Volume 16 Number 5 1988 Nucleic Acids Research

# Close physical linkage of the murine Ren-1 and Ren-2 loci

Kenneth J.Abel and Kenneth W.Gross

Department of Molecular and Cellular Biology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263, USA

Received October 22, 1987; Revised and Accepted January 26, 1988

ABSTRACT

In addition to the Ren-1 gene common to all mice, some inbred strains carry a second copy of the renin structural gene, Ren-2. These two loci are tightly linked genetically on mouse chromosome one. We have used pulsed field gel electrophoresis (PFGE) to study the physical arrangement of the two renin genes in the inbred strain DBA/2. PFGE mapping permitted the construction of a restriction map of the Ren loci spanning roughly 120 Kb. The results indicate that the genes are transcribed in the same relative direction, that Ren-2 lies upstream relative to Ren-1, and that the respective coding sequences are separated by approximately 20 Kb.

## INTRODUCTION

Renin is an aspartyl protease which catalyzes the initial reaction in the processing of angiotensinogen to angiotensin II, a peptide hormone involved in the regulation of blood pressure and electrolyte balance. Renin activity is thought to have originally appeared early in the evolution of vertebrates (1,2). Most mammals examined have a single genetic locus encoding renin, including humans (3,4), rats (5), and a number of inbred and wild-derived mice (5). However, in addition to the Ren-1 locus observed in all mice, certain inbred strains posess a second renin locus, Ren-2 (6-8). This second locus is also present in those wild-derived mice which are closely related to the inbreds, including aboriginal and most commensal mice (5).

Analysis of recombinant inbred strains has shown that Ren-1 and Ren-2 are genetically tightly linked and map near the Pep-3 locus on chromosome one (6). As yet, no recombinants between the two renin loci have been reported. Comparison of nucleotide sequences has shown that the two genes share 96% similarity in their coding regions (9), and the similarity extends both 5' and 3' from the structural gene sequences (7,10,11). These observations are consistent with other studies which suggest that Ren-2 appeared as a result of a relatively recent gene duplication event (6,8). A model for the duplication has been proposed in which during meiosis, unequal crossing-over occurred between mispaired homologs of chromosome one (8). The chromosome bearing two

renin genes was then maintained in a subset of murine subspecies.

The Ren-2 locus encodes an enzyme with apparently equivalent substrate specificity, but with physical properties distinct from, the Ren-1-encoded enzyme (9,12,13). Since the presence of a single renin locus in other mammals argues against generalized selection for additional renin isozymes during vertebrate evolution, a specific physiological role in mice for the enzyme encoded by Ren-2 remains uncertain. However, the duplicated locus accounts for markedly different patterns of tissue- and gene-specific renin expression between inbred mouse strains. Kidney renin mRNA accumulates to roughly equal levels in all strains examined (14), yet strains with two renin genes exhibit an approximately 100-fold increase in submandibular gland (SMG) renin expression relative to strains which have only Ren-1 (6). Also, while Ren-1 and Ren-2 mRNAs are present at nearly equal levels in the kidneys of those strains carrying both genes (15), only Ren-2 appears to be expressed in the SMG of these mice (13,16). There are thus tissue-specific differences in expression characterizing the two loci and alleles at the Ren-1 locus.

The renin genes in mice therefore provide an intriguing situation in which distinct yet highly homologous loci exhibit pronounced diversity in tissue-specific expression. It is conceivable that the duplication resulted in the rearrangement of important cis-acting regulatory elements. Clearly, an understanding of the physical arrangement of the two renin loci might be useful in the identification of those elements.

We have used pulsed field gel electrophoresis (17-19) to characterize the structural organization of the Ren-1 and Ren-2 loci in the inbred strain DBA/2. This technique permits the resolution of very large DNA fragments and has been used in a number of cases to determine the physical distances between linked genes (20-22). Here we report mapping results which indicate the relative orientation and physical distance between the two nonallelic renin genes of mice. We also discuss the possible implications of such an organization with respect to expression and evolution of the Ren loci in mice.

## MATERIALS AND METHODS

### Mice

DBA/2Ros and C57Bl/6J mice were purchased from West Seneca Laboratories.

### DNA Purification

DNA was isolated from liver or spleen according to the procedure of Pellicer et al.(23) with modifications developed by Bennett et al.(24). However, instead of Pronase, Proteinase K was used at 50°C in the buffer described by Maniatis et al.(25).

High molecular weight DNA for PFGE was prepared from adult male DBA/2 spleens as described by Herrmann et al.(26). Spleens were dissected, minced into small pieces, and homogenized in PBS to single cell suspensions with several strokes of a Dounce homogenizer. The cells were washed once and resuspended to a concentration of $4x10^6$ cells per 40 ul PBS. To this suspension was added an equal volume of 1% low melting point agarose (Ultrapure-BRL) in PBS. In our protocol the cell-agarose suspension was drawn up into 1/8" I.D. Tygon tubing and was allowed to set 15 minutes at 4°C. The set suspension was expelled from the tubing, cut into 1-cm plugs, and lysed at 50°C for 48 hours in: 0.5 M $EDTA(Na)_2$ pH 8.0, 1% Sarkosyl, 2 mg/ml Proteinase K. Plugs were washed twice at 50°C in 50 ml TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) plus 40 ug/ml PMSF, then stored in TE/PMSF at 4°C.

