Recombinant DNA: Examples of Present-Day Research

Many words have been spoken and written about DNA—almost all of them speculative, with little factual basis. This issue contains a collection of reports that sample the state of the art in the area of recombinant DNA research. The content is necessarily far from definitive, for the field is young and moving fast. The editors hoped that by assembling this material a more substantial base for discussion would be created, and that a clearer view of the containment problem might emerge. Modest progress has been made toward these objectives. This overview begins by mentioning some of the procedures by which recombinant DNA research is conducted, and then points out some of the highlights of the collection.

Some of the reports in this issue may prove difficult to read because of the technical language. Essentially, however, the concepts are simple. The term recombination as used here is a genetic term. If, for example, two strains of mutant bacteria—one unable to grow without added tryptophan and the other unable to ferment lactose—are crossed a small percentage of the progeny will be "wild-type," that is, able to grow without tryptophan and to ferment lactose. At the molecular level, this recombination between the two strains results because of breakage of the parental DNA molecules and subsequent rejoining to form the recombinants.

The recombinant DNA technology has made it possible to carry out this recombination process of breakage and rejoining of DNA molecules in the test tube. For many years geneticists have used the recombination process to study the nature of genes, but they were confined to studying recombinants of the same or closely related species. The in vitro process now makes it possible, for the first time, to join DNA molecules of unrelated organisms.

Actually recombination between distantly related species may take place in nature. Certainly mechanisms for the recombination of unrelated DNA molecules exist.

The present capability to join DNA molecules in vitro is the result of 25 years of research on the properties of DNA and the enzymes involved in cleaving and replicating it. In this process, the most important enzymes are the restriction endonucleases. These enzymes have the capability to cut DNA at sequence-specific sites. In Roberts' recent review of this subject (*1*), more than 80 such enzymes isolated from diverse strains of bacteria are described. Some of these enzymes produce staggered cuts in the double strands so that single-stranded ends are produced at each end of the fragment. All fragments produced by a given enzyme have the same self-complementary end, so that a single fragment can circularize by base-pairing or it may combine with another fragment to produce a dimer. In the latter case, if the fragments are from different sources, a recombinant molecule is produced. When this technique is used to produce recombinant DNA's, the gaps remaining in both strands are sealed by an enzyme called DNA ligase. In this issue Scheller, Dickerson, *et al.* describe new and more sophisticated methods for joining DNA molecules using synthetic adapter molecules.

It is convenient to refer to the two DNA molecules in a recombinant as the vehicle and the passenger (Fig. 1). The vehicle is a DNA molecule that is capable of self-replication in its host. When the passenger is joined to the vehicle, it behaves as a (sometimes) passive addition and is replicated together with the vehicle. Two general classes of vehicles have been employed in *Escherichia coli*. Plasmids are small, circular DNA molecules that are capable of self-replication independent of the chromosome. Most of the plasmids now in use originated from the plasmid Col El, which is about 1/1000 the size of the *E. coli* chromosome and produces a bactericidal protein—colicin El; this protein kills other bacteria that do not carry the plasmid. The other *E. coli* vehicle is the well-studied bacteriophage lambda (λ). Two reports, those of Blattner *et al.* and Leder *et al.* describe the construction of safe λ vectors for use as vehicles.

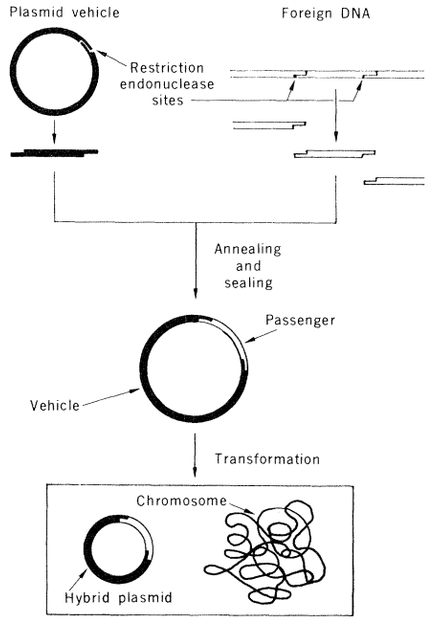

Fig. 1. Scheme of events in recombinant DNA experiments.

