Recent Advances in the Study of Renin and Angiotensinogen Genes: From Molecules to the Whole Body

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The renin-angiotensin system (RAS) plays a key role in the regulation of the circulation and is critically involved in the pathogenesis of several diseases, including hypertension. Renin is synthesized mainly in the kidney and is secreted into the bloodstream. It catalyzes the rate-limiting cleavage of substrate angiotensinogen, which is derived mainly from the liver, to generate angiotensin I. Renin and angiotensinogen genes have been isolated and their structure has been determined by the methods of molecular biology. Renin and angiotensinogen genes are expressed in many tissues, and the tissue-specific regulation of these genes has been studied. The existence of local RASs in contrast to the classical circulating RAS has been suggested, although their exact functional role remains to be determined. Recent molecular analyses have led to a detailed description of the transcriptional mechanism of the renin and angiotensinogen genes, and have made it possible to study the regulation of the expression of these genes in several physiological and pathological states. In addition, several types of transgenic animals have been developed to study the functional importance of the RAS in vivo. Transgenic mice with human renin and human angiotensinogen genes may be a good model of human hypertension. In such mice, the human genes are expressed in the normal tissue-specific pattern, the circulating RAS is activated, and blood pressure is high. Finally, angiotensinogen-deficient mice have also been developed by homologous recombination in mouse embryonic stem cells. These mice do not produce angiotensinogen in the liver. As a result, they have no plasma immunoreactive angiotensin I and are hypotensive. The profound hypotension in these mice indicates the importance of the RAS in maintaining blood pressure. (Hypertens Res 1995; 18: 7-18)

Key Words: renin, angiotensinogen, cloning, gene regulation, transgenic mice, gene targeting, knockout mice

Hypertension is a complex pathological state with a genetic background. It involves the autonomic nervous system and various hormonal vasoactive peptides. Many genes that are important in the regulation of the circulatory system have been identified, but little information regarding their roles in the development of hypertension has been available. By contrast, recent studies have shown that the renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure, fluid volume balance, and other biological phenomena through generation of the active peptide angiotensin II. This peptide has a variety of effects, including vasoconstriction and stimulation of the production and release of aldosterone (Fig. 1). The RAS may have a role in several diseases including hypertension (1-4).

The RAS has been historically viewed as a regulator of the circulatory system. Its primary components are 1) angiotensinogen, a glycoprotein that is the precursor to the angiotensin peptides and is the only known naturally occurring substrate of 2) renin, an aspartyl protease that catalyzes the proteolytic conversion of angiotensinogen to angiotensin I, which is subsequently converted by 3) angiotensin converting enzyme to 4) angiotensin II. Angiotensin II exerts a variety of physiological effects by binding to 5) the angiotensin II receptor. The components of the RAS are produced in different organs and are delivered to their sites of action by the bloodstream. However, evidence accumulated from biochemical and molecular studies of angiotensin physiology raised the possibility that local RASs with different regulatory mechanisms exist as distinct systems. Local RASs may exist and function in the brain, heart, adrenal gland, kidney, blood vessel wall, and adipose tissue. Whether all the components of the RAS are physiologically relevant is controversial, and the exact pathophysiological role of the local RAS remains elusive, but it is interest-

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ing to speculate that a local RAS may increase the effects of angiotensin II on a particular tissue in specific physiological processes (5-10). In this review, we summarize recent advances in the biochemistry and molecular biology of the two major components of the RAS (renin and angiotensinogen), including studies done with transgenic animals. We also refer to a recently published study in which gene targeting of the angiotensinogen gene was used.

### Molecular Level

**Molecular Analysis of Human Renin and Human Angiotensinogen**

**Primary Structure of Human Renin and Its Gene**

Renin is a carboxyl proteinase that catalyzes the hydrolytic release of the decapeptide angiotensin I from angiotensinogen. This reaction is the initial and rate-limiting step in the generation of the bioactive octapeptide angiotensin II (2, 3). Renin is mainly produced in the juxtaglomerular (JG) cells of the kidney (11). In the JG cells, renin mRNA is first translated into preprorenin, which then undergoes removal of a signal peptide from its N-terminal, and is glycosylated during transport through the endoplasmic reticulum to become prorenin. Prorenin either enters the constitutive pathway to be secreted from the Golgi apparatus, or is processed to renin in granules to be secreted through a sophisticated regulatory pathway (12, 13).

