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lucidation of the molecular abnormalities responsible for hypertension is greatly complicated by the multifactorial nature of the disease, which depends on genetically driven physiological components that interact with each other and with environmental factors in complex ways. The development of transgenic animals provides an exciting array of experimental strategies that offer new opportunities for understanding the complex nature and mechanisms of blood pressure homeostasis. In addition, the transgenic approach also offers the opportunity to create animal models with well-defined, systematic genetic alterations that can be used to study normal and abnormal processes involved in blood pressure regulation.

The ability to produce transgenic animals, in which foreign genetic material is incorporated into the germ line cells, is considered a major biological addition, the transgenic approach also offers the mental control that has previously been unobtainable. Because the foreign genetic material is transmitted from generation to generation, it is possible to develop stable colonies of these genetically altered animals. These approaches have been extensively and successfully used in other areas of biology and to study disease processes. For background information, there are a number of excellent, comprehensive reviews on the development and application of transgenic animals.

The purpose of this article is to describe the procedures for generating transgenic animals, to discuss some major experimental opportunities that transgenic animals offer, to summarize a number of scientific advances outside the area of hypertension research, and to elaborate on the current state of the field and future directions as it pertains to normal and altered blood pressure regulation.

Methods

At present there are three general approaches for introducing foreign genes into animals, each offering some distinct advantages, and each suffering from a number of problems. The most frequently and successfully used approach is the direct microinjection of DNA into the pronucleus of fertilized eggs (Figure 1). In some of the eggs the foreign DNA will become stably integrated into the chromosomes. The fertilized eggs are introduced into a surrogate mother and the subsequent offspring are screened to detect successful integration of the DNA construct. This method is technically straightforward and is very efficient in generating transgenic animals that express the foreign DNA. However, the investigator faces difficulties in controlling the number of copies that are inserted into the host DNA and in limiting host DNA rearrangements.

The second method for generating transgenic animals uses retroviral vectors (Figure 1). In this procedure, recombinant DNA techniques are used to insert a gene into retroviruses, which are then used to infect embryonic cells of the developing animal. Two advantages of this procedure are that the investigator can transfer DNA at specific stages of development and that only one copy of the DNA construct is integrated into the host genome. The latter offers a major experimental advantage, should the investigator want to identify the endogenous gene that is disrupted by the insertion of the foreign DNA construct (See Insertional Mutagenesis in the Experimental Strategies section). Problems with using retroviruses include the difficulty in expressing transgenes reproducibly and a limitation in the size of the DNA construct that can be accommodated in a retroviral vector. Additionally, the target for integration must be a replicating cell.

Although these two experimental methods have led to important scientific advances, both suffer from a major disadvantage—the investigator cannot control where the foreign gene will insert in the host DNA and thus cannot predetermine the defect or alteration in function. In addition, the inability to target a gene to a specific chromosomal location may interfere with the transplanted gene's ability to function or may cause unwanted inactivation or activation of endogenous genes.

The third strategy for developing transgenic animals seeks to overcome these obstacles by improving the ability to specify or "target" the gene to be mutated. In this way, the investigator can modify or delete genes with greater precision. The technique, called gene targeting by homologous recombination,
has the potential to permit derivation of precise animal models for human hereditary diseases and to provide higher levels of refinement and resolution in the study of the effects of individual genes on the whole animal (See References 8 and 9 for general reviews).

The technique, which to date is almost exclusively used with mice, consists of several steps and represents a powerful alliance of cellular biology, genetics, and molecular biology (Figure 1). Embryonic stem cells are removed from a mouse during the blastocyst stage of embryogenesis and then are maintained in culture in the pluripotent state. The foreign gene construct is then introduced into these cells, using any of a variety of transfection methods, most commonly electroporation or microinjection. In some of the ES cells the foreign DNA segment will find and replace or add to the homologous host DNA segment. The investigator can then use screening or selection techniques to separate ES cells that have undergone homologous recombination from unmodified cells and cells modified by random integration. Selection procedures now exist that can enrich 2,000-fold for cells containing a targeted mutation. The targeted ES cells, which are pluripotent, are reintroduced into mice blastocysts. The blastocysts are then reimplanted into pregnant mice, which are brought to term. The newborn mice are chimeric because some of the germ line cells are derived from the genetically altered ES cells. These chimeric animals can then be bred to achieve the homozygous state. This approach allows, in principle, the derivation of animals with specific phenotypic characteristics for which in vitro screening and selection methods are available.

