

An Alternative Transcript of the Rat Renin Gene Can Result in a Truncated Prorenin That Is Transported Into Adrenal Mitochondria

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Abstract—Characterization of the local renin-angiotensin system in the rat adrenal zona glomerulosa indicated a dual targeting of renin both to the secretory pathway and mitochondria. To investigate the transport of renin into mitochondria, we constructed a series of amino-terminal deletion variants of preprorenin. One of these variants, lacking the complete signal sequence for the endoplasmic reticulum and 10 amino acids of the profragment, was transported efficiently into isolated mitochondria. The transport was further shown to be dependent on mitochondrial membrane potential and ATP synthesis. Analysis of adrenal RNA revealed the existence of 2 renin transcripts. While one of the transcripts corresponds to the known full-length transcript, the other one lacks exon 1; instead, exon 2 is preceded by a domain of 80 nucleotides originating from intron 1. This domain, as well as the following region of intron 1 being excised, shows all essential sequence elements defining an additional, so-far-unknown exon. The second mRNA possibly derives from an additional transcription start in intron 1 and an alternative splicing process. Translation of this mRNA could result in a truncated prorenin representing a cytosolic form of renin, which is required for transport into mitochondria. This truncated prorenin corresponds exactly to the deletion variant being imported into mitochondria in vitro. (*Circ Res.* 1999;84:337-344.)

Key Words: renin-angiotensin system ■ adrenal gland ■ mitochondria ■ prorenin ■ protein targeting

The renin-angiotensin system (RAS) in the circulation has been well characterized with regard to its role in the regulation of blood pressure and stimulation of aldosterone production. In recent years, findings have accumulated indicating an additional regulation of aldosterone synthesis by a local RAS in the adrenal gland of rats and other mammalian species.¹ In the adrenal cortex of rats, all components of the RAS, renin, angiotensinogen, and angiotensin-converting enzyme, are expressed on the transcriptional level, and the corresponding proteins have been detected as well.^{2,3}

The functional significance of an adrenal RAS is not clear at present. However, in some physiological situations the production of aldosterone is found to correlate better with the adrenal renin level than with the circulating renin concentration. For example, an increase in plasma potassium concentration is associated with suppression of renal renin secretion and decreased plasma renin, whereas adrenal renin is increased.^{4,5} Furthermore, nephrectomy completely eliminates circulating renin, but increases adrenal renin and aldosterone synthesis.⁵ More importantly, the rise in aldosterone production induced by nephrectomy is inhibited by the AT₁-receptor antagonist losartan.^{6,7}

Immunocytochemical and biochemical analyses revealed that renin in the adrenal zona glomerulosa of the rat

is not only localized in intracellular vesicles. Interestingly, renin also has been found within mitochondria, in inclusion bodies of high electron density.^{8,9} As the mitochondrial genome does not contain the gene for renin, the mitochondrial form of this protein must be derived from the nuclear genome and has to be synthesized in the cytosol and imported posttranslationally into the organelles, according to the common concept for the targeting of nuclear-encoded mitochondrial proteins.¹⁰

This raises the question of how renin enters the mitochondria. Renin so far has been known just as a secretory protein, which is cotranslationally targeted to the secretory pathway via the endoplasmic reticulum (ER) and Golgi apparatus. During this pathway, preprorenin is first cleaved to prorenin, glycosylated, and then delivered to storage granules, where it is further processed to active renin. This pathway does not allow a segregation of renin or one of its precursors to the mitochondria, as the cotranslational transport of preprorenin into the ER prevents the occurrence of a cytosolic form of renin, which is required for the transport into mitochondria. In addition, the targeting of nuclear-encoded proteins into mitochondria usually requires the existence of an amino-terminal targeting sequence, which is quite different from the ER signal sequence.¹¹

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These considerations led to the hypothesis that the differential targeting of renin to mitochondria instead of the ER could be achieved by an additional downstream initiation of translation, resulting in the synthesis of a protein that lacks the ER signal sequence and thus escapes the secretory pathway. To investigate the translocation of renin into mitochondria, we constructed amino-terminal deletion mutants of preprorenin corresponding to prorenin, active renin, and several intermediate forms and analyzed their properties in import experiments with isolated mitochondria of the rat adrenal gland. In addition, we characterized different transcripts of the renin gene, thereby providing the molecular basis for the dual targeting of renin to ER and mitochondria in vivo.

Materials and Methods

Construction of Mutants

The full-length rat-renin cDNA¹² was used for the construction of the deletion mutants. The complete insert was subcloned into pBlue-script M13⁻ (Stratagene). Various deletions from the 5'-end were introduced by polymerase chain reaction (PCR) using the antisense primer Ren3 (5'-GCCTAAACTAGGGTCACTGGGTG-3'; nucleotides 1252 to 1275) hybridizing immediately behind the stop codon and a set of sense primers (Gibco BRL) corresponding to different sequences in the 5'-region.