Renin was very difficult to isolate, because of its extremely low concentration in the kidney, and its instability during fractionation. An affinity column specific for renin was developed to resolve these problems (14). With this column, renins were isolated from hog (15), rat (16), and human (17) kidneys, and their properties were characterized. In addition, purified renin was also obtained from bovine anterior pituitary glands (18). Nevertheless, one major problem remained: the quantities of purified renin were so minute that their primary and tertiary structure could not be determined. The methods of molecular biology, including recombinant DNA technology, were applied to solve this problem, and the complete amino acid sequence (19, 20) and gene structure (21) of human renin were determined.

The human renin gene is located within the q42 band of chromosome 1, spans 11.7 kb DNA, and consists of 10 exons and 9 introns (21, 22) (Fig. 2a). All aspartyl proteinases including bovine chymosin and human pepsinogen have 9 exons of similar size, but the human renin gene has another exon (exon 6) with only 9 nucleotides. The primary structure of human (19, 20) and rat (23, 24) renin genes was deduced from their cDNA and genomic sequences, and was compared with mouse Ren-1 and Ren-2 (25-29). The predicted amino acid sequences of human and rat renins consist of 406 and 402 amino acids, respectively, with pre and pro segments (30, 31). The primary structure contains double domains; that is, the amino and carboxyl termini contain areas of similar sequence, forming a two-lobed structure surrounding the active site. The site of the catalytically important aspartic acids in aspartyl proteinases is encoded in exon 3 and another aspartic acid is encoded in exon 8 in the human renin gene (31). The rat renin precursor showed 85%, 82%, and 68% homology to mouse Ren-1, mouse Ren-2, and human renin, respectively. Moreover, certain regions of renin were remarkably conserved between three species, particularly the regions corresponding to the two catalytically important aspartyl residues (31, 32).

**Three-Dimensional (3-D) Structure of Human Renin**

Although renin is an aspartyl protease, its optimal pH is neutral and its substrate specificity is unique among the aspartyl proteinases. These characteristics may be due to its 3-D structure and subtle conformation near the active site. According to the plastic molecular, computer graphic, and X-ray-derived models of its 3-D structure (Fig. 3), human renin is composed of two similar domains separated by a long and deep cleft (33-35). At the end of cleft are located the two catalytically important aspartic acid and substrate-binding sites. The exquisite specificity of renin arises partly from ordered loop regions at the periphery of the binding cleft. Differences in the positions of secondary structure elements (particularly helices) also improve the specificity of renins for angiotensinogen substrates, as
revealed by X-ray analyses of peptide-inhibitor complexes (36, 37).

**Primary Structure of Human Angiotensinogen Gene**

Human angiotensinogen is a hormone precursor of Mr 61,400. The mature form consists of a single polypeptide chain of 452 amino acid residues with 14% carbohydrate content. Angiotensinogen is produced primarily by the liver, and the newly synthesized protein is constitutively secreted by the hepatocytes into the circulation, where it is cleaved by renin to generate angiotensin I (1-3, 38-40). The plasma serves as the major reservoir of angiotensinogen, and changes in the plasma levels are thought to greatly influence RAS activity (41).

The human angiotensinogen gene is 12 kb long, consists of five exons interrupted by four introns (38-42) (Fig. 2b), and maps to chromosome 1 close to the human renin gene within the region of q42 (43). The minimum promoter has been identified as a 76-base-pair region including the TATA box and the first exon (42). The first exon contains 36 bp of untranslated nucleotides that are present in the 5' region of the transcript. The second exon consists of 859 nucleotides in which the signal peptide and angiotensin I are encoded. The third and fourth exons are composed of 268 and 165 nucleotides, respectively. The last exon, exon 5, consists of 796 nucleotides in which two possible polyadenylation signals are present. With respect to the number and location of the introns, the human angiotensinogen gene, as well as those of the mouse and rat, is similar to the genes for human a1-antitrypsin and a1-antichymotrypsin, which are serine protease inhibitors (44).

