Major Approaches for Generating and Analyzing Transgenic Mice

An Overview

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Over the past decade, the development of gene-transfer technology in whole animals has afforded unprecedented opportunities for investigators to probe complex regulatory systems in vivo. Important advances in our understanding of the mechanisms of gene expression and regulation and the development of animal models of human diseases are but two examples of how this technology has affected medical science. Transgenic animals are defined as animals in which a segment of DNA has been physically integrated into the genome of all cells, including the germ line, so that it can be transmitted to offspring as a simple Mendelian trait. The DNA segment generally consists of a whole cloned gene, cDNA, or a novel gene modified by recombinant DNA methodologies. Whole genomic clones of genes are often used to study tissue- and cell-specific expression and regulation or can be used to overexpress a gene product. Alternatively, the coding region of one gene can be fused to the transcriptional regulatory region of another gene, causing it to be expressed in a new spectrum of tissues and cell types. A number of methods can be used to introduce the DNA segment, including direct microinjection of one-cell fertilized embryos, retroviral-mediated transfer, or gene transfer in embryonic stem cells. The technique most often used to generate transgenic animals and perform "gene addition" experiments is direct microinjection. Alternatively, gene deletions or "knockouts" are performed by gene transfer in embryonic stem cells by specifically targeting the site of integration in the genome. This methodology has the potential to be used to generate specific gene mutations and has great applicability for the creation of animal models that emulate human diseases (such as cystic fibrosis). An overview of the technology available for generating transgenic animals, the relative merits of the different gene-transfer methods, and a brief description of some of the questions that can be addressed using transgenic animals are discussed.
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FIG 1. Photomicrographs of a one-cell fertilized mouse embryo visualized with Nomarski DIC optics. The egg is held in place by a holding pipette (h), and the female (f) and male (m) pronuclei are visible. The injection needle (i) is resting outside the cell in A and has microinjected a DNA solution into the male pronucleus of the egg in B. Note the increase in pronuclear circumference in B. Equipment needed to perform microinjections includes a microscope containing a ×10 eyepiece, ×4 and ×40 lenses, and Nomarski DIC or Hoffman optics. Micromanipulators are mounted on each side of the microscope (one each for holding pipette and injection needle) and can be hydraulic or mechanical. Microinjection can be controlled with a syringe or pneumatic pump. Dissecting microscopes with both direct and reflected illumination are needed for egg manipulation and reimplantation; a microforge is needed to polish holding pipettes and implantation pipettes; a pipette puller is needed to reproducibly generate submicron tip injection needles; and a humidity-controlled tissue culture incubator is needed for culturing eggs.

A

B

Preliminary review of these methods, I will briefly describe each technique and their various benefits and pitfalls.

Generation of Transgenic Mice by Microinjection

By far the most common and well-characterized approach for producing transgenic mice is direct pronuclear microinjection of one-cell fertilized embryos. Microinjection is relatively easy to learn and perform, if appropriate equipment is on hand (see legend to Fig 1), and generally results in 5% to 40% of live births being transgenic. Eggs obtained from superovulated female donor mice are injected with an ultrathin drawn glass capillary pipette (<1 μm tip diameter) filled with a solution containing the transgene construct of interest. Approximately 1 to 2 pL of solution is delivered into the male (larger) pronucleus until a visible increase in pronuclear circumference is observed (Fig 1). The surviving eggs are incubated until they divide to the two-cell stage; then they are surgically reimplanted into the oviducts of pseudopregnant foster mothers. Births, a fraction of which are transgenic, occur 19 days later.