The primers used are described below. Primer Ren6 (5'-GGAGGAGGATGCCTCTCTGGGCA-3'; 40 to 62) was used, giving rise to a fragment of 1236 nucleotides. The corresponding coding sequence starts with the 2nd methionine and therefore lacks 5 amino acids of the preprorenin. Primer Ren1 (5'-ACAGCCAGCTTTGGACGAATC-3'; 108 to 132) results in a fragment of 1168 nucleotides, the coding sequence of which yields a protein lacking 36 amino acids. Primer Ren2 (5'-GGGAAATCCTGGAGGAGCGGGGAG-3'; 154 to 177) gives a fragment of 1122 nucleotides, and the corresponding protein shows a deletion of 50 amino acids. Two other constructs are equivalent to prorenin and active renin, respectively. These constructs required the addition of an AUG as start codon, which was introduced with the respective primer. Primer Ren9 (5'-CAGACATGGCCAGCTTTGGACGAATC-3'; 103 to 128; C109-T, A110-G) results in a fragment of 1173 nucleotides; the coding sequence of this fragment corresponds to prorenin. In a similar way, a coding sequence corresponding to active renin was achieved, using primer Ren10 (5'-TGTTATGTCCCCCTGGTCCTC-3'; 239 to 260; C244-T, C245-G), which gives a fragment of 1037 nucleotides.

Amplification was performed using Pwo DNA polymerase (Boehringer Mannheim), and the obtained fragments were cloned into pBlue-script M13⁻ restricted with *EcoRV*. All constructs were verified by sequencing.

In Vitro Transcription and Translation

Plasmids containing the desired fragments were linearized downstream with an appropriate enzyme and transcribed using either T3 or T7 RNA polymerase (Promega). The transcripts were subsequently translated in a rabbit reticulocyte lysate system (Amersham Buchler) in the presence of [³⁵S]methionine (ICN-Biomedicals) for labeling.

Preparation of Mitochondria

Male Sprague-Dawley rats weighing 200 to 300 g were used in the experiments. Rats were killed by cervical dislocation under ether anesthesia. Adrenal glands were removed and trimmed of fat. Adrenal tissue was gently mechanically disrupted by 5 strokes with a motor-driven potter-Elvehjem grinder in homogenization buffer (250 mmol/L sucrose, 10 mmol/L Tris/HCl (pH 7.4), 2 mmol/L EDTA, and 0.1 μmol/L phenylmethylsulfonyl fluoride [PMSF]) as described previously.⁸ The intact organelles were precipitated by differential centrifugation of the homogenate and the following

supernatants with successively increasing speeds of 100g, 300g, and 1000g for 20 minutes each. The pellet obtained by the 1000g centrifugation was washed in homogenization buffer, recentrifuged, and then resuspended in the incubation buffer for the in vitro import assay (see below).

The degree of purification of mitochondria was confirmed by determination of the activity of marker enzymes, malate dehydrogenase for mitochondria, and acid phosphatase for lysosomes by standard procedures.^{8,13}

In Vitro Import Into Isolated Mitochondria

Import of radiolabeled proteins into isolated mitochondria was performed according to Schwarz et al,¹⁴ with some alterations. Isolated mitochondria were resuspended in import buffer (250 mmol/L sucrose, 1% fatty acid-free BSA, 80 mmol/L KCl, 25 mmol/L potassium phosphate, 5 mmol/L magnesium acetate, 1 mmol/L DTT, 1 mmol/L MnCl₂, 2.5 mmol/L NADH, 2.5 mmol/L ATP, 10 mmol/L sodium succinate, and 50 mmol/L HEPES/KOH, pH 7.5) and then divided into aliquots of 120 μL, corresponding to 200 μg of mitochondrial protein. After a preincubation period of 5 minutes at 25°C, 10 μL of radiolabeled precursor was added and the samples were incubated for 30 minutes at 25°C. The import reactions were stopped by adding 400 μL of ice-cold dilution buffer (250 mmol/L sucrose, 80 mmol/L KCl, and 20 mmol/L HEPES/KOH, pH 7.5) and divided, and half of each sample was treated with proteinase K (40 μg/mL) for 15 minutes on ice to distinguish proteins bound to the outer membrane from those imported. Proteinase K digestion was stopped by adding PMSF to a final concentration of 1.6 mmol/L. Mitochondria were reisolated by centrifugation at 1000g and washed with wash buffer (250 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L PMSF, and 20 mmol/L HEPES/KOH, pH 7.5). The pellets were analyzed by SDS-PAGE. Supernatants of the reisolation step were precipitated with 4 volumes of ice-cold acetone, and the precipitated proteins were also subjected to SDS-PAGE to check the efficiency of proteinase K digestion. In some experiments, inhibitors of the mitochondrial electron transport and the oxidative phosphorylation were added to the import assay (inhibitors were, 20 μmol/L oligomycin, 8 μmol/L antimycin A, and 0.5 μmol/L valinomycin).

