendation of the body undertaking the reassessment. Given the differing perspectives required by regulation and the NIH mission to promote health-related research, the regulatory function probably belongs elsewhere in the long run. On the other hand, given the need for careful study of the implications of relying on existing agencies or of establishing a new one, the temporary continued assignment of this responsibility to the NIH may be desirable. This interim solution, if it is adopted, must be

accompanied by additional funding to carry it out effectively.

Considerations of biohazards and physical and biological containment have necessarily had a high priority in this early phase of recombinant-DNA research. Many informed observers believe, however, that these concerns will decline in importance as research continues and experience grows. Therefore although the current furor makes a rational approach to the biohazards question an essential part of any successful recombinant-DNA policy, this approach does not exhaust the longer-term requirements and may even distort them. More crucial in the long run may be several other issues that have been raised directly or indirectly.

For example, in investing in fundamental genetic research that can profit from recombinant-DNA techniques, what relative priorities should be assigned to potential applications? In the past the national strategy in biomedical research has been to invest directly in basic research, without declared objectives, while also investing in specific objectives, allowing some of the latter support to "trickle down" to basic research. Thus an investigator of the interaction of viruses and cells, say, might be alternatively or simultaneously supported by funds for fundamental investigation and by funds intended for promoting the development of an effective therapy for cancer. What should be the priorities among possible practical applications of molecular genetics? Competing lines of inquiry include the microbiological synthesis of drugs, specific human gene therapies, the improved efficiency of photosynthesis, nitrogen fixation by food crops, enhanced agricultural production and so on. There are quite different potential risks and benefits in each of these directions, and all are unlikely to be maximally supported at once. In the new areas that are opening up is a new research strategy called for? If it is, by what procedures should it be formulated and how should it be implemented?

It is widely recognized that there is a logical continuum running from basic research through applied research and development to technological application. It is also recognized that movement along this continuum is neither smooth nor fully predictable and that varying motivations and institutional arrangements operate along its length. Recombinant-DNA techniques are the product of fundamental investigation, supported almost entirely by the partnership of the Federal Government and the universities. For the moment, at least, the techniques are likely to remain useful primarily in that area. The techniques may also be useful for various industrial purposes, however. Given the nature of the original investment as well as the complex issues raised, should technological

uses, at least for a time, be kept under Federal control? Should some of the return from successful applications be employed to recycle the original investment of Federal resources? Should this promising new technology be a prototype for establishing a revolving capital fund to support a more stably financed basic-research effort?

The possibilities of genetic engineering and evolutionary control illustrate the fundamental dilemmas raised by the new capabilities conferred by scientific knowledge. Society has entered an age of intervention, in which the automatic operation of natural processes is increasingly, through informed intervention, brought consciously into the orbit of human purpose. Many events that humanity formerly could regard only as a boon or a scourge—an act of God or of nature—are now the partial product of human decision and intervention. If human beings do not have the capability today to invent new organisms or to initiate life itself, they may soon have that capability. If they cannot today consciously and fully control the behavior of large ecosystems, that power is not far beyond what has already been achieved. The humility of individuals understandably shrinks from awesome powers that were earlier assigned to divine will. It was not, however, the humility of individuals that conferred these emerging capabilities or is called on to control them today. It was the social interaction of individuals, operating through social institutions, that brought us to the present fateful decision making. Imperfect though they are, our social institutions built the platform for the age of intervention.

The policy challenge we face, refracted in the exquisite structure and potential of the double helix, is whether we can create institutions able to transform the fruits of an age of reason into the achievements of an age of intervention. There are voices today urging us not only to eschew conscious intervention but also to distrust and limit the uses and consequences of reason itself. Perhaps it needs to be restated that it was, after all, natural selection that evoked the double helix and all it conveys. Included among the products are human knowledge and judgment, to which has now passed the duty of designing social processes and structures that can cope with the manipulability of the double helix itself.

The concept and control of the double helix signal a new frontier of biocultural progression. A stereoscopic vision that includes both "creative pessimism" and "creative optimism" is now required. Neither alone can do justice to the profound revelations human beings have recently experienced. A single eye is particularly limited in yielding depth and perspective. For the age of intervention at least two are needed.