PFGE Gels/Hybridizations

PFGE gels were run similar to procedures described in (18,19). For FIGE, we use the PULSESTAR system purchased from DNASTAR Inc., Madison, Wisconsin. Gels performed with the FIGE apparatus were 1% agarose in 0.5x TBE (1x TBE = 90 mM Tris base pH 8.2, 90 mM boric acid, 20 mM EDTA), run at 180 Volts using a switching ramp of forward:reverse pulses of 0.9:0.3 seconds through 10.5:3.5 seconds over 24 hours. Our OFAGE apparatus was built according to the design of Carle and Olson (18) by Dan Kar Plastics, Reading, Massachusettes. Gels performed with the OFAGE apparatus were 1.5% agarose in 0.5x TBE, run at 300 Volts for 20 hours with a switch interval of 10 seconds. Size markers for PFGE included ligated oligomers of lambda DNA (18), and embedded intact yeast chromosomes (27). After PFGE, gels were stained and then soaked 30 minutes in 0.25 N HCl. Gels were denatured 60 minutes in 0.5 N NaOH/1.5 M NaCl, then neutralized 60 minutes in 1 M Tris pH 5/ 3 M NaCl. The DNA was transferred 24 hours onto BioRad Zetaprobe nylon filters with 20x SSC. Afterward, blots were rinsed briefly in 50mM $NaPO_4$ pH 7.2 and baked 2 hours at 80°C. DNA was then cross-linked to the filter by exposing the blot to short-wave UV for 2 minutes.

Blots were hybridized according to the method of Church and Gilbert (28). Filters were prehybridized 1 hour at 65°C in 0.5 M $NaPO_4$ pH 7.2, 7% SDS, 1mM $EDTA(Na)_2$, 100 ug/ml denatured salmon sperm DNA. Filters were hybridized at 65°C for 24 hours in the same solution. DNA probes were prepared by the oligonucleotide priming method of Feinberg and Vogelstein (29) to specific activities of $10^9$ dpm/ug. $3-4x10^6$ dpm/ml of denatured probe was included in the hybridization. Washes were done in 40mM $NaPO_4$ pH 7.2, 1% SDS, twice for 5 minutes at room temperature, then one hour at 65°C, followed by one hour in

20mM $NaPO_4$, 1% SDS at 65°C. Hybridization signals were detected by autoradiography with Kodak XAR-5 film at -70°C for several days. Probe was removed for re-hybridization by washing the blot twice for 30 minutes each in 100 mM NaOH at room temperature and then rinsing in distilled water until neutral.

Low Percentage Agarose Gels/Hybridizations

To examine DNA up to 50 Kb in length, digests of 3-5 micrograms of purified liver DNA were loaded onto 0.4% horizontal agarose gels in TAE running buffer (1x TAE = 40mM Tris pH 7.8, 5 mM sodium acetate, 1 mM EDTA) and electrophoresed 40 hours at 40 Volts (Figures 2B, 5B, and 6). Gels were stained, denatured, neutralized, and transferred onto nitrocellulose filters as described (30). Hybridization conditions were similar to those used by Bennett et al. (24), except that the solution contained 10% dextran sulfate and the filters were washed to a final stringency of 0.1x SSC, 0.1% SDS at 65°C. Blots were exposed to Kodak XAR-5 film at -70°C.

Standard Agarose Gels/Hybridizations

Gels shown in Figures 2A, 5A, and 7 were 0.8% agarose in TAE running buffer, run 16 hours at 40 Volts. Transfer and hybridization conditions were as described for nitrocellulose filters above.

DNA Probes

Genomic regions corresponding to each probe are indicated in Figure 1. pDD-1D2 (probe 1) is a full-length DBA/2 SMG renin cDNA clone (14). p3'R/X (probe 2) is a 1.2 Kb EcoRI-XbaI subclone derived from a Ren-2-containing cosmid (10), corresponding to the most 3' flanking sequence homologous in both renin genes. p3'Ren-1 (probe 3) is a 1 Kb SstI subclone which is Ren-1-specific, derived from the 3' flanking region beyond the point of Ren-1/Ren-2 homology. In all cases, subclone inserts were purified by gel electroelution using an ISCO 1750 Sample Concentrator before labelling.

RESULTS

In many instances physical linkage between genes has been determined by the sequential isolation of genomic clones which ultimately overlap with a neighboring gene. However, this technique of "chromosome walking" can be laborious if multiple screenings for overlapping clones are required, and can be slowed by the abundance of repetitive sequences in mammalian genomes. Ren-1 and Ren-2 are known to be genetically closely linked. In the mouse, 0.1 cM is thought to correspond to roughly 100-200 kilobase pairs on average. Assuming that recombination occurs with normal frequency within this region of the chromosome, then the lack of observed recombinants between the Ren loci

