The Era of the Jumping Genes

Pieces shoved in, pulled out, flipping around. That is far from the conventional model of that reliable double helix that carries our genetic information. "There is a new view of DNA," says Melvin Simon of the University of California at San Diego, "where the stability comes from a dynamic situation."

The idea of genes moving around in the chromosome intrigued geneticists at the ICN-UCLA symposium on eukaryotic genetics last week in Park City, Utah. Simon presented data suggesting that a piece of DNA in the bacteria *Salmonella* can swing around to regulate which of two components go into the bacterial flagella, the long, whiplike organelles that propel the cell. Ira Herskowitz of the University of Oregon suggested that one aspect of yeast genetics resembles a cassette recorder. Different genes might move from a storage area and snap in and out of the site where they function.

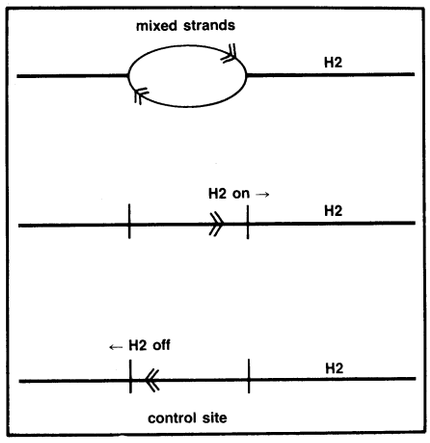
Cells clearly contain more genetic information than they express at any one time. During development, a sequence of different genes functions. The researchers predict that models using the controlled movement of genes will lead to better explanations of development. "Site-specific recombination of DNA is the word," says Simon.

Changes in gene position have been proposed at various times in the past as an important biological mechanism, but the idea was never widely accepted. Joshua Lederberg and T. Iino, who also studied *Salmonella* flagella, suggested 20 years ago that a change in the "local state" of the gene could be involved in development. They concluded, however, that control by proteins that diffuse through the cytoplasm was more likely than control at the gene level. Even earlier, Barbara McClintock of Cold Spring Harbor saw evidence for movement of DNA in the complex regulation of gene expression in maize.

More recently, evidence for gene movement has come from two diverse areas. Bacterial geneticists have discovered DNA regions, called insertion sequences, that change position in bacterial DNA. The idea of plugging in genes has also been considered seriously since 1965 by scientists studying molecules of the immune response. The evidence for gene rearrangement there has become strong (SN: 12/11/76, p. 372).

"It is time for this idea to be looked at again because the techniques are now available," Simon says. "We need to get out the DNA and look at it."

Simon and co-workers Janine Zieg, Michael Silverman and Marcia Hilmen have examined the flagella of *Salmonella*.



Salmonella DNA switch can swing around.

Each bacterium contains the genetic information to make two types of flagellar protein, but only one of the two genes is actually expressed at any time. The signal that determines whether one of these genes (H2) is on or off is located on the DNA right next to that gene. To learn the mechanism of that switch, Simon and collaborators made a recombination DNA plasmid containing pieces of *Salmonella* DNA. The plasmids were inserted into defective *E. coli* bacteria that had no flagella of their own. "The only flagella they could make would be from the *Salmonella* genes," Simon explains. Any bacterium that could swim must have a *Salmonella* flagella gene turned on.

The region of DNA next to a gene usually controls whether the gene is expressed. Other studies have found that proteins bound to that region can prevent or initiate gene expression (SN: 11/12/76, p. 348). Because moving a piece of DNA between bacteria moved the "on-ness" or "off-ness" as well as the flagellar protein gene, Simon suspected that the DNA in the control region was actually different when the gene was turned on and off.

Clear evidence for this hypothesis came from mixtures of DNA. The researchers separated the two strands of DNA from both bacteria in which the H2 gene was on and off. When the strands were mixed they formed into new double helices, but many had a region about 750 nucleotides long, where the strands didn't bind together. These bubbles, seen with an electron microscope, indicated areas where the DNA differed and the nucleotides didn't match. Further experiments showed that the bubble formed right next to the H2 gene. "The bubbles are generated by one strand of DNA that is off and one strand of DNA that is on," Simon proposed.

Simon and his collaborators suggest that the DNA in the bubble region contains a signal for bacterial enzymes to begin reading the DNA in one direction. With the signal piece in one orientation, the flagellar protein will be made. When the signal piece is flipped, the enzymes will move in the opposite direction, perhaps reading instead the gene on the other side of the bubble region.

Simon's data may also be explained by a hypothesis in which different pieces of DNA move in and out of the controlling site. Simon and his colleagues plan to compare the nucleotide sequence of the control region when the H2 gene is in the on and off states to resolve the model. They also hope to use recombinant DNA techniques to connect other genes with known products to the opposite sides of the control site and observe whether the region will flip around to express one or the other of the new genes.

