Control of renin synthesis

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Persson, Pontus B., Angela Skalweit, Ralf Mrowka, and Bernd-Joachim Thiele. Control of renin synthesis. Am J Physiol Regul Integr Comp Physiol 285: R491–R497, 2003; 10.1152/ajpregu.00101.2003.—Studies published recently have considerably enhanced our understanding of the mechanisms controlling renin production. With regard to the control of renin transcription, two enhancer regions have been identified that markedly augment renin synthesis in cell lines. In the absence of this enhancer activity, the basic promoter of the renin gene increases transcription only two- to threefold. The location of one (Jones CA, Sigmund CD, McGowan RA, Kane-Haas CM, and Gross KW. Mol Endocrinol 4: 375–383, 1990) transcription enhancer in the mouse gene is at about −2.7 kb and in humans at roughly −11 kb. A second important region has been identified in a chorionic cell line to be located −5 kb upstream of the transcription start site in humans. Another potentially important regulatory region may lie within −3.9 kb upstream of the −11 kb enhancer, as suggested by several conserved sequences among species in this region. In addition to the control of renin transcription, it seems that renin translation and the stability of renin mRNA are also effectively regulated. This occurs via the 3′-untranslated region, to which several proteins can bind. The binding proteins were identified as hnRNP K and E1, dynamin, nucleolin, MINT homologous protein, and Y-Box 1.

transcription; posttranscriptional regulation; mRNA stability; mRNA-binding proteins; 3′-untranslated region

THE DISCOVERY OF RENIN AND ITS IMPORTANCE

Although the discovery of renin dates back to over a century ago, many of the several functions of renin and important features of its control are only currently being unraveled (46). Tigerstedt and Bergman (52) first postulated a substance from the kidney that increases blood pressure (BP) in 1898 after performing experiments in which kidney extracts were infused intravenously. The first experiments were performed in November 1896, with a cold-water extract from the kidney of a rabbit injected into the jugular vein (30, 52). Today, BP elevation is still regarded as the paramount functional aspect of renin. However, it is clear that this hormone is also involved in many physiological and pathophysiological processes, e.g., during development (9–11, 21, 33), inflammation (1, 5, 29, 53), and even in taste (50). When referring to renin, the commonly known secretory glycoprotein is mostly meant (15), which is expressed, stored, and released in a regulated manner by the juxtaglomerular cells of the kidney. These cells originate from mesenchymal cells unrelated to the endothelial or smooth muscle lineages. During differentiation they acquire smooth muscle markers that are maintained throughout adulthood (36). A number of extrarenal tissues, such as adrenal gland, brain, lung, and heart also express or internalize renin (27). Moreover, rat adrenocortical cells additionally express an alternative renin transcript, termed exon1A renin, which encodes for a truncated prorenin that is imported into mitochondria. Although its function remains unknown, it is interesting that the rat heart only expresses this alternative transcript (27). Today, cultures of renin-producing pulmonary, e.g., Calu-6 cells (18) or renin-expressing mouse kidney cell line (As4.1) (43), which have many characteristics in common with the juxtaglomerular cells, are often used for studying control of expression of the renin gene.