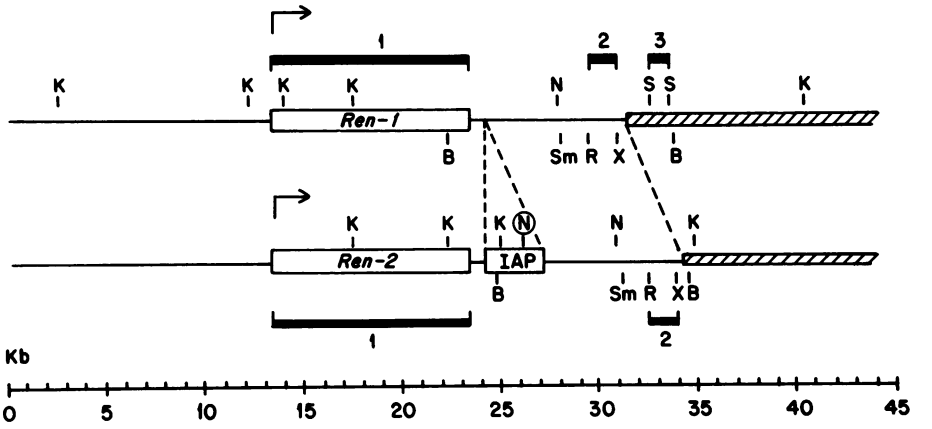


Figure 1. Restriction maps of the Ren-1 and Ren-2 genes in DBA/2 mice. This data has been compiled from Mullins et al.(7), and from our own mapping studies of DBA/2 genomic DNA and a cosmid clone containing Ren-2 (10). Only those restriction sites discussed in the text are included. The circled NotI site is not cleavable in genomic DNA (see text). Cross-hatched bars indicate where 3' flanking sequences no longer demonstrate homology at the level of restriction site mapping. The eight introns associated with each gene are not indicated in the figure. Heavy numbered bars refer to probes described in the text: probe 1 = pDD-1D2, a full-length SMG renin cDNA; probe 2 = p3'R/X, a 3' flanking subclone homologous to both genes; probe 3 = p3'Ren-1, a 3' flanking subclone specific for Ren-1. Arrows indicate the direction of transcription. IAP indicates an intra-cisternal A particle genome (33). Symbols for restriction enzyme sites are as follows: B = BamHI; K = KpnI, N = NotI; R = EcoRI; S = SstI; Sm = SmaI; X = XbaI.

suggests that they are sufficiently close to permit a more detailed linkage analysis. However, no overlap is apparent in the restriction maps of Ren-1 and Ren-2 genomic clones spanning roughly 60 Kb (Figure 1), (5,7). Also, given that we had no prior knowlege of the direction in which a "walk" should be made, we chose to determine physical linkage through large scale restriction mapping of the region of chromosome one containing both renin loci. The information in Figure 1 provided a basis for the linkage studies.

Mapping NotI Sites Near Ren-1 and Ren-2

To obtain a starting point for large scale mapping, a cosmid clone containing roughly 33 Kb of the Ren-2 gene and its flanking sequences (10) was surveyed for sites recognized by rare-cutting restriction enzymes. Such sites either have eight-base pair recognition sequences, or are infrequent because CpG dinucleotides present in the recognition sequences are underrepresented in vertebrate genomes (31,32). Also, methylation of these dinucleotides renders the sites for many restriction enzymes resistent to digestion. No sites for SfiI, SalI, or SacII were found in the cosmid genomic insert. However, two

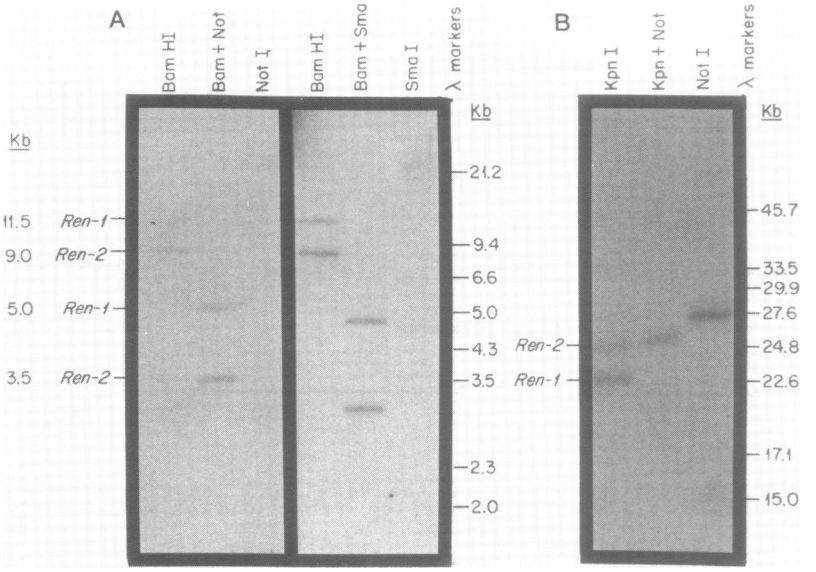


Figure 2. Mapping of cleavable NotI sites within the 3' flanking regions of Ren-1 and Ren-2. BamHI, KpnI, NotI, and SmaI sites are shown in Figure 1. (A). Digests of DBA/2 liver DNA were electrophoresed on a standard 0.8% agarose gel, blotted, and probed with p3'R/X (probe 2, Fig.1). The lengths and renin gene associated with each hybridizing fragment are indicated. Pooled HindIII and HindIII + EcoRI digests of lambda DNA were included as size markers. (B). Digests of DBA/2 liver DNA were electrophoresed on a low percentage agarose gel to resolve relatively large fragments, blotted, and probed with the full-length renin cDNA, pDD-1D2 (probe 1, Fig.1). To obtain resolution of large fragments, smaller hybridizing KpnI fragments are typically electrophoresed off the gel. However, no smaller hybridizing NotI fragments have been observed following electrophoresis under conditions in which all the DNA is retained on the gel. KpnI, NarI, NdeI, SstI, and XhoI single digests of lambda DNA were pooled to provide a set of large size markers.