The recombinant DNA technology is not confined to the *E. coli* host system. It is possible to employ other vehicle-host systems, including mammalian cells in tissue culture. For this purpose, defective derivatives of the DNA tumor viruses SV40 and polyoma can be used as vehicles. These viruses contain circular DNA molecules that are about the same size as the *E. coli* plasmid vehicles. Villarreal and Berg describe their new screening methods for detecting SV40 hybrids.

When the recombination in vitro between vehicle and passenger DNA is completed, the recombinant DNA must be introduced into the host cell where it can replicate. This is done by a process known as transformation. The DNA is added to cells and is taken up at a low frequency. The plasmid vehicles are constructed so that they give the transformed cell a selective advantage. Some vehicles carry antibiotic resistance genes so that only transformed cells can grow in the presence of the antibiotic. It is the transformation process that allows one to "clone" DNA fragments. Since the frequency of transformation is low, a transformed cell is, in general, produced by the uptake of a single vehicle-passenger DNA molecule. The transformed cell multiplies to form a colony. Different colonies contain different passengers. Each colony can be propagated indefinitely, enabling one to store or produce (or both) the passenger DNA molecule at will.

Two popular misconceptions should be cleared up at this point. Cloning as referred to here and in recombinant DNA research in general is totally different from the process developed by Gurdon (*2*), where the nucleus from a somatic cell containing the complete genetic information on one individual (a frog in Gurdon's experiments) is removed and implanted into an enucleated egg. Gurdon demonstrated that this egg could in some cases develop normally to produce a frog. By this technique one could—in principle—propagate an indefinite number of identical individuals. By contrast, in DNA cloning experiments, fragments of passenger DNA represent a minute fraction of the total genetic information of the organism from which they are derived. Clones carrying recombinant plasmids are often referred to as hybrid strains.

If passenger DNA from the mouse is joined to a plasmid vehicle and transformed into *E. coli*, the resulting clones would be referred to as *E. coli*–mouse hybrids. The use of the word hybrid suggests to the layman that a significant mouse character is acquired by *E. coli*. This is, of course, not the case. The hybrid *E. coli* is still very much the organism that it was. The mouse information that it has acquired represents about one-millionth of the genetic information of the mouse—a few genes at most—and is only about one-thousandth of the genetic information of *E. coli*.

In many of the reports in this issue, cloning has been used to isolate and propagate fragments of DNA from diverse sources. When the fragments are isolated from *E. coli* or closely related bacteria, the genes are usually expressed. Thus Zieg *et al.* have isolated the genetic region of *Salmonella* that controls which of two possible flagellar forms will be expressed. The control mechanism that operates in this system is one that had not previously been described. A segment of DNA in the control region can be inverted. The flagellar type that is expressed depends on the orientation of the DNA segment. There are indications that mechanisms similar to this one may operate in higher organisms. It will now be possible to elucidate in detail how this kind of control system works.

Panasenko *et al.* have constructed a hybrid of λ and *E. coli* which carries the DNA ligase gene. This hybrid phage can be used to produce a 500-fold increase of this enzyme in the cell. This work illustrates a practical advantage that can be gained by use of recombinant DNA technology. Thus, the purification of DNA ligase is greatly simplified when there is 500 times as much enzyme per cell.

Many of the reports describe the isolation of DNA fragments from eukaryotes. Beckmann *et al.* have isolated plasmids carrying transfer RNA genes of yeast. McClements and Skalka have cloned the ribosomal RNA of chicken. Lee *et al.* and Scheller, Thomas, *et al.* have characterized fragments of sea urchin DNA, which contain middle repetitive DNA sequences. The organization of these sequences has been determined before by less direct methods. Now examination of cloned fragments confirm that repetitive sequences are interspersed between unique sequences. In one clone it was shown that the nearby repeats are not identical.

Two groups (Liu *et al.* and Wilson *et al.*) have used the enzyme reverse transcriptase to make DNA copies of hemoglobin messenger RNA (mRNA). These DNA fragments were then inserted into plasmids and cloned. The cloned DNA can be used for more convenient sequencing of the mRNA as shown by Liu *et al.* It can also be used as a pure probe to investigate the multiplicity of hemoglobin genes or to isolate the genes themselves in further cloning experiments.