CONTROL OF RENIN TRANSCRIPTION

Plasma renin results from transcriptional control of the propreprorenin gene, posttranscriptional control at the mRNA level, posttranslational processing of the prorenin protein, the uptake of renin into vesicles, and its release (4, 17, 18, 25). Transcriptional control is the key for the regulation of all genes. In general, transcription of the RNA commences when the RNA polymerase binds to the promoter region of the gene. Thus it is important to understand how the RNA polymerase identifies the promoter on the DNA and what determines the activity of the transcriptional process. For many genes, it has become clear that in addition to the DNA sequence of the basic promoter residing just upstream of the cap site, there are additional regulatory elements that activate or repress transcription. Different experimental approaches define a basic promoter of the renin gene between ∼100 (28) and ∼900 bp (7) relative to the cap site. At around ∼30, it contains a canonical TATA box. In contrast to many other genes, the promoter of the renin gene exhibits only weak responses (2- to 3-fold) in reporter gene assays (7, 7, 25). The first implications for the existence of decisive regulatory elements controlling renin gene expression that can explain the two orders of magnitude fold induction came about from transgenic studies indicating a region upstream of the renin gene and the basic promoters controlling the spatial and temporal expression of the mouse renin gene (14, 41, 42, 47). An enhancer of...
transcription was later found by deletion mutagenesis and transient transfection analysis to lie in the mouse from −2866 to −2625 bp (which corresponds to AF140238, the human renin enhancer-like sequence at approximately −11 kb) relative to the initiation of transcription of the mREN gene (55). This enhancer can elicit an 80- to 100-fold increase in basal renin promoter activity in an orientation- and position-independent manner, which is in line with the definition of a classical enhancer of transcription. However, this regulatory sequence appears to be more than just an enhancer: it is a compound regulatory element to which several transcription factors with stimulatory or inhibitory activity can bind. The “enhancer” binds transcription factors at five or more different sites; e.g., at the important cAMP-responsive elements (Fig. 1; 25). Moreover, often these sequences can bind more than one factor (19, 38). For instance, the activity of one of these binding sites is controlled by stimulatory ligands, such as the vitamin A receptor, or inhibitory ligands, e.g., the vitamin D receptor complex (19). The latter seems to explain the clinically important observation that vitamin D₃ supplementation reduces BP in patients with essential hypertension (20). In mammalian renin promoters it has been shown that a critical proximal promoter element located at about −60 corresponds to a recognition sequence for Hox transcription factors binding in association with Pbx cofactors (26).

In addition to the region discussed above, there is evidence for a second important enhancer element −5 kb (in humans) upstream of the basic promoter that has been identified in a chorionic cell model (7), and there may exist even more regions of importance for controlling renin transcription. As a first step to explore possible important regions for the regulation of the human renin gene, we and other groups performed bioinformatic approaches estimating cross species sequence homology upstream of the gene (22, 37). We combined this approach with other independent database information of weight matrices for transcription factor binding sites. We estimated the homology of noncoding DNA between the human, the mouse, and the rat DNA sequences around the renin gene, which are presented as a percent identity plot (Fig. 1) (22). At −11−15 kb upstream of the human renin gene, a 3.9-kb-long block of human DNA human renin was identified containing several conserved elements. Percent identity estimates (35) of human renin gene to the corresponding DNA regions for mouse and rat were then performed, and the human renin DNA block was searched for transcription factor binding sites using Matinspector (31) with matrices for vertebrates.

To combine the information regarding conservation and binding sites, a special algorithm was developed (TFprofile) calculating a binding profile (22). There was no uniform distribution of the binding profile across the human renin block; however, several local peaks do exist, of which one of them is the aforementioned experimentally verified and physiologically very important regulatory region (28, 37, 38, 40). The importance

![Fig. 1. Potential binding sites for factors controlling renin synthesis. Bottom: percent identity 11−15 kb upstream of human renin gene of each aligning sequence from mouse and rat. Middle: binding of transcription factors can occur distant from the coding region. Diagram indicates the potential transcription factor (TF) binding profile, weighted by the conservation across species (human, rat, mouse). Peaks indicate possible regions of binding (adapted from Ref. 22). One of these sequences has been thoroughly characterized as indicated at top (adapted from Ref. 40). Location of binding sites and their designations: Ea, enhancer element A; Ee, enhancer element e for transcription factors. Ea binds NF-Y, Eb and Ec bind RAR/RXR and possibly also the vitamin D receptor (VDR). Ed binds to the CREB/ATF family of transcription factors, whereas Ee binds to e-box proteins USF-1 and USF-2.](image-url)
of the other homologous sequences remains to be assessed.