RNAse Protection Assay

Total RNA was isolated from rat adrenal glands by the method of Auffray and Rougeon,¹⁵ and 200 μg was hybridized to an antisense probe, which was prepared by using a *PstI/KpnI* fragment of 300 nucleotides from the rat renin cDNA subcloned into pGEM3 (Promega). After linearization with *HindIII*, this fragment yielded a rat renin-specific antisense RNA of 297 nucleotides when transcribed with T7 RNA polymerase (Promega) using [³²P]CTP (Amersham) for labeling. RNase protection assays were performed as described previously.¹⁶

Rapid Amplification of cDNA Ends (5'-RACE)

Poly A⁺ RNA was prepared from rat adrenal total RNA using a PolyA Spin mRNA isolation kit (New England Biolabs). 5'-RACE was performed with 1 μg of poly A⁺ RNA using the Marathon cDNA amplification kit (Clontech) according to the manufacturer's instructions, as follows: first- and second-strand cDNA synthesis was followed by the ligation of an adaptor to both cDNA ends after creating blunt ends by T4 DNA polymerase. Amplification of the 5'-fragments of the cDNA was done using the Expand long-template PCR system (Boehringer Mannheim). The renin-specific antisense primers used were Ren3 (5'-GCCTAAACTAGGGTCACTGGGTG-3'; 1252 to 1275) and Ren22 (5'-GCATGATCAACTGCAGGGAGCTG-3'; 923 to 945) for the primary and secondary amplifications, respectively. Two nested sense primers hybridizing to adaptor sequences were provided with the kit. The resulting fragments were restricted with *PstI* and cloned into pBlue-script M13⁻ restricted with *EcoRV/PstI*. The obtained clones were characterized by sequencing.

Preprorenin:
 MGGRMPLWALLLLWTSCFSFLPTDTASFGRILLKKMPVREILEERGVDMTRISA EWGEFIKKSSFTNVTSPVVL T...

D 5: MPLWALLLLWTSCFSFLPTDTASFGRILLKKMPVREILEERGVDMTRISA EWGEFIKKSSFTNVTSPVVL T...

D 26 (Prorenin): MASFGRILLKKMPVREILEERGVDMTRISA EWGEFIKKSSFTNVTSPVVL T...

D 36: MPVREILEERGVDMTRISA EWGEFIKKSSFTNVTSPVVL T...

D 50: MTRISA EWGEFIKKSSFTNVTSPVVL T...

D 71 (active Renin): MSPVVL T...

Figure 1. Amino-terminal amino acid sequences of rat preprorenin and the derived deletion variants constructed for transport into isolated mitochondria. M indicates the additional methionine being introduced in the deletion variants D26 and D71 to allow their translation in vitro.

Results

Transport of Renin Variants

We have postulated that the dual targeting of renin to the ER and mitochondria in the rat adrenal gland requires the existence of both preprorenin and a cytosolic form of renin. In the presumed cytosolic form of renin, the ER signal sequence should be absent, and it should be transported into mitochondria. These requirements first needed to be investigated by an in vitro system. To this end, a series of amino-terminal deletion mutants of renin was constructed by means of PCR, lacking 5, 26, 36, 50, and 71 amino acids, respectively, relative to preprorenin (Figure 1). On the basis of the consideration that an alternative translation start could give rise to a cytosolic form of renin, the construction of the D5, D36, and D50 proteins made use of existing ATG codons within the sequence, which might be used as a translation start instead of the first ATG codon of preprorenin. The D26 and D71 proteins correspond to prorenin and active renin, respectively. These 2 forms occur in vivo; however, to allow their translation in vitro, an ATG codon had to be introduced. Therefore, these 2 variants contain an additional N-terminal methionine, which is not part of the authentic sequence.

All mutant proteins and the full-length preprorenin were analyzed with respect to their possible import into isolated mitochondria of the rat adrenal gland (Figure 2). As a positive control, aldosterone synthase (CYP11B2),^{17,18} a nuclear-encoded cytochrome P450 enzyme involved in aldosterone biosynthesis, which is localized in adrenal mitochondria, was used. This protein was obtained from a cDNA, which we cloned recently.¹⁹ Among the 6 renin variants, the D26 and particularly the D36 protein were transported with considerable efficiency into isolated mitochondria, as was confirmed by protection against exogenous protease. These 2 proteins lack the complete ER targeting sequence of preprorenin. A slight rate of transport could also be observed for the D5 protein, while preprorenin, the D50 protein (not shown), and active renin were not imported into mitochondria.

