usually cold and warm temperatures, as reconstructed from a variety of climatic indicators and records, and the solar activity variations recorded in the ^{14}C data. Are changes in solar magnetic activity in fact related to past climatic changes on the earth? Are climatic shifts themselves an indication of changes in the sun's output of energy?

Not many years ago these questions would have been dismissed by both solar physicists and climatologists as unworthy of serious investigation, but now, even though answers are not yet forthcoming, most investigators would not rule out the possibility. The difficulty is that there is no unambiguous evidence of changes in the sun's output of energy—the solar "constant"—although even changes of 1 or 2 percent would be enough, according to current climatic models, to cause profound changes on the earth. On the other hand, changes in solar magnetic activity, either on an 11-year or a much longer cycle, have not yet been shown to cause climatic changes, whatever their other effects on the terrestrial environment.

Both lines of research are likely to receive more attention. It is now known, for example, that the solar magnetic field controls the flow of matter that eventually escapes the sun as the solar wind. According to Robert Noyes of the Harvard College Observatory, Skylab investigations have shown that the solar wind is emitted from so-called coronal holes—regions of weak magnetic fields in the solar atmosphere. Thus a mechanism linking long-term changes in the solar wind and climate, if one could be found, might indeed implicate solar magnetic activity. Eddy thinks that a more likely explanation will involve changes in the sun's radiated energy, which is emitted not in the corona but lower in the photosphere, where sunspots and other magnetic disturbances also originate. Really accurate measurements of the sun's radiated energy over a long period of time have yet to be done, but it is known that variations in this century have been small and show no correlation with the 11-year magnetic cycle. A possible gradual increase in solar output of about 0.5 percent since 1900 is the only reported change.

Some investigators speculate that convective instabilities on a very large scale within the sun might lead to changes in solar output on a much longer time scale, and might also conceivably cause long-term changes in solar magnetic activity. If so, then solar activity and the terrestrial climate may again experience significant fluctuations. In any case, it begins to look as if *le Roi Soleil* deserves to be remembered as one of history's most ironic misnomers.—ALLEN L. HAMMOND

Molecular Cloning: Powerful Tool for Studying Genes

Few scientific techniques have aroused more speculation and interest than those that permit molecular biologists to artificially join DNA from two different sources to form recombinant molecules. Ordinarily genes are exchanged only between members of the same species as part of the process of sexual reproduction. But the new techniques can join DNA from species as diverse as mammals and bacteria, and then put the recombinant DNA into cells, usually bacteria, where it can reproduce.

Many investigators think that this research may provide the key that will eventually permit practical "genetic engineering." A possible application is the inexpensive commercial production of insulin, human growth hormone, and other medically useful materials in bacteria into which the appropriate genes have been transplanted.

At the same time, research on recombinant DNA has engendered concern that researchers might inadvertently allow bacteria bearing new and unusual genetic combinations to escape from the laboratory and produce adverse effects on human, animal, or plant populations. This concern has in turn engendered a series of committees to produce research guidelines to protect against this eventuality (*Science*, 19 December 1975; 27 February 1976).

At this time the practical applications of recombinant research are still in the future, and the hazards have been realized only—albeit fortunately—in discussion and speculation. What is happening now is that the techniques of the research are enabling investigators to explore the structure and function of genes. This is especially true for the genes and chromosomes of eukaryotic (nucleated) cells because the methods that proved successful with regard to the much simpler chromosomes of prokaryotic (nonnucleated) cells are not easily applicable to eukaryotic chromosomes.

Recombinant DNA techniques are useful for analyzing chromosome structure because they permit investigators to isolate chromosome segments bearing specific genes and to grow the segments in quantities sufficient for study. This is done by a process called molecular cloning in which the DNA to be studied is covalently linked with a suitable vector or carrier that reproduces itself—and the DNA in question—in bacterial cells. The most common vectors are plasmids and bacteriophages. Plasmids are double-stranded, circular pieces of DNA that are found in bacteria. They replicate independently of the bacterial chromosome. Genes for resistance to many antibiotics are carried on plasmids.

The small plasmid designated pSC101 carries the gene for resistance to the antibiotic tetracycline and is frequently used as a cloning vehicle. It was developed for this purpose by Stanley Cohen and Annie Chang of Stanford University Medical School and Herbert Boyer and Robert Helling of the University of California in San Francisco. They found that they could open the plasmid and insert a piece of foreign DNA. Doing this did not destroy the plasmid's ability to enter and reproduce in the bacterium *Escherichia coli*. Nor did it prevent the plasmid from conferring antibiotic resistance on the cells. The latter is important because antibiotic resistance provides an easy means of selecting those bacteria that have acquired the plasmid. Bacteria containing pSC101 will grow in the presence of tetracycline whereas those lacking the plasmid will not.

