Ren-1 and Ren-2 loci are expressed in mouse kidney

gene duplication/primer extension/dideoxynucleotide triphosphate

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ABSTRACT Inbred strains of mice can be categorized into two groups based on the absence or presence of a duplicated copy of the renin structural gene; one-gene strains carry a single renin gene (Ren-1), whereas two-gene strains carry two renin genes (Ren-1 and Ren-2). To investigate the contribution that each locus makes to the composite levels of renin mRNA observed to accumulate in different tissues of two-gene strains, we have developed two assays capable of distinguishing the highly homologous Ren-1 and Ren-2 transcripts. Both methods take advantage of established base sequence differences between Ren-1 and Ren-2 coding regions by using reverse transcriptase-mediated primer extension of oligonucleotide primer/mRNA hybrids in the presence of appropriate dideoxynucleotide phosphates. Using these techniques we found that Ren-1 and Ren-2 mRNAs accumulate in the kidney of two-gene strains to approximately equal levels. These observations are discussed in light of potential mechanisms regulating the tissue-specific expression of the Ren-1 and Ren-2 loci.

Mice are polymorphic for the number of Ren loci. All strains of inbred mice carry the Ren-1 structural gene, which encodes the aspartyl protease renin. Some strains carry an additional renin gene, designated Ren-2, which apparently arose from a relatively recent gene duplication event (1-4, 6). The presence of the Ren-2 gene correlates with the high-submaxillary gland (SMG) renin phenotype (1-3, 7); strains that carry both Ren-1 and Ren-2 exhibit SMG renin levels that are elevated 100-fold as compared with strains which carry only Ren-1.

A considerable body of evidence supports the hypothesis that Ren-2 is the predominantly expressed renin gene in the SMG of two-gene strains. Only renin 2 isozyme activity has been detected in the SMG of two-gene strains (8). Similarly, sequence analysis of several SMG renin cDNA clones derived from two-gene strains indicates that they correspond to Ren-2 transcripts (9). However, RNA blot analysis and isozyme activity assays indicate that the Ren-1 allele of one-gene strains is expressed in the SMG, albeit at considerably reduced levels relative to that of Ren-2 expression in two-gene strains (1, 8).

As the high-SMG renin phenotype is attributable to the differential expression of two nonallelic structural genes (i.e., Ren-1 and Ren-2, see refs. 1-3 and 8), it is important to quantify the relative contributions of each locus to the composite levels of renin expression observed at other sites of synthesis. To date, studies utilizing either alloantibodies specific for the renin 2 isozyme or thermostability differences distinguishing the renin 1 and renin 2 isozymes have suggested that the renin 2 isozyme is not expressed at appreciable levels in the kidney of two-gene strains. Similarly, sequence analysis of two renin cDNA clones independently isolated from kidneys of two-gene animals indicates that they were derived from Ren-1 transcripts (10, 11).

To examine more precisely the gene specificity of expression in various tissues, we have devised methods that permit discrimination and quantification of the highly homologous Ren-1 and Ren-2 mRNAs within total RNA populations. Surprisingly, our results indicate that Ren-2 transcripts are present in the kidneys of two-gene strains and that the abundance of Ren-2 mRNA is approximately equal to that of Ren-1 mRNA. Possible mechanisms mediating the tissue-specific expression of renin genes are discussed in light of these findings and the number of cells actively synthesizing renin mRNA in SMG and kidney.

MATERIALS AND METHODS

Nomenclature. The renin structural genes from the two-gene strain DBA/2J are designated Ren-1(DBA) and Ren-2(DBA). The single renin structural gene present in the one-gene strain C57BL/6J is designated Ren-1(C57). The 38-mer oligonucleotide used for the dideoxynucleotide primer extension experiments hybridizes to a region of identical sequence on Ren-1(C57), Ren-1(DBA), and Ren-2(DBA), at mRNA residues 1039-1076 (see refs. 2, 6, 9, 10, and Fig. 1B). The 70-base pair (bp) primer used for the base-specific incorporation experiments hybridizes at mRNA residues 997-1066 (see refs. 2, 6, 9, 10, and Fig. 3B). Within this region, there are three base-pair differences between Ren-1(DBA) and Ren-1(C57) and Ren-2(DBA).

Animals and RNA Extraction. Male mice 10-16 weeks old were obtained from the West Seneca Laboratories (West Seneca, NY) or from The Jackson Laboratory. Total RNA was prepared by a modification of the method of Cox (12) as described (10).

