EVOLUTION AND VARIATION OF RENIN GENES IN MICE

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ABSTRACT

Inbred strains of mice carry *Ren-1*, a gene encoding the thermostable Renin-1 isozyme. *Ren-1* is expressed at relatively low levels in mouse submandibular gland and kidney. Some strains also carry *Ren-2*, a gene encoding the thermolabile Renin-2 isozyme. *Ren-2* is expressed at high levels in the mouse submandibular gland and at very low levels, if at all, in the kidney. *Ren-1* and *Ren-2* are closely linked on mouse chromosome *1*, show extensive homology in coding and noncoding regions and provide a model for studying the regulation of gene expression. An investigation of renin genes and enzymatic activity in wild-derived mice identified several restriction site polymorphisms as well as putative variants in renin gene expression and protein structure. The number of renin genes carried by different subpopulations of wild-derived mice is consistent with the occurrence of a gene duplication event prior to the divergence of *M. spretus* (2.75–5.5 million yr ago). This conclusion is in agreement with a prior estimate based upon comparative sequence analysis of *Ren-1* and *Ren-2* from inbred laboratory mice.

A number of biologically active polypeptides including renin, epidermal growth factor (EGF), nerve growth factor and several esteroproteolytic enzymes are synthesized and stored in the granular convoluted tubule cells of the mouse submandibular gland (SMG) (BARKA 1980). Differentiation of these cells and synthesis of the polypeptides are regulated by androgen and thyroxine (GRESIK 1980; WILSON *et al.* 1982). Mouse SMG renin activity is under genetic as well as hormonal control (BING and POULSEN 1971; WILSON *et al.* 1981). Some inbred strains of laboratory mice carry a single renin structural gene, *Ren-1*, that encodes the thermostable Renin-1 isozyme. Other strains carry *Ren-1* and a second renin structural gene, *Ren-2*, that encodes the thermolabile Renin-2 isozyme (MULLINS *et al.* 1982; PICCINI, KNOPF and GROSS 1982; PANTHIER, HOLM and ROUGEON 1982; WILSON and TAYLOR 1982). Renin-2 has been purified to homogeneity (COHEN *et al.* 1972) and used to produce an

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alloantibody that blocks Renin-2 but not Renin-1 activity (WILSON and TAYLOR 1982).

Expression of *Ren-2* in mouse SMG is on average 150-fold higher than *Ren-1* in inbred strains. Therefore, Renin-2 is the predominant SMG renin isozyme in mice that carry both genes. The physiological function of SMG renin is not understood. The juxtaglomerular cells of the kidney are the primary source of plasma renin which plays a central role in the regulation of blood pressure. Biochemical and genetic studies indicate that the renin isozyme produced in the kidney is encoded by *Ren-1*, the gene held in common by all inbred strains (INAGAMI *et al.* 1980; WILSON and TAYLOR 1982; PANTHIER and ROUGEON 1983; HOLM *et al.* 1984).

Ren-1 and *Ren-2* are tightly linked (PICCINI, KNOPF and GROSS 1982) and located near the *Pep-3* locus on mouse chromosome *1*. Recombination between *Pep-3* and *Ren-2* has been found in two of 69 informative recombinant inbred strains tested, indicating a recombination frequency of 0.0076 ± 0.0055 (C. M. WILSON, unpublished results). Sequence analysis of renin mRNA showed 96% homology between *Ren-1* and *Ren-2* in the coding regions of these genes (HOLM *et al.* 1984). Striking similarity is also found at the genomic level. Thus, mouse renin genes provide an attractive model in which to investigate the mechanism by which two linked, homologous genes are expressed at dramatically different levels in the same tissues. However, our ability to analyze the mechanism underlying this regulation has been hindered by the limited variation in gene structure and expression that has been fixed in inbred strains of laboratory mice.

