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Kidney and submaxillary gland renins are encoded by two non-allelic genes in Swiss mice

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Two distinct phenotypic groups of inbred strains of mice, with different amounts of submaxillary gland (SMG) renin have been described. We have previously shown that strains with high levels of SMG renin, such as Swiss or AKR mice, have two renin genes, *Rn1* and *Rn2*, per haploid genome, while strains with low levels of SMG, such as BALB/c or C57Bl/6, have only one renin gene. We now report the molecular cloning of cDNA copies of Swiss mouse kidney renin mRNA and present nucleotide sequence data of the recombinant clones. Comparison of these sequences with the sequence of Swiss mouse SMG renin mRNA we have previously reported, demonstrates that Swiss mice express the two non-allelic genes, *Rn1* and *Rn2*.

Key words: gene expression/gene duplication/renin regulatory gene/kidney renin cDNA/DNA sequence

Introduction

Renin plays a key role in blood pressure regulation and salt-water balance, since it catalyzes the first step of the renin-angiotensin-aldosterone cascade. Kidney juxtaglomerular cells constitute the primary source of renin, but renin has also been found in other organs, such as the uterus (Skinner *et al.*, 1968), the brain (Ganten *et al.*, 1971) and the vessel walls (Gould *et al.*, 1964). Furthermore, high levels of renin synthesis have been detected in the submaxillary gland (SMG) of mice (Werle *et al.*, 1962). Biochemical and immunocytochemical studies suggest that SMG renin is closely related to renal renin (Michelakis *et al.*, 1974; Hirose *et al.*, 1977; Malling and Poulsen, 1977).

SMG renin synthesis is under hormonal control by androgens (Oliver and Gross, 1967; Wilson *et al.*, 1977) and differences in SMG renin content between inbred strains of mice have been described (Wilson *et al.*, 1978). Strains high in SMG renin are presumed to carry the *S* allele of the renin regulatory gene *(Rnr)* and strains low in SMG renin the *B* allele. We have previously shown that strains carrying the *B* allele, such as BALB/c, C57Bl/6 or C3H mice have one renin gene per haploid genome, while those having the *S* allele, such as AKR or Swiss mice, have two renin genes (Panthier *et al.*, 1982b).

This correlation between a high level of SMG renin activity and a renin structural gene duplication led us to propose a model for the genetic control of SMG and kidney renin activities: mice bearing the *B* allele have only one renin gene, named *Rn1*, which is expressed mainly in kidney but also at a very low level in the SMG. Mice bearing the *S* allele have two renin genes, the former *Rn1* gene and a second renin gene, named *Rn2*, which is actively expressed in the SMG in response to androgenic hormones.

Since there is no definitive evidence that kidney and SMG renins in mice bearing the *S* allele are respectively the products of the two non-allelic genes *Rn1* and *Rn2*, we have compared the kidney and SMG renin mRNA from Swiss mice. Kidney cDNA fragments were cloned and partial nucleotide sequences of these recombinant clones were determined and compared with the previously reported sequence of a SMG renin cDNA fragment (Panthier *et al.*, 1982a). Here we present evidence that SMG and kidney renins in Swiss mice are the products of the two non-allelic *Rn1* and *Rn2* genes.

Results

Partial purification of kidney renin mRNA

We have previously shown that probes derived from SMG renin mRNA allow identification of kidney mRNA in stringent conditions (Rougeon *et al.*, 1981) and that kidney renin mRNA and SMG renin mRNA have the same length, 1600 nucleotides. Furthermore, as shown in Figure 1, the kidney and SMG renin precursors are of the same mol. wt., ~45 000 daltons as determined previously (Rougeon *et al.*, 1981; Panthier *et al.*, 1982a).

It has been shown that there is no difference in plasma renin concentration among different mouse strains. However, we have noticed that the high SMG renin synthesis in Rnr *S* mice is associated with a lower level of renin mRNA sequences in kidney in the male than in the female (Rougeon *et al.*, 1981). Therefore, we isolated total poly(A)-containing RNA from female mice.

Total poly(A)-containing RNA was prepared from the kidneys of 7-week-old Swiss females and fractionated on a 5–20% sucrose gradient. Aliquots of each fraction were treated with glyoxal in dimethylsulfoxide and subjected to electrophoresis on 1.1% agarose gel. The RNA was blotted to nitrocellulose and hybridized to ^{32}P-labeled pRn1-4. Aliquots of each fraction were also translated *in vitro* in the rabbit reticulocyte lysate system and the translation products were analyzed on a SDS-12.5% polyacrylamide gel (data not shown). Fractions, which were shown by both methods to contain mRNA encoding kidney renin, were pooled and used in the following experiments.