**Experimental Approaches**

In the broadest possible context transgenic animal technologies allow the investigator to introduce foreign genetic material to express new genetic information or to alter or abolish the expression of endogenous genes. These alternatives provide a broad array of experimental approaches for unraveling complex biological phenomena by studying selected events in intact biological systems at the molecular, biochemical, cellular, and physiological levels. Examples of some of the opportunities that transgenic animals provide are given below.

**Understanding the Control and Regulation of Gene Expression**

Transgenic animals can be used to assist in understanding the most intricate and fundamental elements involved in the control of gene expression—for example, in investigations focusing on the identification of DNA sequences (cis elements) responsible for tissue-specific and temporal-specific gene expression and the biochemical components (transacting factors) that bind to these DNA sequences. The transgenic approach is particularly useful as an adjunct to studies of cells in culture or when appropriate cell lines are not available. Indeed, this approach affords the potential to assess elements important to expression in any tissue throughout ontogeny. For example, an investigator who wants to determine the DNA regulatory elements responsible for tissue-specific expression of a particular protein can develop transgenic animals using a carefully designed series of DNA constructs containing different lengths of DNA sequences that are adjacent to the protein coding sequences. Each construct contains a different length of flanking sequence linked to a “reporter gene,” which enables the investigator to detect when, where, and to what extent the putative regulatory sequences are activated. (DNA constructs that combine nucleotide sequences from different genes are often referred to as “hybrid” genes or “chimeric” genes.) If the chimeric gene contains an oncogene (such as the
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SV40 T-antigen), gene expression can often be detected by the development of tumors; if the chimeric gene contains the DNA sequence for an enzyme, gene expression can be determined by assays on gels; if the chimeric gene codes for a structural protein, gene expression can be determined by immunohistochemical procedures. Given that the insertion site of the transgene in the genome is not controlled, it is generally important to establish multiple founder lines containing a particular construct to ensure that position effects are not influencing expression patterns. Using this, and other approaches, research workers have identified promoter regions, enhancer regions, and promoter-enhancer interactions for genes that encode a wide variety of proteins, have provided greater insight into the regulation of developmental processes, and have elucidated mechanisms by which neural and hormonal factors alter gene expression.\(^4,5,7\)

Alteration of Levels of Endogenous Substances

As DNA constructs can be made with protein coding sequences for endogenous substances, the investigator can develop and study transgenic animals with altered levels of the protein of interest. Judicious choice of cis elements (generally the promoter region) allows the investigator to target the expression to virtually all tissues, or alternatively, to selected tissues. The classic example of the former is the use of the metallothionein promoter to achieve high levels of expression of human growth hormone.\(^10\) In another example, it was possible to demonstrate that overexpression of the low density lipoprotein (LDL) receptor eliminates LDL from the plasma by developing transgenic mice with a DNA construct consisting of the complete coding region of the human LDL receptor linked to the metallothionein promoter.\(^11\) Examples of DNA sequences that have been used to deliver genes to selected tissues with great precision include the promoter from surfactant C to target the lung,\(^12\) and the promoters from crystallins to target different cells in the lens.\(^13,14\) It has also been possible to target the brain, liver, kidney, muscle, spermatids, pancreas, connective tissue, and certain hematopoietic tissues.\(^4,5\)