The extensive homology between *Ren-1* and *Ren-2* in coding and noncoding regions is consistent with either a gene duplication event during the speciation of the mouse or a relatively recent deletion of one of these genes from an ancestral duplication. Prior analyses of renin genes have, in fact, led to conflicting proposals that a duplication of the ancestral *Ren* gene occurred after divergence of *M. spretus* (2.25–5.5 million yr ago) (PANTHIER, HOLM and ROUGEON 1982) or, conversely, that a duplication of *Ren-1* and *Ren-2* could have occurred prior to the species separation of rat and mouse and may have been followed by a deletion event (HOLM *et al.* 1984).

In this paper we describe the results of a phylogenetically based survey of wild mice. The aims of this study were twofold: to investigate the evolution of the gene duplication in the mouse and to identify additional genetic variants that could be used to study the regulation of renin gene expression.

MATERIALS AND METHODS

Stocks of mice: Most of the stocks we examined are maintained by V. CHAPMAN at Roswell Park Memorial Institute. Several breeding schemes are used. Random breeding colonies (denoted by an R) are maintained by ten to 15 pairs of mice breeding in each generation. Brother-sister mating is avoided, and no set of parents contributes more than two progeny to the breeding of the next generation. Some stocks are maintained as small colonies in which brother-sister matings are avoided (denoted by *). Other stocks are partially inbred (denoted by a T) or have been inbred for more than 20 generations (I). Since a number of these stocks were derived from animals trapped by R. SAGE, for convenience we have continued to use the nomenclature proposed by

MARSHALL and SAGE (1981) for the different species rather than the biochemical group nomenclature of THALER, BONHOMME and BRITTON-DAVIDIAN (1981). The *M. caroli* (R) and *M. castaneus* (R) stocks were derived from animals trapped by J. MARSHALL (National Museum of Natural History, Washington, D.C.); *M. molossinus* (T) from animals trapped in Japan, obtained from a colony at the Ontario Cancer Institute; and *M. hortulanus* (Pancevo) (R) from animals trapped by R. SAGE (Museum of Vertebrate Zoology, Berkeley, California). The *M. spretus* (Spain/France) (R) stock was derived from animals trapped in France and Spain and supplied separately to V. CHAPMAN by F. BONHOMME (Universite Montpellier, Montpellier, France) and by R. SAGE.

The *Mus musculus* stocks tested were derived from animals trapped in the following locations: Northern Jutland, Denmark (R); Brno, Czechoslovakia (*); Belgrade, Yugoslavia (*) and Skive, Denmark (T).

For the purpose of this paper the following stocks are classified as *M. domesticus*: PAC (I), derived from animals trapped and inbred by J. CONNER in Philadelphia; MOR-2 (I), derived from animals trapped in Ohio by J. BRUEL and inbred by T. SHOWS and V. CHAPMAN at Roswell Park Memorial Institute; (Azrou) (*) from animals trapped in Azrou, Morocco, by R. SAGE. We also examined F_1 animals constructed by crossing wild-trapped animals of *M.d. praetextus* (Jerusalem) with C57BL/6 and *M.d. praetextus* (Egypt) with C3H/HeHa. These mice were trapped by R. SAGE and sent to Roswell Park Memorial Institute, where they were crossed with the inbred strains.

Frozen tissues from a male and a female of each of the following species were provided by M. POTTER (National Institutes of Health): *M. cervicolor cervicolor, M. cervicolor popaeus, M. (Coelomys) pahari, M. cookii* and *M. spretus* from animals trapped in Morocco. Tissues from *Peromyscus maniculatus, Peromyscus leucopus* and *Peromyscus polionotus* were provided by W. D. DAWSON (The University of South Carolina).

Two inbred laboratory strains DBA/2J and C57BL/6J obtained from The Jackson Laboratory and a random-breeding colony HA/ICR maintained by V. CHAPMAN at Roswell Park Memorial Institute were also examined.

Enzyme assays: Cytosol was prepared from fresh or frozen submandibular glands or kidneys as previously described (WILSON *et al.* 1977). Saliva was collected from animals injected intraperitoneally with 0.1–0.2 ml of 0.5 mg/ml acetyl-β-methyl choline chloride (Sigma) to induce salivation. Samples were frozen until assayed.