Molecular cloning of kidney renin cDNA fragments

As shown by *in vitro* translation of mRNA isolated from SMG and kidney (Figure 1) and by hybridization of SMG and kidney mRNA with a renin probe (Rougeon *et al.*, 1981), the amount of mRNA that hybridizes to the renin cDNA clone appears to be much lower in kidney that in SMG, We estimate the level of renin mRNA in kidney to be 1/100 to 1/1000 of that in SMG of male Swiss mice.

Complementary DNA was synthesized from 25 μg of partially purified kidney renin mRNA as a template by reverse transcription, made double-stranded (ds) and sized by sucrose gradient centrifugation. The ds cDNA was then inserted into the *Pst*I site of pBR322 by the oligo(dC/dG) tailing method. Transformation of *Escherichia coli* C600 with the hybrid

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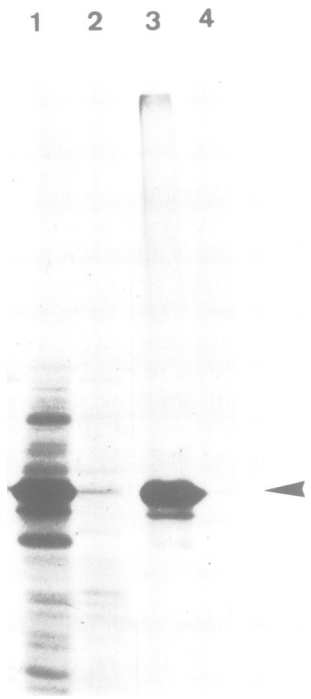

Fig. 1. Identification of renin precursor from SMG and kidney of Swiss mice. Total poly(A)-containing RNA from SMG **(lane 1)** or kidney **(lane 2)** were translated in a reticulocyte cell-free system. Aliquots of the translation products from either SMG **(lane 3)** or kidney **(lane 4)** were immunoprecipitated with anti-renin antibodies. [^{35}S]Methionine-labeled products were analyzed on a SDS-12.5% polyacrylamide gel.

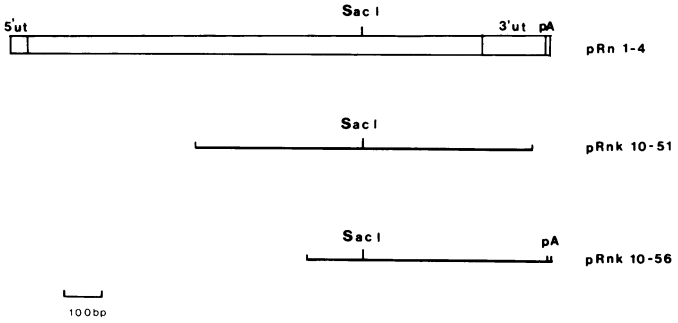

Fig. 2. Restriction maps of pRnk 10-51 and pRnk 10-56 cDNA clones. The upper map shows the location of the *Sac*I restriction site in the SMG renin cDNA clone pRn 1-4 (Panthier *et al.*, 1982b). The two lower maps show the extent of pRnk 10-51 and pRnk 10-56 kidney renin cDNA fragments. The presence of a poly(A) extension at one end of pRnk 10-56, as revealed by nucleotide sequence analysis, indicated that the cDNA sequence of this clone contains the complete 3′ untranslated region (3′ UT) of kidney renin mRNA.

plasmids resulted in ~10 000 recombinant clones harboring kidney ds cDNA inserts. The recombinant clones were screened by *in situ* hybridization under higher stringency conditions with a ^{32}P-labeled SMG renin cDNA as a probe (Panthier *et al.*, 1982b). Two recombinant clones, named pRnk 10-51 and pRnk 10-56, that hybridized with the SMG renin cDNA, were obtained.

Partial nucleotide sequence analysis

Restriction maps of clones pRnk 10-51 and pRnk 10-56 were made. The results revealed the presence of a unique *Sac*I site in both sequences (Figure 2). A *Sac*I site was also described in the two recombinant clones isolated from the SMG of Swiss mice (Panthier *et al.*, 1982a). To examine whether differences between renal and SMG renins could be seen at the mRNA level, we carried out a partial sequence analysis of the region located on the 5′ side of the *Sac*I site in the pRnk 10-51 and pRnk 10-56 clones.

Figure 3 compares the obtained kidney renin partial mRNA sequence and the previously reported sequence of SMG renin mRNA (Panthier *et al.*, 1982a). The kidney sequence shows a close homology with the SMG sequence, but the comparison reveals a few differences; i.e., three bases differ at positions indicated in Figure 3. Two of these alterations lead to no change of the amino acid in the encoded protein and one leads to change of a Glu residue *versus* an Ala residue. Figure 4 shows parts of the denaturing polyacrylamide gels used for sequencing this region, of both the kidney and SMG renin cDNAs; framed bases indicate nucleotides that differ in these sequences.