**POSTTRANSCRIPTIONAL REGULATION OF RENIN SYNTHESIS**

A second important regulatory step of renin synthesis seems to occur after the transcription processes: it is the posttranscriptional control at the mRNA level. From a quantitative point of view, this appears to be a very potent regulatory target. As demonstrated by previous studies, the actions of cAMP, a well-known inducer of renin synthesis, extend beyond its stimulatory effect on transcription of the renin gene in that it also prolongs renin mRNA half-life, thereby elevating renin synthesis (4, 18, 44, 54). In Calu-6-cells, renin mRNA levels increase up to 100-fold in response to forskolin stimulation. Under the conditions of blocked transcription, the metabolic half-life of renin is markedly augmented (44). Thus there can be little doubt of the existence of posttranscriptional renin mRNA stabilization, which contributes to developmental or cAMP-based upregulation of renin synthesis. However, it remains unknown whether the efficiency of the translation process itself, i.e., the yield of preprorenin from a certain amount of mRNA, can also be controlled. Moreover, potential mediators of mRNA stability and perhaps also translational efficiency have only recently been identified (45).

Posttranscriptional modulation often takes place by interactions between proteins and nucleotides at sequences located before or after the actual coding regions, i.e., on either the mRNA 5' or 3'-untranslated regions (UTR). If the sequences are very short, as is the case for the renin 5'-UTR, extensive mRNA-specific control via mRNA-binding proteins is less likely at this point. The 5'-UTR contains only 32–46 nucleotides (dependent on species), which makes it likely that ribosome binding may be the pivotal or sole purpose of this UTR. This interpretation is underscored by the sequence alignments for preprorenin mRNAs, showing that the 5'-UTR is not highly conserved between the four cloned species. In contrast to the 5'-UTR, the human renin 3'-UTR consists of nearly 200 nucleotides that reveal striking conservation across mammalian renin mRNAs (45). Cytidine-uridine-rich (CU-rich) sequence elements are interspersed throughout the 3'-UTR (Fig. 2). These are motifs that can bind proteins relevant for the control of mRNA stability and translational efficiency. In particular, hnRNP proteins E1 and K bind to these domains (24). This is similar to mRNAs coding for lipoygenase, 1A1-collagen, or erythropoietin (13). These CU-rich motifs have been called differentiation control element (DICE) and were identified in many other mRNA 3'-UTRs (32). Another basic determinant involved in mRNA stability is the 3'-terminal poly(A); however, notably, deadenylation/readenylation does not seem to play a role in cAMP-dependent control of renin mRNA stabilization (44).

**IDENTIFICATION OF RENIN mRNA BINDING PROTEINS**

To see whether the renin mRNA 3'-UTR can bind proteins of potential regulatory impact, we performed electromobility shift assays (EMSA). This was done with in vitro-transcribed 32P-labeled RNA and cytoplasmatic protein extracts (S100) of renin-synthesizing Calu-6 cells. The EMSA experiments show stable complex formation between cytoplasmatic proteins and renin 3'-UTR (Fig. 3).

In a recent study to further shed light on the nature of interacting proteins of potential regulatory significance, we employed ultraviolet cross-linking and RNA-affinity chromatography with subsequent matrix-assisted laser desorption/ionization time of light mass spectrometry (MALDI-TOF-MS) identification (45). Furthermore, bound proteins were analyzed by immunological techniques. The cross-linking pattern of Calu-6 cytoplasm to renin 3'-UTR and, more importantly, RNA-affinity chromatography with Calu-6 cytoplasm via biotinylated transcripts combined with MALDI-TOF-MS analysis, lead to the unambiguous identification of six proteins: hnRNP E1 (synonyms α-CP or PCBP, NP 006187), hnRNP K (NP 002131),...