Renin activity, thermostability and alloantibody sensitivity were determined as previously described (WILSON and TAYLOR 1982). One unit of renin activity is defined as the amount of renin that produces 1 nanomole of angiotensin I per minute. Thermostability of renin activity was determined by diluting cytosol or saliva in 0.05 M sodium acetate, pH 5.5, containing 0.25 mg of crystalline bovine serum albumin/ml to a final concentration of 0.0015–0.003 units/ml and heating 100-μl aliquots for 10 min at 60°. Activity remaining was assayed and is presented as percent of activity in the unheated control. To determine the sensitivity of renin activity to an alloantibody that is specific for the Renin-2 isozyme, SMG cytosol was incubated overnight at 10° with antiserum at a final dilution of 1:50. Activity that remained is presented as percent of activity in a sample incubated in the absence of antiserum. SMG cytosol from C57BL/6J and DBA/2J mice were included in each determination of thermostability or alloantibody sensitivity to indicate standard responses for Renin-1 and Renin-2 isozymes, respectively. Values reported are the average of at least two determinations.

Renin concentration was estimated by radial immunodiffusion using goat antiserum that does not distinguish between Renin-1 and Renin-2 isozymes. In every sample, renin concentration paralleled enzymatic activity.

EGF was measured by the direct radioimmunoassay of HOATH *et al.* (1983), except that 200 μl of 25% polyethylene glycol was used to separate bound and free moieties. The primary EGF antibody was kindly provided by S. B. HOATH and D. A. FISHER. Protein was determined according to LOWRY *et al.* (1951) using crystalline bovine serum albumin as the protein standard.

Pep-3 alleles were determined by the method of LEWIS and TRUSLOVE (1969) using L-leucyl-L-alanine as substrate or a modification of this method using cellulose acetate plates (Titan III, Helena Laboratories) for the electrophoretic separation and L-leucyl-L-tyrosine as substrate. For our samples we used both hemolysates and either SMG, kidney or testes cytosol. Cytosol was prepared by homogenization of a small piece of tissue in 0.25 M sucrose, 0.02 M imidazole, pH 7.6, followed by a 20-min centrifugation at 25,500 × *g* in a Sorvall SS-34 to remove debris.

Genomic DNA preparation and analysis: Liver genomic DNA was prepared by the following method (P. HOWLES, personal communication). Fresh or frozen livers were homogenized in 5 ml of 0.3 M sucrose, 0.25% Triton, 10 mM EDTA, 50 mM Tris-HCl, pH 7.5, and filtered through gauze; 5 ml of 2 M sucrose, 0.1% Triton, 10 mM EDTA, 50 mM Tris-HCl, pH 7.5, were added and mixed by inversion. The mixture was layered onto a cushion of 6.5 ml of 2 M sucrose, 0.1% Triton, 10 mM EDTA, 50 mM Tris-HCl, pH 7.5, in a 4 × ⅝ inch cellulose nitrate tube and centrifuged for 70 min at 25,000 rpm, 4°, in a Beckman SW-28 rotor. After centrifugation, the liquid was aspirated off down to the cushion and the remaining liquid poured off the pellet of nuclei. Remaining liquid was removed by wiping with a tissue, and then 1 ml of 100 mM EDTA, 10 mM Tris-HCl, pH 7.5, was added. The tube was swirled to lift the pellet off the bottom, and then 5 μl of boiled RNase A (20 mg/ml) were added. The tube was incubated for 30–60 min at 37°, after which 415 μl of 100 mM EDTA, 10 mM Tris-HCl, pH 7.5; 30 μl 10% SDS; and 55 μl of Proteinase K (5.5 mg/ml) were added. The mixture was incubated 3–15 hr at 55° and then extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) and twice with chloroform-isoamyl alcohol (24:1). The organic phases were drawn off from the bottom. The aqueous layer was then extracted twice with isoamyl alcohol and desiccated to remove some of the remaining isoamyl alcohol. The aqueous layer was dialyzed against two changes of sterile 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. Restriction endonuclease digestions of liver DNA were performed under the conditions recommended by the manufacturer. After agarose gel electrophoresis, the fragments were transferred to nitrocellulose membrane as described by SOUTHERN (1975). The filters were hybridized with a ^{32}P-labeled probe ($1–2 \times 10^8$ cpm/μg) prepared from the SMG renin cDNA clone pSM479, using the procedure described by MANIATIS, FRITSCH and SAMBROOK (1982), except that we use a 10× nick translation buffer of 500 mM Tris-HCl, pH 7.8, 50 mM $MgCl_2$, 100 mM β-mercaptoethanol and 500 μg/ml of bovine serum albumin. The construction of this clone has been described by PICCINI, KNOPF and GROSS (1982). Subsequent work in our laboratory has shown that this clone encodes at least part of the last 3′ exon of the *Ren-2* gene (exon 9) but does not extend significantly into exon 8 (D. P. DICKINSON, unpublished observations).

