Gene Transfer in Mammalian Cells: Mediated by Chromosomes

The availability of systems for transferring genes between bacterial cells is one of the major reasons bacterial geneticists have achieved great success in gene mapping. Mammalian geneticists have not enjoyed such a tool, although they have made considerable progress in mapping mammalian—including human—genomes within the last decade. Now a new technique, which may be the mammalian equivalent of one of the bacterial gene transfer systems, has appeared. It is called chromosome-mediated gene transfer because it uses purified chromosomes to transfer genetic information from one mammalian cell to another. Investigators think that the technique will greatly facilitate the mapping of the human genome.

Previous research had shown that isolated chromosomes would penetrate into cultured mammalian cells, but there was little or no evidence that the genetic information they carried would be expressed in the new environment. Then in 1973, O. Wesley McBride of the National Cancer Institute (NCI) and Harvey Ozer, now at the Worcester Foundation for Experimental Biology, demonstrated that both replication and expression of chromosomal genes can occur after the uptake of mammalian chromosomes.

To demonstrate this, they first incubated metaphase chromosomes isolated from Chinese hamster cells with a mutant line of mouse cells. (By the metaphase stage of mitosis the nucleus has disappeared, and the chromosomes, which are in their most compact form, are lining up across the center of the cell.) The mutant mouse cells lack an enzyme called hypoxanthine-guanine phosphoribosyl transferase (HPRT) that the Chinese hamster cells contain. Cells that lack this enzyme are unable to grow in a particular culture medium designated the HAT medium because hypoxanthine, amethopterin, and thymidine are three of its essential components.

When the cells that had been incubated with the chromosomes were cultured in the HAT medium, most died, but a few—1 in 10^6 to 10^7—divided and formed colonies. Thus, McBride and Ozer concluded that the growing cells had acquired the missing enzyme. Using electrophorectic and chromatographic techniques, they then showed that the newly acquired enzyme was identical to the one produced by Chinese hamster cells and unlike the mouse enzyme. This is important because the mutant gene that renders the mouse cells deficient in HPRT occasionally reverts spontaneously to the wild, enzyme-producing type. However, McBride and Ozer say that it is highly unlikely that reversion could explain the presence in mouse cells of an enzyme that appears identical to the one characteristic of Chinese hamster cells.

Several investigators have now confirmed the observation that genes transferred between mammalian cells on isolated chromosomes are expressed in the recipients. For example, Frank Ruddle of Yale University and Klaus Willecke, now at the Institut für Genetik in Cologne, Germany, used the technique of McBride and Ozer to transfer the human HPRT gene to mouse cells, which then began to produce the human enzyme. They also estimated the amount of the donor's genetic information maintained in the recipient cells in a functional form.

The Ruddle group had previously shown that the human HPRT gene is located on the X chromosome between the genes for the enzymes glucose-6-phosphate dehydrogenase and phosphoglycerate kinase, a distance estimated to represent no more than 1 percent of the human genome. Since Ruddle and Willecke found that neither of these genes was expressed in mouse cells producing human HPRT, they concluded that only a small chromosomal fragment, less than 1 percent of the genome, had been transferred to the mouse cells. McBride and John Burch, now at Washington University Medical School, have performed a similar experiment with comparable results. Investigators of chromosome-mediated gene transfer generally agree that the chromosomal segments maintained in the recipient cells are small.

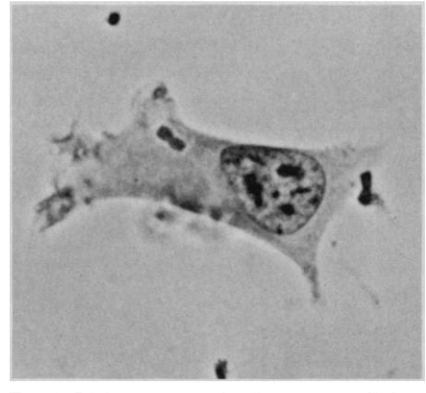

Fig. 1. Light micrograph of a mouse cell that was incubated with isolated chromosomes. The cell appears to contain a chromosome located within a vacuole. [Source: O. Wesley McBride, National Cancer Institute]

The picture being developed is that, after attachment of the chromosomes, the cell membrane of the recipient cells invaginates at the site of attachment and engulfs the chromosomes. Light and electron micrographs show chromosomes, apparently in vesicles, within the cells shortly after they are incubated with purified chromosomes (Fig. 1). However, McBride says that it is difficult to determine by microscopy whether a chromosome is inside a cell and not just adhering to the surface; the best evidence for gene transfer is the presence in the recipient cell of the gene product.

