TRANSGENIC MICE IN IMMUNOBIOLOGY

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Summary

Strategies to generate transgenic mice with defined genetic alterations have evolved rapidly. Highly sophisticated strategies have been developed in which the activity of a selected gene can be spatially and temporally controlled. A large amount of information has been gained by utilizing transgenic mice in the study of complex biological processes such as the immune system. This article describes advances in the technologies available to manipulate the mouse genome. Moreover, applications of these technologies in the investigation of basic immunology and in modeling immunological disorders are outlined.

1. Introduction

There has been a revolution in the development of strategies used in the manipulation of the mouse genome. These technologies represent fascinating tools with which to investigate biological processes in mammals (see Mammalian Cell Culture and Genetic Engineering of Mammalian Cells). Although the ability of the mouse to model human diseases has been criticized, a consensus exists that the majority of physiological and chromosomal events are mediated in a similar manner in both species. Since both
species also share similarities in their anatomy, this can be utilized in the examination of developmental processes in humans. Relevant in vivo models are essential in developing strategies to prevent or treat human diseases as in vitro systems can never provide the complex and tightly regulated environment of organs and cells present in the living organism. Furthermore, sophisticated biological processes such as the immune system cannot be investigated in lower organisms (e.g., bacteria, nematode worms, or fruit fly), they must be analyzed in organisms that possess such processes.

Several human immunological diseases are caused by chromosomal aberrations such as gene deletions, duplications, inversions, translocations, and point mutations (see Methods in Gene Engineering). These events can impair the function of the gene product directly, or they can interfere with gene regulation. Chromosomal defects can be spontaneously induced, but exposure to external factors, e.g., X-ray radiation and certain chemicals can substantially increase the frequency of gene aberrations. Gene-manipulated mice with defined alterations in their genome can be exploited in modeling gene-mediated human diseases. Such mice are also useful in the investigation of which biological processes are required to respond efficiently against pathogens, or to examine homeostasis in the organism. This review describes advances in the transgenic technologies available to manipulate the mouse genome. Moreover, applications of these techniques in the investigation of basic immunology and immunological diseases are outlined.

2. Strategies to Generate Transgenic Mice

Several mouse strains with spontaneous mutations have been identified over the years and permanent colonies of these mutant strains have been produced, but because of the stochastic nature and the low frequency of the spontaneous mutation this approach is not suitable for the generation of specific gene defects. Exposure to certain chemicals and radiation has also been used to increase the mutation frequency in mice, but the unpredictable nature of the mutation process makes this approach difficult to use in an efficient way. In the early 1980s, investigators introduced cloned DNA directly into fertilized mouse eggs by microinjection and were able to generate transgenic mice with stable integration of foreign DNA into the host genome. The limitation of this approach was that transgene integration was a random process (see Transgenic Animals). The revolution in gene targeting occurred in the late 1980s by an ingenious combination of two different techniques: 1) the generation of the pluripotent embryonic stem (ES) cells in culture and 2) the development of in vitro gene targeting methodology in mammalian cells by homologous recombination. Thereafter tremendous progress was made in the generation of gene-manipulated mice. In early 1991, there were seven targeted mutations described in the literature whereas a mere six years later more than 700 mutations had been reported. Strategies to manipulate the mouse genome have evolved rapidly, utilizing more and more sophisticated techniques that can be applied in the study of complex biological processes.

2.1. Gene targeting with homologous recombination

The defining property of ES cells is their stable maintenance of a pluripotent differentiation potential, as demonstrated by the expression cell markers for the
pluripotent cell but not those of differentiated cells. ES cells can be cultured in large numbers which permits the genetic modifications of these cells in culture. ES cells also have a remarkable ability to colonize the host embryo, which is crucial in transferring genetic information from ES cells into the germline. Advances in controlling ES cell differentiation have also provided insights into cell biology and the determination of cell fate.

In homologous recombination, the enzymatic machinery of the recipient cells replace homologous endogenous DNA sequences with exogenous DNA sequences. By utilizing specific targeting vectors that are transferred into ES cells, the desired endogenous gene can be permanently eliminated, resulting in gene knockout cells (Figure 1A). Alternatively, targeting vectors can be designed to transfer foreign DNA into the desired site in the host DNA without disrupting the endogenous gene resulting in knocking cells (Figure 1B).