Hybridization and wash conditions were similar to those used by PICCINI, KNOPF, and GROSS (1982), except that we used 2–3 washes of 2× SSC, 0.1% SDS, 0.1% sodium pyrophosphate, followed by 1–2 washes of 1× SSC, 0.1% SDS, 0.1% sodium pyrophosphate. Hybridization signals were detected by autoradiography with Kodak XAR-5 film at −70° using one or two Kodak intensifying screens.

RESULTS

Survey of wild-derived mice

SMG renin activity and thermolability: The activity and thermolability of renin in SMG cytosol or in saliva can be used as a marker for the presence or absence of the *Ren-2* gene in inbred laboratory mice. Adult mice from inbred strains such as C57BL/6J that carry only *Ren-1* produce low levels of the thermostable Renin-1 isozyme (less than 1 unit/mg of protein in SMG cytosol). Strains such as DBA/2J that carry *Ren-2* in addition to *Ren-1* produce higher levels of the thermolabile Renin-2 isozyme (WILSON *et al.* 1981). (Typical SMG renin activity and thermostability values for the two types of inbred strains are given in Table 2A.)

Results of a survey of a number of stocks of wild-derived mice are presented in Table 1. Many of these animals did indeed produce low levels of thermostable SMG renin activity. These mice were tentatively classified as carrying a single *Ren-1*-type gene. A second group of mice had relatively high levels of thermolabile SMG renin. These were tentatively classified as carrying a *Ren-2*-type gene in addition to *Ren-1*. In most instances the entire population of each stock fell into the same category. Some individuals from the *M.d. praetextus*

TABLE 1

Survey of SMG renin activity and Pep-3 *genotype in wild-derived mice*

Renin thermostability[a]	Species/stock/strain	No. of animals/total no. examined	Renin activity[b]	*Pep-3* genotype
Stable	*M. caroli*	18/21	—[c]	ND
		2/21	Low	Unidentified
		1/21	High	Unidentified
	M. cervicolor cervicolor	2/2	High	Unidentified
	M. cervicolor popeus	2/2	Low	Unidentified
	M. (Coelomys) pahari	2/2	Low	Unidentified
	M. cookii	2/2	Low	b/b
	M. castaneus	1/3	Low	a/b
	M. domesticus praetextus (Egypt)[d]	5/5	—[c]	b/b
	M. domesticus praetextus (Jerusalem)[e]	4/7	—[c]	a/c
Labile	*M. musculus*			
	(Denmark)	13/16	—[c]	b/b
		3/16	High	b/b
	(Brno)	1/4	—[c]	
		3/4	High	b/b
	(Skive)	2/5	—[c]	
		3/5	High	b/b
	(Belgrade)	6/9	—[c]	b/b
		3/9	High	b/b
	M. castaneus	2/3	High	b/b
	M. molossinus	1/1	High	a/a
	M. domesticus praetextus (Jerusalem)[e]	3/7	—[c]	a/b
	M. domesticus			
	(Azrou)	3/3	High	b/b
	(PAC)	2/3	—[c]	
		1/3	High	b/b
	(MOR-2)	2/2	—[c]	ND
Intermediate	*M. spretus* (France/Spain)	13/28	—[c]	b/b
		15/28	High	b/b
	M. spretus (Morocco)	2/2	High	b/b
	M. hortulanus (Pancevo)	10/10	High	b/b

ND = not done.