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**CU-rich sequence elements**

**Fig. 2.** Scheme of human renin mRNA. Positions of the cytidine-uridine-rich (CU-rich) domains in the 3′-region are indicated in gray.
56-kDa MINT homologous protein (NP 0055816), Y-box-binding protein YB-1 (NP 035862), nucleolin (NP 005372), and dynamin (NP 056384). To further verify the MALDI-TOF-MS results, SDS gels were probed using four available antibodies. The results confirm the MALDI-TOF-MS identification of YB-1, hnRNP E1, dynamin, and nucleolin. The putative renin 3′-UTR binding proteins are not restricted to particle-free cytoplasm (S300), but were also identified in two functional forms of mRNA containing mRNP complexes, i.e., polysomes and postpolysomal mRNPs. HnRNP proteins E1 and K are among these proteins that can bind to renin 3′-UTR as could be shown by adding recombinant protein in a purified form. These hnRNP proteins are nuclear RNA-binding proteins; however, they also modulate mRNA function in the cytosole (16, 23) via a specific RNA recognition motif, the KH (K-homology) domain. The three KH domains of E1/E2 and K target pyrimidine-rich RNAs, mediating their translational regulation and stability.

MODULATION OF RENIN mRNA STABILITY

One of the most prominent examples for proteins involved in 3′-UTR-mediated control of mRNA stability and translational efficiency is perhaps hnRNP E1/K proteins that cause the extremely high metabolic stability of the globin mRNAs by assembly of E1/K proteins to pyrimidine-rich sequences in α/β-globin mRNA 3′-UTRs (6, 13, 56) and translational inhibition via the CU-rich DICE control element in lipoxygenase mRNA (23, 24, 32). Remarkably, human renin mRNA 3′-UTR also contains oligo (C)-clusters, which are essential for E1 and K binding, and is a prerequisite for stabilizing the mRNA (51). Because it has been shown, at least for hnRNP K, that the efficiency of mRNA binding is controlled by cAMP-dependent phosphorylation via ERK kinases (12), this might provide the link to understand the observed increase in renin mRNA half-life via cAMP (44).

As in several previous investigations by other groups (4, 44), we tested the half-life of renin mRNA in response to cAMP induction, a stimulus known to stabilize renin mRNA. In addition to the assessment of renin mRNA amounts (Northern blots) and renin protein (RIA), the concentrations of four renin mRNA binding proteins were also determined (Western blotting; 45). Forskolin increased the renin mRNA level ~10- to 20-fold and renin total protein by a factor of 15. Intriguingly, the level of renin mRNA binding proteins also increased between three- and sixfold. To further verify the effect of renin mRNA binding proteins on mRNA stability, in vitro RNase degradation assays were performed. They showed that binding proteins caused a marked protection of renin mRNA integrity, i.e., under these conditions, renin mRNA half-life increased roughly threefold.

Our results on renin mRNA binding proteins and their influence on mRNA half-life are derived from experiments using Calu-6 cells, a nonrenal pulmonary cell line with enhanced renin expression. Future work will clarify to what extent these mechanisms apply to renal juxtaglomerular cells. However, we are confident that the discovered basic mRNA/protein interactions reflect important mechanisms in renin expression control.

MODULATION OF RENIN mRNA TRANSLATION EFFICIENCY

The effect of renin mRNA binding proteins on mRNA stability does not exclude a second level of their potential impact, that is, on translational efficiency. To see if renin mRNA 3′-UTR influences the translation behavior, in vitro transcription/translation studies were performed.

Figure 4 indicates that the translation efficiency of renin mRNA is dependent on an intact 3′-UTR, the putative binding region of the regulatory proteins. In Fig. 4A, the cell-free production of preprorenin in re-
Reticulocyte lysates was quantified with the complete renin mRNA. Remarkably, the preprorenin yield was only one-tenth as high as in the intact mRNA when the 3′-UTR was deleted, indicating that the 3′-UTR seems to modulate the amount of protein being produced. As a second, albeit indirect, approach to test the existence of specific proteins that modulate renin translation efficiency, the aforementioned experiment was repeated in wheat germ lysate (Fig. 4B). Assuming that wheat germ cells contain a different set of regulatory proteins than mammalian cells, there should be no proteins in the lysate of these cells that can specifically bind to the 3′-UTR of the renin mRNA. Consequently, there should be no differences in the amount of protein transcript produced. This is indeed seen in Fig. 4B.