		BIOLOGICAL CONTAINMENT (FOR <i>E. COLI</i> HOST SYSTEMS ONLY)		
		EK1	EK2	EK3
PHYSICAL CONTAINMENT	P1	DNA from nonpathogenic prokaryotes that naturally exchange genes with <i>E. coli</i>  Plasmid or bacteriophage DNA from host cells that naturally exchange genes with <i>E. coli</i> . (If plasmid or bacteriophage genome contains harmful genes or if DNA segment is less than 99 percent pure and characterized, higher levels of containment are required.)		
	P2	DNA from embryonic or germ-line cells of cold-blooded vertebrates  DNA from other cold-blooded animals and lower eukaryotes (except insects maintained in the laboratory for fewer than 10 generations)  DNA from plants (except plants containing known pathogens or producing known toxins)  DNA from low-risk pathogenic prokaryotes that naturally exchange genes with <i>E. coli</i>  Organelle DNA from nonprimate eukaryotes. (For organelle DNA that is less than 99 percent pure higher levels of containment are required.)	DNA from nonembryonic cold-blooded vertebrates  DNA from moderate-risk pathogenic prokaryotes that naturally exchange genes with <i>E. coli</i>  DNA from nonpathogenic prokaryotes that do not naturally exchange genes with <i>E. coli</i>  DNA from plant viruses  Organelle DNA from primates. (For organelle DNA that is less than 99 percent pure higher levels of containment are required.)  Plasmid or bacteriophage DNA from host cells that do not naturally exchange genes with <i>E. coli</i> . (If there is a risk that recombinant will increase pathogenicity or ecological potential of host, higher levels of containment are required.)	
	P3	DNA from nonpathogenic prokaryotes that do not naturally exchange genes with <i>E. coli</i>  DNA from plant viruses  Plasmid or bacteriophage DNA from host cells that do not naturally exchange genes with <i>E. coli</i> . (If there is a risk that recombinant will increase pathogenicity or ecological potential of host, higher levels of containment are required.),	DNA from embryonic primate-tissue or germ-line cells  DNA from other mammalian cells  DNA from birds  DNA from embryonic, nonembryonic or germ-line vertebrate cells (if vertebrate produces a toxin)  DNA from moderate-risk pathogenic prokaryotes that do not naturally exchange genes with <i>E. coli</i>  DNA from animal viruses (if cloned DNA does not contain harmful genes)	DNA from nonembryonic primate tissue  DNA from animal viruses (if cloned DNA contains harmful genes)
	P4		DNA from nonembryonic primate tissue  DNA from animal viruses (if cloned DNA contains harmful genes)	

"SHOTGUN" EXPERIMENTS USING *E. COLI* K-12 OR ITS DERIVATIVES AS THE HOST CELL AND PLASMIDS, BACTERIOPHAGES OR OTHER VIRUSES AS THE CLONING VECTORS

SOME EXAMPLES of the physical and biological containment requirements set forth in the NIH guidelines for research involving recombinant-DNA molecules, issued in June, 1976, are given in this table. The guidelines, which replaced the partial moratorium that limited such research for the preceding two years, are based on "worst case" estimates of the potential risks associated with various classes of recombinant-DNA experiments. Certain experiments are banned, such as those involving DNA from known high-risk pathogens; other experiments, such as those involving DNA from organisms that are known to exchange genes with *E. coli* in nature, require only the safeguards of good laboratory practice (physical-containment level P1) and the use of the standard K-12 laboratory strain of *E. coli* (biological-containment level EK1). Between these extremes the NIH guidelines prescribe appropriate combinations of increasing physical and biological containment for increasing levels of estimated risk. (In this table containment increases from upper left to lower right.)

EXPERIMENTS IN WHICH PURE, CHARACTERIZED "FOREIGN" GENES CARRIED BY PLASMIDS, BACTERIOPHAGES OR OTHER VIRUSES ARE CLONED IN *E. COLI* K-12 OR ITS DERIVATIVES

Thus physical-containment levels P2, P3 and P4 correspond respectively to minimum isolation, moderate isolation and maximum isolation. Biological-containment level EK2 refers to the use of new "crippled" strains of K-12 incorporating various genetic defects designed to make the cells' survival outside of laboratory conditions essentially impossible. Level EK3 is reserved for an EK2-level host-vector system that has successfully passed additional field-testing. Because of the very limited availability of P4 facilities and because no bacterial host-vector system has yet been certified by the NIH as satisfying the EK3 criteria, the recombinant-DNA experiments now in progress in the U.S. with *E. coli* host systems are with a few exceptions limited to those in the unshaded boxes. Experiments with animal-virus host systems (currently only the polyoma and SV40 viruses) require either the P3 or the P4 level of physical containment. Experiments with plant-virus host systems have special physical-containment requirements that are analogous to the P1-to-P4 system.