Use of pSC101 as a cloning vehicle is permitted under the proposed guidelines for research on recombinant DNA because tetracycline resistance is already widespread among *E. coli* strains found in nature, and experiments with pSC101 will not result in any extension of the resistance capability of *E. coli*. The guidelines prohibit the transfer of genes conferring drug resistance to microorganisms not known to acquire such resistance naturally because the clinical effectiveness of the antibiotic in question might be compromised.

In order to open the circular pSC101 molecule and insert the additional DNA, Cohen and Boyer used the restriction enzyme Eco RI. Restriction enzymes are bacterial enzymes (Eco RI is isolated from *E. coli*) that recognize specific base sequences on double-stranded DNA and cut both strands (*Science*, 4 May 1973).

The discovery of restriction enzymes has greatly facilitated the production of recombinant DNA's because several of the enzymes, including Eco RI, break the two strands of DNA in such a manner that the cuts are staggered. This generates single-stranded "sticky" or cohesive ends with base sequences that are complementary to

one another. Under appropriate conditions these ends will hydrogen-bond to one another and they can be covalently rejoined with DNA ligase, an enzyme that repairs breaks in DNA. Pieces of DNA from different sources can also be covalently linked, provided that the pieces were originally formed by splitting the parent molecules with the same restriction enzyme. Moreover, the pieces can be recovered after cloning by treating the recombinant molecule with the restriction enzyme. It will split the DNA at the same sites where the pieces were joined.

Cohen and Boyer found that there is just one site for Eco RI on pSC101. Consequently the enzyme produces a linear molecule with two sticky ends. Combination with another DNA with two cohesive ends can generate a circular plasmid composed of both DNA's.

Recombinant molecules can also be produced without using restriction enzymes. An alternative process involves generation of sticky ends by enzymatically adding adenine nucleotides to one end of both strands of one of the DNA's to be joined and thymine nucleotides to one end of both strands of the other. Since adenine and thymine are complementary bases, the two DNA's can hydrogen bond to one another and be covalently linked by DNA ligase.

However the recombinant plasmids are constructed, the next step of the cloning procedure is transformation of the bacterial host. In transformation, appropriately treated bacteria take up individual plasmids which can then reproduce.

A number of investigators, including Cohen and Laurence Kedes of Stanford University Medical School, Donald Brown of the Carnegie Institution of Washington in Baltimore, and Ronald Davis and David Hogness, both working independently at Stanford, are now using pSC101 to clone DNA's from various sources for structural studies. Brown, for example, is studying the genes specifying the structure of a small RNA found in ribosomes. In the frog chromosome, this structural gene alternates with a region of spacer DNA. The structural gene plus the spacer form one repeating unit, thousands of copies of which are present in the frog genome. Because so many copies are present, these genes can be isolated. Brown says, however, that cloning the isolated DNA greatly facilitates the acquisition of enough to study—and saves the lives of a lot of frogs.

For these experiments, Brown uses the Hind III restriction enzyme that is isolated from *Hemophilus influenzae* strain d. This enzyme, which is one of those that generate cohesive ends, makes one cut in pSC101 at a site very close to where Eco RI splits the plasmid. It also cleaves the repetitive DNA that Brown is studying into segments approximately equal in length to the repeating unit of the repetitive gene sequences.

Brown partially digests this DNA with Hind III in order to get fragments equal in length to four or five repeating units and then inserts these fragments into pSC101 for cloning. Complete digestion of the cloned recombinant molecules with Hind III will split the molecule at all of the enzyme-sensitive sites and produce four or five fragments of frog DNA plus the linear plasmid. Thus, Brown is able to analyze the range of lengths of the repeat units. He finds that adjacent units can differ in length. Structural analyses of this type can help to distinguish which of the several models proposed to account for the evolution of repetitive DNA is correct.

"Shotgunning"

Most genes are present in the genome in single copies and cannot be readily isolated. But another recombinant DNA technique—called "shotgunning"—provides a method for cloning any gene for which there is an appropriate selection or detection method. In shotgun experiments an entire genome is digested with a restriction enzyme and the fragments are combined with a suitable carrier for cloning.