![Diagram](image.png)

Figure 1. Generation of knockout and knockin cells by homologous recombination. A) Targeting vector contains sequences homologous to endogenous gene and a selection cassette (neo) inserted into an exon. Homologous recombination event replaces genomic sequences by vector sequences and disrupts endogenous exon by neo. B) Knockin strategy. DNA of interest will be inserted in-frame in an endogenous exon to be
targeted. The transgene will be expressed in the desired place of the host DNA without disrupting the endogenous gene.

Because the frequency of the homologous recombination event is low, investigators have devised strategies to enrich cells in which homologous targeting events have taken place. The most frequently used approach is a “positive-negative” selection strategy (Figure 2). This procedure has two components: a) a positive selectable gene to enrich recipient cells that have incorporated the targeting vector somewhere in their genomes, and b) a negative selectable gene to eliminate cells in which the targeting vector has been incorporated randomly into nonhomologous sites in the genome. To achieve this kind of selection, the transfected ES cells are cultured in a medium containing a drug for positive selection which kills cells that lack the protective drug resistance gene, derived from the targeting vector, and a drug for a negative selection destroying cells containing a negative selectable gene that is incorporated into genomes only if the targeting vector is incorporated at nonhomologous sites. This strategy guarantees an enrichment of targeted versus nontargeted ES cells by up to 50-fold and is the approach used to generate most of the gene-knockout mice reported so far.
Figure 2. Positive-negative selection strategy for enrichment of targeted ES cells. A) In a homologous recombination event, only a positive selectable gene (neo), derived from the targeting vector, is incorporated into the desired place in the genome, whereas a negative selectable gene (HSV-) is not incorporated. This generates targeted cells that are resistant both to a drug for positive selection and a drug for negative selection. B) In a random integration event both a positive selectable gene (neo) and a negative selectable gene (HSV-) are incorporated into the genome. This generates cells that are resistant to a drug for positive selection but are sensitive to a drug for negative selection.
Figure 3. Generation of gene targeted mice. The first step involves introduction of targeting vector into ES cells. A positive-negative selection strategy is used to enrich the targeted ES cells. Selected targeted ES cells are injected into blastocysts which are in turn surgically transferred into the uterus of foster mothers generating chimeric progeny.

Chimeric mice are crossed with wild type mice to establish a stable germline transmission of transgene. The progeny derived from the chimeras and wild type mice are characterized and a stable mutant mouse line carrying the transgene is generated.
The next step in the generation of gene-targeted mice includes in vitro microinjection of selected ES cells which contain the desired gene modification into mouse preimplantation embryos, termed as blastocysts (Figure 3). Blastocysts are in turn surgically transferred into the uterus of hormone-treated foster mothers, resulting in generation of chimeric progeny (Figure 3). Chimeric mice have mosaic-like tissues composed of a random distribution of wild type cells and knockout cells. To facilitate isolation of the chimeric progeny with a high degree of gene-targeted cells, the ES cells and the recipient blastocysts are derived from mice with different coat color alleles. This allows evaluation of the extent of chimerism by the coat color chimerism. Chimeric mice are then crossed with wild type mice to establish a stable germline transmission of the modified genome (Figure 3). The progeny derived from the chimeras and wild type mice are characterized and the mice with gene modification are selected. These mice are used to generate the stable mutant mouse line that carries the desired gene modification in their germline.

Bibliography


**Biographical Sketch**

**Harri Alenius** has an M.Sc. from the Department of Cell and Molecular Biology, University of Jyväskylä, and a Ph.D. from the Medical School of the University of Tampere, where he is also a Docent. He is the permanent Chief of the Laboratory of Immunotoxicology at the Finnish Institute of Occupational Health. Dr. Alenius was a Research Fellow at the Medical College of Wisconsin, US, and a postdoctoral fellow at Children’s Hospital, Harvard Medical School, Cambridge, MA, US. He has written more than 50 articles in the field of Allergy and Immunology, and is a reviewer for *The Journal of Immunology*, *Int. Arch. of Allergy and Applied Immunology*, *J. Allergy Clin. Immunol.*, *Clin. Exp. Allergy*, and *Allergy*.