A characteristic of import of many mitochondrial proteins is its dependence on the membrane potential and ATP synthesis.^{20,21} Inhibition of mitochondrial electron transport and ATP generation therefore could be expected to impair the transport of the D5, D26, and D36 proteins. Import experiments in the presence of specific inhibitors of the mitochondrial electron transport and ATP synthesis, antimycin A, oligomycin, and valinomycin, revealed a substantial decrease of the transport of the D26 protein and a nearly complete inhibition of the import of the D5 and D36 proteins, as well as CYP11B2 (Figure 2).

Most, but not all, mitochondrial proteins are synthesized with an amino-terminal targeting sequence, which is removed by proteolytic cleavage on import into the mitochondrial matrix, as was seen for CYP11B2 (Figure 2). In our import experiments, we did not observe a processing of the imported renin variants, as their apparent molecular weight remained constant. This could be explained possibly by the existence of an internal targeting signal rather than an amino-terminal cleavable targeting sequence.

Renin mRNA in the Kidney and Adrenal Gland

In our in vitro experiments, we could demonstrate that renin variants lacking the presequence of preprorenin are imported into isolated mitochondria, especially the D36 protein, which starts with the first ATG codon of exon 2. This raised the question whether the corresponding protein can be generated in vivo by using a start codon distinct from the translation start of preprorenin. The only known transcript of the renin gene includes the coding sequence of the ER-targeting signal. This transcript could give rise to different proteins with or without the ER-targeting signal by using different ATG codons for initiation of translation. Another possibility could be given by the existence of an alternative transcript, which does not include the coding sequence of the ER-targeting signal.

Therefore, total RNA isolated from rat kidney and adrenal gland was analyzed by RNase protection assay. Hybridization was performed with a cRNA specific for the 5'-region of rat renin mRNA, covering nucleotides 12 to 124 of the first exon, the complete second exon, and the first 28 nucleotides of exon 3. In the kidney, this cRNA yielded a single fragment of 290 nucleotides, corresponding to the full-length transcript (Figure 3). In the adrenal gland, an additional fragment of ≈ 180 nucleotides was found, which might be equivalent to a second, shorter mRNA.

To further investigate the adrenal renin mRNA, 5'-RACE was performed to determine the structure of both transcripts. Fragments derived from the 5'-region of the mRNA were obtained by specific amplification of rat adrenal cDNA using 2 sets of nested primers, each consisting of a renin-specific antisense primer and a sense primer complementary to an adaptor ligated to the cDNA. Analysis of the cloned 5'-RACE fragments by sequencing revealed the existence of 2 distinct types of mRNA (Figure 4). These 2 transcripts show considerable differences in their 5'-region. One of the transcripts corresponds to the full-length mRNA, beginning with the known transcription start of exon 1. The other transcript represents a so-far-unknown variant. In contrast to the full-