Many investigators think that shotgun experiments have a high degree of potential hazard because the total genome of any organism contains genes that could be harmful to man if expressed in *E. coli*, a common inhabitant of the human intestine. The guidelines for recombinant DNA research require precautions to prevent the escape from the laboratory of bacteria carrying recombinants formed in shotgun experiments. The guidelines are more stringent for experiments with the genomes of higher organisms, such as warm-blooded vertebrates, than for those with the genomes of lower organisms. For this reason, current shotgun experiments are performed with the genomes of cold-blooded vertebrates and insects.

Shotgunning produces a large number of different recombinants; locating which, if any, carry the wanted gene can be a formidable task. The situation is somewhat simplified, however, if the gene specifies a function that can serve as a basis for selection. Boyer, Donald Helinski of the University of California at San Diego, and Charles Yanofsky of Stanford, used this approach to clone the tryptophan operon of a bacteriophage. (This operon includes the genes that direct the synthesis of enzymes needed for the synthesis of tryptophan, an amino acid.) The ColE1 plasmid served as their cloning vehicle, and a strain of *E. coli* that lacks the capacity to synthesize tryptophan was the host. Bacterial clones that acquired the tryptophan operon could then be selected by growing them in a tryptophan-free medium.

Under normal conditions, an *E. coli* cell has about 25 copies of the ColE1 plasmid, whereas only six to eight copies of pSC101 are found in one bacterial cell. Moreover, when replication of the *E. coli* chromosome is inhibited by chloramphenicol, ColE1 continues to replicate until 1000 or more copies accumulate. This property should make ColE1 a very useful vehicle for cloning and amplifying the concentration of a gene. However, chloramphenicol inhibits protein synthesis, including that of proteins specified by plasmid genes.

In the absence of a selective mechanism, the investigator needs a method to detect either the gene or the gene product. Genes can be detected by hybridization with the complementary radioactive DNA or RNA, if these are available. One of the major problems is that it may be necessary to screen a large number of bacterial clones to find out whether any have the wanted genes. Various techniques have been used to minimize the work required. The colony hybridization technique developed by Hogness is one.

The colony hybridization method involves culturing bacteria transformed by recombinant DNA on sterile filters on agar plates. When the colonies develop, duplicate cultures are made. Then the bacteria on the filter are lysed and treated so that the DNA is fixed, in the original location, to the filter. The radioactive DNA or RNA probe can be hybridized to the DNA on the filter and detected by autoradiography. The corresponding clone (or clones) on the duplicate cultures serve as a source of material for further studies. Hogness has used this technique to isolate the genes for ribosomal RNA's from the fruit fly. He says that it can be applied to the isolation of any gene, even those present in a single copy, if there is an appropriate probe.

Sometimes it is possible to detect the gene product itself. Stanley Falkow and his colleagues at the University of Washington School of Medicine are studying enterotoxins produced in strains of *E. coli* that carry certain plasmids. These bacteria cause severe diarrhea in very young calves and pigs and may also cause a traveler's diarrhea (Montezuma's revenge) in man.

One of the enterotoxins is known to be heat-stable, but little else is known of its nature or mode of action. The enterotoxin is usually detected by a biological assay such as its production of toxic effects in the intestines of suckling mice. Falkow, with Boyer, formed recombinants between fragments of the plasmid carrying the enterotoxin gene and pSC101 and cloned them in *E. coli*. The investigators detected the clone carrying the right recombinant DNA

by the assay in suckling mice. This experiment does not violate the research guidelines because it involves transfer of the enterotoxin gene between strains of *E. coli* and not the introduction of a new gene into the species.

Falkow hopes to be able to use the cloned DNA to identify the gene product and learn how it works. He has found, for example, that minicells (see below) bearing the recombinant plasmid contain five or six proteins not observed in minicells having only the carrier plasmid. The next step is to identify which of these proteins are involved in synthesis of the toxin.

Minicells are formed by a mutant strain of *E. coli* that cannot synthesize DNA but that does bud to form small cells without chromosomes. Because these minicells are so much smaller than normal bacterial cells the two can be easily separated by centrifugation. Plasmids are taken into the minicells during budding and function there. Thus investigators can study processes controlled by plasmids in the absence of those under chromosomal direction.

The bacterium *E. coli* is quite simple compared to nucleated cells, and more is known about it than about any other cell type. Consequently, the ability to put eukaryotic genes into *E. coli* should give molecular biologists a way to approach some of the many unanswered questions about the expression of these genes and how the expression is controlled. To do this, investigators must first find out whether prokaryotic enzymes will recognize and respond to the signals that control transcription and translation of the eukaryotic genome. This is also important because, if they do not, a product such as insulin will not be synthesized by bacteria unless the recombinant DNA molecule also includes the appropriate prokaryotic control signals at the correct locations.