Discussion

We have previously reported the almost complete nucleotide sequence of the SMG renin cDNA from Swiss mice (Panthier *et al.*, 1982a) and have shown that animals, such as Swiss mice with high levels of SMG renin, have two renin genes while animals with low levels of SMG renin have only one renin gene (Panthier *et al.*, 1982b). Here we describe the molecular cloning of Swiss kidney renin cDNA fragments, and partial nucleotide sequences of these recombinant clones. The comparison of these sequences with the previously reported sequence of renin mRNA isolated from the Swiss mouse SMG demonstrates without ambiguity that Swiss mice, bearing the *S* allele, express two non-allelic renin genes. First, both SMG and kidney cDNA sequences have been deduced from two independent clones to exclude the possibility of sequence variation introduced during the cloning procedure. Secondly, the inbreeding coefficient of the Swiss strain we used allows us to exclude allelic variations at the renin locus. Thus, the two renin-related sequences, we have called *Rn1* and *Rn2* in our model (Panthier *et al.*,

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          270                                 280                                 290
GLY VAL SER VAL GLY SER SER THR LEU LEU CYS GLU GLU GLY CYS GLU VAL VAL VAL ASP THR GLY SER SER PHE ILE
GGG GTG TCT GTG GGG TCT TCC ACC CTA CTG TGT GAA GAA GGC TGT GAG GTA GTG GTG GAC ACT GGT TCA TCC TTT ATC   SMG
--- --- --- --- --- --- --- --- --G --A --- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- ---
GLY VAL SER VAL GLY SER SER THR LEU LEU CYS GLU GLU GLY CYS ALA VAL VAL VAL ASP THR GLY SER SER PHE ILE   KIDNEY
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Fig. 3. Partial nucleotide sequence of mouse kidney cDNA as deduced from the sequence of plasmids pRnk 10-51 and pRnk 10-56. The nucleotide sequence of part of kidney renin cDNA was determined on both strands using the method of Maxam and Gilbert (1980). The amino acid sequence is that predicted from the nucleotide sequence. The two upper lines give the predicted amino acid and nucleotide sequences of the SMG renin chain and cDNA. The two lower lines give the nucleotide and predicted amino acid sequences of kidney renin cDNA and renin chain. Only nucleotides which differ from the SMG renin cDNA are shown. Homologous positions are indicated by dashes.