The other suggestion of genes moving within chromosomes comes from studies of yeast. Like the *Salmonella,* yeast cells express one or the other of two clearly distinguishable genes, the genes that determine their mating type. During sexual reproduction, cells of opposite types, *a* and *alpha,* fuse to form a diploid state. "We are asking what makes an *a* cell an *a* cell and what makes an *alpha* cell an *alpha* cell," Herskowitz told a crowded workshop.

Earlier experiments showed that about one in a million yeast cells switch mating types, for example an *a*-type yeast may give rise to a perfectly stable, "garden variety" *alpha* type, as Herskowitz described it. This sort of switching is greatly increased by the presence of a gene called H0. Because the H0 cells change mating types almost every generation, they are useful to the study of the switching.

At first Herskowitz and collaborators Jeffrey Strathern, James Hicks and Jasper Rine thought that a flip-flop model, like that described by Simon, might apply to yeast mating types. The control region would sit between an *a* and an *alpha* gene. When yeast cells with a mutation in only the *alpha* gene switched mating types, as expected, they produced cells with a perfectly good *a* gene product. But a further experiment indicated that the situation was more complex. When on subsequent divisions those *a* cells switched again, they could give rise to perfectly good *alpha* cells. "We propose that a yeast cell contains extra copies of *alpha* information and of *a* information," Herskowitz says. "At the mating type locus a promoter allows expression of the block of information next to it. Elsewhere there are silent blocks of information. Copies of the silent information get plugged into the mating type locus." Herskowitz calls this model the cassette hypothesis.

Song, signs and spite spice DNA talks

A rousing chorus of "We Shall Not Be Cloned" from the back of the auditorium led off the first evening of a three-day public forum on recombinant DNA this week at the National Academy of Sciences. In the intermittently stormy session that followed, outbursts from audience and participants interrupted prepared remarks read by the scheduled speakers.

The meeting was planned to aid informed public discussion of the dilemma of recombinant DNA research. Both scientists and members of the public, however, disagreed sharply on just what ought to be under consideration. Some wanted to debate what safety measures should be required in laboratories, whereas others still wanted to discuss whether the research should be done at all.

The tension of the meeting was foreshadowed at the afternoon kick-off news conference where Mayor Alfred Vellucci of Cambridge, Mass., suddenly stole the show. Speaking from within the crowd of reporters, Vellucci declared that he had not been invited to the forum but had come on his own to ask whether a city has the right to control genetic engineering and regulate experiments in laboratories within its limit. "We have to watch what the hell crawls out of those laboratories," he proclaimed.

Styles clashed early in the evening meeting. After biologist Daniel E. Koshland of the University of California at Berkeley and David A. Hamburg, president of the Institute of Medicine, spoke of the deep concern that has arisen over recent genetic research innovations, Hamburg turned the microphone over to Jeremy Rifkin of the People's Business Commission (formerly the People's Bicentennial Commission). The protest group had demanded changes in the agenda to include questions of patents, of business ties of the speakers and of the absence of religious leaders in the program. As Rifkin headed toward the stage, supporters sprang up with banners saying "Public debate before private profit" and "We will create the perfect race—Adolph Hitler." Rifkin read selections from scientists James D. Watson, Joshua Lederberg and James Bonner that suggested cloning of human beings would be possible and desirable in the near future. Rifkin summed up his view of the future of genetic research with "You ain't seen nothing yet" and returned to his seat among shouts of "open it [the agenda] up" and rhythmic clapping.

Hamburg returned to the podium and patiently explained the mechanisms for including all points of view in the meeting, although he said the basic agenda could not be changed. Members of the audience interrupted Hamburg and each other, until it seemed uncertain the meeting could continue. Hamburg finally turned the meeting over to Maxine Singer of the National Cancer Institute, the first scheduled speaker. The audience remained quiet during her description of the history of recombinant DNA regulation.

David Callahan of the Institute of Society, Ethics and the Life Sciences was the next speaker. He used an extended analogy comparing the public and the scientists to a couple in need of marriage counseling. From what followed in the forum, however, it was clear that the squabble also involved a decidedly split personality in at least one spouse.

The division between scientists surfaced when geneticist Jonathan King of the Massachusetts Institute of Technology attacked Singer's talk as a whitewash of a "technocratic coup." At several points King was interrupted by scientists in the audience shouting, "That's not true, Jon." At the end of his talk King raised the question of money: "Who is funding the people who are taking into their hands the genetic future of the human race?"

After several questions from the audience, the speakers on stage responded to the issue of funding. Two reported they were funded by government and nonprofit groups, Daniel Nathans of Johns Hopkins refused to answer and Erwin Chargoff broke the tension by replying that he was retired and had no corporate relationships whatever, he was sorry to say.