NotI sites were found in the 3' flanking region: one site within the previously identified IAP element which is adjacent to Ren-2 but is absent from the corresponding position in Ren-1 (33), and the second site 4 Kb downstream of the IAP site (Fig. 1). Since the recognition sequence for NotI contains two CpG dinucleotides, the ability of these sites to be cleaved in genomic DNA was tested by Southern blot hybridization.

Single and combined NotI and BamHI digests of DBA/2 liver DNA were probed with the subclone p3'R/X (probe 2, Fig. 1), which is derived from the Ren-2 cosmid but is homologous in the 3' flanking regions of both genes. The hybridization shown in Figure 2A indicates that the downstream NotI site is cleaved to completion. Additional Southern data show that the NotI site in

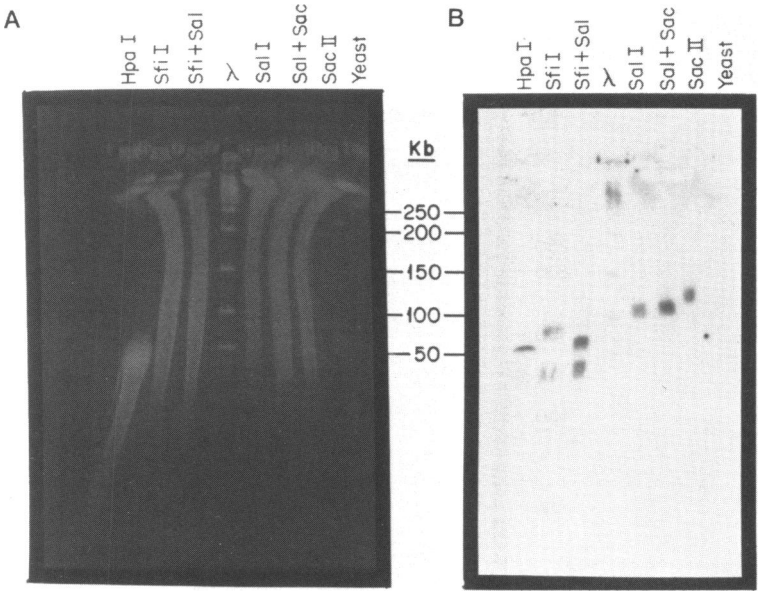


Figure 3. Southern analysis of the DBA/2 Ren loci using pulsed field gel electrophoresis. Digests of embedded spleen DNA were subjected to orthagonal field alternation gel electrophoresis (OFAGE) as described in Materials and Methods. (A). Photograph of the ethidium bromide-stained gel. Ligated oligomers of lambda DNA and intact yeast chromosomes were included as size markers. Approximate sizes of the lambda ladder bands in kilobases are indicated. (B). Southern hybridization results after transfer of the DNA from the gel in (A) using the full-length renin cDNA probe.

the IAP element is not cleaved in genomic DNA (not shown). A single, homologously positioned NotI site is also cleavable in the 3' flanking region of Ren-1. The ability of the previously mapped SmaI sites to be cleaved in genomic DNA helped to more accurately position the NotI sites. The large hybridizing fragments generated by NotI and SmaI alone are just detectable in this exposure.

Low percentage agarose gels which resolve DNA fragments up to 50 Kb in length were used to analyze the NotI fragment(s) containing the renin genes. A fragment of approximately 28 Kb was detected by Southern hybridization of NotI-digested DBA/2 genomic DNA. Figure 2B shows KpnI and NotI single and combined digests of DBA/2 DNA probed with a full-length SMG renin cDNA, pDD-1D2 (probe 1, Fig.1) (14). KpnI alone generates relatively large hybridizing fragments; the 25 Kb Ren-2 fragment extends upstream from a KpnI site in exon 3 and the 23 Kb Ren-1 fragment extends downstream from a homologous exon 3 site. The Kpn/Not double digest again demonstrates cleavage

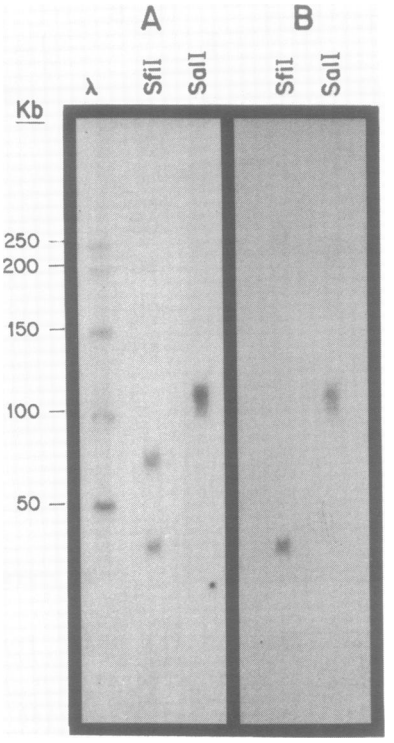


Figure 4. Ren-1 and Ren-2 lie within separate SfiI fragments. Electrophoresis of SfiI- and SalI-digested spleen DNA was performed with the FIGE apparatus as described in Materials and Methods. After Southern transfer, the blot was hybridized with the renin cDNA (A). The same blot was then stripped of probe and re-hybridized with p3'Ren-1, a probe specific for Ren-1 (B). Approximate sizes of the lambda ladder bands in kilobases are indicated.

of the NotI site in the 3' flanking region of Ren-1. NotI does not cleave within the large KpnI fragment extending upstream from Ren-2.

