Still More About Gene Transfer

New genes can be introduced into the germ lines of mice, but expression of the transferred genes in the mice and their progeny is harder to achieve

"What has come as a surprise to many is how easy it is to incorporate foreign genetic material into the genome of a mammal," said Beatrice Mintz at a recent workshop on the Expression of Cloned Genes in Development.* The remark signals the rapid progress being made in producing for developmental studies new strains of mice that carry foreign genes in their germ lines.

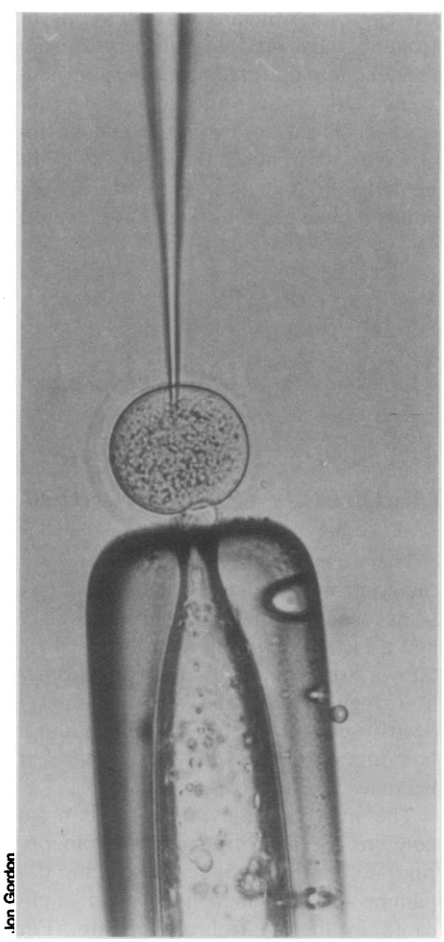
The past year or two have seen the first results of experiments in which cloned genes were injected into fertilized mouse eggs, which were then implanted in foster mothers and allowed to develop (*Science*, 28 August 1981, p. 996). Although some of the mice that developed carried the foreign DNA, the big question was whether the DNA would be stably integrated into the recipient's genome and transmitted to future generations. If it was—and if the genes were functional—developmental biologists could study how a specific gene is turned on and off in various tissues as they differentiate.

That germ line transmission of injected genes can be achieved was made clear by workshop presentations by Mintz, whose laboratory is at the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia; Frank Costantini of the College of Physicians and Surgeons of Columbia University; and Ralph Brinster of the School of Veterinary Medicine, University of Pennsylvania. The past year's experience showed that 20 to 30 percent of the mice that develop from injected eggs may carry the foreign DNA in their genome and transmit it to their offspring.

In one series of experiments Mintz, with Timothy Stewart and Erwin Wagner, injected eggs with a cloned DNA molecule containing both the human β-globin gene and a viral gene coding for the enzyme thymidine kinase (tk gene). Two of the 62 animals that eventually developed carried foreign DNA sequences. One carried part of the human β-globin gene and transmitted the segments to about 50 percent of its offspring—the expected result for a gene carried on just one member of a homologous chromosome pair. The other animal carried intact human and viral genes but did not transmit them to any progeny, possibly because it was a mosaic that had the genes in some cells, but not in others, including the germ cells.

More recently, the Fox Chase workers injected mouse eggs with the human growth hormone (hGH) gene. Six of the 20 animals produced acquired intact hGH sequences, the number and arrangement of transferred sequences varying from animal to animal. The results suggested that the foreign DNA had been integrated into the recipients' genomes. Mintz says, "Five of the six animals transmitted their own characteristic gene patterns to their progeny. Again, about 50 percent of the offspring had the foreign DNA sequences, as expected. The sixth animal seems to have been a mosaic."

Costantini, who described work performed with Elizabeth Lacy in Christopher Graham's laboratory at the University of Oxford, reported that 9 of the 24 mice that developed from eggs injected with the rabbit β-globin gene carried the foreign sequences. One animal had no progeny, but the other eight transmitted the rabbit sequences to about 50 percent of their progeny. Costantini says, "We conclude that we can derive long-term strains of mice carrying the gene in different chromosomal locations."



Jon Gordon

Injecting a fertilized mouse egg with DNA.

In another series of experiments, Costantini and Lacy used a hybrid gene, consisting of control and structural sequences from the 5′ end of the mouse β-globin gene that had been fused to a human β-globin gene segment. Only 3 of 35 mice that developed from eggs injected with the hybrid gene carried foreign DNA. "This is lower than in the first experiment," Costantini remarks, "but still a frequency you can live with."

Finally, Brinster, who collaborates with Richard Palmiter of the University of Washington, also uses a hybrid gene, this one consisting of the 5′ control sequences from a mouse gene coding for the protein metallothionein and the viral tk gene. Brinster and Palmiter find that 20 to 30 percent of the mice that develop from injected eggs carry the tk gene sequences and transmit them to their progeny.

At least in experienced hands, then, introduction of foreign genetic material into the mouse germ line no longer seems to present a problem. But a big question still remains. Will the foreign genes be expressed in these new mouse strains? And here the results have been mixed.

Brinster and Palmiter find that about half of the animals carrying the hybrid gene actually produce the viral enzyme. "Most of the time I expect to have mice that contain the gene and express the gene," Brinster remarks.