Three observations have led to the suggestion that the angiotensinogen gene is involved in the pathogenesis of essential hypertension: a genetic linkage in hypertensive siblings, the prevalence of two molecular variants of the human angiotensinogen gene (M174 and T235) in hypertensive patients, and a significant relationship between the level of plasma angiotensinogen and one of the two variants of the gene (T235) (45-47). Furthermore, a molecular variant of the angiotensinogen gene was found to be closely associated with pregnancy-induced hypertension (48). These interesting results indicate that some molecular variants of the human angiotensinogen gene may contribute to the susceptibility of an individual to the development of hypertension.

**Cell and Tissue Level**

**Regulation of Renin and Angiotensinogen Genes**

Many abnormal physiological responses and abnormal values of biochemical variables have been reported in patients with essential hypertension and in animal models of genetic hypertension, but the etiology and pathogenetic mechanisms of essential hypertension remain enigmatic. It is unclear which of the abnormalities are primary (causative) and which are secondary (the result of hypertension). Physiological and biochemical responses are regulated by a variety of factors at multiple biological levels. Thus, molecular and cellular biological research can contribute significantly to our understanding of the basic mechanisms underlying the regulation of blood pressure and the pathogenesis of hypertension. As described above, the genes involved in the RAS have been cloned and analyzed with the methods of molecular biology. The results of such analyses suggest that the regulation of the
expression of these genes is involved in the pathogenesis of hypertension, and a detailed analysis of the molecular regulation and activity of the RAS would further our understanding of the abnormalities that can result in hypertension. Therefore, in this section we will discuss the molecular mechanisms that regulate the expression of the renin and angiotensinogen genes.

**Tissue Distribution and Regulation of Renin mRNA Expression**

The expression of the renin gene is regulated in a tissue-specific manner, and most circulating renin is produced in the kidney. In the kidney, the JG cells synthesize renin, store it, and release it into the circulation (2, 3, 11-13). However, the availability of cDNA probes for renin, and more sensitive and specific polymerase chain reaction (PCR) techniques, have enabled researchers to detect low-level expression of renin mRNAs in the adrenal glands, testis, ovaries, liver, and brain (49, 50). In contrast to earlier reports, results of two very recent studies indicate that renin mRNA is expressed only at very low levels or is absent in the heart and vascular walls, and renin in these tissues may come from the circulation (51, 52). The 5'-flanking region of the human renin gene contains several identifiable promoter and enhancer regions as well as regulatory elements, which combine to regulate tissue-specific and biological expression of the gene (2, 3, 32, 53).

The 5'-flanking sequences of the mouse, rat, and human renin genes have significant homology, and the highly homologous promoter regions of these renin genes may be involved in regulating the expression of the renin genes in the kidney (32, 53). Transgenic studies have shown that the 5'-flanking region of the human renin gene directs tissue-specific expression in the JG cells of the kidney and in several other tissues (54, 55), and that 5'-flanking sequences of the mouse renin gene (Ren-2) direct tissue-specific and development-specific expression of the reporter SV40 T antigen gene in transgenic mice (56, 57). Transient transfection of embryonic kidney-derived cells was used to identify two transcriptionally important promoter elements (RU-1; -224 to -138, and RP-2; -75 to -47) in the mouse renin gene (Ren-1C) (Fig. 4), and the combination of these elements was found to be responsible for cell-type specific transcriptional activity of the renin gene in transfected kidney cells (58, 59).

The biosynthesis of renin in the kidney is concomitantly regulated by many other factors, including sodium chloride balance, blood pressure, sympathetic nerve activity, and angiotensin II (2, 3, 51). Expression of renin mRNA in various tissues appears to be differentially regulated. For example, sodium depletion or β-adrenergic receptor activation was found to increase renin mRNA expression in the kidney and adrenal glands but not in the submandibular or genital glands (60). In spontaneously hypertensive rats (SHR), a constitutive increase in renin mRNA was found in extrarenal tissues, despite an early increase and a late decrease in the kidney, and these data led Samni et al. to propose that extrarenal expression of mRNA is involved in hypertension (61). However, extrarenal tissues secrete prorenin, but not active renin, and there is no evidence that it is ever converted to renin in the circulation (52). Renal tissue is the only tissue known to be able to convert prorenin to renin, and to secrete active renin into the circulation. A growing number of investigators have concluded that the kidney is the only source of cardiovascular tissue renin (52, 62). Therefore, understanding of the mechanism of renin gene expression in the kidney would be very important.