Female donor mice are hormonally superovulated by the administration of pregnant mares' serum (PMS, 10 U IP) and human chorionic gonadotropin (hCG, 10 U IP). The timing of these injections is fairly critical; the PMS and hCG injections must be separated by 48 hours, and the hCG injection must precede an endogenous luteinizing hormone surge. The females are bred to male mice overnight, and fertilization is confirmed by the presence of a vaginal mucous plug in the morning. The oviducts are surgically removed, and the eggs are flushed into tissue culture media. A typical egg yield is 15 to 20 per oviduct; a variable fraction of which are unfertilized, exhibit polyspermy, or are otherwise uninjectable. The pseudopregnant foster mothers are generated by breeding female mice with vasectomized or genetically sterile males. A schematic representation of the events described above is presented in Fig 2. Details of these procedures, including a comprehensive review of the equipment and protocols, have been published previously.

DNA segments delivered into the nucleus by microinjection integrate at random in the genome, and although multiple insertion sites have been reported, they generally insert at a single site per diploid genome. Finding multiple copies (in tandem head-to-tail arrays)
of the transgene at the insertion site is a routine occurrence, with the copy number ranging from one to several hundred (Fig 3A). Unfortunately, transgene expression is rarely proportional to copy number. In addition, the random insertion of DNA can have several detrimental consequences that need to be addressed in a typical transgenic experiment. For instance, the tissue- and cell-specific expression profile exhibited by the transgene can be altered as a result of juxtaposition of the transgene near transcriptional regulatory elements controlling another gene. The result, termed a "position effect" or "position artifact," can be manifested by higher or lower expression than normal, partially inappropriate, or completely inappropriate tissue-specific expression. Examination of multiple independent transgenic lines—each generated by an individual, and therefore unique, insertion—allows investigators to distinguish these position artifacts from true expression.

FIG 2. Schematic shows time course of events during production of transgenic mice. Injection of pregnant mares' serum (PMS) is designated as day 1. Human chorionic gonadotropin (hCG) is injected on day 3, and matings between donor mice and male studs of the same strain are set up overnight. On day 4, embryos are removed from donors, briefly cultured, microinjected, and cultured overnight until they divide to the two-cell stage. Foster mothers are bred to vasectomized male studs or genetically sterile males on day 4. Reimplantation of the two-cell stage-injected embryos is on day 5. Mice are born 19 days later (embryonic day 21, E21). More details on the time course of this procedure have been previously reported.

FIG 3. Identification of transgenic mice, with an example of Southern blotting (B), dot blotting (C), and polymerase chain reaction (PCR) (D) shown for differentiating transgenic mice containing the human renin gene from negative littermates. A: Schematic map shows the organization of human renin transgenes in the genome. Multiple copies of the human renin gene are arranged in a head-to-tail array. Two complete and two partial human renin gene units are shown for simplicity. The restriction endonuclease sites used in the Southern blot are shown (B, BamHI; E, EcoRI). The position of the hybridization probe is indicated by the closed bar; sizes of the fragments detected by the probe are indicated. B: Southern blot analysis of genomic DNA isolated from tail biopsies and digested with BamHI (left) and EcoRI (right). In both blots the large molecular weight bands result from fusion between the 3' end of one transgene unit and 5' end of the adjacent unit. The endogenous band in both blots is barely visible at this exposure. C: Dot blot hybridization of undigested DNA loaded onto nitrocellulose using a manifold is hybridized with a partial human renin cDNA probe. Identification of positive and negative transgenic mice is indicated. The copy number of the transgene in this case easily allows us to differentiate transgene sequences from endogenous gene sequences. D: PCR analysis of crude tail DNA preparations. PCR was performed using primers that specifically hybridize to the human renin gene and 35 rounds of amplification. Identification of positive transgenic mice and negative littermates is indicated. The lower band in each lane is the unextended oligonucleotide primers.
A second possible consequence of random integration is the potential to induce a mutation caused by the disruption of an essential gene. Although these mutations can often go unnoticed until the animals are bred to homozygosity (because only one chromosome of the diploid set is affected), one needs to be aware of this possibility when designing a breeding scheme for propagating a transgenic line. A variety of transgene-induced mutations have been reported in the literature.12-14 These mutations often have been extremely interesting and have sparked further study (see below).