In any event, not even fragments of the donor chromosomes can be seen in recipient cells producing the donor enzyme. Investigators think that digestive enzymes found in lysosomes (small sacs containing enzymes that attack a wide variety of biological molecules) break down the chromosomes that have been taken up. But occasionally a small portion of a chromosome escapes unscathed, possibly because the chromosomal proteins help to protect it, and is maintained in a functional state in the cell, which continues to divide.

The location of this transferred genetic material is unclear, especially immediately after transfer, but accumulating evidence indicates that it may eventually be integrated into the host cell genome. Several investigators have shown that the transferred material is at first unstable but eventually becomes stable. For example, Jerome Eisenstadt and Gerald Degnen of Yale University Medical School found that about 10 percent of mouse cells carrying a transferred gene for HPRT lose the ability to produce the enzyme in each generation. But in a small fraction of the cells (about 1 in 100,000 per generation), stabilization occurs. If large cell populations are serially cultured in selective HAT medium, the cells with the unstable gene form will disappear and be replaced by those with the stable form.

The evidence for integration includes the demonstration by McBride and Raghbir Athwal of NCI that the human HPRT gene can be serially transferred

first to mouse cells and then from the mouse cells to Chinese hamster cells. For the second transfer, the Chinese hamster cells are incubated with chromosomes from mouse cells in which the transferred human gene is stable. Although McBride and Athwal concede that the transferred genetic material might be tightly—but not covalently—bound to mouse chromosomes, they think that the rigorous methods used to purify the chromosomes would remove such material.

In a similar manner, Demetrios Spandidos and Louis Siminovitch of the University of Toronto have serially transferred a gene conferring resistance to the drug methotrexate from one line of Chinese hamster cells to two different lines. However, the genetic material does not appear to integrate into a specific chromosome. Chromosomes can be fractionated on the basis of size into three groups. Spandidos and Siminovitch found that the gene for methotrexate resistance in the original cell line is located on a chromosome in the middle-sized group. When they fractionated the chromosomes from the recipients of the first transfer and used them for the second transfer, chromosomes of all three classes produced recipients bearing the gene. Spandidos and Siminovitch concluded that integration had occurred at more than one site.

More direct evidence for both integration and its nonspecific nature was acquired by Ruddle and R. E. K. Fournier, also of Yale. They first prepared "microcells" from mouse cells carrying the human HPRT gene in a stable form. Microcells have very small nuclei and just a few chromosomes, only a small fraction of the normal complement. Ruddle and Fournier then fused the microcells with Chinese hamster cells lacking HPRT and selected for acquisition of the enzyme by culturing the hybrids (which contain the genetic material of three species) in HAT medium. Finally, they showed that the presence of the human HPRT gene was correlated with the presence of a different murine chromosome in each of three hybrid clones thus selected.

Although the possibility exists that chromosome-mediated gene transfer might one day be applied to achieve genetic engineering, its potential in this regard is unclear. Thus, at present, the prospect that the technique will facilitate gene mapping accounts for much of the interest in the procedure. As with bacterial gene-transfer systems, the relative distances between genes on the same chromosome can be assessed by measuring the frequency with which they are transferred together. Genes that are close to one another are more likely to remain together after transfer than those that are far apart.

Investigators have already confirmed that two genes known to be closely linked are cotransferred. The genes for the enzymes thymidine kinase and galactokinase are located near one another on human chromosome 17. Willecke and his colleagues in Cologne demonstrated that, when mouse cells are incubated with human chromosomes, the two genes are cotransferred about 25 percent of the time. McBride and Ruddle have observed a similar frequency of cotransfer with preparations of Chinese hamster chromosomes.

Spandidos and Siminovitch now have evidence that three genes thought to function in the same biochemical pathway are closely linked on the same chromosome. The genes are the one for methotrexate resistance plus two others that function in the folic acid pathway. They found that these latter two genes cotransfer with the gene for methotrexate resistance. Siminovitch points out that there is very little information about the location of mammalian genes coding for enzymes that act in the same biochemical pathway. In bacteria, such genes are often next to one another and their expression is subject to common controls. Siminovitch says that the finding that three genes in the folic acid pathway cotransfer suggests that some mammalian genes might be arranged in a similar manner, although he and Spandidos do not yet have enough information to prove or disprove this conjecture.