Scientists again attacked each other in the final question period, after Nathans and Chargoff spoke on potential benefits and risks of the genetic research. Stanley Cohen of Stanford reminded those who were afraid products of recombinant DNA experiments would upset evolution that evolutionary wisdom had created bubonic plague and cancer. Cohen charged that Chargoff deplored the scientific knowledge that comes from experimentation. Chargoff countered, "I am willing to bear God's scourges, but do I have to bear Dr. Cohen's?"

"The traveling circus" is the name some scientists have applied to the continuing debates around the country on the topic of recombinant DNA research by these same participants in the controversy. With federal legislation looming and local legislation sprouting, the show will go on. The rest of the Academy meeting promised to be lively, but its chances of resolving the recombinant DNA dilemma seem as low as the probability of cloning a Frankenstein. □

Herskowitz and co-workers have further found that the yeast cells with the H0 gene exhibit a specific pattern of switching. "Experienced cells (those which have undergone at least one cell cycle) give rise to switched cells 80 percent of the time, whereas inexperienced cells switch rarely if at all," they observed. This difference suggests a very simple type of cell differentiation.

Although there are still other models that explain his data, Herskowitz finds the cassette mechanism the most promising, especially when it is extended to higher organisms. "Many cell types at certain points in development could exhibit sequential insertion of cassettes." □

Yeast and mold genes work in bacteria

Both the hopes of the advocates of gene splicing research and the fears of the opponents require that a transplanted gene function in its new environment. Early research showed the effectiveness of genes moved between different types of bacteria, but the big step comes between bacteria and the higher forms of life. Most of the new genetic techniques proposed for research and practical applications involve forcing a bacterium to make the product of a higher organism's gene.

To the cell biologist the most basic division between forms of life separates bacteria and blue-green algae (prokaryotes) from all other organisms (eukaryotes), ranging from microorganisms to man. Prokaryotes have no cell nuclei and their genes are located in a naked strand of DNA. All eukaryotes have cell nuclei which contain the genes bound with other DNA and protein into chromosomes.

It is now becoming clear that the differences between prokaryotes and eukaryotes will not prevent successful gene transfer in the laboratory. Last week at the ICN-UCLA symposium on eukaryotic genetics in Park City, Utah, researchers presented evidence that genes from yeast and from mold can direct protein manufacture in bacteria.

John Carbon of the University of California at Santa Barbara reports that at least four genes from baker's yeast function in bacteria *Escherichia coli*. He chose yeast because many of its enzymes are similar in function to those of *E. coli*. Of all eukaryotic genes, those of yeast seemed the most likely to work in bacteria.

In experiments with Barry Ratzkin, Carbon demonstrated that yeast genes can relieve bacterial deficiencies. Using recombinant DNA techniques, the researchers constructed rings, or plasmids (SN: 6/21/75, p. 404), containing short segments of yeast DNA. In four cases they found that with a particular plasmid, mutant bacteria could survive in the absence of a previously required amino acid. To-

gether the bacteria and plasmid manufactured the missing enzyme. These results are published in the February PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

In different experiments Kevin Struhl, John R. Cameron and Ronald W. Davis used a virus to package yeast genes and move them into a bacterium. The Stanford researchers also found that one of the yeast genes allowed the bacterium to grow in the absence of a previously required amino acid (PROCEEDINGS 73:1471, 1976).

More experiments were needed to prove that the transplanted yeast genes were directly responsible for the new enzyme, rather than somehow reversing the effect of the bacterial mutation. In their most recent work, Carbon and Ratzkin examined enzyme activity of plasmid-containing bacteria that had previously lacked an enzyme for synthesis of the amino acid leucine. The normal bacterial enzyme is stable in the cold, but the yeast enzyme can be completely destroyed after 40 minutes on ice. The researchers found that the enzyme from deficient bacteria with the plasmid was quite sensitive to cold. "Therefore it has the properties of yeast," Carbon concludes.

At first, the deficient bacteria with the plasmids grew more slowly than normal bacteria, but after three days many grew at the normal rate. "The take-home lesson is that *E. coli* are amazingly versatile," Carbon says. "They can take a segment from eukaryotic DNA and make it work with high efficiency."

Similar experiments using the red bread mold *Neurospora* have produced preliminary evidence that its genes can also function in a bacteria, reports J. W. Jacobson of the University of Georgia. A plasmid containing mold DNA allowed bacteria of a deficient strain to survive in medium not containing certain essential amino acids. Biochemical analysis of normal bacteria and deficient bacteria with the plasmid showed several differences in the relevant enzyme. "We still need to do more experiments," Jacobson says.