Generating Transgenic Mice by Retroviral Transfer and Embryonic Stem Cells

An alternative method for generating transgenic animals is infection of embryos with retroviral constructs. A number of retroviral vectors are available that allow genes to be inserted and packaged into viral particles.15,16 These viral particles can be used to infect a wide range of cell types, including embryos. Infection can occur through direct exposure to retroviral particles or by cocultivation with virus-producing cell lines. One advantage of this technique is the ability to infect preimplantation embryos at the 1-cell through the 8- or 16-cell stage. This has applicability to cell-lineage studies, which can benefit from the purposeful generation of mosaic transgenic founder animals. Mosaic animals do not contain a copy of the transgene in every cell, and although mosaicism does occur in transgenic mice produced by microinjection, the process is unpredictable and stochastic. However, it is important to realize that the utility of the technique to study lineage relationships in mosaic animals is limited to the founder generation because all subsequent generations will contain transgene DNA in all cells. An additional advantage of retroviral-mediated transfer as compared with microinjection is the relative ease by which DNA sequences flanking the position of the transgene insertion can be cloned. The reason for this is that transgenes delivered by retroviral infection integrate into the genome in a single copy. The retrieval of flanking sequence, which can be complicated by the presence of multimers of the transgene in transgenics produced by microinjection, is the first and most important step in identifying and cloning genes mutated by transgene insertion. Disadvantages of the technique include the need to manipulate and produce viral particles and a limitation on the size of the transgene genome being packaged (in the 8-kb range).

Both microinjection and retroviral-mediated gene transfer suffer from the inability to specifically target the chromosomal location of the insertion event. An alternative to both of these methods is now gaining in popularity because of the ability to precisely target transgene insertion. This procedure involves the use of embryonic stem (ES) cells and constructs designed to homologously recombine into the mammalian genome. A review of these methods is described in an accompanying article in this issue of Hypertension.17 Briefly, ES cells are pluripotent stem cells derived from the inner cell mass of the mouse blastocyst. These cells can be cultivated under conditions that prevent their differentiation but retain their potential to repopulate the entire embryo. Furthermore, these cells can be genetically manipulated by transfection or infection with constructs designed to homologously recombine (exchange genetic information) with regions of identical sequence on the chromosome. The greatest advantage of this methodology is the ability to specifically mutate (create a null mutation in) a gene located in the host genome. Because the cells are pluripotent, genetically modified ES cells can be reintroduced into preimplantation mouse blastocysts, where they become part of the inner cell mass. These blastocysts can be reimplanted into the uterus of a foster mother, where they will develop into genetically mosaic animals. The mosaicism is due to a mixture of parental blastocyst-derived and ES cell-derived tissues. The extent of mosaicism can be easily visualized if the ES cells and host blastocysts are derived from mice with different coat colors. The resultant mosaics have a mixture of colors, and the extent of mosaicism is roughly reflected in the relative contribution of the two colors. Transmission of the disrupted genes to offspring can occur if the germ line of the mosaic animal is ES cell-derived, and germ line transmission of the ES cell genome results in mice with a homogenous coat color, that is, ES cell-derived.

The greatest disadvantage of this technique is the effort required to differentiate and detect a small number of homologous recombinants in a vast excess of random integrants, although modifications in the design of the recombinant vectors can add an element of selectivity to the process. For example, investigators have had success using the herpes simplex virus thymidine kinase (HSV-TK) gene to enrich for specific targeting events. The HSV-TK gene, when expressed, renders the cells sensitive to the drug gancyclovir, which kills TK+ cells. If the gene is placed at either or both ends of the targeting construct, it will remain intact and integrate along with the transgene during random integration events but will be deleted if the transgene integrates by homologous recombination. If a selectable marker such as neomycin resistance is used as part of the targeting construct, then selecting for neomycin and gancyclovir resistance will enrich the overall population (10- to 100-fold) for specific targeting events and therefore increase the chances of detecting the rare homologous recombinants.