Apparently the same 28 Kb NotI fragment is observed using 5' and 3' flanking probes homologous to both genes, including p3'R/X (not shown). The KpnI mapping studies preclude a head-to-head arrangement with both genes contained on a single NotI fragment of only 28 Kb. Another possibility is that either Ren-1 or Ren-2 is contained within a 28 Kb fragment which links the 3' region of one renin gene to the homologous region of the other gene. Either both genes lie within NotI fragments of nearly equal size which were not resolved on this gel, or the NotI fragment containing the other gene must be very large. Fragments which are very large are often difficult to detect

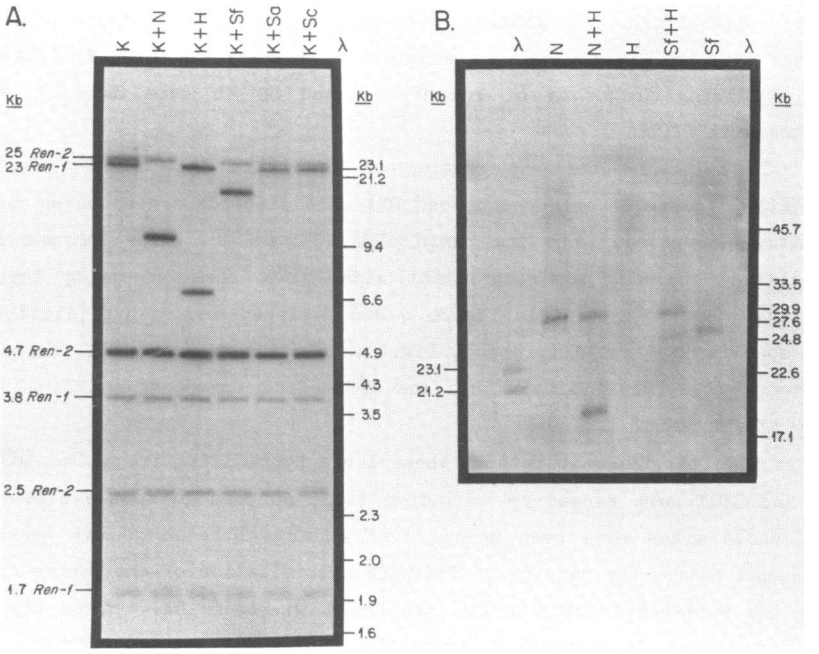


Figure 5. (A).Restriction site mapping near the DBA/2 Ren loci by Southern analysis. Digests of isolated spleen DNA using KpnI alone or in combination with additional restriction enzymes were electrophoresed on a standard 0.8% agarose gel, blotted, and probed with the renin cDNA (probe 1). (B). Southern hybridization of DBA/2 DNA digested with HpaI alone or in combination with NotI or SfiI. Digests were electrophoresed on a low percentage agarose gel to resolve relatively large fragments, blotted, and probed with the renin cDNA. K = KpnI; N = NotI; H = HpaI; Sf = SfiI; Sa = SalI; Sc = SacII.

due to poor transfer efficiency. No additional attempts, for example acid depurination, were made to enhance detection of very large fragments in these or in following low percentage agarose gels since their lengths generally exceed the upper limits of resolution for these gels.

Large Scale Restriction Mapping

Pulsed field gel electrophoresis (PFGE) was used to determine the distance between Ren-1 and Ren-2. The goal of the PFGE analysis was to identify a single hybridizing fragment, using a probe homologous to both loci, which would indicate the maximum distance between them. Using DBA/2 spleen DNA embedded in agarose, field inversion gel electrophoresis (FIGE) initially suggested that both genes were contained on single SalI and SacII fragments of approximately 100 Kb. Orthagonal field alternation gel electrophoresis (OFAGE) was then set up to resolve digests of spleen DNA within a relatively

smaller size range. Southern hybridization using the renin cDNA probe revealed that both genes lie on a single HpaI fragment of 60-70 Kb (Figure 3). Two hybridizing fragments of roughly 25 and 80 Kb were detected in DNA digested with SfiI.

To determine the organization of the renin loci within these SfiI fragments, digests of embedded spleen DNA were electrophoresed using the FIGE apparatus and probed with the renin cDNA (Figure 4). The lengths of the hybridizing fragments are consistent with those observed using the OFAGE apparatus. When the blot in Figure 4 was stripped and re-hybridized with a probe specific for Ren-1 (probe 3, Fig.1), only the smaller SfiI fragment was detected. Thus, Ren-1 lies within the 25 Kb SfiI fragment, and Ren-2 within the 80 Kb fragment.

To identify the endpoints of these large hybridizing fragments, sites for HpaI and SfiI were mapped by including these enzymes in double digests with KpnI, whose sites have been mapped over a large stretch of DNA surrounding each renin gene. The results of Southern hybridization of the double digests, using the full-length cDNA probe, are shown in Figure 5A. Since this probe does not detect flanking KpnI fragments extending upstream from Ren-1 or downstream from Ren-2, restriction analysis was also performed on genomic clones containing these regions. Together, these approaches permitted a rapid survey for new sites within approximately 80 Kb of Ren-associated DNA. A HpaI site was localized 3 Kb 5' to the first exon of Ren-2. One SfiI site was mapped in the 3' flanking region of Ren-1, 5 Kb downstream of the Ren-1-associated NotI site. Another SfiI site was mapped in a genomic subclone containing Ren-1 5' flanking sequences. The distance between these SfiI sites is approximately 25 Kb, consistent with the length of the Ren-1-containing SfiI fragment observed in the PFGE analyses. No cleavable SalI or SacII sites were found in close proximity to either gene using this approach. However, the PFGE results using OFAGE indicate a SalI site roughly 20 Kb from one end of the large SfiI fragment containing Ren-2.

