Editorial Comment

Transgenic Animals in Hypertension Research
New Approaches to Old Questions

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Techniques for introducing eukaryotic DNA sequences into the genomes of fertilized mammalian embryos have been available for more than 10 years. The production of transgenic animals (primarily mice) from these embryos has provided a valuable tool for the study of molecular, cellular, and whole animal physiology in the recipient. The power of the technique derives from the fact that the expression of the transgene can be followed developmentally for extended periods of time, in most cases for the entire lifetime of the recipient, in a setting that minimally perturbs the remainder of the animal's genome. In addition, because the transgene is incorporated into the recipient's germ line as well as somatic cells, it is transmitted to its offspring with a high degree of fidelity, thereby allowing for detailed molecular or physiological studies as the strain is propagated over subsequent generations.

To date, transgenic animals have been used to address a variety of different biological questions with considerable success. They have been used to study the details of tissue-specific gene expression and have accurately identified cis-acting enhancer sequences responsible for conferring this specificity on target genes. They have also been used to locate cis-acting DNA elements that confer sensitivity to environmental stimuli (e.g., glucocorticoid or heavy metals) on contiguous coding sequences. They have been used to determine the effects of protein (e.g., hormone) overproduction in transgene-bearing animals and, in some instances, to replace a defective gene in the recipient.

Chimeric transgenes linking tissue-specific regulatory elements to oncogene coding sequences have been used to foster the development of malignancies in selected tissues, in some cases as a prelude to establishing a transformed cell line retaining the phenotype of the targeted cell. Finally, the transgenic approach has been used to eliminate cells of a particular phenotypic lineage from the recipient animals. This has been accomplished by linking cell-specific or tissue-specific regulatory elements either to toxins, interferon, or major histocompatibility class II gene coding sequences. Cell-specific expression of these proteins results in the death of the targeted cells and, by inference, interruption of the physiological activities that they control.

Several important papers using the transgenic approach to study genes related to cardiovascular control have been published in the last 3 years. Recently, this approach has been brought to bear more directly on issues relevant to hypertension research in a study by Mullins et al and in a study by Steinhelper et al in the present issue of Hypertension. Mullins et al reported the results of an experiment in which they introduced the mouse ren-2 gene into fertilized rat embryos. Interestingly, the transgenic founder animals and subsequent offspring developed significant increases in arterial blood pressure (100–145 mm Hg). The Mullins article was noteworthy for a number of reasons. First, it appeared that the adrenal gland in the host animal expressed the transgene with particularly high avidity. Second, although plasma prorenin levels were high, plasma renin, angiotensin I, and to some degree, angiotensin II levels were reduced relative to their nontransgenic littermates. Plasma aldosterone levels, on the other hand, were increased approximately twofold in the hypertensive animals. Although not conclusive, these data suggest that local activation of an adrenal renin-angiotensin cascade and enhanced aldosterone production might contribute to the development or maintenance of hypertension in these animals. If this hypothesis proves true, it lends support to the importance of such a system in the local regulation of aldosterone production. As noted by Leckie, the biochemical profile of these rats is not typical of human patients with essential hypertension. The profile, at least superficially, more closely resembles that of primary hyperaldosteronism due to idiopathic adrenal hyperplasia (IAH), a disorder typified by suppression of plasma renin and angiotensin levels but which retains sensitivity to exogenous stimuli. Interestingly, these patients, like the transgenic rats of Mullins et al, are sensitive to converting enzyme inhibition despite presumed suppression of the renal renin-angiotensin system. If a link between adrenal renin overproduction and IAH is established in human patients, these rats...
may prove to be a useful model to decipher the pathophysiology, and perhaps the pathogenesis, of that syndrome.

An issue not resolved in Mullins et al. relates to the sources of the high levels of circulating prorenin. Some of the prorenin may represent adrenal prohormone that has for some reason escaped the processing event. Equally possible sources are the other sites of putative extrarenal renin production in the normal animal including the gonads and vascular endothelial and smooth muscle cells. Given the levels of blood pressure seen in these rats, the presence or absence of high levels of renin gene expression in vascular tissue is a particularly important issue awaiting resolution. It should also be pointed out that although the adrenal glands are clearly affected in these transgenic rats, it is not clear that the hypertension is solely dependent on overproduction of aldosterone or another adrenal hormone. It is conceivable that adrenal malfunction may represent only one piece of the puzzle, while other sites of extrarenal ren-2 expression (e.g., vascular cells) play an equal or perhaps greater role in promoting the elevation in blood pressure. Similarly, although plasma renin levels are obviously reduced in the rats with established hypertension, the data do not exclude a role for renal ren-2 gene expression in the development of the hypertensive phenotype. Suppression of renal renin production as the blood pressure increases would then shift responsibility for maintaining the hypertension to other mechanisms (e.g., extrarenal renin production). More detailed studies of the developmental profile of renal ren-2 transgene expression and plasma renin levels, particularly as they relate to the onset of hypertension, should lead to a definitive resolution of this question.