Another common problem is the low frequency of germ line transmission of the ES cell genome. Nevertheless, the potential to use the power of selective mutation to the mammalian system outweighs such technical difficulties. This is certainly borne out in the recent development of important human disease models in mice.18 An extensive review of factors influencing homologous recombination in ES cells is available.19-21

Analysis of Transgenic Animals: Differentiating Transgenic Offspring From Nontransgenic Littermates

In practice, transgenes integrate into the genome as little as 5% or as much as 30% to 40% of the time. Therefore, transgenic animals must be identified by assaying for the presence of the transgene in the genome. Three general methods have been used to assay for the presence of transgene sequences: restriction enzyme analysis, DNA sequencing, and Southern blotting. Nontransgenic DNA, and the polymerase chain reaction (PCR) (examples of each are shown in Fig 3). Typically, the trans-
gene becomes part of the genome of all tissues, allowing DNA to be purified from a small tail biopsy sample without the need for an invasive surgical procedure. Southern blotting, while being the most labor intensive of the assays (because of the need for highly purified DNA), is certainly the most stringent test for transgene insertion. The most important use for Southern blotting is when restriction fragment length polymorphism analysis is required to differentiate the transgene from a closely related endogenous gene already present in the mouse genome. We routinely perform Southern blotting to identify putative transgenic founders.

Dot blotting (the application of denatured genomic DNA directly to a solid membrane support) followed by hybridization can be performed if the transgene sequences are unique to the mouse genome, such as a bacterial reporter gene or viral oncogene. Homologous sequences can be detected on dot blots if they are present in sufficient copy. In the latter, the hybridization intensity is used to differentiate transgenic from non-transgenic mice.

More recently, the application of PCR has been used to rapidly identify transgenic animals. Because even crude DNA preparations yield PCR amplification products, investigators are able to go from tissue sample to results in a single day. The major requirement for successful PCR is highly specific primer sequences that are capable of differentiating transgene sequences from endogenous gene sequences. Again, this is easy if the transgene is unique to the genome. Minor sequence polymorphisms, such as those present between different alleles of a gene or interspecific genes, can easily be used successfully to differentiate homologous sequences. In this case, polymorphic residues are placed at the 3' end of the oligomers, resulting in destabilization of the hybrid formed between the oligonucleotide primer and a mismatched sequence. Application of PCR to identify transgenic mice containing the human renin gene is illustrated in Fig 3.

Experimental Design: What Type of Construct Should I Design?

Transgenic animal experiments are classically gene addition experiments and can take several forms, including, among others, overexpression of protein products in normal and abnormal sites of synthesis, the use of reporter genes for identifying cell types expressing a gene, and mapping of transcriptional regulatory elements controlling gene expression. The design of the transgene constructs outweighs all other considerations in determining the types of questions that can be addressed in transgenic animals. Fig 4 illustrates several general classes of constructs that can be designed. The remainder of this article will focus mainly on transgenic mice generated by microinjection and gene addition experiments.

Investigators have often been faced with the problem of determining the function of a protein in regulating or mediating a physiological response. One of the ways this has been addressed is by studying the physiological consequences of acute or chronic protein overproduction in a whole animal model. Indeed, this has been and continues to be an important question in whole animal physiology. Although short-term effects can be examined by direct administration of a protein to an animal, elucidating long-term effects can be much more laborious because of difficulties in long-term delivery (days, weeks, or even months) of a protein in vivo. Transgenic animals can be designed to act as "chronic protein delivery systems"; in essence, this is the quintessential question addressed in a transgenic animal model. There are several approaches through which this can be accomplished.