Need for Marker Genes

In order to map genomes with the aid of chromosome-mediated gene transfer, investigators need marker genes with known chromosomal locations and for which there are selection methods. The latter are required to identify the few cells that acquire the marker gene. At present, only a few human genes fulfill both of these conditions. They include the one for HPRT on the X chromosome and that for thymidine kinase on chromosome 17. Investigators would like to develop more marker genes of this type.

But an even more interesting suggestion has been made by Ruddle and Fournier. They say that, because transferred genetic material is stably integrated in a random fashion in recipient cell chromosomes, researchers may be able to introduce markers such as the HPRT and thymidine kinase genes into chromosomes that lack them. Thus, a series of clones, each bearing the new marker at a different chromosomal location, could be produced. The position of recipient cell genes, relative to that of the markers, could then be determined.

Investigators would also like to increase the frequency of gene transfer, which is usually very low. Spandidos and Siminovitch say that their technique is two to three times more efficient in this regard than that of McBride and Ozer. Other researchers are now trying to confirm that observation.

Eisenstadt says that he once thought that the transfer frequency between cells of the same species might be higher than that between cells of different species. But when he and Degnen transferred the HPRT gene from one line of mouse cells to another, the resulting transfer efficiency was comparable to those determined by other investigators for interspecific transfers.

The results of the experiments of Eisenstadt and Degnen did differ from those of other experiments in a significant way, however. They observed that the specific activity of HPRT was substantially—as much as 50 or 60 times—greater in cells in which the gene was unstable than in either the donors or in recipients in which the gene was stable. The specific activities of the enzyme in the last two groups of cells were about the same. Most other investigators found the specific activity of this enzyme to be reduced after gene transfer.

According to Eisenstadt and Degnen, one possible explanation for their observations is that multiple gene copies are present before stabilization, but only one copy becomes integrated into the recipient chromosomes. Another explanation is that the control of the unintegrated form is different from that of the stable integrated gene.

The technique of chromosome-mediated gene transfer is just now being adopted by a number of laboratories, and its full potential for genetic analysis has not yet been reached. There is a chance that before it is, the technique will be superseded by recombinant DNA technology, which can also be applied to genome mapping. Investigators think this unlikely for several reasons, however. Recombinant DNA procedures usually involve bacteria or viruses, a circumstance that has contributed to fears that some kind of pathogenic "super-bug" might be created and has led to the imposition of controls on the research.

For example, polyoma virus and SV40 might possibly be used as vehicles for introducing new genes into cultured mammalian cells. According to the guidelines for recombinant DNA research recently adopted by the National Institutes of

Health, experiments of this type have to be performed in at least a P3 laboratory and many require P4 facilities, which are not yet available in the United States. (P3 is the second highest and P4 is the highest level of physical containment specified by the guidelines.) Chromosome-mediated gene transfer does not involve infectious agents and thus does not share this liability.

Moreover, Ruddle points out that the two techniques ought to be complementary rather than competitive. Gene transfer on chromosomes can be used to determine relative gene positions on the chromosome, whereas recombinant techniques are capable of providing much finer details of molecular structure. The result of a combined effort could be a highly precise picture of mammalian genomes.—JEAN L. MARX

Synchrotron Radiation: Large Demand Spurs New Facilities

From biologists to solid state physicists, researchers have taken to using the intense radiation emitted by electrons orbiting in synchrotrons and storage rings for a wide range of spectroscopic and x-ray diffraction experiments. Interest in this unique ultraviolet and x-ray light source has grown so fast that there are long waiting lines to get on the existing facilities, and projections are for a rapidly escalating demand for them over the next decade. Good news for researchers, therefore, is that the Administration's budget for fiscal 1978 includes funds to build an entirely new synchrotron radiation center at the Brookhaven National Laboratory and to substantially upgrade two existing ones at Stanford University and the University of Wisconsin.

Although it began in Europe in 1964 with the opening of the DESY synchrotron near Hamburg, the age of synchrotron radiation in the United States began in earnest about 8 years ago when the Tantalus I electron storage ring at the University of Wisconsin's Synchrotron Radiation Center at Stoughton became available for full-time use as a light source (Fig. 1). Tantalus I produces light from about 40 angstroms, which is near the boundary between ultraviolet and x-ray wavelengths (also called the "soft" x-ray region), to the ultraviolet. The operating energy of the storage ring is 240 Mev. Within the last 2 years, a similar ring (SURF II) came into operation at the National Bureau of Standards, but its radiation is somewhat less intense.