## The Author

CLIFFORD GROBSTEIN is professor of biology and vice-chancellor of university relations at the University of California at San Diego. He received his Ph.D. in zoology from the University of California at Los Angeles and then worked as an instructor of zoology at Oregon State College. In 1946, after spending three years as an aviation physiologist in the Air Force, he joined the National Cancer Institute, where he worked as a research biologist for 10 years. For nine years thereafter he was professor of biology at Stanford University. He joined the faculty at San Diego in 1965, and from 1967 through 1973 he was dean of the School of Medicine and vice-chancellor of health sciences.

## Bibliography

- THE MANIPULATION OF GENES. Stanley N. Cohen in *Scientific American*, Vol. 233, No. 1, pages 24-33; July, 1975.
- MOLECULAR BIOLOGY OF THE GENE. J. D. Watson. Addison-Wesley Publishing Co., Inc., 1976.
- NATIONAL INSTITUTES OF HEALTH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES. Public Health Service, U.S. Department of Health, Education, and Welfare, June 23, 1976.
- RECOMBINANT DNA RESEARCH: BEYOND THE NIH GUIDELINES. Clifford Grobstein in *Science*, Vol. 194, No. 4270, pages 1133-1135; December 10, 1976.
- NOTES OF A BIOLOGY-WATCHER: THE HAZARDS OF SCIENCE. Lewis Thomas in *The New England Journal of Medicine*, Vol. 296, No. 6, pages 324-328; February 10, 1977.
- RECOMBINANT DNA: FACT AND FICTION. Stanley N. Cohen in *Science*, Vol. 195, No. 4279, pages 654-657; February 18, 1977.



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# Amid Gene Editing Worry, A Return To Biotech's 1st "Asilomar Moment"

UNDERWRITERS AND PARTNERS

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OPINION



**Victor McElheny**  
March 26th, 2015

@xconomy

Like Us

Biotechnology leaders—and the rest of us—should “count to 10” as they read **the March 19 call in *Science*** to consider limits on using revolutionary new gene-editing techniques for germline gene therapy. The techniques are powerful and simple to use and key scientists, **worried about misuse**, want us to pause, confer, and set limits. But we must also recall the risks on the other side: turmoil and distrust of science.



SHARE

As the writer of the first newspaper story about recombinant DNA gene-transfer back in 1974, and one who covered the famous international conference at Asilomar in February 1975 (both for *The New York Times*), I think we should remember how life sciences passed through several very parlous years four decades ago, where major potential human benefits walked a knife edge between outright bans and an evolving set of rules that allowed an industry to struggle and grow.

Public discussion of such issues is required in a democratic society, but we must ensure a rational discussion in today's even more contentious public forums.

Last week's letter to *Science* by leading biologists, urging discussion of regulating so-called CRISPR-Cas9 gene-editing technology, creates a new “Asilomar Moment.” Appealing to the community of researchers in the field, the authors are following a pattern set in biotechnology and life sciences 40 years ago.

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The manifesto focused on the new opportunities for altering genes so that the changes can be passed on to future generations. It was entitled, "A prudent path forward for genomic engineering and germline gene modification." Its 18 signers asserted, "A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed."

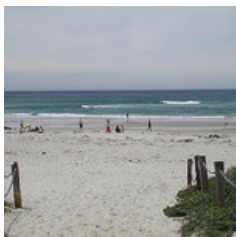
They wrote of "unparalleled potential for modifying human and nonhuman genomes," to cure genetic diseases in humans and to "reshape the biosphere." They warned of consequent "unknown risks to human health and well-being."

As in 1974, when gene-splicing or gene-transfer techniques sped from lab to lab, the authors noted that the new "gene editing" techniques that emerged in 2012 are spreading widely. The trends led them to meet in Napa, California, in January to hammer out their case, just as their predecessors did in David Baltimore's office at MIT on April 17, 1974.

A Nobel Prize winner in 1975 for his work with retroviruses, Baltimore is the former president of Rockefeller University, California Institute of Technology, and the American Association for the Advancement of Science. He was also lead author of last week's Science letter. Among the signers were Harvard University's George Church and Jennifer Doudna of the University of California at Berkeley. Both are pioneers in developing CRISPR-Cas9 for use in human disease therapy.