The first is overproducing a gene product in its normal sites of synthesis. For this to be achieved, one needs to be able to direct the synthesis of the gene product to the appropriate cells, using transcriptional regulatory elements active in that cell type and the protein coding region of the gene being produced. For all essential purposes, complete genomic clones that contain all exons and introns and sequences extending upstream (5') and downstream (3') of the gene already have the information required to accomplish this. Transgenic mice containing genomic clones that encode the mouse and human renin genes have been successfully used to target expression to the appropriate spectrum of tissues and cells, with overexpression of the mRNA and protein occurring in several tissues. 23-27

Examples of human renin expression in transgenic mice are shown in Fig 5 and also have been previously reported. 27 The data shown in the figure are from transgenic mice containing a human renin genomic segment including all 10 exons and introns and sequences extending approximately 900 bp upstream and 400 bp downstream of the gene. Fig 5A and 5B show sample data on human renin transgene expression in the adrenal gland of three different lines of transgenic mice. Both mouse and human renin messages are visualized in A, but only human renin mRNA is detected in B (see legend). As can be seen, human renin mRNA is clearly expressed in the adrenals of the transgenic animals, and no human renin is observed in the adrenal glands from nontransgenic DBA/2J (D) or C57BL/6 (B) mice. Adult C57BL/6 mice do not express adrenal renin, 23 and the genetic background of the transgenics is based on a strain with a similar adrenal renin expression profile. Fig 5C shows a sample of human renin expression in the kidney of a transgenic mouse and a nontransgenic littermate. The assay is a differential primer extension assay capable of differentiating the closely homologous mouse and human renin mRNAs based on minor sequence polymorphisms. 23 Approximately equal levels of mouse and human renin mRNAs are expressed in kidney. This is consistent with the observation that approximately equal amounts of active mouse renin and active human renin were released into the systemic circulation (Fig 5D). These mice are currently being used as models to examine the regulation of human renin gene expression and secretion and also to determine if they will provide a model for examining the regulation of human renin expression during experimental or genetic hypertension.

Clones comprising only a cDNA (DNA copy of an mRNA) fused to regulatory sequences from a gene (minigenes) can also be used to target cell-specific expression, although the use of cDNA clones has been less predictable than the use of genomic clones. This is apparently due to the observation that the presence of spliceable intron sequences is required for high-level expression and proper maturation of an mRNA. The use of a DNA cassette containing a cloned heterologous
### Construct Type | Explanation | General Structure | Function
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Genomic | DNA segment from genomic clone containing 5' and 3' flank, all exons and introns | ![Genomic construct](image1) | Study tissue- and cell-specific expression; Overproduce gene product in normal sites of synthesis; Transgenes should respond to signals which normally regulate expression of gene |
"mini-gene" | DNA segment containing 5' flanking sequence fused to cloned cDNA and artificial intron + poly A site. | ![Mini-gene construct](image2) | Essentially the same as for genomic constructs; Constructs are generally expressed better if artificial intron and poly A addition sites are added; Most important when genomic clone spans large distance. |
Heterologous-Promoter | DNA segment containing promoter element from another gene fused to the protein coding region of interest | ![Heterologous-promoter construct](image3) | Used to impart a new tissue- and cell-specific expression profile on the gene being expressed; By using a well-characterized heterologous promoter a gene's mRNA and product can be redirected to a new spectrum of tissues and cells. |
Reporter Fusion | DNA segment containing 5' flanking region of interest fused to reporter gene. | ![Reporter fusion construct](image4) | Used to examine the cellular sites of gene expression and mapping transcriptional regulatory elements in the 5' flanking region of a gene; Expression of bacterial genes may benefit from intron and poly A site. |
Oncogene Fusion | DNA segment containing 5' flanking region of interest fused to oncogene | ![Oncogene fusion construct](image5) | Similar to reporter gene in that promoter element directs synthesis of oncogenic product to specific cells. Oncogene can be used as reporter. Most often used to target dysplastic or neoplastic growth to specific cells and for isolating novel cell lines |

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**Fig 4.** Summary of the various types of constructs that can be designed for use in transgenic mice. A schematic representation of each construct is included, in which the hypothetical gene of interest has four exons (horizontal hatching) separated by introns in the genomic context but spliced together in the minigene context. The promoter for this gene is indicated by a thin line labeled 5' flank. A heterologous promoter is so labeled and indicated by a thick line. The intron–poly A segment used in the minigene, heterologous promoter, and reporter constructs can be any of a number of DNA cassettes containing a cloned intron and polyadenylation recognition site. The various DNA segments are assembled using standard molecular cloning techniques. CAT, chloramphenicol acetyltransferase.

