# MOLECULAR MEDICINE

## TRANSGENIC ANIMALS

ALAN R. SHULDINER, M.D.

THE photograph of a giant transgenic mouse created by the overexpression of growth hormone, which appeared on the cover of *Nature* in 1982, represented a landmark in molecular biology. The technology for producing transgenic animals was made possible by the coalescence of fundamental discoveries in several disciplines over a period of decades. These discoveries led to important insights into the hormonal control of reproduction in mammals and the development of techniques to harvest, manipulate, and reimplant eggs and early embryos — techniques that are used today by in vitro fertilization clinics throughout the world. Another important development was the use of recombinant DNA technology to isolate, characterize, and cut and paste segments of DNA in virtually any configuration. Although transgenic techniques have been applied to several species, including sheep, cows, goats, chickens, and fish, mice are by far the most successfully and widely used. In this article, I will describe how transgenic animals are produced and used in biomedical research. I will focus on the introduction of exogenous DNA into a fertilized egg for the subsequent expression of a protein product. Modifications of this method are now being used to disrupt, or knock out, the expression of endogenous genes — a topic that will be covered in a subsequent article.

The first step in producing transgenic animals is to construct the DNA to be transferred — the transgene — so that the desired gene product will be expressed in the desired location. This step is accomplished with conventional recombinant DNA techniques. Typical transgenes contain nucleotide sequences that correspond to the gene of interest, with all the components necessary for efficient expression of the gene, including a transcription-initiation site, the 5′ untranslated region, a translation-initiation codon, the coding region, a stop codon, the 3′ untranslated region, and a polyadenylation site (Fig. 1). A critical component of the transgene is the promoter, or regulatory region, that drives transcription. The transgene can be expressed in many tissues of the transgenic animal by using a promoter from a ubiquitously expressed gene, such as that of $\beta$-actin or simian virus 40 T antigen. Alternatively, with tissue-specific promoters, the transgene can be expressed in a given location. For example, selective expression in fat cells is possible with the adipocyte P2 promoter, and expression in muscle can be accomplished by using the myosin light-chain promoter; the amylase promoter confines expression to the acinar pancreas, and the insulin promoter restricts expression of the transgene to islet beta cells.

Introduction of the transgene into the mouse genome requires fertilized mouse eggs (Fig. 1). Injection of gonadotropins (typically, a mixture of pregnant-mare serum gonadotropin and human chorionic gonadotropin) into a female mouse induces hyperovulation, which, followed by natural mating with a fertile male, provides the source of the eggs. The fertilized eggs are harvested before the first cleavage and placed in a petri dish. The DNA construct (usually about 100 to 200 copies in 2 pl of buffer) is introduced by microinjection through a fine glass needle into the male pronucleus — the nucleus provided by the sperm before fusion with the nucleus of the egg. The diameter of the egg is 70 $\mu$m and that of the glass needle is 0.75 $\mu$m; the experimenter performs the manipulations with a binocular microscope at a magnification of 200×. The injected eggs are cultured to the two-cell stage and then implanted in the reproductive system of a female mouse. A total of 25 to 30 injected embryos are usually implanted. After 19 to 20 days of gestation, pups are born.

Typically, 15 to 30 percent of the injected embryos will proceed to term, and 10 to 20 percent of these full-term embryos will have integrated the transgene into their germ-line DNA. These transgenic pups (called founders) are identified by testing their genomic DNA (usually obtained from the tails of the pups) for the transgene by Southern blot analysis or the polymerase chain reaction. Typically, 1 to 200 copies of the transgene are incorporated in a head-to-tail orientation into a single random site in the mouse genome. Since injection and integration occur before the first cell division, all cells of the founders, including the germ cells, will be heterozygous for the transgene.

It is critical to determine whether the transgene is being expressed in the appropriate location. This requires a quantitative assay of the tissue (or tissues) of interest for the messenger RNA or protein (or both) that corresponds to the transgene. The level of expression of the transgene, which can vary greatly among transgenic animals, depends on many factors, including the intrinsic efficiency of the transgene promoter, the number of copies of the transgene that were incorporated, and to some extent, the site of integration within the mouse genome.

Once expression of the transgene has been confirmed, heterozygous founders are mated with nontransgenic mice, and the resulting heterozygous siblings are mated with each other to generate mice that are homozygous for the transgene. These animals constitute a transgenic line. Transgenic lines that have incorporated different numbers of copies of the transgene at different integration sites are usually produced and studied to establish that a given phenotype is indeed due to overexpression of the transgene and not to artifacts of the manipulations.

From the Division of Gerontology and Geriatric Medicine, Department of Medicine, Johns Hopkins University School of Medicine, 5501 Hopkins Bayview Cir., Rm. 5A42, Baltimore, MD 21224, where reprint requests should be addressed to Dr. Shuldiner.