cAMP is an important mediator of renal physiological responses to various stimuli (63-66), and intracellular levels of cAMP greatly influence the synthesis and secretion of renin in the kidney, as revealed by in vitro and in vivo studies (2, 3, 32, 67-69). cAMP increases the level of renin mRNA by stimulating transcription (59, 70, 71), and by stabilizing the mRNA after transcription (72, 73). In other studies, the upstream 5'-flanking region of the mouse renin genes (Ren-1D and Ren-2) activated transcription from a heterologous promoter in transfected chorion-derived cells, and a cAMP-responsive element (CRE) (Ren-1D; -619 to -597 of the transcriptional start site, Ren-2; -670 to -648) was identified (70, 74). The proximal promoter region from -75 to +16 of the mouse renin gene (Ren-1C) also mediated the transcriptional activation by cAMP in embryonic kidney-derived cells, and the RP-2 element (-75 to -47) in the mouse renin gene (Ren-1C) promoter, which overlapped the TATA-like region, was found to be the major contributor to the regulation of the mouse renin promoter activity by cAMP in transfected kidney cells (59, 71). The RP-2 and RU-1 elements synergistically mediated cAMP-inducibility. Electrophoretic mobility shift assay showed that nuclear factors, which were distinct from AP-1, AP-2, and CREB/ATF family transcription factors, interacted with the RP-2 element, although the cAMP-protein kinase pathway might be involved in the cAMP-mediated trans-activation of the mouse renin pro-
moter by the RP-2 element (71). Furthermore, the nuclear factors in the JG cells bound to the RP-2 element, and nuclear factors binding to the RP-2 element were found to be regulated by cAMP in vivo (71) (Fig. 4). A recent DNA transfection study in primary choriocarcinoma cells revealed that the first 110 base pairs of the human renin promoter region can direct cAMP-induced transcription (75), and showed that proximal CRE-specific (−234 to −214) and pituitary-specific factor (Pit-l)-like sequences (76) can act together to confer cAMP responsiveness (77). These results suggest that the combined action of the several transcriptional factors binding to the proximal promoter region plays an important role in cAMP-mediated regulation of the expression of renin genes. Other studies showed that transcription of the renin gene might be regulated by an early response gene product AP-1 (c-Jun/c-Fos) (78, 79) and a tumor suppressor RB protein (80). Finally, because sequences extending through at least the first 100 base pairs of intron I are highly conserved between species, this region may also play a role (81).

Tissue Distribution and Regulation of Angiotensinogen mRNA Expression

Angiotensinogen is the unique substrate of renin in vivo in the RAS, and it is well accepted that the primary source of plasma angiotensinogen is the liver (2, 3, 38–41). In contrast to renin, angiotensinogen is constitutively secreted into the circulation after it is synthesized in hepatocytes. One important question is whether angiotensinogen regulates renin reactions or is solely an extracellular reservoir of angiotensin peptides. Recent studies have suggested that angiotensinogen has an active regulatory function in both circulating blood and local tissues under physiological and pathophysiological conditions (2, 9, 10, 39). A kinetic analysis of the renin reaction has indicated that plasma angiotensin II production is sensitive to small changes in both renin and angiotensinogen concentrations in the circulation (39). Epidemiological research and recent genetic linkage analyses also led to the proposal that plasma angiotensinogen has a regulatory role: plasma angiotensinogen and blood pressure were found to be strongly correlated (45, 46, 82–84). Regulation of the expression of the hepatic angiotensinogen gene has been intensely studied by many investigators. Steroid hormones are major modulators of hepatic angiotensinogen synthesis, as revealed by in vitro and in vivo studies (2, 3, 38–41). Glucocorticoids, thyroid hormones, and estrogens positively regulate hepatic angiotensinogen synthesis at the transcriptional level, probably through those parts of the 5′-flanking regions of the angiotensinogen gene that respond to steroid hormones (38–42, 85). Angiotensin II, the product of the RAS, also stimulates expression of the angiotensinogen mRNA by a positive feedback loop (86). In addition, the expression of rat angiotensinogen gene was reported to be induced during inflammatory responses (87, 88). Recent studies on the regulation of the gene in the liver during the acute response led to the identification of a hormonally induced enhancer unit consisting of two glucocorticoid-responsive elements and a cytokine-responsive element in the upstream 5′-flanking region of the rat angiotensinogen gene (89, 90). However, human angiotensinogen mRNA in the liver failed to respond to LPS in transgenic mice (91), and the exact role of angiotensinogen in inflammation remains to be determined (88).