intron linked to a polyadenylation recognition site may help eliminate such problems (Fig 4). Expression of a mouse angiotensinogen minigene has been previously reported in transgenic mice. A limiting factor in overproduction of a gene product using its endogenous promoter elements may be the tight restriction imposed on the level of gene transcription, which is caused by the presence of regulatory signals in the promoter designed to prevent uncontrolled overproduction of the final protein product. For example, overproduction of renin in the kidney can potentially induce a cascade of events whereby elevated levels of circulating angiotensin II feed back on the kidney and attenuate expression of the renin gene. Regulatory input may also be received as a result of physiological perturbation. Using the same example, increased arterial pressure caused by elevated circulating angiotensin II could trigger baroreceptors and cause a reduction in expression of the renin gene in the kidney. Transgenic rats containing a mouse renin genomic construct exhibit a markedly elevated arterial pressure and severely attenuated level of renal renin mRNA. An alternative method for overproducing a protein is to place the protein coding region of the gene under the control of a heterologous promoter element that is unresponsive to regulatory inputs controlling expression of the gene. Overproduction of atrial natriuretic factor in the liver of transgenic mice was achieved by fusing the coding region of atrial natriuretic factor to the liver-specific transthyretin promoter. The results were constitutive overproduction of atrial natriuretic factor in liver, elevated circulating levels of the hormone, and chronically lowered arterial pressure. Similarly, expression of the rat renin and rat angiotensinogen genes under the control of the metallocrin promoter resulted in elevated expression of both genes in liver, elevated levels of both proteins in plasma, and mild but chronic hypertension. Currently, the limiting factor in directing the synthesis of any protein to any cell type is the availability of well-characterized, cell-specific regulatory elements (promoters). Certainly, as additional genes are identified and cloned, our ability to target specific cell types will improve.

Of course, of equal or potentially greater import is protein underproduction. ES cell technology, discussed
above, while having the ability to knock out expression of a gene in all tissues, is not tissue selective. A “tissue-specific knockout” utilizing a targeted antisense RNA has been performed successfully in transgenic mice.34 Antisense RNA is generated by placing the coding region of a gene in the opposite orientation with respect to a cell-specific promoter. Theoretically, the antisense RNA hybridizes to the normal mRNA and either renders it translationally inactive or targets the duplex for degradation; if targeted to a specific cell type, this could address important questions regarding the function of a gene product in a single tissue or set of tissues. In practice, however, such experiments have proved to be extremely difficult. Factors influencing the success of an antisense experiment probably include the availability of a powerful cell-specific promoter, the level of antisense RNA production (needed in extreme molar excess), and the stability of the antisense mRNA. Also, in the case of an inhibition of translation, the antisense mRNA has to traverse the nucleus and accumulate in the cytoplasm where protein synthesis occurs. Perhaps the development of improved tools, such as ribozymes (catalytic antisense RNAs), will eventually make these experiments more feasible.

Our ability to express mRNAs in specific cell types in transgenic animals has allowed us to specifically target proteins, such as bacterial genes and viral oncogenes, that are not normally expressed in mammals. This has been an extremely powerful technique, providing us with an unprecedented opportunity to study gene expression in vivo, identify expressing cell types, develop novel cell lines, and emulate human disease. Reporter genes generally encode innocuous gene products that can easily be detected through a biologic or histochemical assay and have been extensively used in tissue culture for many years. The most popular reporter gene in transgenic mice has been E. coli lacZ encoding β-galactosidase. β-Galactosidase expression can be detected easily in tissues by virtue of its unique mRNA but more importantly by its catalytic activity. A number of synthetic substrates are available, which when cleaved by β-galactosidase, liberate color, making it an extraordinarily sensitive reporter. A number of questions can be addressed when β-galactosidase is expressed in transgenic mice using cell-specific promoter elements: (1) In what cell types is a promoter active? (2) Does the cell specificity of the promoter change throughout ontogeny? (3) How is the gene regulated? Transcriptional regulatory elements can also be mapped using this approach by analyzing a collection of β-galactosidase constructs differing in the amount of 5′ flanking sequences used to target expression. Examples of transgenic mice containing the β-galactosidase gene have been previously reported35-36; other reporter genes, such as chloramphenicol acetyltransferase and luciferase, among others, have also been used successfully.37,38