Baltimore and another signer, Nobel Prize winner Paul Berg of Stanford, were principal organizers of the February 1975 world conference at Asilomar in Pacific Grove, California, that considered management of risks from the then-new techniques of recombinant DNA.

The gene-transfer techniques were pulled together by Herbert Boyer of UCSF and Stanley Cohen of Stanford. Their methods were reported to nucleic acid researchers at a Gordon conference in New Hampshire in June 1973. Concerned about risks, attendees voted to send a letter to Science and to ask the National Academy of Sciences for a study. Berg headed the resultant committee that met in Baltimore's office in April 1974. Recommending a moratorium on three types of experiments until the Asilomar conference could consider the matter, the committee's letter was made public July 18, 1974.



Asilomar Beach, CA.

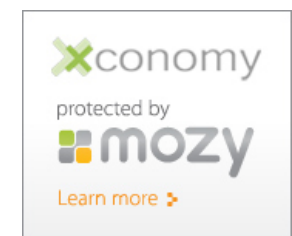
I learned of Boyer and Cohen's work in April 1974. It was published in May 1974, in the Proceedings of the National Academy of Sciences. That was when my Times story appeared on the front page of the national edition. Niels Reimers, Stanford's director of technology licensing, told me later that this spurred him to talk to Cohen about what became the famous Boyer-Cohen patent.

I recall the Asilomar conference amid the pines on the Pacific shore as an incredibly tense week, while young scientists whose careers were in the balance watched their seniors thrash out a final statement. Only two voices warned that regulating risks that could not be quantified in advance risked bringing police into the lab. They were Nobel winners James D. Watson of Harvard, co-discoverer

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with Francis Crick of the DNA double helix, and Joshua Lederberg of Stanford, co-discoverer of mating in bacteria.

In retrospect, Asilomar is viewed as an example of responsible action by scientists entering a new field of first importance to humanity. They gathered to consider the risks of particular types of experiments before undertaking them, and they hammered out detailed principles that should govern regulation of those experiments. As intended, many of these Asilomar principles were embodied in rules issued by the Director of the U.S. National Institutes of Health in June 1976. These rules were relaxed over succeeding years as laboratory experience accumulated and dangers of genetic engineering failed to materialize.

But there was widespread and fierce opposition to the emerging biotechnology. The NIH issued those guidelines on the very day of a heated, televised meeting of the city council of Cambridge, MA, which debated a total ban on all recombinant DNA or gene-splicing research. The ban would have fallen on both Harvard and MIT. A second, calmer meeting of the council set up a study commission, which six months later recommended that Cambridge adopt the NIH guidelines as a city ordinance, which the city council did.

The tense debate in Cambridge was repeated in other cities across the country, and also at the state and federal levels. In New York, the legislature passed a restrictive bill. Only after furious lobbying by such biologists as Norton Zinder of Rockefeller University, did New York Governor Hugh Carey veto the bill in the summer of 1977 as a restriction of academic freedom.

For a considerable time, it looked as if a U.S. commission analogous to the ones regulating atomic energy would be set up for biology. Sponsored by Senator Edward M. Kennedy, the legislation ultimately died in a committee of the U.S. House of Representatives, in part because of animosity between the committee's chair and the chair of the subcommittee shaping the bill. Kennedy put off action in September 1977, shortly before NIH's Recombinant DNA Advisory Committee (RAC) began to relax the rules.

But researchers' and investors' fear that a patchwork of regulation would cripple biotechnology in the United States did not disappear right away. Biologist Thomas Maniatis of Harvard left his home lab to work on the techniques in tighter-security conditions at Cold Spring Harbor Laboratory in New York. Others went abroad. Biogen, founded in 1978, put its first major lab in Geneva, Switzerland.

This was a time of intense concern about environmental dangers from the chemical industry in particular and science in general. It took some years for biologists to gain respect among local state, and federal officials for their sense of responsibility in the recombinant DNA maelstrom of the mid-1970s. But politicians did accept that biotechnology was a significant new industry that other countries, like Japan, might seize if America dropped the ball.

Although biotechnology established itself as economically important and focused on hitherto-untreated diseases, there was also growing polarization of opinion over issues like abortion. Clearly the new respect for life sciences had its limits. There were many years of later interference with research on stem cells (in which Congress repeatedly passed bans on federal funding).


The momentous events around Asilomar made for a precarious time for the life sciences, and should operate as a caution in how the debate on gene editing is carried on, lest it be derailed by irrationality.

Asilomar Beach photo courtesy of [Michelle](#) via Creative Commons license.


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