The article by Steinhelper et al. reported in the present issue, focuses on another gene product involved in the control of cardiovascular function, the atrial natriuretic factor (ANF). A line of mice was produced that carry a chimeric transgene linking the 5' regulatory sequences of the mouse transthyretin promoter, a strong, constitutively active promoter in liver tissue, to mouse ANF coding sequences. The resultant transgenic mice produced large amounts of immunoreactive ANF, which was shown to circulate in plasma. The major site of synthesis of the transgene messenger RNA (i.e., liver) and the developmental appearance of the transgene protein product followed those predicted for the transthyretin promoter. Most importantly, the mean arterial pressures of the ANF transgene-bearing animals were a significant 28 mm Hg lower than those of their nontransgenic littermates. This suggests that persistently high levels of the ANF peptide are capable of reducing arterial blood pressure, irrespective of potential desensitization of peripheral ANF receptors or reduction of intravascular volume. This is an important finding given the conflicting data reported from studies using long-term ANF infusion in normal animals (see Steinhelper et al.). A number of issues remain to be explored in Steinhelper's mice. One relates to the etiology of the hypotensive effect (i.e., whether it results from chronic volume contraction with reduced cardiac output or rather, as the authors imply, from a direct effect at the level of the heart and peripheral vasculature). A second question relates to the issue of proANF processing. In the atrial myocardium most immunoreactive ANF is stored in the secretory granule as the prohormone. Although the exact location of the processing event remains undefined, it is thought that conversion of proANF to ANF takes place either cosecretionally as the granular contents are released from the atrial cell or shortly thereafter, before the prohormone leaves the heart. Steinhelper et al. report that a fraction (~33%) of circulating immunoreactive ANF in their transgenic mice segregates with the lower molecular weight fractions containing mature ANF by size-exclusion chromatography. The remainder is larger molecular weight material compatible with the prohormone. The latter finding is not surprising if one accepts that the processing event is linked to the secretory granule. Hepatic cells lack secretory granules and use predominantly the constitutive secretory pathway to release their products. The real questions here relate to the sources of mature ANF and to the nature and location of the processing event in these transgenic mice. Does the hepatocyte have at least a limited capacity to effect the conversion of proANF to ANF or does processing take place outside the hepatic cell? It will be important to determine whether "processing" of the transgene product has a physiological equivalent (e.g., processing in the cardiac extracellular compartment) or whether it represents an anomaly related to the site and quantity of the protein that is expressed. It will also be important to determine whether the processing event in these animals occurs at the same position in the prohormone as it does in the atrial myocyte. This is an important issue as many smaller carboxy terminal fragments of proANF, including fragments that are not products of the normal processing mechanism, retain immunological and biological activity.

In summary, the studies described above have provided us with some tantalizing glimpses into unexplored areas of blood pressure regulation and, perhaps more importantly, have yielded some unexpected findings. These transgenic animals, and others that are certain to follow, offer promise of a unique and fresh approach to questions associated with malregulation of cardiovascular homeostasis. Avenues for future exploration are virtually unlimited. Identification of other transgenes associated with a hypertensive phenotype could be incorporated into the ren-2 transgene-bearing animals using conventional breeding techniques. Selected addition of such genes might eventually produce animal models more closely approximating human essential hypertension. The transgene approach may also afford the potential for studying the developmental modulation of hypertension through introduction of a second "anti-hypertensive" transgene, such as that reported by...
Steinhelper et al. into an established genetically hypertensive background. Finally, the transgenic approach may allow for the selective ablation of gene expression associated, in either a positive or a negative sense, with the development of hypertension. In the past, such ablative approaches have either been nonselective or have targeted a specific cell type for elimination. Recent advances in the field of homologous recombination suggest that individual genes may soon be selectively targeted for mutagenesis with consequent loss of function but without damage to the uninvolved genome or the host cell. This technique, if adaptable to transgenic systems, would provide the precision required to dissect out the individual contributions of candidate genes to the regulation, or malregulation, of blood pressure.

It is ironic in a sense that the recent shift of emphasis away from whole animal research toward the recombinant techniques of modern biology has now come full circle, albeit with an updated version of the “whole animal”. This marriage of the “old” with the “new” promises serious reevaluation of longstanding questions related to cardiovascular regulation and an exciting future for hypertension research.

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