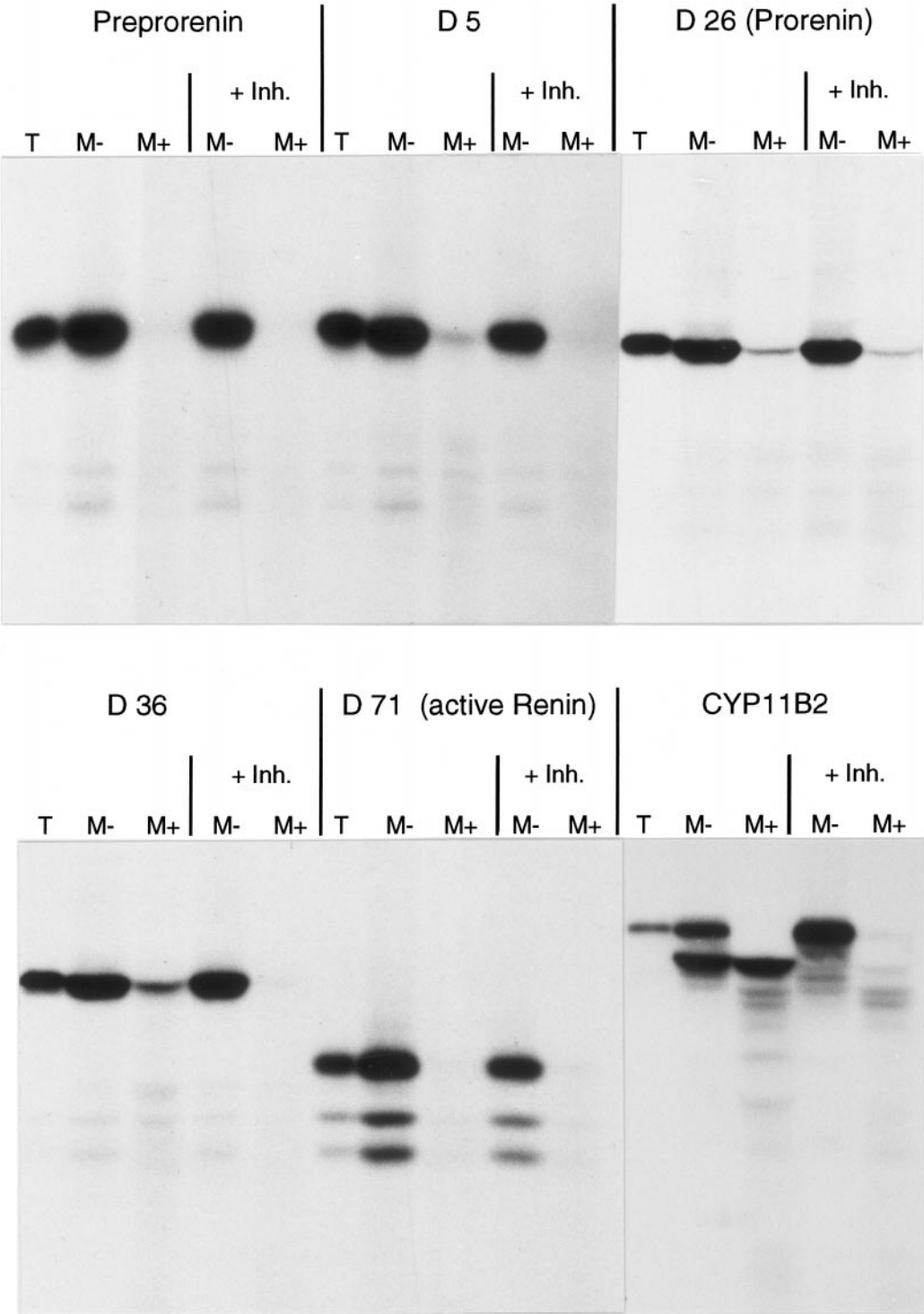


Figure 2. In vitro transport into mitochondria of rat preprorenin, the amino-terminal deletion variants ($n=4$), and rat aldosterone synthase ($n=2$). Isolated mitochondria of the rat adrenal gland were incubated with the in vitro-translated, ^{35}S -labeled proteins (lane T; $0.1\ \mu\text{L}$ was loaded). After incubation, samples were divided; half of the mitochondria were treated with proteinase K (lane M+), and the other half remained untreated (lane M-) to distinguish bound and imported proteins. In parallel experiments, $20\ \mu\text{mol/L}$ oligomycin, $8\ \mu\text{mol/L}$ antimycin A, and $0.5\ \mu\text{mol/L}$ valinomycin as inhibitors of the mitochondrial electron transport and oxidative phosphorylation were added (+Inh.).

length mRNA, this transcript lacks exon 1. Instead, in the largest of the analyzed clones, exon 2 is preceded by a domain of ≈ 80 nucleotides, originating from intron 1. Intron 1 comprises the nucleotides 125 to 4590, according to Fukamizu et al.²² The region of intron 1 present in the renin

mRNA consists of nucleotides 3833 to 3911. The intervening sequence being excised comprises nucleotides 3912 to 4590. Thus, the sequence of nucleotides 3833 to 3911 could represent a so-far-unknown additional exon, which we termed exon 1A, while the intervening region could be considered as