Primer Preparation. The 38-mer oligonucleotide primer was synthesized by phosphoramidite chemistry on an Applied Biosystems oligonucleotide synthesizer. The primer was 5' end-labeled with [γ-32P]ATP (7000 Ci/mmol; 1 Ci = 37 GBq; ICN) and T4 polynucleotide kinase (Bethesda Research Laboratories) as described (13). The full-length labeled oligonucleotide was isolated from 20% polyacrylamide/7 M urea gels (14) by electroelution. The 70-bp primer was constructed by selected restriction endonuclease digestion of our full-length SMG renin cDNA clone, pDD-1D2 (10). Plasmid DNA was isolated as described (15, 16). Selected restriction fragments were electroeluted from preparative agarose gels and used for the base-specific incorporation reactions.

Dideoxynucleotide Primer Extension Analysis. The procedure for the primer extension reactions was modified from that of Field et al. (17). Six thousand to 20,000 dpm of the end-labeled 38-mer oligonucleotide primer (estimated to give a 100-fold primer/transcript ratio) was hybridized to various amounts of total RNA in 40 μl of 80% deionized form-

Abbreviations: SMG, submaxillary gland; dNTP, dideoxynucleotide triphosphate; bp, base pair(s).
amide/12 mM Tris-HCl, pH 7/0.56 M NaCl. The samples were denatured at 68°C for 15 min and then incubated at 42°C overnight. The hybrids were then ethanol precipitated and dissolved in 50 μl of 50 mM Tris-HCl, pH 8.1/2 mM dithiothreitol/5 mM MgCl₂/40 mM KCl. Three ddNTPs and one dideoxynucleotide (ddNTP) were added to a final concentration of 0.2 mM. Then, 15 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) was added and the reaction mixture was incubated at 42°C for 90 min. RNA was hydrolyzed by the addition of 50 μl of 0.4 M NaOH and incubation at 42°C for 2 hr. Samples were precipitated by the addition of 1 ml of 95% ethanol/12.5 mM Tris-HCl, pH 7, containing 3 μg of calf thymus DNA per ml, washed twice with 75% ethanol, and analyzed on 20% acrylamide/7 M urea sequencing gels (14).

**Base-Specific Incorporation Analyses.** The base-specific incorporation reactions were carried out as follows. The 70-bp primer (0.1 ng) was hybridized to various amounts of total RNA in 40 μl of 80% deionized formamide/12 mM Tris-HCl, pH 7/0.56 M NaCl. The samples were denatured at 68°C for 15 min and then incubated at 42°C overnight. The hybrids were ethanol precipitated and dissolved in 50 μl of 50 mM Tris-HCl, pH 8.1/2 mM dithiothreitol/5 mM MgCl₂/40 mM KCl. Three ddNTPs were added to a final concentration of 0.2 mM and one [α-32P]dNTP (3000 Ci/mmoll; Amersham) was added to a final concentration of 0.15 μM. Then, 15 units of AMV reverse transcriptase was added and the reaction mixture was incubated at 42°C for 90 min. RNA hydrolysis, precipitation, and analysis were as described above, with the exception that 12% polyacrylamide/7 M urea gels were used to analyze the products.

**RESULTS**

**Dideoxynucleotide Primer Extension Analysis.** The strong homology observed for Ren-1 and Ren-2 coding sequences (=97%, refs. 2, 3, 6, and 10) has expedited direct quantification of the respective transcript levels in tissues known to accumulate renin mRNA. The lack of substantial regions of base sequence difference between the genes has prohibited the preparation of gene-specific hybridization probes. Similarly, no unequivocal size differences characteristic of the transcripts are apparent from 5′ and 3′ nucleic S1 analyses or primer extension analyses (17, 18). We have devised two assays that take advantage of established single-base sequence differences between the Ren-1 and Ren-2 coding regions to distinguish their respective transcripts. These assays use primer extension methodology in the presence of dideoxynucleotides to quantitate the relative ratio of Ren-1 and Ren-2 transcripts in total organ RNA.