[a] Thermostability was determined by heating saliva or SMG cytosol as described in the MATERIALS AND METHODS. Renin that retained more than 60% of the original activity was classified as stable, less than 10% as labile. Intermediate values varied from 20 to 50%.

[b] Cytosol activity less than 1 unit/mg of protein is classified as low. We have seen no inbred mouse of either sex that has thermostable renin and activity higher than this value.

[c] Renin thermostability was determined only in saliva from these animals in order to preserve them for breeding purposes. Renin activity levels are not reported because factors other than the concentration of renin in the SMG may influence concentration in saliva.

[d] F_1 animals from a cross between wild-trapped animals and C3H/HeHa.

[e] F_1 animals from a cross between wild-trapped animals and C57BL/6.

(Jerusalem) and the *M. castaneus* colonies fell into each category, suggesting polymorphism at the *Ren-2* locus in these stocks.

Putative structural gene variants: Unique combinations of thermolability and activity levels were found in a third group (Table 1) comprised of *M. spretus*

(France/Spain), *M. spretus* (Morocco) and *M. hortulanus*. Levels of SMG renin activity in mice from these stocks were similar to those found in mice that carry both *Ren-1* and *Ren-2*. Results of restriction enzyme analysis of DNA from these animals presented later in this paper confirmed that they carry two renin genes. However, SMG renin activity from these wild-derived stocks was more stable when heated than Renin-2 from inbred laboratory mice and less stable than Renin-1 (Tables 1 and 2). SMG renin activity from *M. spretus* (France/Spain) was blocked by an alloantibody specific for Renin-2 from inbred laboratory mice (Table 2B). Although we cannot rule out the possibility that altered thermostability of SMG renin in this case is due to differences in cytosolic factors, these results are consistent with the hypothesis that the *M. spretus* (France/Spain) stock carries a *Ren-2*-like gene encoding a structural variant of Renin-2.

SMG renin activity from *M. hortulanus* animals was only partially blocked by concentrations of Renin-2-specific alloantibody that completely blocked Renin-2 from inbred laboratory mice (Table 2B) but was completely blocked by heterologous antiserum that cross-reacts with Renin-1 and Renin-2 (data not shown). When SMG cytosol from DBA-2J mice and *M. hortulanus* were mixed prior to incubation with the alloantibody, results were additive (data not shown) indicating that cytosolic factors are not influencing the immunological reaction.

The simplest explanation for these results is that, as postulated for *M. spretus*, *M. hortulanus* carries a *Ren-2*-like gene that encodes a Renin-2 structural variant. However, these results would also be expected if *M. hortulanus* SMG renin was an equal mixture of inbred-type Renin-1 and Renin-2 isozymes. At present we cannot distinguish these alternatives.

Putative variation in expression of renin genes: Most *M. caroli* mice tested produced relatively low levels of thermostable SMG renin consistent with the expression of a single *Ren-1* gene (Tables 1 and 2C). Results of restriction enzyme analysis of DNA from these animals presented later in this paper confirmed this assumption. However, a single male *M. caroli* mouse (Table 1 and Table 2C, male 1) had markedly higher levels of SMG renin activity (whether expressed as units per milligram of tissue weight or units per milligram of protein) than the highest values we have previously found in inbred male laboratory mice carrying a single renin structural gene (WILSON *et al.* 1981; C. M. WILSON unpublished data). Levels of kidney renin in this animal were similar to those of normal low SMG renin *M. caroli* animals (Table 2C).