Initiation of transcription of at least one kind of eukaryotic DNA—mouse mitochondrial DNA—does occur on the eukaryotic portion of the recombinant molecule in *E. coli* minicells. Cohen and David Clayton, also at Stanford University School of Medicine, formed recombinants between the whole mitochondrial chromosome, which is a circular DNA molecule, and the pSC101 plasmid and cloned them in minicells. They found that portions of the mitochondrial genome were transcribed into RNA's, and the pattern of the RNA's formed indicated that the transcription originated at sites within the mitochondrial DNA itself. Cohen points out, however, that these transcription signals to start do not appear to be the normal ones of the mitochondrial DNA.

Cohen and his colleagues also examined protein synthesis in minicells containing the recombinant molecule and compared it with that of minicells having only pSC101. They found that the recombinant transformed cells contained peptides not produced by the pSC101 transformation and concluded that the peptides were translated from DNA transcripts of the mitochondrial DNA. However, these peptides differed markedly from those normally formed under the direction of the mitochondrial chromosome. Thus, at this time there is no evidence that DNA from higher eukaryotic organisms will be expressed normally in bacteria.

Although most investigators are now using plasmids as cloning vehicles, bacteriophages can also serve in this capacity provided that they are suitably modified. Bacteriophage DNA is larger than most plasmids and will usually be split in more than one place by restriction enzymes. This would obviously complicate the process of constructing a workable recombinant. Mutants with only one or two enzyme-sensitive sites are needed. Moreover, nonessential DNA must be deleted to make room for additional DNA; otherwise, the recombinant would not fit in the protein coat of the phage. Finally, these alterations must not destroy the capacity of the phage to infect and reproduce in bacteria.

Three investigators, Davis, Kenneth Murray of the University of Edinburgh, and Alain Rambach of the Pasteur Institute, have altered bacteriophage lambda DNA so that it meets these criteria. This DNA normally has five sites that are cleaved by Eco RI. The investigators have been able to produce mutants with only one or two. Davis, for example, constructed a mutant with two cleavage sites. Digestion with Eco RI therefore produces three fragments. The small central fragment includes no genes needed for phage propagation. It can be separated from the two larger end fragments. These have the genes required for infection and reproduction in the host bacterium, but when joined together, they form a molecule that is too small to be active. However, if another piece of DNA is inserted between them, a viable phage results. The fact that the phage cannot reproduce unless the DNA is of the correct length provides a mechanism for selection of recombinant molecules. Davis has used this modified phage to isolate ribosomal RNA genes of yeast.

One of the advantages of using bacteriophage lambda DNA is that this genome has been extensively mapped and its control regions identified. It may be possible to construct a recombinant molecule between eukaryotic DNA and a modified lambda DNA in such a way that the expression of the eukaryotic material is controlled by phage genes.

Recently, Philip Leder and his colleagues at the National Institute of Child Health and Human Development further modified the phage developed by Davis in order to make it even more suitable for use as a cloning vehicle. They introduced one mutation that allows the growth of large quantities of the virus in a relatively small volume. Two additional mutations improve the safety of the vector by reducing the possibility of its encountering a suitable host in nature.

Many problems, including the current inability to demonstrate that eukaryotic genes are expressed in bacterial cells, will have to be solved before recombinant DNA techniques find practical application. Most investigators, however, are optimistic that the difficulties can be overcome. Meanwhile, molecular biologists have a powerful tool with which to explore the riddles of gene structure and function.

—JEAN L. MARX

Laser Enrichment: Time Clarifies the Difficulty

For several years, laser techniques have stood out as the most exotic and promising new methods of uranium enrichment. The idea is that monochromatic laser beams can be used to separate isotopes on the basis of miniscule chemical differences long thought to be too small to exploit. The promise is that laser methods could potentially save as much as half the cost and 90 percent of the energy used in present enrichment methods. Many observers have feared that laser methods would be so easy to implement that they might also, as one weapons scientist said, enable people to "build bombs in their basements."

The status of laser enrichment technology has been obscured by the secrecy imposed on the research, in the government weapons laboratories at Los Alamos and Livermore and at the Avco-Exxon Nuclear laboratories where a large amount of industrial research is done. Perhaps the clearest perspective to date was presented last month at an American Physical Society meeting in New York, where representatives from all three laboratories