Gene Transfer Is Coming on Target

The ability to put transferred genes into specific genomic sites may improve the chances of achieving gene therapy and is providing a new way of studying gene action in development

RESEARCHERS ARE NOW LEARNING to put foreign genes into cells, not just anywhere in the recipient genome but exactly where they want them. This new capacity for targeting a transferred gene to a specific site on a chromosome may improve the chances of achieving effective gene therapy for human hereditary diseases, such as sickle cell anemia.

Before these recent developments, it was not possible to control where transferred genes would end up in the genome. They could go anywhere and the chances of a gene's hitting its normal location by accident are small. "The best we could do is put a gene into cells and stand back and pray," says Paul Berg of Stanford University. If the gene inserted at the wrong site, it might, for example, inactivate an essential cellular gene or turn on a cancer-causing oncogene.

The principal problem encountered to date, however, has simply been lack of expression of the transferred genes. When researchers have tested proposed protocols for gene therapy in animals, they have generally found that the transferred genes either did not work at all or else made too little of their protein products to do any good. This probably occurs because the transferred genes do not carry all the regulatory sequences they need for normal expression.

Targeted gene transfer may solve this problem. As Berg points out, "Repairing a gene in situ leaves it intact with all its regulatory sequences. Repairing a gene is far more desirable than pumping genes in wherever they go."

Moreover, targeted gene transfer can be used to introduce specific mutations into mice, as well as to repair gene defects. "The potential now exists to generate mice of any desired genotype," says Mario Capecchi of the University of Utah in Salt Lake City. This could be useful for producing animal models of human genetic diseases and for studying the role of genes in development.

An early demonstration that transferred gene sequences could be directed to a specific site in the genome of mammalian cells came about 3 years ago from Oliver Smithies, who was then at the University of Wisconsin in Madison. Smithies and his colleagues showed that human β-globin gene sequences could be inserted into the β-globin gene of the recipient cells by "homologous recombination."

This procedure requires that the vector used to introduce the new gene into cells carry nucleotide sequences identical to those of the DNA at the chromosomal site where the researcher wants the gene to integrate. The regions of shared nucleotide sequence essentially help the vector to find the desired location and exchange its genetic material with the DNA there. All of the gene targeting work now going on depends on homologous recombination in some form.

The demonstration of the targeted transfer of the β-globin gene attracted a great deal of interest. "People really didn't think [targeted gene transfer] would be possible in an a genome as complicated as the human genome," says Smithies, who has recently moved to the University of North Carolina School of Medicine in Chapel Hill.

Moreover, the β-globin gene is the one defective in sickle cell anemia and β-thalassemia, and therefore a potential target for gene therapy. Immediate application to gene therapy was not possible, however, because of the low frequency of the targeted transfer. Only about one cell in one million integrated the transferred β-globin gene in the endogenous β-globin site.

Over the past year or two, however, researchers have been learning how to improve the frequency of targeted gene transfer and move it into the realm of practicality. This requires finding ways of selecting the relatively few cells that acquire a transferred gene in the correct genomic location from among the many that do not.

Oliver Smithies

A chimeric mouse *made by injecting embryonic stem cells of a black strain into a white mouse embryo.*

For a handful of genes, selection can be based on the gene activity and is relatively easy. This is true, for example, for the gene encoding the enzyme hypoxanthine phosphoribosyl transferase (HPRT), which is the one defective in the fatal human hereditary condition called Lesch-Nyhan disease. Selection can also be aided by including an antibiotic resistance gene, which enables cells to grow in the presence of an antibiotic that would otherwise kill them, with the sequences to be transferred. In addition, some investigators are using the polymerase chain reaction, a newly developed and highly sensitive method of analyzing DNA, to screen directly for cells that have acquired transferred gene sequences in the desired site.

In any event, about a year ago, Capecchi and Kirk Thomas, also of the University of Utah, showed that they could use targeted gene transfer to insert foreign DNA into the HPRT gene, thereby disabling it. In a similar vein, Smithies and his colleagues showed that they could not only inactivate the HPRT gene but could also repair it in cells in which it was already defective.

For the HPRT experiments, the Capecchi and Smithies groups used mouse embryonic stem cells. After serving as recipients for targeted gene transfer, these cells can be put back into mouse embryos to produce chimeric animals in which both the embryo cells and the manipulated embryonic stem cells contribute to tissue formation. Both the Capecchi and Smithies groups have made chimeric mice with embryonic stem cells in which they have modified the HPRT gene by targeted gene transfer.

With luck, the manipulated embryonic stem cells will contribute to the production of sperm and eggs in the chimeras, thus allowing the development of pure lines of mice bearing the altered gene. The researchers are now awaiting the results of breeding experiments to see if this has happened.

Other investigators, including Alexandra Joyner and Janet Rossant of the Mount Sinai Hospital Research Institute in Toronto and Peter Gruss and Andreas Zimmer at

the Max Planck Institute for Biophysical Chemistry in Göttingen, West Germany, have used gene targeting to knock out certain homeobox-containing genes in mouse embryonic stem cells. Homeobox genes play important roles in fruit-fly development, but the functions of the mammalian genes are not known.