It can be seen that there is plenty of activity in this field. DNA's from diverse sources are being cloned, and the cloning technique is being used to study questions of gene organization and control. With all of this work, are we in a better position to answer the question that is being asked by the public with increasing frequency? Are these organisms dangerous? It is perhaps impossible to design an experiment that can give an unambiguous answer to this important question. In one sense, the workers themselves have been guinea pigs—there have been no reports of illness associated with infection by a strain of bacteria containing cloned DNA. Cameron and Davis have looked carefully to determine whether or not a fragment of DNA from yeast or *E. coli* could confer an advantage to a λ bacteriophage vehicle which carries it. They found that foreign DNA fragments often inhibit growth of the phage, but in no case did a fragment confer an advantage. This agrees with the impression formed by many workers that some DNA fragments—especially from bacteria—may be "unclonable," suggesting that they have a lethal effect on the host. Thus, we can believe that it is probably not possible to create a strain that would overgrow the laboratory and head for town as depicted in movies of the 1950's.

One way to assess the danger of inadvertent creation of a pathogen is to design "worst case" experiments that can test the ability of *E. coli* to act as a pathogen. An example of such an experiment would be to test whether strains of *E. coli* carrying polyoma DNA as a passenger can cause tumors in mice (polyoma is a virus which can do this). This experiment is being planned at the National Institutes of Health (NIH), but it must take place under the highest containment conditions (P4), and therefore it will be some time before the results will be available (*3*). The pathogenic character of a species is the result of complicated interactions between the organism and its environment. The "worst case" experiments represent a way to test the possibility that *E. coli* K12 can be converted into a pathogen—at least in special cases. One hopes that more of these experiments will be conducted.

The NIH guidelines are the result of the compromise between the need to protect the public from conjectural hazards and to provide them with the benefits that can be gained from fundamental knowledge in biology. The guidelines provide a sliding scale of biological and physical protection that increases for experiments with higher organisms.

Two reports in this issue deal with the construction and testing of bacteriophage λ vehicles that have been awarded the EK2 designation (medium biological containment) by the NIH advisory committee (*4*). The standards used in evaluating these vehicles have been rigorous and imaginative. They require that the EK2 vehicle pass a series of "worst case" tests that ensure that the chance of propagation of an escaped particle is less than 10^{-8}. These vehicles make it possible to carry out experiments with mammalian DNA under P3 conditions.

The ability to clone and propagate DNA fragments from higher organisms is a genetic technique for which there was no previous counterpart. It was simply impossible to design an experiment for obtaining the gene for the α chain of hemoglobin, for example. Two new technologies—recombinant DNA research and DNA sequencing—now make it possible to examine the structure and organization of eukaryotic genes on a scale not imaginable 5 years ago.

The following reports describe the beginning of this research. Five years from now we will have taken a quantum jump in our understanding of chromosome structure and gene organization.

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References and Notes

1. R. J. Roberts, *Crit. Rev. Biochem.* **3**, 123 (1976).
2. J. B. Gurdon, *J. Embryol. Exp. Morphol.* **10**, 622 (1962).
3. **P1** containment specifies sterile techniques such as hand washing, decontamination of the work area, and exclusion of food and smoking materials. Waste must be decontaminated before disposal. **P2** containment is similar to P1, except that more stringent procedures must be followed and access to the laboratory must be limited to authorized personnel who have been advised of the hazard. In **P3** containment, in addition to the above, experiments requiring P3 must be done in a negative pressure laboratory in an isolated room separated from other work areas. All procedures involving opening of vessels must be performed in a biological safety cabinet. Gloves must be worn. No recombinant organisms (or molecules) are permitted to leave the P3 in viable form, except in a sealed, unbreakable, externally decontaminated container. **P4** is an elaborate containment for handling the most dangerous pathogens known.
4. Biological containment is as follows. **EK1** requires, for example, the use of *E. coli* K12, a strain generally conceded to be unable to colonize the human bowel. **EK2** systems require, for example, the use of genetically altered K12 strains, so that they will not grow under usual conditions for *E. coli*. **EK3** is the use of an EK2 system and, in addition, requires the system be tested in animals for safety.

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