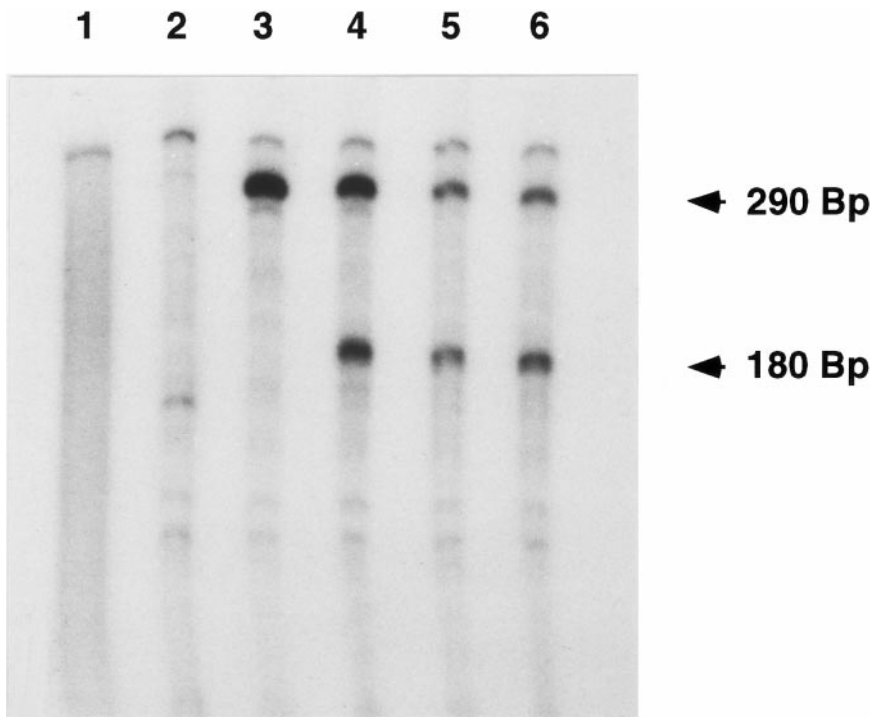


Figure 3. Expression of the renin gene in the rat adrenal gland. RNase protection assay was performed by hybridizing a renin-specific probe (lane 1) to 20 μ g of tRNA as a negative control (lane 2), 2 μ g of RNA from rat kidney (lane 3), and 200 μ g of rat adrenal RNA (lanes 4 to 6) isolated from different animals.

a separate intron. This hypothesis is supported by the fact that the sequences at the putative splice sites correspond well to the known consensus elements; nucleotides 3912/3913 and 4589/4590 are in agreement with the GT/AG consensus sequence of 5'- and 3'-splice sites, respectively (Figure 4). The subsequent nucleotides, 3914 to 3917, at the 5'-end of

the putative intron also correspond to the known consensus sequence GURRGU, which has been described as an essential element in the splicing reaction.²³⁻²⁵ Furthermore, nucleotides 3909 to 3911, the first 3 nucleotides upstream of the putative 5'-splice site, are in accordance with the consensus element YRG, which is typically found at the 3'-end of an

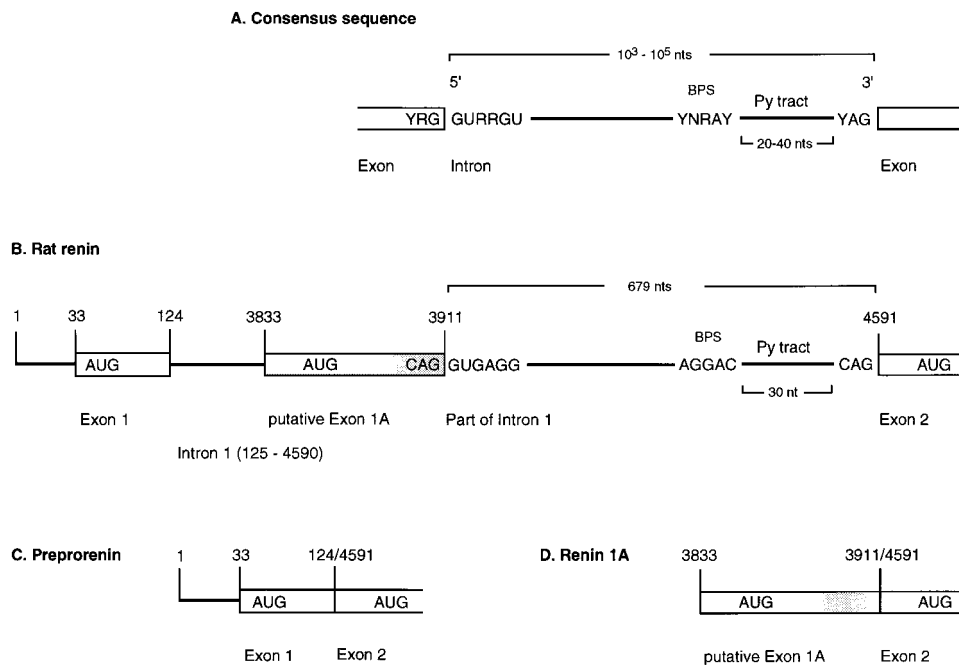


Figure 4. A, Consensus elements at 5'- and 3'-splice sites²⁵ for comparison with the putative exon 1A/intron sequences of the alternative rat renin transcript. Shown is the 5'-region of the rat renin primary transcript (B) with the derived full-length mRNA (C) and the alternative mRNA (D). Numbers denote the nucleotides in the genomic sequence according to Reference 22. AUG codons are shown; the shaded area shows the putative exon 1A within intron 1. R indicates purine; Y, pyrimidine; N, any nucleotide; BPS, branchpoint sequence; and Py tract, a pyrimidine-rich tract between the branchpoint sequence and the AG dinucleotide. Exons and intron 1 are not drawn to scale.

exon. At the 3'-end of the intron, the splice acceptor site corresponds well to the consensus sequence, as expected.

Therefore, the renin mRNA variant that we found in the adrenal gland might be the result of an alternative splicing event, using a so-far-unknown splice donor site. Further upstream of nucleotide 3833, the intron sequence shows no elements defining a splice acceptor site; thus, the start point of this mRNA may be derived from an alternative transcription start in intron 1.