Relative Orientation of the Ren Loci

Since both genes appear to lie within a single HpaI fragment, the presence of one HpaI site 5' to Ren-2 suggests that Ren-1 resides on the 3' side of Ren-2. The close spacing suggests that the genes may reside on adjacent SfiI fragments. To test these possibilities, low percentage agarose gels were used to analyze double digests of DBA/2 liver DNA cut with HpaI and either NotI or SfiI (Figure 5B). The cDNA probe detects the 28 Kb NotI fragment and a second fragment in the Hpa/Not double digest of 19 Kb. The

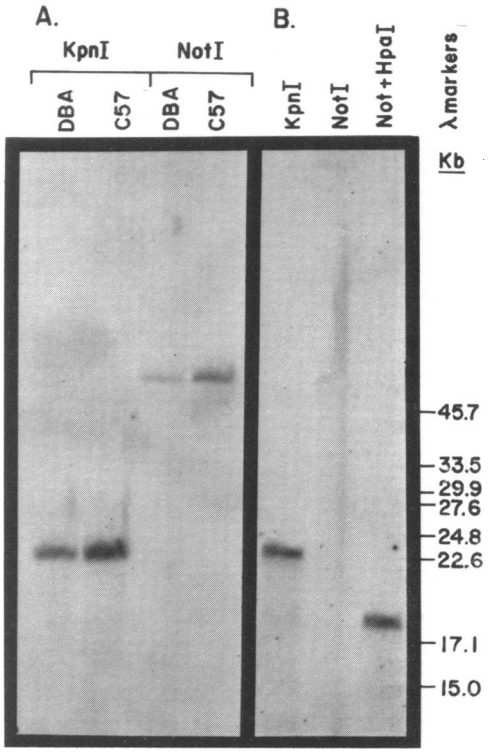


Figure 6. Southern analysis of the Ren-1 3' flanking region. (A) and (B). Digests of DBA/2 DNA were electrophoresed on low percentage agarose gels, blotted, and probed with p3'Ren-1 (probe 3, Fig.1). Digests of genomic DNA from the single renin gene-inbred strain C57Bl/6J are included where indicated. Only the 23 Kb KpnI fragment associated with Ren-1 is observed using this probe. The NotI-digested DNA did not resolve well in (B).

size of this second fragment matches that predicted for a fragment generated by the HpaI site and the downstream, cleavable NotI site which flank Ren-2. Ren-1 must therefore lie within the 28 Kb fragment. These NotI and Hpa/Not fragments are thought to be adjacent because, as discussed earlier, the 28 Kb NotI fragment is also detected with the probe p3'R/X. This probe hybridizes 3' to the Ren-2-associated NotI site. Although p3'R/X is homologous to both genes, Figure 6A shows that the 28 Kb NotI fragment does not lie on the 3' side of Ren-1. NotI digests of DBA/2 DNA electrophoresed on low percentage agarose gels were probed with p3'Ren-1, the 3' flanking probe specific for Ren-1. Hybridizing NotI fragments of roughly 50 Kb were detected in DNA from both DBA/2 and C57Bl/6, an inbred strain with only a single renin gene.

These observations support the placement of the 28 Kb NotI fragment containing Ren-1 on the 3' side of Ren-2. They also indicate a tail-to-head

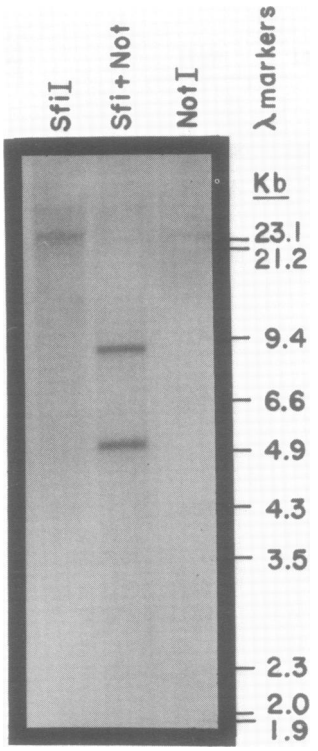


Figure 7. Southern analysis of SfiI and NotI single and combined digests of DBA/2 DNA. Digests were electrophoresed on a standard 0.8% agarose gel, blotted, and probed with p3'R/X (probe 2). The 5 Kb fragment reflects the distance between the NotI and SfiI sites mapped within the Ren-1 3' flanking region. The 9 Kb fragment links the NotI and SfiI sites in the Ren-2 (3') and Ren-1 (5') flanking regions, respectively.

arrangement for the two renin genes, that is, that Ren-1 and Ren-2 are transcribed in the same relative direction. The probe p3'Ren-1 also detects a fragment of 18 Kb in a Hpa/Not double digest (Fig. 6B) which positions the 3' end of the large HpaI fragment containing both genes. Listed in a 5' to 3' order, the fragments Hpa/Not (19 Kb, Ren-2), NotI (28 Kb, Ren-1), and Not/Hpa (18 Kb, Ren-1 3') have a combined length of 65 Kb. This length agrees well with the HpaI hybridization pattern seen in the PFGE Southern blot.