A 38-mer oligonucleotide primer suitable for dideoxynucleotide primer extension was synthesized. The oligonucleotide, which is fully complementary to mRNAs derived from either Ren-1 or Ren-2 (residues 1039–1076; see Materials and Methods and Fig. 1B), was labeled at the 5′ end by using T4 polynucleotide kinase and [γ-32P]ATP. Five bases upstream from the 3′ end of the primer on the mRNAs is a base-pair difference, with a guanine residue in both Ren-1(C57) and Ren-1(DBA) transcripts and an adenine residue in Ren-2(DBA) transcripts (see Fig. 1B and Materials and Methods). The next guanosine residue in all 387 transcripts is 12 bases further upstream. Primer extension in the presence of ddCTP and dATP, ddGTP, and dTTP should result in the addition of 5 bases to primers hybridized to either Ren-2(C57) or Ren-2(DBA) transcripts. The differentially truncated transcripts can then be distinguished by size on a DNA sequencing gel.

The results of dideoxynucleotide primer extensions using the 38-mer primer and ddCTP, dATP, ddGTP, and dTTP are shown in Fig. 1. A Ren-2(DBA)-specific primer extension oligonucleotide was generated by using SMG RNA from the two-gene strain DBA/2J as a template (Fig. 1A, lane 1). Reverse transcription proceeds until the first guanine residue 17 bases upstream from the primer is encountered. Incorporation of ddCTP at this position resulted in chain termination with the concomitant production of a 55-base oligonucleotide. A Ren-1(C57)-specific primer extension oligonucleotide was generated by using SMG RNA from the one-gene strain C57BL/6J as a template (Fig. 1A, lane 3). In this instance, reverse transcription produced a 43-base oligonucleotide, since the first upstream guanine residue in Ren-1(C57) transcripts lies only 5 bases from the 3′ end of the primer. When RNA isolated from tissue known not to transcribe renin mRNA was analyzed, no Ren-1- or Ren-2-specific oligonucleotides were generated (for example, C3H/HeHa liver RNA; Fig. 1A, lane 4). This experiment indicates that the 38-mer primer can distinguish Ren-1 and Ren-2 transcripts. Therefore, the primer was used to analyze kidney RNA from the two-gene strain DBA/2J. The results are shown in Fig. 1A (lane 2). Both Ren-1(DBA)- and Ren-2(DBA)-specific oligonucleotides were generated by dideoxynucleotide primer extension, indicating that Ren-1 and Ren-2 are expressed in the kidney of DBA/2J mice at approximately equivalent levels (densitometer tracings indicate that Ren-1 and Ren-2 transcripts are present at a ratio of ~2:1). This result was also observed for kidney RNA from other two-gene strains (e.g., LG/J, SJL/J, and AKR/J; ref. 7 and unpublished data).

Further evidence that the oligonucleotides generated were specific for renin mRNA was obtained by substituting ddTTP for ddCTP in the primer extension reaction, which should result in chain termination at a base held in common in the Ren-1(C57), Ren-1(DBA), and Ren-2(DBA) sequences. This
Fig. 2. Dideoxynucleotide primer extension analysis of renin transcripts from inbred and F1 animals. (A) To demonstrate truncation of reverse transcripts at a common site on primers hybridized to Ren-1 or Ren-2 mRNAs, 0.8 μg of total RNA from DBA/2J SMG (lane 1) or 80 μg of total RNA from DBA/2J kidney (lane 2), C57BL/6J SMG (lane 3), or C3H/HeHa liver (lane 4) was hybridized to 10,000 dpm of the labeled 38-mer oligonucleotide primer and elongated by using reverse transcriptase in the presence of ddTTP, dATP, ddCTP, and ddGTP. (B) To demonstrate the effect of renin gene dosage on the accumulation of Ren-1 and Ren-2 transcripts in the kidney, 80 μg of total RNA from DBA/2J (lanes 3 and 5) or (C57BL/6J × AKR/J)F1 (lane 4) kidney was analyzed by dideoxynucleotide primer extension in the presence of ddCTP, dATP, dTTP, and ddGTP. (A) Under similar conditions, Ren-2-specific Ren-J(C57) mRNA/primer and Ren-1(C57) mRNA/primer hybrids will incorporate two labeled dTTP residues and one ddCTP residue, resulting in the formation of a radiolabeled 73-base oligonucleotide. Under similar conditions, Ren-2(DBA) mRNA/primer hybrids will not incorporate any isotope. Conversely, if [α-32P]dCTP, ddATP, ddTTP, and ddGTP are used in the primer extension reaction, Ren-1(C57) mRNA/primer and Ren-1(DBA) mRNA/primer hybrids will incorporate one labeled dCTP residue, one ddTTP residue and thus generate a radiolabeled 72-base oligonucleotide (Fig. 3B).