These are preliminary indications for a possible regulatory role of the 3′-UTR for an immediate control function in translation of renin mRNA. Whether the isolated 3′-UTR binding proteins actually can modulate translational behavior remains to be determined.

RENIN mRNA–PROTEIN INTERACTION IN THE CONTEXT OF mRNA-MEDIATED POSTTRANSCRIPTIONAL CONTROL

Taken together, the series of techniques identified six proteins with high affinity for human renin mRNA 3′-UTR: hnRNP proteins E1 and K, dynamin, nucleolin, YB-1, and MINT homologous protein. All contain RNA- or general nucleotide-recognition motifs and have been shown before to be involved in one of the processes for which the significance of mRNA 3′-UTRs is discussed today, that is, mRNA stability, translational efficiency, or intracellular localization. For example, nucleolin, an abundant 76-kDa protein of the nucleolus, also found in the cytoplasm, has been implicated in RNA maturation, ribosome assembly, nuclear/cytoplasmatic RNA transport, mRNA stabilization, mRNP assembly, and masking (8). In Alzheimer’s disease, nucleolin seems to stabilize mRNA of amyloid precursor protein that then accumulates within brain structures. This increased mRNA half-life is brought about by binding to the 3′-UTR of APP mRNA (57), perhaps in analogy to human renin mRNA. Another parallel to human renin mRNA binding to nucleolin is the mRNA stabilization of interleukin-2 (IL-2) mRNA (3). This occurs jointly with YB-1, as for renin, where mRNP complexes contain a combination of nucleolin and YB-1.

As discussed above, the group of hnRNP proteins E and K are important mediators of mRNA stabilization and translational control, particularly studied in the context of the globins and lipoxygenases (6, 13, 23, 24, 32, 56). Renin mRNA is a new important example where they are involved (45). A further similarity between the control of renin and a better understood regulatory system is the common occurrence of YB-1 and nucleolin in the mRNP complex of FMRP mRNA. FMRP is an RNA-binding protein (also a KH-domain protein like hnRNP proteins E and K) involved in fragile X mental retardation syndrome, one of the most common forms of inherited mental retardation. The 3′-UTR of FMRP mRNA binds its own protein product along with YB-1 and nucleolin, thereby stabilizing FMRP mRNA (2). Y-box-1 protein is a member of a larger group of proteins binding to RNA as well as DNA. Some Y-box proteins modulate transcription, whereas others, such as YB-1, are more important for cytoplasmatic processes such as stabilization of the mRNA structure. Moreover, YB-1 may affect translation (48). As suggested by the combined occurrence with nucleolin, YB-1 seems to interact with other RNA-regulatory proteins. In addition to the cooccurrence...
with nucleolin, hnRNP K is a suggested interaction partner of YB-1 in two-hybrid screens (39). In the renin-producing juxtaglomerular cells, YB-1 may also play a role in intracellular positioning of human renin mRNA: YB-1 interacts with actin microfilaments (34) and a mentionable portion of mRNAs and polysomes of the cell are associated with the cytoskeleton. Thus YB-1 may help to direct mRNA from the nucleus to its correct location in the cytoplasm (49).

Perspectives

The diversity of renin actions is reflected by its complex control. The release of renin from the vesicles in juxtaglomerular cells is determined by stretch (arterial pressure), sympathetic nerves, and salt (macula densa mechanism). The refurbishment of renin to the vesicles underlies a multifaceted control, which occurs at the levels of transcription and translation. Although important regions for binding transcription factors have already been identified, the conservation of nucleotide sequences throughout different species suggests that there might be even more binding regions with importance for the control of renin transcription. Renin mRNA stability appears to be the result of the interaction between several regulatory proteins, most of which are well known in other systems. Along with modulation of mRNA half-life, the translation efficiency seems to play a key role in determining the amount of renin to be produced.

DISCLOSURES

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REFERENCES