Understanding Cellular Functions by Targeted Genetic Ablation

This approach offers insight into the function of specific cell types, provides a way to dissect the complex interactions between different cells, and permits the elucidation of cellular lineages. The strategy involves the creation of transgenic animals that contain a hybrid gene consisting of a carefully tailored DNA sequence dictating tissue-specific expression that is linked to a toxin coding sequence. Specific cell types are eliminated because, although all cells of the organism contain the transgene, only cells that recognize the tissue-specific sequences will express the toxin. For example, transgenic mice carrying an elastase promoter linked to the gene coding for the A chain of diphtheria toxin produce mice lacking a normal pancreas because the toxin is expressed in pancreatic acinar cells.\(^15\) Diphtheria toxin-A produces transgenic mice with lens defects when linked to promoters from different crystallins,\(^13,14\) yields transgenic mice devoid of pulmonary epithelial cells when linked to promoter sequences from surfactant C,\(^12\) and produces dwarf mice when linked to promoter sequences from rat growth hormone.\(^16\)

Identification of New Genes and Proteins by Insertional Mutagenesis

When a foreign DNA construct integrates into the host DNA it can modify or abolish the function of an endogenous gene. Using this procedure to generate mutant animals is highly advantageous because the investigator can identify, clone, and characterize the gene that is disrupted. This is possible because the foreign DNA sequence can serve as a probe for isolating the integration site and its flanking sequences.\(^1,5,17\) The flanking sequences can then be used as a probe to isolate the endogenous gene from nonmutated individuals. Thus, it is possible to select for a particular altered physiological phenotype, study the biological consequences that result from insertional mutagenesis, and recover and characterize the gene that has been mutated and whose altered function or absence is now responsible for the new phenotype. Examples of some phenotypes produced in this manner include limb formation defects,\(^18,19\) situs inversus,\(20\) neurological defects,\(^21\) and rupture of major blood vessels due to collagen defects.\(^4\)

Developing New Animal Models of Human Diseases

The ability to create new animal models by disrupting or altering the expression of endogenous genes, by introducing defective human genes, or by adding genes that will be expressed inappropriately offer a new host of exciting possibilities for understanding basic pathogenic mechanisms of important diseases. Examples of disease models made possible by transgenic animal technologies include AIDS,\(^22\) some forms of diabetes,\(23-25\) hepatocarcinogenesis,\(^26\) onco genesis,\(^27\) neonatal hepatitis,\(^28\) some forms of inflammatory human diseases,\(^29\) glomerulosclerosis,\(^30\) osteogenesis imperfecta,\(^31\) some forms of demyelinating diseases,\(^32\) and sickle cell anemia.\(^33,34\)

Sickle cell anemia is an excellent illustration of a complex, multisystem disorder where the lack of an appropriate animal model has frustrated prospects for understanding the factors that initiate sickling and the pathophysiology of the disease, even though the molecular basis is understood in the most exquisite detail (the substitution of valine for glutamic acid on the β-globin chain at position 6). Recently, however, transgenic mouse models\(^33,34\) of sickle cell anemia have been developed. If pathophysiological events in these models parallel those in humans, then there will be numerous opportunities to understand the reasons why patients are
more prone to severe infection, the origins of sequestration crises, the interactions between vascular endothelium and sickled erythrocytes, and the clinical heterogeneity of this disease.35

**Developing New Therapeutic Approaches**

Besides facilitating research into the basic mechanisms of disease, transgenic animals also provide a model system for the design and testing of new therapeutic strategies, including somatic cell gene therapy. In addition, transgenic animal models can be used to test drugs for their levels of toxicity and to devise new diagnostic procedures. Again, sickle cell anemia is an example of a disease where the basic defect is well understood at the molecular level, but whose lack of a suitable animal model impedes progress in the development of effective therapies. Today, more than 30 years after the elucidation of its molecular basis, there is still no definitive treatment available.36 The recent availability of transgenic animal models33,35 now makes it feasible to test drugs that might prevent sickling and to develop strategies for better treatment and perhaps even a cure.