Fig. 3. Base-specific incorporation of renin transcripts. Eighty or 0.8 μg of total RNA from DBA/2J SMG (lanes 1 and 2, respectively) or 80 μg of total RNA from DBA/2J kidney (lane 3), C57BL/6J SMG (lane 4), or DBA/2J liver (lane 5) was hybridized to 1 nmol of the 70-bp primer and elongated in the presence of [α-32P]dCTP (Ren-2 specific, A1), [α-32P]dATP (Ren-1 specific, A2) or [α-32P]dATP (negative control, A3). (B) Hybridization of the 70-bp primer to Ren-1 or Ren-2 transcripts.

experiment is shown in Fig. 2. The primer extension reaction was carried out in the presence of ddTTP, dATP, dTTP, and ddCTP. Under these conditions, reverse transcription proceeds until the first adenine residue is encountered, resulting in the addition of four bases for both Ren-1 and Ren-2 transcripts. As shown, RNA from DBA/2J SMG, DBA/2J kidney, and C57BL/6J SMG gave rise to identical oligonucleotides 42 bases long, while RNA from C3H/HeHa liver failed to generate any renin-specific extension products (Fig. 2A, lanes 1–4).

The dideoxynucleotide primer extension analysis should be quite quantitative as minimal requirements are placed on the reverse transcriptase reaction. This was tested directly by analyzing kidney RNA from F1 animals derived from crosses between one-gene and two-gene inbred parents. Such animals have two copies of Ren-1 but only one copy of Ren-2. As a first approximation, we might expect the ratio of Ren-1 to Ren-2-specific oligonucleotides to increase for kidney RNA of F1 animals relative to that of the two-gene inbred parent, provided that the mRNA accumulation is proportional to the gene copy number and that Ren-1(DBA) and Ren-1(C57) alleles exhibit similar expression levels. The results obtained using the 38-mer primer and ddCTP, dATP, ddGTP, and dTTP are shown in Fig. 2. As shown in a (C57BL/6J × AKR/J)F1 animal the ratio of Ren-1 to Ren-2-specific oligonucleotides increases relative to that of DBA/2J, indicating that the technique is capable of detecting dosage effects for the renin genes. We have also observed similar ratios for Ren-1- and Ren-2-specific oligonucleotides in (C57BL/6J × DBA/2J)F1 and (C57BL/6J × SWR/J)F1 animals (unpublished).

Base-Specific Incorporation Analysis. To verify our findings, we have devised a variation of the dideoxynucleotide primer extension assay that is simple and potentially more sensitive. This technique utilizes a primer that readily permits detection of differential incorporation of radiolabeled nucleotides upon copying of template. Specifically, we have exploited an Alu I restriction site polymorphism between Ren-1(C57 or DBA) (A-A-C-T) and Ren-2(DBA) (A-G-C-T) at residues 995–998 of the mRNA (see refs. 6, 10, and Fig. 3B). A 70-bp restriction fragment was generated by Alu I/Rsa I digestion of our full-length Ren-2(DBA) cDNA clone, pRDD-1D2 (10). When this blunt-ended fragment was used as a primer, the 3' end of the strand complementary to the mRNA hybridized adjacent to the A/G base-pair difference at residue 996 of the renin transcripts. If [α-32P]dCTP, dATP, ddCTP, and ddGTP are used in a primer extension reaction, both Ren-1(C57) mRNA/primer and Ren-1(DBA) mRNA/primer hybrids will incorporate two labeled dTTP residues and one ddCTP residue, resulting in the formation of a radiolabeled 73-base oligonucleotide. Under similar conditions, Ren-2(DBA) mRNA/primer hybrids will not incorporate any isotope. Conversely, if [α-32P]dCTP, ddATP, ddTTP, and ddGTP are used in the primer extension reaction, Ren-1(C57) mRNA/primer and Ren-1(DBA) mRNA/primer hybrids will incorporate one labeled dCTP residue, one ddTTP residue and thus generate a radiolabeled 72-base oligonucleotide (Fig. 3B).
The results obtained using the 70-bp primer are shown in Fig. 3. When the Ren-2-specific nucleotides \( [\alpha^{32P}]dCTP, ddATP, ddTTP, \) and ddGTP were used in the primer extension reaction, hybridization of the primer to DBA/2J SMG RNA [which contains primarily Ren-2(DBA) transcripts] gave a radiolabeled oligonucleotide (Fig. 3A1, lanes 1 and 2) but hybridization of the primer to C57BL/6J SMG RNA [which contains only Ren-1(C57) transcripts] or to DBA/2J liver RNA (which contains no detectable renin mRNA) did not give a labeled product (Fig. 3A1, lanes 4 and 5). However, when the primer was hybridized to DBA/2J kidney RNA, a radiolabeled fragment was generated, indicating that Ren-2(DBA) transcripts were present (Fig. 3A1, lane 3). As expected, when the Ren-1-specific nucleotides \( [\alpha^{32P}]dTTP, \) ddATP, ddGTP, and ddCTP were used in the primer extension reaction, hybridization to DBA/2J kidney RNA and C57BL/6J SMG RNA generated labeled oligonucleotides, indicating the presence of Ren-1(DBA) and Ren-1(C57) transcripts, respectively (Fig. 3A2, lanes 3 and 4). Hybridization of the primer to DBA/2J liver RNA did not produce any Ren-1-specific labeled oligonucleotides (Fig. 3A2, lane 5). Finally, when \( [\alpha^{32P}]dATP \) and the appropriate ddNTPs were used in the primer extension reaction, none of the RNAs analyzed produced renin-specific labeled oligonucleotides (Fig. 3A3).