Together these results indicate that bacteria may respond to genes from yeast, mold and perhaps other higher organisms more readily than had been expected. □

Carbon dioxide laser: Fusion at last

Scientists have been somewhat skeptical about the practicality of the carbon dioxide laser's ability to induce thermonuclear fusion. Although the CO_2 laser has a high repetition rate and high efficiency, its wavelength, 10.6 microns, was considered too long to attain fusion. Researchers at Los Alamos Scientific Laboratory nevertheless argued that wavelength was not nearly as important as theory had suggested (SN: 11/27/76, p. 340). Last week they announced their two-beam CO_2 laser has achieved fusion reactions resulting in an energy release of 14 MeV per reaction.

Laser-beam fusion has had considerable success using glass lasers doped with neodymium, which produce light with a short wavelength of 1.06 microns. In both systems, laser light is directed at a fuel pellet containing a mixture of deuterium and tritium gas. The energy of the light explodes the pellet's outer shell, causing an implosion of the shell inside and compressing the gas mixture to fuse the atomic nuclei. Scientists had believed that the long wavelengths of the CO_2 laser would waste most of its energy exciting a few electrons on the shell and preheating the target.

The Los Alamos team found this not to be so. Instead, the laser light distributed itself fairly evenly over all the electrons resulting in considerably less preheating of the target.

Heading the project at Los Alamos were Sidney Singer, project leader, and Gene McCall, alternate division leader of the Laser Research and Technology Division. Their targets were standard glass microballoon pellets, measuring about 200 microns in diameter. Maximum power output reached 0.4 terawatt with a pulse length of 1.2 nanoseconds.

The achievement of fusion at Los Alamos appeared to challenge the work on glass lasers continuing at Lawrence Livermore Laboratories. Although glass lasers have been successful their "coolability" has posed a problem. Glass lasers heat up very quickly and take an extremely long time to cool down, making their use in reactor applications impractical. Carbon dioxide lasers, on the other hand, maintain a continual gas flow and therefore remain relatively "cool." Thus, the finding that the short wavelengths of the glass lasers need not be essential to achieve fusion implies that CO_2 lasers may have passed up glass lasers in the race for controlled fusion. No one at Lawrence Livermore or ERDA, however, cared to speculate on such a prospect. Officials at ERDA say they feel it is too soon to begin thinking about abandoning any route to fusion, glass, CO_2 or other.

Just who wins the race to fusion will ultimately depend on the amount of funds each project receives. President Carter has cut some $80 million from the budget for all types of fusion research, $12 million of which came from the Los Alamos 100-kilojoule six-beam laser originally scheduled for completion in 1981. The researchers had hoped that they could scale up the two-beam laser to a 10-kilojoule system by 1978 to produce one percent of the energy required for breakeven. By 1982, the researchers thought they might use the 100-kilojoule system to achieve breakeven, where energy of the fusions produced equals the energy required to cause the fusions. If the budget cuts go through, the schedule would slip at least one year, assuming the funding is replaced the following year. □

Ozone: A world plan of action

A world plan of action on the ozone layer was recommended this week by an international meeting in Washington convened by the United Nations Environmental Program. The meeting grew out of widespread concern that human activities could lead to significant reduction in the protective ozone in a few decades. For nine days, representatives of 30 nations considered reports on research by individual countries, the World Meteorological Organization and the International Civil Aviation Organization. "There was substantial agreement on what we know scientifically," says Edward Epstein, head of the U.S. delegation. The participants considered chlorofluorocarbon emissions from aerosol spray cans to be a matter of serious concern but concluded that the current aircraft emissions probably have a negligible effect on the ozone layer. No conclusion could be reached on the ozone depletion role of volcanoes, solar activity, nuclear explosives or nitrogen fertilizer.

The representatives suggested a three-part program for future study of the ozone layer: study the chemical reactions of the layer and monitor changes around the world, investigate the impact of ozone depletion on humans and the biosphere and evaluate the costs of policies that might reduce ozone depletion.

The participants also requested that UNEP set up a facility to compile and redistribute ozone-related information.

The meeting did not include any discussion of potential regulatory actions.□

MJS christened Voyager

The twin spacecraft of the Mariner Jupiter-Saturn mission (SN: 1/1/77, p. 10), due to be launched this summer, have been renamed Voyager 1 and 2 instead of Mariner 11 and 12. Officials of the National Aeronautics and Space Administration considered more than 100 names solicited from project and headquarters personnel, public affairs officials and the press. The winner was selected despite the fact that Voyager was also the name of a proposed Mars mission that was cancelled in 1968 as being too costly. One awkwardness is that although Voyager 1 will be launched 12 days before Voyager 2, it will be the second of the two to reach Jupiter and Saturn, promising years of confusion. Officials are considering reversing the numbers. □