Making Mutant Mice by Gene Transfer

Foreign genes that have been injected into mouse eggs can produce developmentally interesting mutations in the resulting animals

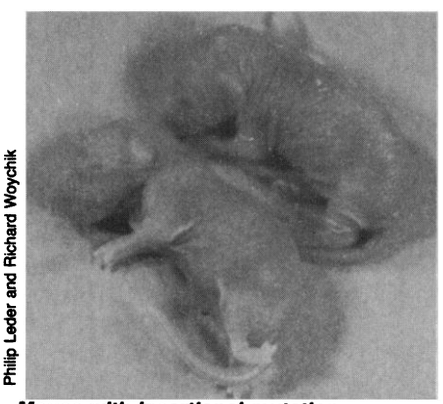
One approach now being used in an attempt to get a handle on the hitherto elusive genes that control development in mice is an outgrowth of experiments in which foreign genes are introduced into the germlines of the animals, thereby creating new strains of "transgenic" mice, as they are called. These experiments were primarily developed for studying the factors that control the expression in the developing embryo of the tranferred genes themselves. But investigators are also finding that the foreign DNA can cause mutations by inserting itself into the recipient's own genes. Some of these insertions have resulted in developmental abnormalities and the foreign DNA can then serve as a tag for isolating the affected genes.

A recent example of a developmental mutant that was produced in this way comes from Philip Leder, Timothy Stewart, and Richard Woychik of Harvard Medical School. According to Leder, who described the results last month at a symposium* on The Molecular Biology of the Cancer Cell, the insertional mutation results in abnormal limb development in mice—and is apparently the recreation of a previously known mutant called "limb deformity."

The foreign DNA used for these experiments was a modified version of the human *myc* gene, one of two dozen or so oncogenes that have been implicated in the formation of cancerous tumors. About a year ago, Stewart and Leder produced tumor-prone mice by injecting fertilized eggs with the *myc* gene and allowing them to develop in foster mothers.

The investigators went on to ask whether the transferred gene might have produced any interesting mutations by interrupting one of the recipient's genes. The kind of mutations they were looking for would not be detectable in the original transgenic animals because they would have the *myc* insert in only one of the two copies of any affected gene and would still retain a functional gene. The Harvard workers thus bred the transgenic mice to produce homozygous animals with comparable *myc* inserts in both gene copies.

The breeding experiment succeeded. One line of the resulting animals turned out to have deformed, flipper-like limbs. Both the hind legs and forelegs of these mice are much shorter than normal. Closer examination showed that the two bones of the lower limbs had fused into one. In addition, the feet are webbed and have three toes instead of the usual five, also as a result of bone fusions.



Philip Leder and Richard Woychik

Mouse with insertional mutation

The mouse in the foreground has deformed limbs as a result of a myc *gene insertion in its genome. The other animal shows the normal limb structure.*

The transferred *myc* gene had apparently interrupted a mouse gene needed for normal limb development. "That gene's function is evidently to ensure the development of the limb bud and in particular of the limb skeleton," Leder explains.

A search of the literature on developmental limb abnormalities in mice produced a surprising, but very encouraging, result. A mutant called "limb deformity," which was discovered in 1960 at Oak Ridge National Laboratory and independently about 5 years later at the Jackson Laboratory, displays the same set of abnormalities as those caused by the *myc* insertion. Moreover, another mutation, which was identified nearly 30 years ago at the Biological Station of Roswell Park Memorial Institute by L. Strong and L. Hardy, produces exactly the opposite effects. The lower limbs of affected mice have extra bones and their feet have more than five toes.

The properties of the limb deformity and luxoid strong mutations are in some ways reminiscent of certain homeotic mutations in *Drosophila* and the flatworm *Caenorhabditis elegans* in which the amount of a gene product determines the ultimate developmental fates of cells. The limb deformity mutation, like that caused by the *myc* insertion, is recessive and presumably caused by loss of the product of the affected gene. In contrast, the luxoid strong mutation is dominant. It may alter control of the limb deformity gene, perhaps leading to increased synthesis of the product.

The indications are that the limb deformity and insertional mutations affect the same gene. Leder and Woychik, in collaboration with Peter D'Eustachio of New York University Medical Center, have mapped the *myc* insert location to a position on mouse chromosome 2, very near, if not at, the location for the limb deformity gene. The luxoid strong gene also maps to that region. In addition, the investigators have mated their mutant mice with limb deformity mutants obtained from the Jackson Laboratory. The results indicate that the two mutations behave as alleles—that is, as variants of the same gene. Finding an insertional mutation that produces such a specific developmental defect, especially one that was already known, required luck, Leder points out. "It is like winning the Massachusetts State Lottery."

Leder and Woychik have isolated the DNA segments flanking the *myc* gene insertion and will determine their nucleotide sequences. If their luck holds, this might provide information about the product of the limb deformity gene and perhaps also provide clues to the function of the luxoid strong gene. However, it is often much easier to isolate a gene than to find out what it does.

Other investigators who are doing mouse egg injections have also found that they can produce developmental mutants in this way. For example, Beatrice Mintz, Luis Covarrubias, and their colleagues at the Fox Chase Cancer Center have identified two independent insertional mutations that cause the deaths of homozygous mouse fetuses shortly after they implant in the uterus. Richard Palmiter of the Howard Hughes Medical Institute at the University of Washing-

*The symposium was held at Harvard Medical School on 13 May. It was dedicated to the memory of Harry D. Williams II, who founded the American Business Cancer Research Foundation.

ton, Ralph Brinster of the University of Pennsylvania School of Veterinary Medicine, and their colleagues have found another type of insertional mutation that apparently disrupts sperm formation by male mice.