**DISCUSSION**

We have devised two assays capable of distinguishing the highly homologous transcripts derived from Ren-1 and Ren-2 to more clearly define and quantitate the respective contributions of each of these loci to the composite levels of renin mRNA accumulation observed for different tissues. Using these techniques we have found that both Ren loci are active in kidneys of two-gene strains of mice (Figs. 1 and 3). This result came as somewhat of a surprise because, to date, the published data have favored the hypothesis that Ren-1 is the predominantly, if not exclusively, expressed renin gene in the kidney of two-gene strains (8, 10, 11). Alloantibody raised against the renin 2 isozyme fails to inactivate the renin activity present in kidney extracts of two-gene strains (8). Similarly, thermostability analysis of the kidney renin activity of two-gene strains has suggested that it is of the thermostable renin 1 type (8). In addition, sequence analysis by our group (10) and others (11) of two independently isolated kidney renin cDNA clones from two-gene strains has shown that the cDNAs are derived from Ren-1 transcripts.

Although these previous experiments strongly suggest that Ren-1 is the predominantly expressed renin gene in the kidney of two-gene strains, they do not, for several reasons, unequivocally demonstrate that Ren-2 is silent. First, the protein activity studies utilized alloantibody of relatively low affinity and thus could readily have failed to detect a minor renin 2 isozyme component in unfraccionated extracts of two-gene-strain kidneys (8). Alternatively, the absence of N-linked glycosylation of the renin 2 isozyme (9, 19) could conceivably render it less stable than the renin 1 isozyme and thus lower the steady-state level of the protein, precluding its detection. Finally, sequence analysis of a limited number of two-gene kidney renin cDNA clones does not provide a statistically valid proof that Ren-2 is silent at the level of transcription. Although there is precedent for gene-specific translational regulation of two highly homologous transcripts (for example, the rat insulin genes; ref. 20), we believe it is more likely that renin 2 isozyme activity has not previously been detected in kidneys of two-gene strains due to limitations of the assays used in those studies. In support of this, recent analysis of nonglycosylated proteins enriched from kidney extracts has suggested that a renin 2-like activity is present (Carol Wilson, personal communication). At present it is unclear as to how the steady-state levels of protein detected relate to the mRNA levels observed by us.