Discussion

On the basis of previous observations, showing localization of rat adrenal renin in cytoplasmic vesicles as well as in mitochondria, we were interested to find a possible mechanism of this dual targeting and to obtain further evidence regarding the presence of renin within mitochondria.

As renin is usually known to be targeted to the secretory pathway, whereby the occurrence of a cytosolic renin is prevented, our first aim was to define the postulated cytoplasmic form of renin that can be targeted to the mitochondria and provides the structure necessary for translocation. From our series of N-terminal deletion variants, we conclude that a form of renin lacking 36 amino acids, namely the complete ER signal sequence and 10 amino acids of the profragment, meets these requirements. This D36 protein was transported into mitochondria with higher efficiency than were 2 other variants, D26 and D5, which comprised the complete profragment or the complete profragment as well as part of the ER signal sequence, respectively. Compared with CYP11B2, transport of D36 appeared to be less efficient. This might be due to the fact that renin, in contrast to CYP11B2, is expressed in only a small number of cells in the zona glomerulosa^{7,26}; therefore, it may be only a small population of mitochondria in the adrenal gland that is able to import renin. The full-length preprorenin, as well as the shorter proteins D50 and active renin, were not imported at all. These results indicate that the region between amino acids 36 and 50 might comprise the necessary mitochondrial targeting sequence, or at least part of it. Although the longer variants, D5 and D26, contain the sequence required for mitochondrial import, their additional N-terminal amino acids, especially the ER-targeting sequence, may impair the translocation process, whereas in the shorter proteins, D50 and active renin, essential sequence elements appear to be missing.

The exact structure and localization of the mitochondrial targeting signal within the renin sequence remain to be investigated. The region between amino acids 36 and 50, although apparently indispensable for import, differs from mitochondrial targeting sequences in that it contains some negatively charged amino acids, besides 3 arginine residues. Interestingly, the N-terminal targeting sequences of other nuclear-encoded proteins located in adrenal mitochondria, CYP11B1, and CYP11B2, as well as cytochrome P450 side-chain cleavage enzyme (P450SCC), also include negatively charged amino acids.²⁷ Neither the CYP11B1 and CYP11B2 targeting signals nor the N terminus of the D36 protein clearly show the potential to form an amphiphilic helix. We did not observe a proteolytic processing of the imported D36 protein, which is in contrast to most nuclear-

encoded mitochondrial proteins; we cannot exclude, however, that part of the targeting signal resides within the protein as an internal signal, but there is only little information available concerning the properties of internal targeting signals.^{28,29} There are a number of mitochondrial proteins known that contain an internal targeting sequence that is not cleaved (eg, cytochrome c, cytochrome c heme lyase, and the BCS1 protein).^{28,30,31} Proteins bearing an internal targeting sequence are usually known to be located in the mitochondrial inner membrane or intermembrane space. Renin, in contrast, has been detected within dense bodies, which are likely to be located in the mitochondrial matrix, and therefore it presumably has to be transported across the inner membrane. This is in agreement with our observation that import of the D36 protein is dependent on the presence of a membrane potential, which is required for translocation of proteins into the mitochondrial matrix.^{20,21}

A proteolytic processing of the imported D36 protein may also be required to generate active renin. However, considering some previous findings, it is quite possible that this form of renin is already enzymatically active. Apart from the fact that mitochondrial renin has been demonstrated to be biochemically active,⁸ in human plasma as well as in plasma of other mammalian species truncated forms of prorenin have been characterized, showing partial or full renin activity.^{32,33} The *in vitro*-translated D36 protein also shows enzymatic activity, which is inhibited by the renin-specific inhibitor CH732³⁴ (data not shown). Further investigations are required to prove renin activity of D36 protein *in vivo*.

The results of our *in vitro* transport experiments suggested the existence of a cytosolic form of renin, which could be derived either from the full-length mRNA by using a downstream initiation of translation or from a shorter transcript, lacking the sequence coding for the ER targeting signal. Different transcripts of the rat renin gene so far have not been described. An initiation of transcription and possibly translation upstream of the known start of preprorenin, although reported for mice and humans,³⁵⁻³⁷ was not found for the rat renin gene.³⁸ Moreover, an additional upstream translation start would result in the synthesis of a protein still containing the ER signal sequence, which would be targeted to the secretory pathway.

Analysis of renin mRNA in the rat adrenal gland indeed indicated the existence of 2 different transcripts. Whereas by RNase protection assay, using a renin-specific cRNA hybridizing to the 5'-region, a single fragment was detected in the kidney, RNA isolated from the adrenal gland yielded a second, shorter fragment. For detailed analysis of these mRNAs we performed 5'-RACE, which revealed the existence of 2 renin transcripts with completely different 5'-regions. Besides the known full-length transcript, we found an additional mRNA lacking exon 1, but instead containing a region of 80 nucleotides, having its origin in intron 1. The intervening sequence of intron 1, which has been excised, shows all essential sequence elements defining a so-far-unidentified splice site. Thus, we conclude that this renin mRNA represents an alternative spliced transcript, in which exon 1 appears to be replaced by another, so-far-unknown exon, termed exon 1A.