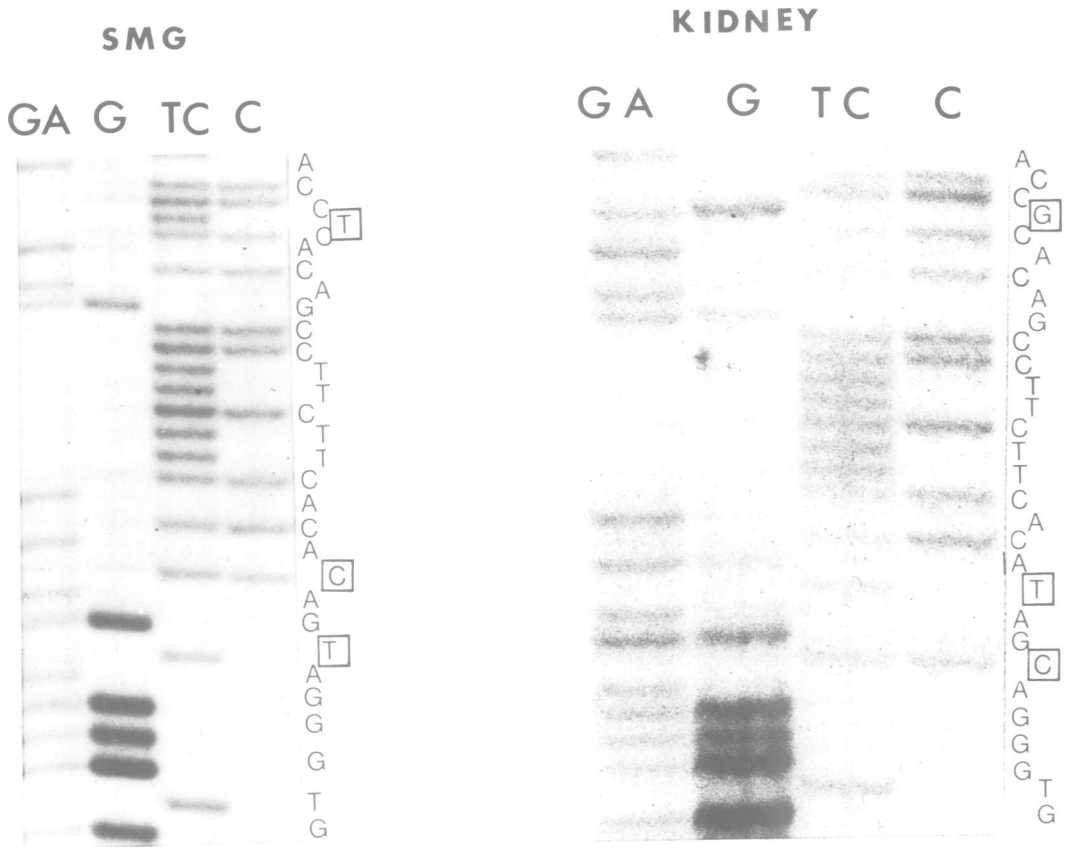

Fig. 4. Autoradiographs of parts of the sequencing gels used in deriving the corresponding SMG **(Gel A)** and kidney **(Gel B)** partial cDNA sequences. The sequences shown were confirmed by sequencing the complementary strands (data not shown). Nucleotides which differ are framed.

1982b), correspond to two active non-allelic genes. Kidney renin mRNA is the transcriptional product of the *Rn1* gene while SMG renin mRNA is the transcriptional product of the *Rn2* gene. Nevertheless, we cannot completely exclude the possibility that *Rn1* or *Rn2* are expressed at low levels in SMG or in kidney, respectively.

Mullins *et al.* (1982) recently reported the molecular cloning of two distinct renin genes from DBA/2 mouse and presented partial nucleotide sequence data of a renin gene, named *Ren-A*, encompassing the nucleotide sequence of the Swiss kidney cDNA we present in this report. The sequence of Mullins *et al.* (1982) shows a complete identity with the partial sequence of Swiss kidney cDNA. Thus, it seems likely that the *Ren-A* and the *Ren-B* copies of DBA/2 renin gene, respectively, correspond to the *Rn1* and *Rn2* copies of Swiss renin gene.

It is well known that kidney and SMG renin activities are independently regulated. Synthesis of renin in SMG is stimulated by α-adrenergic mechanisms (Bing and Poulsen, 1979) and by testosterone (Oliver and Gross, 1967); in contrast, release of renin from kidney is predominantly stimulated by β-adrenergic receptor (Bing and Poulsen, 1977; Meyer *et al.*, 1977). Furthermore, unlike the release of kidney renin, the release of SMG renin is not controlled by the plasma level of angiotensin II (Bing and Poulsen, 1975). Such a situation is expected for two non-allelic genes that are regulated differently by tissue specific mechanisms.

Wilson and Taylor (1982) reported that SMG renin activity from mice carrying the *S* allele is thermolabile, while kidney renin activity from mice carrying either the *S* or the *B* allele is thermostable. Moreover, Inagami *et al.* (1980) have reported that SMG renin is not glycosylated in contrast to kidney renin. The differences we present between kidney and SMG renin cDNA fragments demonstrate that kidney and SMG renins are two proteins having different primary sequences, thus different physico-chemical properties are expected. The determination of the sequence of the *Rn1* gene, in progress in our laboratory (Holm *et al.*, in preparation), should answer these questions.

Materials and methods

Animals

Kidney of 7–8 week-old inbred Swiss mice, obtained from Institut Pasteur, were used as a source of renin mRNA.

DNA and enzymes

The sources of DNA and enzymes were as described (Rougeon *et al.*, 1981).

Isolation of poly(A)-containing RNA and renin mRNA purification

Conditions for extraction and fractionation of total poly(A)-containing RNA have been described (Auffray and Rougeon, 1980).

In vitro protein synthesis and poly(A)-containing RNA electrophoresis, transfer and hybridization

In vitro translation of mRNA was performed by using the mRNA-dependent reticulocyte lysate translation system as described by Pelham and Jackson (1976). Poly(A)-containing RNA were electrophoresed, transferred to nitrocellulose and hybridized in conditions previously described (Rougeon *et al.*, 1981).

Construction and screening of cDNA-pBR322 hybrid molecules

Synthesis of ds S1 nuclease-resistant cDNA and its insertion into the *Pst*I site of pBR322 by the oligo(dC/dG) tailing method were carried out as described (Auffray *et al.*, 1980). Host bacteria C600 (rk^-, mk^-) were transformed and colonies were screened by *in situ* hybridization with nick-

translated cDNA insert of recombinant clone pRn 1-4 as a probe (Panthier *et al.*, 1982b).

Nucleotide sequence determination

The nucleotide sequence of part of cDNA inserts was determined using the partial chemical degradation method of Maxam and Gilbert (1980).

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