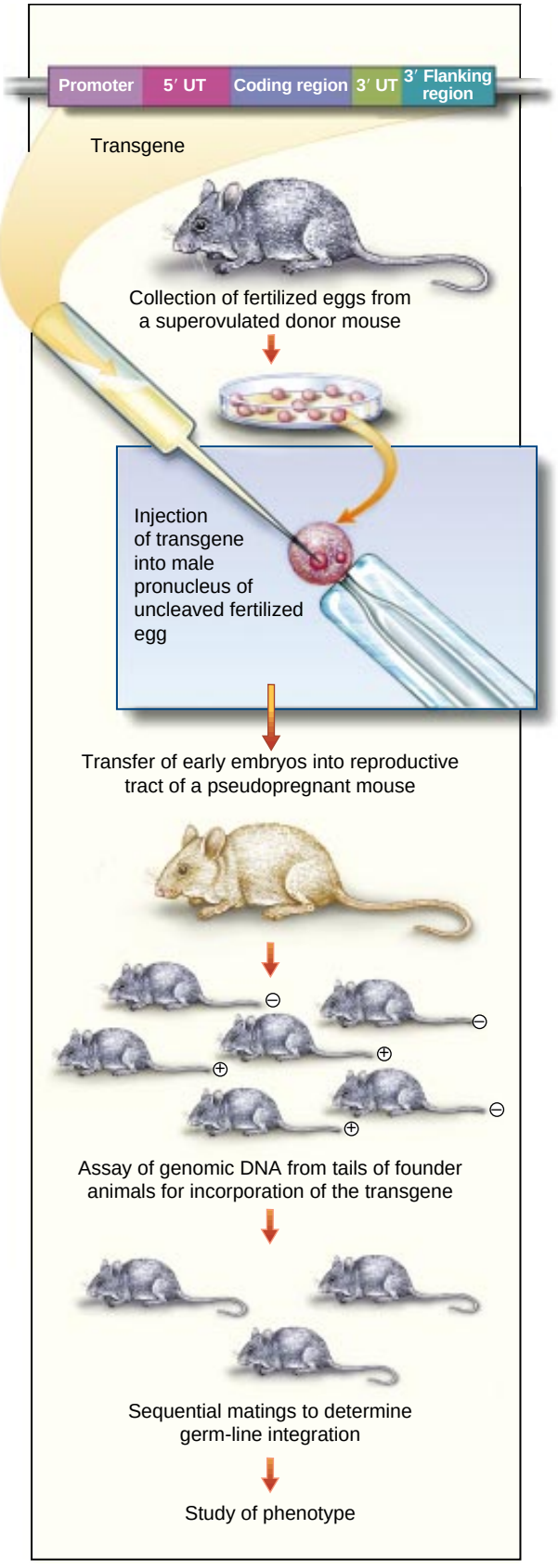


Figure 1. Generation of Transgenic Mice

The transgene containing the DNA sequences necessary for the expression of a functional protein is injected into the male (larger) pronucleus of uncleaved fertilized eggs through a micropipette. The early embryos are then transferred into the reproductive tract of a mouse rendered "pseudopregnant" by hormonal therapy. The resulting pups (founders) are tested for incorporation of the transgene by assaying genomic DNA from their tails. Founder animals that have incorporated the transgene (+) are mated with nontransgenic mice and their offspring are mated with each other to confirm germ-line integration and to establish a line of homozygous transgenic mice. Several transgenic lines that have incorporated different numbers of transgenes at different integration sites (and thus express various amounts of the protein of interest) are usually studied. UT denotes untranslated.

Transgenic animals have been used for simulating diseases and testing new therapies. For example, transgenic animals engineered to overexpress a mutant form of the gene for $\beta$-amyloid protein precursor (the *APP* gene) have neuropathological changes very similar to those in persons with Alzheimer's disease. This model supports a primary role of the *APP* gene in the development of Alzheimer's disease, an idea that heretofore was controversial. The model also provides an opportunity to test methods for the prevention or delay of Alzheimer's disease.

Transgenes that express cytotoxic molecules (so-called toxigenes) have been used to extirpate specific tissues. In one such model, a transgene was created in which the gene for diphtheria toxin was placed under the control of a promoter that drives expression specifically in brown adipose tissue. Expression of diphtheria toxin resulted in the extirpation of the tissue. These animals were extremely obese and had defects in thermogenesis — results that support an important role of brown adipose tissue in the regulation of energy balance. The animals also had insulin resistance and diabetes mellitus, making them an appropriate model for studying the relations among obesity, insulin resistance, and glucose intolerance.

A promising new animal model for determining the extent of DNA damage (mutagenesis) caused by chemical toxins has been developed. The model exploits the transgenic expression of lacZ, a bacterial enzyme whose activity can be measured in vitro with a simple colorimetric assay. LacZ transgenic mice are exposed to the putative mutagen. Relevant tissues are then harvested, DNA is prepared from them, and the lacZ transgene is cloned into a plasmid vector and expressed in bacteria. The frequency of mutations in the lacZ transgene, which corresponds to the number of bacterial colonies that lack lacZ activity, is correlated with the mutagenic capability of the chemical given in vivo. This model has important applications in toxicology, studies of carcinogenesis, research on the role of DNA damage and repair in various diseases, and studies of the normal process of aging.

These are just a few examples of applications of transgenic models in biomedical research. They illustrate the in vivo extension of recombinant DNA technology for the study of specific molecules in diseases and for the production of animal models that can accelerate the discovery of new treatments. Transgenic technology helps set the stage for somatic gene therapy in humans.

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## Recommended Reading

Brinster RL, Palmiter RD. Introduction of genes into the germ line of animals. Harvey Lect 1984-85;80:1-38.

Gossen JA, de Leeuw WJ, Vijg J. LacZ transgenic mouse models: their application in genetic toxicology. Mutat Res 1994;307:451-9.

Lowell BB, S-Susulic V, Hamann A, et al. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 1993;366:740-2.

Pattengale PK, Stewart TA, Leder A, et al. Animal models of human disease: pathology and molecular biology of spontaneous neoplasms occurring in transgenic mice carrying and expressing activated cellular oncogenes. Am J Pathol 1989;135:39-61.

*Padua, Italy* JON PRITCHARD, F.R.C.P.