There are several possible explanations for this observation, the most interesting of which is that it reflects tissue-specific enhanced expression of *Ren-1*. To rule out some other possibilities, we compared the properties of SMG renin from the putative gene expression variant and two typical *M. caroli* mice (males 2 and 3, Table 2C). High SMG renin activity in *M. caroli* male 1 was associated with a parallel increase in renin protein concentration estimated by radial immunodiffusion with heterologous antiserum that precipitates both Renin-1 and Renin-2 (data not shown). SMG renin activity from all three *M. caroli* mice resembled Renin-1 immunologically and was not blocked by an alloanti-

TABLE 2

Properties of renin from inbred laboratory mice and wild-derived mouse stocks that harbor putative variants in renin structural genes and gene expression

Species/strain/stock	Sex	SMG renin activity				Kidney renin (units/mg protein)	SMG EGF (μg/mg protein)
		Units/mg protein	Units/mg tissue	Thermostability (% remaining)	Alloantibody sensitivity (% remaining)		
A. Inbred Laboratory Strains[a]							
C57BL/6J	Female	0.02	0.002				
	Male	0.34	0.023	60	91		
DSA/2J	Female	13.5	0.97				
	Male	68.9	3.5	11	9		
B. Putative variation in renin structural genes[b]							
M. spretus (France/Spain)	Male	43.8 ± 3.0	3.9 ± 0.6	31 ± 4	14 ± 1		
M. hortulanus	Male	41.4 ± 5.0	3.8 ± 0.6	21 ± 0.4	41 ± 3		
C. Putative variation in renin gene expression[c]							
M. caroli	Male 1[d]	6.7	0.48	66	97	0.05	1.9
	Male 2	0.53	0.040	68	99	0.07	1.3
	Male 3	0.45	0.026	71	86	0.07	2.0
M. cervicolor cervicolor							
	Female[d]	5.0	0.39	63	92	0.15	0.04
M. cervicolor popaeus	Male[d]	1.8	0.15	75	91	0.28	0.55
	Female	0.64	0.040	62	91	0.09	0.13
	Male	0.38	0.028	80	94	0.23	2.2

[a] C57BL/6J is the type strain for mice that carry the single *Ren-1* gene and produce only the thermostable, alloantibody-insensitive Renin-1 isozyme. DBA/2J is the type strain for mice that carry both *Ren-1* and *Ren-2*. At least 99% of SMG renin from these mice is the thermolabile, alloantibody-sensitive Renin-2 isozyme. SMGs from six to 12 inbred mice were pooled to prepare cytosol.

[b] Average values ± SEM obtained from four mice.

[c] Values from individual animals.

[d] Putative gene expression variants.

body specific for Renin-2 (Table 2C). Furthermore, levels of SMG EGF were similar in these animals, indicating that the tenfold difference in SMG renin activity does not reflect a general increase in all products of the SMG granular convoluted tubule cells.

Both *M. cervicolor cervicolor* animals tested also had unusually high levels of thermostable SMG renin activity when compared with inbred mice that produce only Renin-1 and compared with two mice from the closely related subspecies *M. cervicolor popaeus*. Kidney renin levels in all of these animals were similar. Other properties of SMG renin from *cervicolor* mice were typical of the Renin-1 isozyme from inbred mice. Restriction site analysis of renin genes in both subspecies confirmed the presence of a single gene like *Ren-1*. It is noteworthy that both *M. cervicolor* animals came from the same breeding pair that was a second generation brother-sister mating.

SMG renin and EGF are markedly higher in male inbred laboratory mice than in females, presumably because of the effect of androgen on differentiation of SMG convoluted tubule cells. SMG EGF in *cervicolor* SMG showed the same sexual dimorphism (Table 2C). However, SMG renin activity and EGF concentration did not vary concordantly, indicating that the elevated renin levels in *M. cervicolor cervicolor* were not due to some generalized effect on the convoluted tubule cells.