Some oncogenes, by virtue of their uniqueness in the mammalian genome, also can be used as a reporter. Transgenic mice containing the mouse renin promoter fused to SV40 T antigen were previously used to map regulatory elements in the renin gene.39,40 More significant, however, is the ability to target an oncogene to specific cells, causing, in many cases, a dysplastic or neoplastic phenotype to result. These types of studies have been instrumental in examining the mechanisms of tumorigenesis and developing animal models for the study of tumor progression.41-43

FIG 5. Examples of tissue-specific expression analysis of transgenic mice containing a human renin genomic clone are shown.27 A and B: Northern blot of adrenal gland RNAs from various adult transgenic mice (Tg+) and nontransgenic DBA/2J (D) and BCF (B) mice. The blot in A is probed under conditions that allow us to detect both human renin and endogenous mouse renin mRNAs. BCF mice do not express renin in the adrenal gland at this age. The genetic background of the transgenic mice is BCF. The blot in B is probed under conditions that allow us to detect only human renin mRNA. See Reference 27 for further details. C: Differential primer extension analysis of kidney RNA from a transgenic (Tg+) mouse and nontransgenic (Tg−) littermate. The assay is capable of generating specific bands correlating to the expression of the human and mouse renin gene. No human renin mRNA is detected in the kidney of the nontransgenic animal. Approximately equal levels of human and mouse renin mRNA are expressed in the transgenic kidney. D: Bar graph shows plasma renin activity assay. Plasma was isolated from transgenic and nontransgenic animals and subjected to a plasma renin activity assay using a substrate cleavable by both human and mouse active renin. Total: Combined human and mouse active renin activity in transgenic mice. Mouse: Level of mouse active renin activity in transgenic and nontransgenic mice. Human: Level of active human renin activity in transgenic mice. The level of human and mouse renin was extrapolated by subtracting the plasma renin activity of nontransgenic mice (data not shown). We previously demonstrated that the presence of the human renin transgene has no effect on the expression or release of active mouse renin.27
The accumulation of an oncprotein in a cell can predispose it to a series of genetic modifications, leading to a fully tumorigenic phenotype. These cells, once amplified in a tumor, are often immortalized or fully transformed and therefore can be propagated permanently in vitro. The renin promoter SV40 T antigen transgenic mice alluded to above have been used to isolate a renin-expressing kidney cell line that exhibits many characteristics of juxtaglomerular cells.46-48 Similarly, atrial tumors and atrial cardiomyocyte lines have been established from transgenic mice containing the ANF promoter fused to T antigen.49 A concern in such experiments is that the transformation event leading to the immortalized phenotype can affect the terminal-differentiated character of the cells. An important question that must be addressed in these experiments is how the resultant cell lines emulate their in vivo counterparts. Recently, conditionally active oncogenes, such as temperature-sensitive mutants of SV40 T antigen, have been used to limit the effects of the transformation process.48

Conclusion

Transgenic animals have provided and continue to provide an important resource for cardiovascular research. Novel animal models exhibiting hypertension and atherosclerosis are providing new data on the pathogenesis of these diseases and the role played by specific genes. Recently, the power of molecular genetics has been used to identify genes linked to hypertension. Transgenic animals will unquestionably become an important tool for testing the significance of allelic gene variants. Combined with the ability to specifically mutate genes involved in cardiovascular regulation, the use of transgenic animals will undoubtedly lead to our greater understanding of genotype and phenotype in the cardiovascular system.

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References


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