In addition, Rudolf Jaenisch of the University of Hamburg and his colleagues have produced a lethal developmental mutation by introducing a virus into early embryos. The mutation, which results in the deaths of the embryos around the 12th day of gestation, was caused by integration of the viral genome in a gene for collagen, one of the body's major structural proteins.

Neither the Mintz nor the Palmiter-Brinster groups have as yet determined the functions of the genes interrupted by the inserts, although they have been able to pull out the mouse sequences flanking the DNA inserts. According to Mintz, integration of the foreign gene, one coding for human growth hormone, produced some unanticipated complications. "In both mutants, there have definitely been rearrangements in the recipient mouse DNA," she explains. "I believe that this is more prevalent than had been expected." The rearrangements may include deletions as well as insertions and inversions of the DNA.

The relative ease with which the DNA alterations occur during these experiments suggest, Mintz points out, that similar rearrangements might have given rise to some of the many mutations known to occur in the mouse. Examination of the bithorax complex, a set of genes that help to control *Drosophila* development, has shown that such major DNA rearrangements are common causes of mutations. Whatever role the rearrangements play naturally, their occurrence during insertional mutagenesis will make unraveling the gene functions much more difficult.

In addition to the general problem of determining gene function, especially when the gene has rearranged, there is also the possibility that an insertional mutation may cause an apparent developmental defect without actually interrupting a developmentally specific gene. For example, it could disrupt a gene needed for one of the cell's common synthetic or energy-producing pathways.

Nevertheless, Mintz remains convinced that insertional mutagenesis is a good way of homing in on the genes that control mouse development. She estimates that incorporation of transferred DNA into one of the recipient animal's genes occurs in up to 20 percent of the animals that develop from injected eggs. That is a sufficiently high frequency to make it worthwhile to do the breeding experiments needed to detect a developmental mutation, especially if the investigator is already doing egg injections. "What has come out of the work so far," Mintz explains, "is an indication that insertional mutagenesis is not a rare event, and with just some luck you may be able to get something interesting." And that is what the Leder group appears to have done.—**JEAN L. MARX**

Additional Reading

1. R. Jaenisch *et al.*, *Cell* **32**, 209 (1983).
2. J. L. Marx, *Science* **226**, 823 (1984).
3. R. D. Palmiter, T. M. Wilkie, H. Y. Chen, R. L. Brinster, *Cell* **36**, 869 (1984).
4. T. A. Stewart, P. K. Pattengale, P. Leder, *ibid.* **38**, 823 (1984).
5. E. R. Wagner, L. Covarrubias, T. A. Stewart, B. Mintz, *ibid.* **35**, 647 (1983).

How Do Proteins Find Mitochondria?

They have signaling sequences, investigators find, that tell them where to go

Liver cells are packed wall to wall with hundreds of mitochondria—the oval shaped organelles that are responsible for the cells' energy metabolism. Other cells may have as few as a dozen or so mitochondria. But every animal cell contains these organelles and every cell presents researchers with a problem: Nearly all of the several hundred proteins contained in the mitochondria are synthesized not in the mitochondria but in the cell's cytoplasm. How, then, do these proteins find their way to the mitochondria and, once there, how do they end up in the correct portion of this organelle, which has inner and outer membranes and a convoluted interior?

The answer seems to be that most proteins destined for mitochondria are synthesized as precursor proteins carrying with them specific amino acid sequences that are recognized by the organelles and that allow the proteins to be taken up by the mitochondria. In most cases, these sequences, called leader sequences, are cleaved after the proteins enter the mitochondria.

So crucial are the leader sequences to mitochondrial import that when researchers purposely put these sequences on non-mitochondrial proteins, they, too, are taken up by mitochondria. But this finding raises further questions, such as, What is it about the leader sequence that the mitochondria recognize and how does this system compare to systems used to direct proteins to other parts of the cell? Several groups of cellular and molecular biologists are actively working on these problems, motivated not just by the fact that technology allows them to address these issues but also by their belief that an understanding of how proteins are imported to mitochondria may have real clinical significance.

Leon Rosenberg of Yale University School of Medicine explains: "One thing I'm sure about is that we'll find that certain diseases of mitochondrial proteins will be diseases of traffic rather than diseases of enzyme activity. The proteins will not function in the mitochondria because they don't get there." Geneticists have described 30 to 40 inherited metabolic disorders characterized by specific deficiencies of imported mitochondrial enzymes. "Until recently, we thought of mutations leading to such enzyme deficiencies in classic terms, that is, as a point mutation, an elongation, or a deletion," Rosenberg says. "Now here's another possible metabolic basis of these diseases—proteins that don't get where they're supposed to go." Defects in the import of mitochondrial proteins might also explain acquired disorders. For example, Reye's syndrome is an acquired disease of mitochondria.

The study of how proteins are imported to mitochondria began in earnest around 1979 when Gottfried Schatz and his colleagues in Basel, Switzerland, and Rosenberg and his associates began to establish that these proteins are synthesized as precursors, that their leader sequences are cleaved in the mitochondria, and that these cleavages occur after the proteins are translated. A second phase, which focuses on understanding the molecular biology of the import of mitochondrial proteins, began in 1982 when Gunter Blobel and his colleagues at Rockefeller cloned the gene for the yeast mitochondrial enzyme cytochrome