Renin mRNA accumulation in two-gene-strain SMG constitutes \(-1%\) of the poly(A)\(^+\) RNA of the organ (10). By a number of criteria already stated (see above), the majority of this mRNA derives from the Ren-2(DBA) locus. Renin mRNA accumulation in one-gene-strain SMGs corresponds to \(-0.01%\) of the total organ poly(A)\(^+\) and derives necessarily from the Ren-1 locus [Ren-1(C57) allele]. Renin mRNA accumulation in either one- or two-gene strain kidneys also comprises \(-0.01%\) of the total organ poly(A)\(^+\) RNA (10) and, as reported here, both the Ren-1 and the Ren-2 loci can contribute roughly equivalently to this accumulation. However, the fraction of cells actively contributing to the accumulations observed for total organ RNA in these tissues is markedly different. In the SMG, renin is synthesized in the granular convoluted tubule cells, which comprise 20–30% of the gland (21). In the kidney, renin is synthesized in the juxtaglomerular cells, which constitute \(<1%\) of cells of this organ (this figure is obtained by dividing the estimated number of juxtaglomerular cells by the estimated number of cells per kidney; ref. 5). When transcript accumulation is normalized to the number of cells synthesizing renin, we see that Ren-2 transcript accumulation in the SMG granular convoluted tubule cells is roughly equivalent to the composite renin transcript accumulation in the kidney juxtaglomerular cells (Table 1). Moreover, our results show that in two-gene strains Ren-2 can be expressed in the kidney nearly as well as Ren-1. In fact, when viewed from this standpoint it is Ren-1(C57) [and probably Ren-1(DBA)] mRNA accumulation in SMG that is considerably reduced (by a factor of \(-100\) relative to Ren-1(C57) and Ren-1(DBA) expression in kidney or Ren-2(DBA) expression in kidney or SMG.

Although it is difficult to assess mechanisms governing regulation from data on mRNA abundance alone, our observations raise an interesting possibility—namely, that the elevated levels of Ren-2(DBA) expression in the SMG are not due to any positive or enhanced up-regulation of the Ren-2(DBA) promoter relative to that of Ren-1(DBA) but in fact

<table>
<thead>
<tr>
<th>SMG renin expression</th>
<th>C57BL/6J (one gene)</th>
<th>DBA/2J (two gene)</th>
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<tr>
<td>Predominant isozyme</td>
<td>Renin 1</td>
<td>Renin 2</td>
</tr>
<tr>
<td>% renin mRNA relative to DBA/2J SMG signal</td>
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<td>100*</td>
</tr>
<tr>
<td>% SMG cells synthesizing renin</td>
<td>20–30</td>
<td>20–30</td>
</tr>
<tr>
<td>Relative signal/cell synthesizing renin mRNA</td>
<td>3–5</td>
<td>300–500</td>
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<td>Renin transcript detected</td>
<td>Ren-1</td>
<td>Ren-2</td>
</tr>
<tr>
<td>Kidney renin expression</td>
<td>Predominant isozyme</td>
<td>Renin 2</td>
</tr>
<tr>
<td>% renin mRNA relative to DBA/2J SMG signal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>% kidney cells synthesizing renin</td>
<td>0.1–1</td>
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</tr>
<tr>
<td>Relative signal/cell synthesizing renin mRNA</td>
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</tr>
<tr>
<td>Renin transcript(s) detected</td>
<td>Ren-1</td>
<td>Ren-1, Ren-2</td>
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*This level is estimated to be 1–2% of total organ poly(A)\(^+\) RNA (10).
1Calculated as follows: % DBA/2J SMG signal \( \times (100/\% \) cells synthesizing renin).
2Calculated as follows: (estimated number of juxtaglomerular cells)/(estimated number of cells per kidney).
3At approximately equal levels.
could be due to the inability to turn off or repress the Ren-2(DBA) gene in this tissue. Obviously, other common sequences and/or cellular factors must also be able to modulate renin expression as both Ren-1(DBA) and Ren-2(DBA) are apparently completely transcriptionally silent in other tissues (for example, liver).

In light of this, it is of interest to note that the 5' flanking sequences of Ren-1(DBA) and Ren-2(DBA) are strikingly similar, with the exception of the insertion of a repetitive DNA element (and subsequent minor sequence scrambling) over a limited region on the Ren-2(DBA) copy (17). This sequence organization is also conserved among Mus subspecies that harbor duplicated Ren loci and exhibit high SMG renin levels (ref. 4; unpublished data). Thus, a plausible working model encompassing these observations might be that Ren-1(DBA) and Ren-2(DBA) exhibit expression in kidney because both loci harbor cis-acting information necessary to effect expression in this tissue environment, while the limited sequence perturbations observed in the Ren-2(DBA) 5' proximal region may in fact be ultimately responsible for the abundant expression of Ren-2(DBA) in SMG.

Although we have used the primer extension assays to quantitate the contributions made by separate loci to mRNA populations, they should be equally applicable for measuring allele specific contributions. For example, suitable sequence differences exist between the Ren-1(C57) and Ren-1(DBA) alleles (6, 18) to permit direct quantitation of their respective mRNAs in kidney. If the assays are used in conjunction with appropriate genetic crosses, they should be useful in genetically defining allele-specific differences.

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