The researchers may be able to dissect the roles of the mammalian homeobox genes, by making mice in which the genes have been inactivated. The Toronto and Göttingen workers have produced chimeric mice with the altered embryonic stem cells, and are carrying out breeding experiments to see if the mutated homeobox genes have made it into the germline of the chimeras.

Meanwhile, at the International Genetics Congress that was held in Toronto in August, Berg described his group's recent progress in achieving targeted gene transfer. In one set of experiments, Maria Jasin of the Berg laboratory introduced a bacterial gene into a line of cells carrying a copy of the genome of simian virus 40 (SV40). The bacterial gene and the vector used to transfer it were designed in such a way that the gene was supposed to be expressed only if it inserted into the SV40 DNA. Jasin found that about half the cells that expressed the bacterial gene had it integrated in the desired SV40 site, although other integration sites also conferred activity on the gene.

In another set of experiments, David Strehlow of the Berg group showed that plasmid and cellular DNA could mutually exchange genetic material to correct defective antibiotic resistance genes. "The two genes talk to each other and repair each other's defects," Berg says.

Most of the genes that researchers are going to want to correct or inactivate, as the case may be, do not offer ready selection opportunities, such as those provided by the HPRT and antibiotic resistance genes. Also at the genetics congress, Capecchi described a method that should allow the targeting of any gene in the genome.

The method depends on the use of two selectable genes, one positive and one negative, that are incorporated into the gene transfer vector. The vector is designed so that the negative selection gene can go into the genome only at incorrect sites, allowing those cells to be killed while positive selection can also be applied to identify cells that have the correct integration site. The Capecchi group has used the method to inactivate a mouse homeobox gene and also an oncogene, both of which are otherwise not selectable. All in all, researchers should soon have the ability to apply targeted gene transfer to modify whatever genes they want.

■ JEAN L. MARX

Similar Experiments, Dissimilar Results

Two recent experiments added to the confusion about how high-temperature superconductivity works by reporting inconsistent measurements and coming to opposing conclusions

CONFLICTING RESULTS APPEARED this past week from two experiments designed to test how high-temperature superconductors work. Two groups of researchers, one at AT&T Bell Labs and the other at Argonne National Laboratory, performed similar experiments but obtained different data and came to opposing conclusions. The results reflect the confusion that still exists about the mechanisms behind high-temperature superconductivity.

At issue is why the recently discovered high-temperature materials become superconducting—lose their resistance to electrical current—when cooled past a certain critical temperature. Until 1986, the only known superconductors had to be cooled to at least 23 K (23° above absolute zero, or −418°F), and many scientists thought it might be impossible to find superconductors that worked at significantly higher temperatures. But 2 years ago, IBM's Georg Bednorz and Alex Muller found superconductivity at 30 K in a Ba-La-Cu-O material. The discovery set off a flurry of research that produced several superconductors with much higher critical temperatures, and the current record is 125 K (−235°F) in a Tl-Ba-Ca-Cu-O material.

The problem is that the standard theoretical explanation for superconductivity does not seem to apply to the new materials. Scientists have offered several alternative theories, and experiments such as the Bell Labs and Argonne work are aimed at testing them, with the goal of determining which (if any) of the competing explanations reveal why the new materials work.

The accepted theory for low-temperature superconductors is the Bardeen-Cooper-Schrieffer theory (BCS). BCS traces superconductivity to an interaction between conduction electrons and quantum vibrations (or phonons) in the lattice of atoms that make up the superconductor. This electron-phonon interaction causes the conduction electrons to travel through the superconductor in pairs rather than singly, and this pairing combined with a second effect causes electrical resistance to disappear if the material is cooled to a low enough temperature.

Experiments on Y-Ba-Cu-O superconductors that have a critical temperature of 90 K indicate that the electrons in these materials do travel in pairs but that an electron-phonon interaction plays little or no role in causing the pairing. The recent discovery of a 30 K Ba-K-Bi-O superconductor provided an opportunity to learn more about what is going on, since the new material is not a copper oxide like all other high-temperature superconductors but is still superconducting at relatively high temperatures. The Bell Labs and Argonne groups both looked at the Ba-K-Bi-O material to get clues about the mechanism behind its superconductivity.

To sort out which team is right, the experiment will probably have to be repeated by other groups.

Both groups used a standard test for electron-phonon interactions called the isotope effect test. If electron-phonon interactions are behind the superconductivity of a material, then the critical temperature should vary with the strength of the phonons, or lattice vibrations. One can modify the frequency (and thus the strength) of the lattice vibrations by changing the mass of the atoms in the lattice, which can be done by substituting one isotope for another in the atoms of the material. (Different isotopes of an element have different masses.) Although there are various subtleties, the basic idea is that if electron-phonon interactions play a role, then changing isotopes in a superconductor should modify its critical temperature in a certain, predictable way.

The Argonne and Bell Labs researchers both performed this test on the Ba-K-Bi-O superconductor by substituting ^{18}O for ^{16}O and looking for changes in the critical temperature.

The Argonne group, led by Dave Hinks, found a large isotope effect—the critical