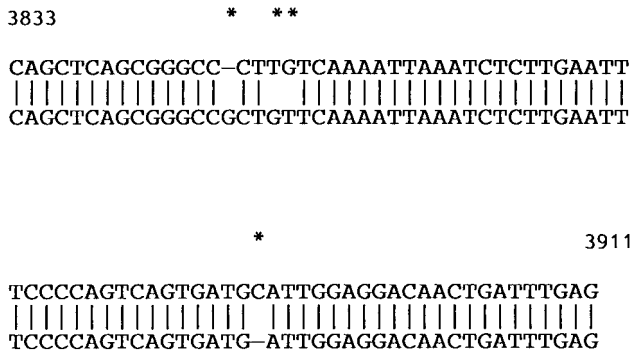


Figure 5. The region of intron 1 of the rat renin gene found in the splice variant. Shown is a comparison of the published sequence of Fukamizu et al²² (top lines) and the sequence determined in this work (bottom lines). Asterisks indicate differences; numbers denote the nucleotides in the genomic sequence.

Because of the method of cDNA synthesis, it is not clear yet whether the start point at nucleotide 3833 we found represents the true 5'-end of this mRNA. As most of the clones derived from the full-length mRNA indeed correspond to the known transcription start, we suppose that the sequence of our mRNA variant either is complete or lacks only a few nucleotides. Upstream of the 5'-end of our mRNA variant, there are no sequence elements equivalent to a splice acceptor site. Therefore, an alternative start of transcription within intron 1 might be used, although promoter elements in the upstream region are not found.

Interestingly, the putative exon sequence includes an additional ATG codon (nucleotides 3886 to 3888). According to the published genomic sequence,²² this ATG codon would be within the reading frame. Consequently, translation of this mRNA could result in a protein including an additional N-terminal sequence. Our sequenced clones, however, differ in 4 positions from the published sequence (Figure 5). To confirm these findings, we also cloned and sequenced part of intron 1 from rat genomic DNA, including the corresponding region, which was in agreement with the sequence determined from the 5'-RACE clones. Since we found a deletion of a cytosine at position 3889, the additional ATG codon would be out of the reading frame. Thus, translation could start from the first ATG codon of exon 2, resulting in a protein being unable to enter the secretory pathway and remaining intracellular. Such a protein corresponds exactly to our D36 protein, which was shown to be transported into isolated mitochondria most efficiently.

In our in vitro translation system, the first ATG codon in exon 2 appears to be used with the same efficiency as the start codon of preprorenin (Figure 2). None of the start codons of either preprorenin or one of the deletion variants shows considerable agreement with the GCCACCAUGG consensus motif for initiation of translation.³⁹ For example, the first start codon of preprorenin corresponds only in positions -4 and +4 to the consensus, the first ATG in exon 2 corresponds in position -3, and the ATG codon in exon 1A agrees in position -5. In vitro translation of a renin transcript containing exon 1A results in the D36 protein (S.C. et al, unpublished data). These observations suggest that the respective ATG codon in

exon 2 might also be used as a translation start in vivo, which has still to be proved by purification and N-terminal sequencing of the protein.

Obviously, the observed different transcription and splicing process of the rat renin gene seems to be tissue specific, as this mRNA variant is found in the adrenal gland but not in the kidney. From sequence considerations, an intracellular form of renin has also been proposed for the human renin.³⁷ In the human renin gene, a putative promoter is found within the first intron, which might yield either a renin precursor that lacks the ER signal sequence and the amino-terminal half of the profragment or a precursor containing a different hydrophobic leader sequence. Thus, a differential targeting within the cell could also be regarded as possible for the human renin. An alternative splice process, as we observed for the rat renin transcript, apparently would not take place in the human mRNA.

The results presented here support previous findings of renin within mitochondria. Further investigations are required to explain the role of mitochondrial renin. Essential steps of aldosterone biosynthesis take place within the mitochondria of the zona glomerulosa. So it is tempting to speculate that intramitochondrial renin might be involved in the regulation of steroid biosynthesis. Interestingly, mitochondrial renin as well as the number of dense bodies in the adrenal gland of the rat are increased by nephrectomy, in association with an increase in aldosterone production.^{5,8} Furthermore, under these conditions, aldosterone production is still dependent on local generation of angiotensins and is inhibited by losartan even after eliminating the circulating RAS.^{6,7} However, besides a possible role in the regulation of aldosterone production in the mitochondria, other functions of the cytosolic renin are also conceivable.

Acknowledgments

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