Recent studies of transgenic rats with human angiotensinogen genes (92, 93) and genetic linkage analyses of the human angiotensinogen gene in patients with high blood pressure (45–48) have led to the proposal that the basal transcriptional mechanism of the angiotensinogen gene is involved in the pathogenesis of hypertension. At present, although several reports on the basal transcription of the angiotensinogen gene have been published, the molecular relationship between the regulation of transcription and the pathogenesis of hypertension is unclear. Several studies showed that the 5′-flanking region of the mouse, rat, and human angiotensinogen gene is important for tissue-specific and cell type-specific expression of the gene in vitro and in vivo (42, 94–98). Interestingly, the 750-bp promoter element from the immediate 5′-flanking region directed most, but not all, tissue-specific and hormonal regulation of the angiotensinogen minigene in transgenic mice (97). In addition, the proximal promoter region from −96 to +22 of the mouse angiotensinogen gene conferred hepatocyte-specific transcriptional activity, and two cis-acting elements (AGE2: −96 to −52 and AGE3: −6 to +22) were found to confer to this specificity (99) (Fig. 5). Two nuclear factors, AGF2 and AGF3, were identified by electrophoretic mobility shift assay. AGF2 bound to AGE2 in a hepatocyte-specific manner, whereas AGF3 interacted with AGE3 in all cell lines examined. DNase I footprint analysis indicated that the palindromic sequences are involved in AGF2 binding, and that the exon I region is critical for AGF3 binding. Furthermore, substitution mutation analysis and heterologous promoter assay showed that the cooperative interaction between these proximal and core promoter elements was essential for transcriptional activation of the mouse angiotensinogen gene in hepatocytes. These proximal and core promoter elements were also involved.

![Fig. 5. Schematic representation of transcriptional activation of the mouse angiotensinogen promoter. AGEs, angiotensinogen gene-activating elements; TATA, TATA box; TBP, TATA box binding protein; +1, transcriptional start site.](image-url)
in the adipogenic differentiation-induced expression of the angiotensinogen gene (100, 101). All of these studies indicate that the 5'-flanking sequences play a major role in the regulation of angiotensinogen gene expression.

Analysis of the distribution of angiotensinogen mRNA by molecular biological methods has shown that the angiotensinogen gene is expressed in many tissues, including those of the brain, spinal cord, aorta, kidney, adrenal gland, atria, spleen, and adipose tissue. As in plasma, angiotensinogen appears to play a role in regulating local angiotensin II production. A study of the kinetics of the renin reaction in tissue extracts showed that synthesis of angiotensin II depended on the local availability of angiotensinogen (102). In another study, the levels of angiotensin II in the kidney were found to correlate well with both angiotensinogen gene expression and with angiotensinogen concentration in the kidney, which supports the idea that angiotensinogen is involved in local control of angiotensin II production (103). Similar to expression of renin mRNA, levels of angiotensinogen mRNA appear to be regulated differently in different tissues. Sodium has different effects on hepatic and renal angiotensinogen mRNA (60). Angiotensinogen mRNA is expressed in the medial smooth muscle and the periaortial fat of the aortic wall, and the levels of medial angiotensinogen mRNA were found to be locally regulated by a low-sodium diet and sympathetic activation, whereas those in the periaortial fat were not (104). Furthermore, expression of the angiotensinogen gene in adipocytes was found to be regulated by nutrition, and blood pressure was found to be modulated by fasting and refeeding in a way parallel to the adipocyte angiotensinogen mRNA level, even with no apparent change in the hepatic angiotensinogen mRNA level (105).