Usually the production of the enzyme is very low, even undetectable, unless the animals are first treated with sublethal doses of cadmium or nontoxic levels of zinc. These metals normally induce metallothionein synthesis, and apparently the transferred gene, which carries metallothionein control sequences, responds in a similar fashion. Moreover, the pattern of responses in different tissues resembles that of metallothionein itself. The induced enzyme activity is

*The workshop was held on 29 and 30 September at the National Institutes of Health, Bethesda, Maryland.

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high in liver and kidney and low in brain. As liver and kidney may be more efficient than brain in permitting access of the heavy metal to the cell interior, the apparent cell specificity of the response may be more reflective of the tissue's characteristics than of the gene's. Brinster notes, "Although the pattern of expression is what you would expect of metallothionein, we can't really say yet that it is expressed in a tissue-specific way."

Some puzzles remain about the expression of the hybrid metallothionein -tk gene in mice. For one, the degree of its expression is not proportional to the number of copies per cell. Like the other investigators, Brinster and Palmiter often find multiple copies of the transferred gene per cell. Usually these copies are arranged in a head-to-tail tandem array. But tissues from animals that have many copies per cell do not necessarily make more enzyme than tissues from animals that have only a few copies per cell, and may make less.

For another, the ability to make the viral tk enzyme may not be stably inherited. Offspring carrying the gene sequences may express the enzyme to a greater, lesser, or the same degree as their parents. If the parent was a mosaic, with the foreign gene in only some of its cells, the level of expression of the gene might appear higher in the tissues of its progeny, who would carry the gene in all their cells. Both Mintz and Costantini and Lacy also found evidence for mosaicism in animals produced by egg injection experiments. The loss of expression is more difficult to explain, however.

The Mintz group, in a very early experiment, found production of the viral tk enzyme in one of five fetuses that developed from injected eggs and carried the foreign gene. But preliminary efforts to detect expression of transferred hemoglobin genes have proved less successful. Mintz, Stewart, and Wagner did not find the human β-globin gene sequences to be transcribed in mice that had acquired them.

Costantini and Lacy detected transcripts of the rabbit β-globin gene in muscle tissue from two mice and possibly in testis from another. But all other tissues, including those of the hematopoietic system, which normally make hemoglobin, gave negative results. Nor did they find evidence of transcription of the hybrid gene, even though it carried mouse sequences thought to be involved in initiating gene transcription. "The tentative conclusion," Costantini says, "is that this gene isn't working any better than the rabbit gene."

The gene works in cultured mouse cells, however. Tom Maniatis of Harvard University, who supplied Costantini and Lacy with the hybrid gene, reported at the meeting on work done in collaboration with Richard Axel of the College of Physicians and Surgeons of Columbia University. They transferred the gene into cultured mouse erythroleukemia cells, where the gene was transcribed. The transcription could be increased by treating the cells with dimethyl sulfoxide, as is that of the endogenous mouse gene, although the level of transcription of the transferred gene is much lower. "There seems little doubt that the cell-specific induction of the globin gene can be achieved and studied," Maniatis concluded.

Several theories, which are not mutually exclusive, have been put forth to explain the difficulties in obtaining expression of genes transferred into mice. Methylation of the foreign sequences might occur during early embryonic life and prevent their expression. Detlev Jähner from Rudolf Jaenisch's laboratory at the Heinrich-Pette Institüt of the University of Hamburg, Germany, presented results showing that methylation prevents the expression of viral DNA that is incorporated into the genomes of mice early in development.

The position of the integrated foreign gene sequences is another factor that may influence their transcription. Generally, the transferred DNA integrates randomly throughout the genome, rather than in the normal location for the particular gene. Transferred genes may be missing remote control sites needed for the normal initiation of their transcription.

Control of genes such as the globin gene may be complicated and easily disrupted. Brinster notes, "The β-globin gene is one of the more fastidious genes. It is cell-specific and developmentally specific, expressed only in certain cells at certain times." Still, the gene transfer work is moving rapidly ahead, and reliable expression seems to be an advance that will come with time.

—JEAN L. MARX

Brain Receptors for Appetite Discovered

Amphetamines and their derivatives bind to the receptors, and the strength with which they bind is directly related to their ability to suppress appetite

Three neuropharmacologists have found what appear to be appetite receptors in the brain. Steven M. Paul and Bridget Hulihan-Giblin of the National Institute of Mental Health and Phil Skolnick of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases report their work in this issue (p. 487). The finding should enable pharmaceutical firms to search quickly for new drugs that suppress the appetite without producing undesirable side effects. It also may explain how amphetamines work and may lead to a coherent theory of how appetite is regulated.

There has been no lack of research on the control of appetite but the field has so far been muddled by a surplus of data and a lack of unifying hypotheses. It is known, for example, that eating is somehow regulated by the hypothalamus—destroy part of the hypothalamus and a laboratory animal will overeat until it becomes obese.

The hypothalamic area of the brain contains the neurotransmitters epinephrine, norepinephrine, serotonin, and dopamine—all of which have been implicated in the control of appetite. For example, several groups of researchers have reported that when they inject norepinephrine directly into animals' brains, the animals overeat and that serotonin injections cause the animals to undereat. A few years ago Sarah Leibowitz of Rockefeller University found that when she injected dopamine or epinephrine directly into animals' brains, they undereate.

In addition, drugs that alter the concentrations of these neurotransmitters alter eating patterns. Tricyclic antidepressants cause patients to gain weight apparently, Leibowitz says, by increasing the amount of norepinephrine in their

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