The cDNA probe detects the 25 Kb SfiI fragment containing Ren-1 (Fig.5B). In a combined Hpa/Sfi digest a second fragment of roughly 29 Kb is observed which must contain Ren-2. Given the arrangement suggested by the NotI results, the length of this second fragment matches that predicted for a fragment generated by the HpaI and SfiI sites in the 5' flanking regions of

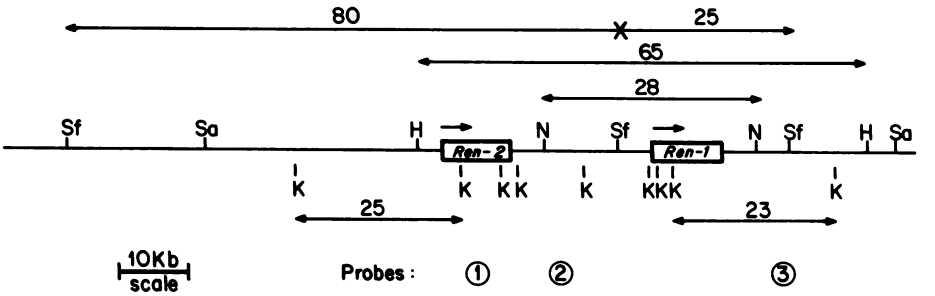


Figure 8. Restriction map of the region of chromosome one containing the Ren loci, showing the physical arrangement of Ren-1 and Ren-2 in DBA/2 mice. The genes are transcribed in the same relative direction as indicated by arrows above each gene. Hybridizing fragments discussed in the text are denoted using lines with arrowheads at both ends which identify boundary restriction sites. Numbers above the lines refer to the lengths of the fragments in kilobase pairs. Circled numbers identify the approximate positions of the DNA sequences used as probes. Symbols for restriction enzyme sites are the same as in Fig. 4.

Ren-2 and Ren-1, respectively. Lastly, the overlap between maps of the two loci can be seen by the ability of SfiI to cleave within the 28 Kb NotI fragment detected by p3'R/X (Figure 7). Probing a NotI/SfiI double digest with p3'R/X yields a fragment of 5 Kb which is predicted from the NotI and SfiI sites mapped on the 3' side of Ren-1, and a fragment of roughly 9 Kb which represents the link between the 3' region of Ren-2 and the 5' region of Ren-1. A map of the region of mouse chromosome one containing the Ren loci is shown in Figure 8.

## DISCUSSION

In addition to the murine renin genes there exist a number of examples in which gene duplication is accompanied by variation in the tissue-specific or developmental patterns of expression between the multiple gene copies (eg. alpha-amylase <34>, growth hormone/prolactin/placental lactogen <35>, and globins <rev. in 36>, albumin/alpha-fetoprotein <37>, respectively). Characterizing the physical relationships between gene copies has provided insights into mechanisms of gene duplication, and the sequences involved in the regulation of expression of different copies.

The mapping results presented here establish physical linkage between the two renin genes in DBA/2 mice. These results indicate that Ren-2 lies upstream relative to Ren-1, that both genes are transcribed in the same relative direction, and that a span of 20 Kb separates the coding regions of

both genes. Knowledge of the arrangement of the genes should now simplify the isolation of genomic clones which link Ren-1 and Ren-2.

This organization prompts speculation on the evolution and expression of the Ren loci in mice. The duplicated copy may have arisen by unequal crossing-over between chromosomes bearing the ancestral Ren-1 gene (8). Presumably, recombination occurred between short directly repeated sequences present at each end of the renin gene. The observations that some inbred strains and commensal mice have a unique renin gene are consistent with either a recent duplication occurring subsequent to the speciation of the mouse or a recent deletion of one of the genes from an ancestral duplication (5,8,9). Either possibility implies that the rearrangement was not fixed in the population during evolution of the mouse. The arrangement of gene-NotI-SfiI is seen for both Ren-1 and Ren-2, with possible subsequent insertions and/or deletions to account for minor spacing differences. This motif may therefore reflect the underlying unit of duplication, and suggests that the crossover may have occurred between the original SfiI site and what is now Ren-1.

This model suggests a rearrangement of the 5' flanking region of Ren-1 with the introduction of the duplicated copy upstream. If the rearrangement is responsible for the difference in SMG expression of the two loci, then it is possible either that Ren-1 lost important sequences which promote SMG expression or that sequences which down-regulate transcription were introduced upstream of Ren-1. However, observations of little or no renin activity in the SMG of other species (38) leave open the possibility that this locus has no history of abundant expression in this gland. The expression differences between the genes might otherwise be explained by the introduction of Ren-2 into a new context of sequence or chromatin structure which influences SMG expression. In the future, it will be important to examine the structural arrangements of the Ren loci in one-gene inbred strains and wild-derived mice. Such studies may provide insights into the history of genetic rearrangements involving the renin genes in mice, and help to define the relationships between renin gene structure and expression.

ACKNOWLEDGEMENTS

We are grateful to Drs. Curt D. Sigmund and John J. Mullins for valuable criticism during preparation of the manuscript. We wish to thank Dr. Dave Burt (Harvard Medical School, Boston, MA) for providing genomic subclones of Ren-1 flanking sequences. We also thank Dr. Joel Huberman for providing the yeast strain A364a. This work was supported by a grant from the National Institutes of Health (GM30248).