**Providing New Cell Lines**

In vitro experiments can be seriously limited by the availability of suitable lines of cultured cells. Some of the strengths of using cultured cells include the ability to control precisely the environment of the experimental preparation and to perform long-term experiments, the use of homogeneous cell populations with predictable characteristics, the introduction of molecular biological techniques that allow the expression of selected gene products, a shorter time frame for performing experiments, and a reduction of the expensive animal and labor costs required to continually generate primary cultures. Transgenic animals offer a potential route for immortalized cell lines. One approach is to use a DNA construct in which promoter/enhancer sequences that are functional in the tissue of interest are linked to an oncogene, such as the T-antigen of SV-40. Tumors develop in tissues that activate the oncogene, and the investigator can then isolate cells to maintain in culture. For example, atrial tumor cells were produced in transgenic animals by linking the SV-40 T-antigen to the 5' and 3' flanking sequences of the mouse protamine 1 gene37 or to the transcriptional regulatory regions of atrial natriuretic peptide.38 If it is possible to arrest oncogene expression (such as using temperature-sensitive mutant genes) and to promote differentiation of desired cellular phenotypes, one may witness a new era of hypertension research made possible by a plethora of new renal, adrenal, vascular, and cardiac cell lines.

**New Ways to Make Clinically Active Proteins**

Recombinant DNA techniques allow the production of large amounts of therapeutically active substances, such as human growth hormone, human insulin, and tissue plasminogen activator. These procedures use bacteria, which may limit the extrapolation of this technology to other important proteins because prokaryotic cells may not contain the appropriate enzymes for posttranslational modifications central for biological activity. In addition to providing a readily accessible source of starting material for purification of these compounds, transgenic animals have the appropriate enzymatic systems for posttranslational modification. For example, investigators have demonstrated the feasibility of the transgenic animal approach by creating a mouse that synthesizes and excretes tissue plasminogen activator in milk.39 This was accomplished by using a hybrid gene containing DNA sequences responsible for tissue-specific expression in mammary glands and the DNA coding sequences for human tissue plasminogen activator.

**Current Progress and Future Directions**

Transgenic animals offer exciting new avenues for the systematic dissection of the genetic, molecular, biochemical, cellular, and physiological mechanisms involved in the pathogenesis of heterogeneous and complex diseases such as hypertension. The most significant advances using these technologies are in the areas of oncology, immunology, neurology, and developmental biology.1-8 Recently, more widespread application of these approaches is leading to important progress in the area of hypertension research. Current efforts focus on the production of transgenic mice to delineate the DNA sequences and cellular factors and mechanisms responsible for tissue-specific and development-specific expression of renin,40-45 angiotensinogen,46 and atrial natriuretic peptide,38 and to dissect autocrine/paracrine effects from endocrine mechanisms.40-46 There are also recent reports37,48 of a severely hypertensive transgenic rat model that has the mouse ren-2 gene incorporated in its genome and a hypertensive mouse model that is doubly transgenic for the rat renin and angiotensinogen genes under the control of mouse metallothionein I promoters. The latter is of special interest because elevated blood pressure requires the presence of both transgenes. The rat model represents new opportunities to elucidate cardiovascular mechanisms because these animals do not overexpress active renin in the kidney and have low levels of plasma renin and because rats are more readily amenable to experimental manipulation for physiological studies than are mice and have historically been the animal of choice for hypertension research. The most recent example of the application of transgenic animals49 uses a hybrid gene composed of the transthyretin promoter and the atrial natriuretic peptide structural gene. Adult transgenic mice have chronic, elevated levels of atrial natriuretic peptide and lower arterial pressure in the absence of significant changes in heart rate, plasma and urinary electrolytes, water intake, and urine volume. Development of these transgenic animal models provides
an example of a new approach to study cardiovascular homeostasis.

Increased and broader use of the full complement of transgenic animal approaches, such as the ones described in this report, will provide many opportunities for creative explorations. Representative areas might be: to investigate the molecular, biochemical, cellular, and physiological function of individual gene products involved in blood pressure homeostasis and their interaction with other gene products under physiological and pathophysiological conditions; to develop new animal models with precise genetic defects that alter blood pressure regulation; to identify and elucidate the regulatory mechanisms of neural, hormonal, and other systems that are relevant to normal and abnormal blood pressure control; to use genetic ablation techniques to unravel the complex interactions of systems composed of multiple cell types, such as the relations among the array of cells that comprise the blood vessel wall; and to develop new therapeutic strategies. Such endeavors will lead to important breakthroughs in our understanding of the etiology and pathogenesis of hypertension, to innovative and novel therapeutic strategies, and to better insights into key fundamental mechanisms important to all biology.

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