Further evidence for differences in regulation comes from studies of gene expression in various pathological states, including hypertension. Brain angiotensinogen mRNA was found to be higher in SHR than in Wistar-Kyoto rats, even with no difference in liver mRNA levels (106). Although the angiotensinogen mRNA level was extremely low in ventricles of normal hearts, mRNA significantly increased in hypertrophied left ventricles in an in vivo model of pressure-overload cardiac hypertrophy (107), and stretching increased angiotensinogen mRNA expression in primary cultured cardiomyocytes in vitro (108). In models of heart failure, the levels of heart and kidney angiotensinogen mRNA were specifically high (109, 110). In addition, balloon injury can activate angiotensinogen gene expression in the medial layer of the aorta, which suggests that angiotensinogen has a role in the myointimal proliferation that occurs after such injury (111). Furthermore, a variant of the angiotensinogen gene has been found to be related to coronary atherosclerosis, as evaluated by coronary angiography (112). Future studies are expected to focus on the details of the pathophysiological role of angiotensinogen synthesized in cardiovascular tissues.

Whole-Body Level

Transgenic Mice with the Human Renin Gene

Transgenic mice with the human renin gene were produced by microinjection of 15-kb DNA molecules, with up to 3 kb of 5'-flanking sequence and 1.2 kb of 3'-flanking sequence (122, 123). The transgenes have been shown to be stably transmitted to progeny. The human renin gene in a transgenic mouse was expressed preferentially in the kidney. Human renin mRNA was also detected at low levels in many tissues, including those of the brain, heart, lung, pancreas, spleen, stomach, testis, and thymus. In the transgenic mouse, human active renin is synthesized mainly in the kidney (122). Moreover, immunohistochemical analysis with a monoclonal antibody specific for human renin showed that the transgenic mouse kidney it can be found in the JG cells of afferent arterioles (54, 55). Thus, expression of the human renin gene in transgenic mice is both tissue-specific and cell-specific. However, these transgenic mice had no abnormal phenotype (i.e., they were not hypertensive).

Transgenic Mice with the Human Angiotensinogen Gene

Two lines of transgenic mice with integrated copies of a 14-kb human DNA fragment containing the angiotensinogen gene, which includes 1.3 kb of 5'- and 3'-flanking regions, have been produced and analyzed (95). In both lines, a considerable quantity of the correctly initiated and processed human angiotensinogen mRNA was detected in the liver, and it was also detectable in the heart. The levels of mRNA for the transgene found in the kidney were comparable to those in the liver. This result was not expected because the kidney is normally a relatively minor source of angiotensinogen. However, the transgenic mice with the human angiotensinogen gene were not hypertensive.

Transgenic Mice with Both the Human Renin and the Human Angiotensinogen Genes

As described above, transgenic mice with either the human renin gene (R/) or the human angiotensinogen gene (−/A) did not develop hypertension despite the normal tissue-specific expression of these transgenes. Therefore, transgenic mice with both
human renin and human angiotensinogen genes (R/A, dual carrier) were produced by mating R−/− mice with −/A mice (93).

Blood pressure in the R/A mice was higher (129.1 ± 7.1 mmHg) than in R−/− mice (97.0 ± 7.3 mmHg), −/A mice (98.0 ± 5.0 mmHg), and −/− mice (97.1 ± 7.4 mmHg). The higher blood pressure was accompanied by a higher value of plasma renin activity (PRA) than in R−/−, −/A, and −/− mice. These differences in blood pressure and PRA were probably caused by the higher levels of angiotensin I and angiotensin II in the plasma of the R/A mice, which resulted from the reaction between human renin and human angiotensinogen in mouse plasma. Since the amino acid sequences of human angiotensin I and human angiotensin II are exactly as same as those of mouse angiotensin I and mouse angiotensin II, respectively, these results indicate that the human RAS operates in mouse plasma independently of the mouse RAS. This is consistent with the very strict species specificity shown in vitro with renin and angiotensinogen isolated from humans and mice.