REFERENCES
1. Bentley,P.J. (1982) Comparative Vertebrate Endocrinology, 2nd edn. pp.55-58 and 303-337, Cambridge University Press, New York.
2. Wilson, J.X. (1984) Endocrine Reviews 5, 45-61.
3. Soubrier,F., Panthier,J.J., Corvol,P., and Rougeon,F. (1983) Nucl. Acids Res. 11, 7181-7190.
4. Chirgwin,J.M., Schaefer, I.M., Rotwein,P.S., Piccini,N., and Gross,K.W., and Naylor,S.L. (1984) Som. Cell Mol. Gen. 10, 415-421.
5. Dickinson,D.P., Gross,K.W., Piccini,N., and Wilson,C.M. (1984) Genetics 108, 651-667.
6. Piccini,N., Knopf,J.L., and Gross,K.W. (1982) Cell 30, 205-213.
7. Mullins,J.J., Burt,D.W., Windass,J.D., McTurk,P., George,H., and Brammar,W.J. (1982) EMBO J. 1, 1461-1466.
8. Panthier,J.J., Holm,I., and Rougeon,F. (1982) EMBO J. 1, 1417-1421.
9. Holm,I., Ollo,R., Panthier,J.J., and Rougeon,F. (1984) EMBO J. 3, 557-562.
10. Field,L.J., Philbrick,W.M., Howles,P.N., Dickinson,D.P., McGowan,R.A., and Gross,K.W. (1984) Mol. Cell Biol. 4, 2321-2331.
11. Panthier,J.J., Dreyfus,M., Tronik-Le Roux,D., and Rougeon,F. (1984) Proc. Natl. Acad. Sci. USA 81, 5489-5493.
12. Inagami,T., Murakami,K., Yokosawa,H., Matoba,T., Yokosawa,N., Takii,Y., and Misono,K. (1980) in Enzymatic Release of Vasoactive Peptides, Gross,F. and Vogel,H.F. Eds., pp. 7-30, Raven Press, New York.
13. Wilson,C.M. and Taylor,B.A. (1982) J. Biol. Chem. 257, 217-223.
14. Field,L.J., McGowan,R.A., Dickinson,D.P., and Gross,K.W. (1984) Hypertension 6, 597-603.
15. Field,L.J. and Gross,K.W. (1985) Proc. Natl. Acad. Sci. USA 82, 6196-6200.
16. Panthier,J.J., Foote,S., Chambraud,B., Strosberg,A.D., Corvol,P., and Rougeon,F. (1982) Nature(London) 298, 90-92.
17. Schwartz,D.C. and Cantor,C.R. (1984) Cell 37, 67-75.
18. Carle,G.F. and Olson,M.V. (1984) Nucl. Acids Res. 12, 5647-5664.
19. Carle,G.F., Frank,M., and Olson,M.V. (1986) Science 232, 65-68.
20. Barlow,D.P., Bucan,M., Lehrach,H., Hogan,B.L.M., and Gough,N.M. (1987) EMBO J. 6, 617-623.
21. Lawrance,S.K., Smith,C.L., Srivastava,R., Cantor,C.R., and Weissman,S.M. (1987) Science 235, 1387-1390.
22. Hardy,D.A., Bell,J.I., Long,E.O., Lindsten,T., and McDevitt,H.O. (1986) Nature 323, 453-455.
23. Pellicer,K., Wigler,M., Axel,R., and Silverstein,S. (1978) Cell 14, 133-141.
24. Bennet,K.L., Lalley,P.A., Barth,R.K., and Hastie,N.D. (1982) Proc. Natl. Acad. Sci. USA 79, 1120-1124.
25. Maniatis,T., Fritsch, E.F., and Sambrook,J. (1982) Molecular Cloning: A Laboratory Manual pp.450, Cold Spring Harbor Press, Cold Spring Harbor, NY.
26. Herrmann,B.G., Barlow,D.P., and Lehrach,H. (1987) Cell 48, 813-825.
27. Gardiner,K., Laas,W., and Patterson,D. (1986) Som. Cell Mol. Gen. 12, 185-195.
28. Church,G.M. and Gilbert,W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991-1995.
29. Feinberg,A.P. and Vogelstein,B. (1983) Analyt. Biochem. 132, 6-13.
30. Southern,E.M. (1975) J. Mol. Biol. 98, 503-517.
31. Schwartz,M.N., Trautner,T.A., and Kornberg,A. (1962) J. Biol. Chem. 237, 1961-1967.
32. Russell,G.J., Walker,P.M.B., Elton,R.A., and Subak-Sharpe,J.H. (1976) J. Mol. Biol. 108, 1-23.
33. Burt,D.W., Reith,A.D., and Brammar,W.J. (1984) Nucl. Acids Res. 12, 8579-8593.
34. Hagenbuchle,O., Bovey,R., and Young,R.A. (1980) Cell 21, 179-187.

35. Niall,H.D., Hogan,M.L., Sayer,R., Rosenblum,I.Y., and Greenwood,F.C. (1971) Proc. Natl. Acad. Sci. USA 68, 866-869.
36. Maniatis,T., Fritsch,E.F., Lauer,J., and Lawn,R.M. (1980) Ann. Rev. Gen. 14, 145-178.
37. Jagodzinski,L.L., Sargent,T.D., Yang,M., Glackin,C., and Bonner,J. (1981) Proc. Natl. Acad. Sci. USA 78, 3521-3525.
38. Morris,B.J., deZwart,R.T., and Young,J.A. (1980) Experientia 36, 1333-1334.