A big boost for American researchers came in 1974 when the Stanford Synchrotron Radiation Project opened its doors. The high energy of the SPEAR storage ring at Stanford (now at a maximum of about 4 Gev) permitted usable fluxes of x-rays with wavelengths as short as a third of an angstrom. Interest in the use of these so-called "hard" x-rays has mushroomed so fast that, in less than 2 years, the Stanford project has drawn the largest group of users of any of the American synchrotron radiation facilities.

Last year, synchrotron radiation received a bigger boost when the National Research Council issued a report recommending that the present 7 hard x-ray and 16 soft x-ray and ultraviolet experimental stations available in the United States be increased to 60 and 40 stations, respectively, to meet researchers' needs during the next 10 years. The pending budget request for new synchrotron radiation facilities was specifically designed to answer this projected need, providing for more than 80 new stations.

The Energy Research and Development Administration (ERDA) has asked for $24 million to be spent during the next 4 years on the construction of a National Synchrotron Light Source at the Brookhaven National Laboratory. Annual operating costs thereafter are expected to run about $2.1 million, not including research funds. The Light Source will actually consist of two storage rings. A large ring will store electrons at energies up to 2.5 Gev and will provide hard x-rays, while a smaller 700-Mev ring will serve users in the soft x-ray and ultraviolet regions. Scientists from both Brookhaven and the outside will compose an executive committee that selects the experiments to be run from the proposals submitted by researchers. Brookhaven scientists also plan to establish a strong in-house capability in the use of synchrotron radiation. Thus, although the facility is conceived to be a national one, in-house research could claim as much as one-half of the time available on the two storage rings, according to Mark Wittels of ERDA.

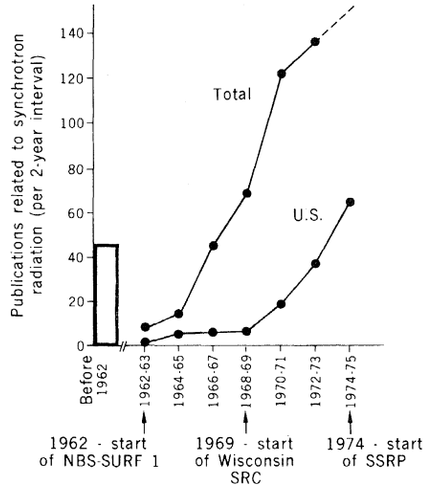

Fig. 1. Number of scientific publications in refereed journals reporting experiments using synchrotron radiation in 2-year intervals. The 5-year lag of American science behind foreign efforts has been attributed to a corresponding lag in the availability of synchrotron radiation facilities in the United States. [Source: Solid State Sciences Committee, National Research Council]

At the same time, the National Science Foundation (NSF) has requested funds to nearly triple the experimental capacity of the Stanford project. The plan is to build a second experimental hall for synchrotron radiation at a cost of $6.7 million. After it is completed in 1980, the new hall will primarily be used for hard x-ray experiments.

The future of the Stanford project is closely linked to the schedule of high energy physics research at the Stanford Linear Accelerator Center, which operates SPEAR. Unlike Tantalus I and SURF II, the SPEAR storage ring is mainly used for high energy physics. A much larger storage ring for high energy physics is being built at Stanford and will be completed sometime around 1980. When the larger ring is finished, there is what William Oosterhuis of NSF calls a pretty firm agreement with accelerator center officials that SPEAR will become available exclusively for synchrotron radiation research about half the time, thus greatly increasing experimental productivity.

The NSF would also like to build a new storage ring at Wisconsin to replace Tantalus I. To be called Aladdin, the new ring would have a higher energy of 750 Mev, so that useful intensities of synchrotron radiation could be obtained farther into the soft x-ray region with wavelengths as small as 10 angstroms. This region of the spectrum is especially interesting to many researchers, not only because it is relatively unexplored, but also because it is where elements with low atomic numbers that are associated with organic compounds absorb x-rays. The estimate is that Aladdin would cost $2.9 million to build over a 3-year period.—ARTHUR L. ROBINSON