Three inhibitors of the RAS were used to confirm the effect of the transgene products on blood pressure in transgenic mice (Fig. 6). An angiotensin-converting enzyme inhibitor (captopril) and a selective antagonist directed at the AI1 receptor (DuP 753) (124) reduced the blood pressure in R/A, R−/−, −/A, and −/− mice. However, a human renin-specific inhibitor (ES-8891) (125) reduced the blood pressure only in R/A mice. These results indicate that the increase in blood pressure in R/A mice is due to the reaction between human renin and human angiotensinogen.

SHR have been widely used as a low-renin model of human essential hypertension because of their normal or low PRA levels (126). Despite their usefulness as a model, the genetic basis of the development of hypertension in SHR remains unclear. In contrast to artificial selection and production of such animals, several transgenic animals with the RAS component genes have been created with microinjection methods (92, 94, 118-121). Of these animals, three types develop high blood pressure: transgenic rats with the native mouse Ren-2 gene (118), transgenic mice with the rat renin and rat angiotensinogen genes under the control of mouse metallothionein promoter (120), and those with the rat angiotensinogen gene (92). However, the injected mouse or rat genes lacked an appropriately regulated pattern of expression in the transgenic animals, and the onset of high blood pressure in these transgenic animals is still not fully understood (127-129). Whether the transgene products were directly involved in the pathogenesis of hypertension in these animals remains unknown, because the effects of specific inhibitors of the function of transgenic renin have not been examined. In contrast, the expression of human renin and human angiotensinogen genes in R/A mice is regulated in a physiologically normal fashion, and the interaction of the resulting renin and angiotensinogen clearly plays an important role in the elevation of blood pressure. Therefore, this line of transgenic mice is the first model of human high-renin hypertension.

**Angiotensinogen-Deficient Mice**

The most recent and exciting approach to hypertension is gene targeting: the disruption of a particular
gene by homologous recombination in embryonic stem (ES) cells (130-132) (Fig. 7). A target vector containing a desired mutation of a gene is inserted into ES cells by electroporation. After homologous recombination in the target locus, ES cells with the mutated gene are screened, and then the ES cells are injected into eight-cell embryos of recipient mice. The body of each chimeric mouse consists of cells derived from the altered ES cells and cells derived from the ICR host embryo. If ES cells differentiate into germ cells in the chimeric mice, mice in which all the cells have the mutated gene can be obtained by crossing chimeric mice with wild-type ICR mice. Then, male chimeric mice with the mutated gene (heterozygous gene in Fig. 7) are mated with similar female chimeric mice. Thus, heterozygous mutant mice (+/− genotype in Table 1) lacking the angiotensinogen gene in one allele are produced first, and are used to make homologous mutant mice (−/− genotype in Table 1) lacking the angiotensinogen gene in both alleles (133, 134).

These homologous mice (134) clearly have chronic hypotension, with 33.5, 20.8, and 14.3 mmHg lower systolic, mean, and diastolic blood pressures than their wild-type littermates (Table 1). There is no significant difference in blood pressure between heterozygous mutant and wild-type mice. The plasma concentration of angiotensinogen was 58% lower in the heterozygous mutants and was below the lowest detectable level in the homologous mutants. Angiotensin I was also undetectable in plasma of homologous mutant mice. Transgenic mice with high blood pressure (93) and the angiotensinogen-deficient mice (134) should provide valuable insights into the regulation of blood pressure and the pathogenesis of hypotension and hypertension.

**Conclusion**

Recent advances in molecular and developmental biology have made possible the study of the physiological and pathophysiological role of the RAS both in vitro and in vivo at the level of gene regulation and signal transduction. The RAS is critically involved in hypertension, cardiac hypertrophy, vascular remodeling, and restenosis after angioplasty, but further studies are clearly needed to completely understand the functional importance of the

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**Fig. 7.** Gene targeting. ES cells, embryonic stem cells.
local RAS in normal and pathological conditions and the probable relationships between genetic polymorphism and these pathological states. Furthermore, the very recently developed technique known as gene targeting can be used to modify or "knock out" individual component genes of the RAS. Gene targeting may provide revolutionary and exciting findings regarding the nature of the RAS in vivo, and may lead to new types of therapy, including gene therapy.

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