Transgenic Hypertensive Animals Carrying Genes for the Components of the Renin-Angiotensin System. Akiyoshi Fukamizu and Kazuo Murakami. Institute of Applied Biochemistry, University of Tsukuba, Ibaraki 305.

Epidemiological studies show that both atherosclerosis and essential (primary) hypertension rank among the most common causes of cerebrovascular, cardiac and renal pathology. Other diseases such as diabetes markedly predispose the individual to developing hypertension and thereby accelerates the course of cardiac disease, peripheral vascular disease, stroke, retinopathy and nephropathy. Therefore, hypertension is a significant community health problem and understanding the causes of high blood pressure is of paramount importance. Basically, blood pressure is defined as a function of cardiac output and total peripheral resistance. These two parameters are governed by the contractile state of the heart and the cross-sectional diameter of the arteriolar resistance vessels, the latter of which depends greatly on vascular smooth muscle tone. Both cardiac function and vascular tone are controlled to a major degree by neuronal and hormonal effector systems, such as the renin-angiotensin system, the kinin-kallikrein system, endothelin and atrial natriuretic peptide, which are thought to be involved in blood pressure regulation in a complex fashion.

Of these effector systems, the renin-angiotensin cascade has long been considered to play an important role in the regulation of blood pressure, fluid volume balance and other biological responses, through the production of an octapeptide, angiotensin II, which acts as a vasoconstrictor and which additionally promotes aldosterone release from the adrenal gland. The significant role that the renin-angiotensin system plays has been assessed by a large number of experiments involving the use of primary cell cultures, including vascular smooth muscle cells and adrenal granulosa cells. Although the participation of the renin-angiotensin system in several disease states including hypertension has long been suspected, *in vitro* analyses fall short of being an accurate representation of hypertension in the living organism. For example, tissue culture experiments cannot assess the influence of blood supply along with various humoral and cell-mediated factors. Furthermore, previous studies have reported that genetic susceptibility forms a significant component in the pathogenesis of hypertension, and it has been estimated that approximately one-third of human blood pressure variance is genetically determined. Among several proposed candidate genes in the pathogenesis of hypertension, the renin gene appears particularly relevant. Clinical observation has revealed that overproduction of renin due to a renin-secreting tumor or renal arterial stenosis elevates blood pressure and that the blockade of the renin-angiotensin system by angiotensin-converting enzyme inhibitors is highly effective in controlling the blood pressure of the patients with essential hypertension. Therefore, the generation of transgenic animals carrying genes for renin or its substrate, angiotensinogen, has been recently sharply under focus.

Two groups have reported the generation of transgenic animals with elevated blood pressure. Ohkubo *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* **87**, 5153-5157, 1990) have produced transgenic mice carrying the rat renin or rat angiotensinogen genes, both of which are placed under the control of the mMT-I promoter. Only transgenic mice possessing both of these two genes exhibited significantly elevated blood pressure. Mullins *et al.* (*Nature* **344**, 541-544, 1990) have developed transgenic rats harboring the mouse *Ren-2* gene along with its native promoter. This gene, found only in certain strains of mice, is active under normal circumstances in the kidney, the submandibular and the adrenal glands. In the rat, the transgene was highly expressed in the adrenal gland, but its expression was suppressed in the kidney as well as in the submandibular gland. Nevertheless, these rats demonstrated fulminant hypertension which was transmissible into subsequent generations. Interestingly, the administration of an inhibitor of angiotensin-converting enzyme which processes the inactive angiotensin I into the bioactive peptide, angiotensin II, was sufficient to reduce the elevated blood pressure of rats bearing the *Ren-2* gene along with mice carrying the rat renin and rat angiotensinogen genes.

In order to assess the role of the interaction of human renin with human angiotensinogen in the development of high blood pressure, we have recently constructed the chimeric renin-angiotensin cascade in mice comprising both human renin and human angiotensinogen as well as the endogenous angiotensin-converting enzyme and angiotensin II receptor, by cross-mating separate lines of transgenic mice carrying either the human renin or human angiotensinogen genes (*J. Biol. Chem.* **268**, 11617-11621, 1993). Although each single gene carrier did not develop hypertension despite the observed normal tissue-specific expression of the transgenes, dual gene strains exhibited a chronically sustained increase in blood pressure. Administration of a human renin-specific inhibitor (ES-8891) was effective in reducing the elevated blood pressure only against the cross-mated hybrid mice, but treatment of an angiotensin-converting enzyme inhibitor (captopril) and a selective antagonist (DuP 753) directed at the angiotensin II receptor decreased the basal level of blood pressure even in single gene carriers as well as in dual gene mice. These results clearly demonstrated that the sustained increase in blood pressure of the hybrid mice was initiated by the interaction between the products of the two human genes.

The most efficient and commonly used method in transgenic studies is the direct microinjection of DNA into the pronucleus of 1-cell embryos. Despite its common acceptance, this approach has limitations in that researchers are neither able to control the precise integration site of the transgene nor control the copy number of the gene inserted. Workers in future studies of the role of genes encoding endocrine hormones will therefore probably turn to the gene targeting method where the integration site and the copy number are known. Nevertheless, both techniques will likely continue to be utilized to further build on our present knowledge in the quest for understanding the complete working mechanism of the endocrine system, a quest which is already just commencing for endocrinologists and molecular biologists alike.