Pep-3 *as a marker for renin heterozygosity*: Renin activity in the mouse SMG is under complex hormonal, as well as genetic, control. Therefore, it is impossible to differentiate unambiguously mice that are homozygous at the *Ren-2* locus from those that carry only one copy of *Ren-2* on the basis of Renin-2 activity. *Pep-3*, an independent gene closely linked to the *Ren-1*, *Ren-2* loci on chromosome *1*, encodes at least three electrophoretic variants of Peptidase-3. Linkage disequilibrium between *Pep-3* and *Ren-1*, *Ren-2* has been noted in inbred laboratory strains (WILSON *et al.* 1978). *Pep-3*^{a}, or more rarely, *Pep-3*^{b}, has been found in strains that carry only *Ren-1*; *Pep-3*^{b} or *Pep-3*^{c} in strains that carry both *Ren-1* and *Ren-2* (STAATS 1980; DICKINSON and GROSS 1982).

Heterozygosity at the *Pep-3* locus frequently indicates heterozygosity at the *Ren-2* locus. The presence of different *Pep-3* alleles in a population may indicate the presence of different *Ren* alleles, and *Pep-3* may also serve as a useful marker for identifying mice in breeding experiments. Our strategy for screening populations, therefore, included determination of the *Pep-3* genotype.

The *Pep-3* genotypes of screened animals are shown in Table 1. Under our assay conditions, Peptidase-3 could not be identified unambiguously in *M. caroli*, *M. cervicolor* and *M. pahari*, which are all distantly related to inbred laboratory strains of mice (CALLAHAN and TODARO 1978). The remaining animals examined carried *Pep-3* alleles indistinguishable from those found in inbred laboratory mice, and the majority were homozygous for *Pep-3*^{b}.

The *M.d. praetextus* (Jerusalem) stock contained some animals that carried *Pep-3*^{b} linked to *Ren-1*, *Ren-2* and others with *Pep-3*^{c} in the apparent absence of *Ren-2*. We did not detect the *Pep-3*^{c} allele in other wild-derived mice. The inbred stock of *M. molossinus* that we examined carried *Pep-3*^{a} in the presence of *Ren-2*. To our knowledge, this is the first time these combinations have been described. However, although these *Pep-3* alleles are electrophoretically

indistinguishable from the analogous inbred strain alleles, it is conceivable that they result from different structural modifications that produce the same effect.

The occurrence of *Pep-3*^{a} in the absence of *Ren-2*, although common in inbred laboratory strains, was observed only once in this survey, in an animal from an *M. castaneus* stock (Table 1, thermostable SMG renin activity, *Pep-3*^{a}/*Pep-3*^{b} and Figure 2A, no. 2, lane g). Conversely, the occurrence of *Pep-3*^{b} in the absence of *Ren-2*, which is relatively rare in inbred laboratory strains, was found in both the *M. castaneus* and *M.d. praetextus* (Egyptian) stocks.

We took advantage of these results to construct homozygotes of *M.d. praetextus* (Jerusalem) *Ren-2*^{null}, *Pep-3*^{c} and *M. castaneus Ren-2*^{null}, *Pep-3*^{a} and *Ren-2*^{null}, *Pep-3*^{b} for use in subsequent genomic analysis.

Renin gene copy number

The results from an initial survey of wild-derived mice strongly suggested that most of the stocks we examined could be divided into two groups analogous to those found in inbred strains; that is, they produced either low levels of thermostable SMG renin or relatively high levels of thermolabile SMG renin. By analogy with inbred strains, we anticipated that mice in the first group carry a single copy of the renin gene and mice in the second group carry two copies. Some stocks appeared to contain both types. Other stocks harbored individuals that did not fit into either category. The simplest explanation for the unusual properties of SMG renin from *M. spretus* and *M. hortulanus* is the presence of an inbred strain-like gene duplication in these mice with the *Ren-2*-like gene encoding a protein variant. In the case of the two *M. cervicolor cervicolor* animals and *M. caroli* male 1 (Table 2C), the most straightforward explanation is that these animals carry variant forms of the single *Ren-1*-like gene that result in increased renin expression in the SMG but not in the kidney. To further clarify these results, we determined the genomic composition of individuals from stocks by Southern blot analysis. As shown by PICCINI, KNOPF and GROSS (1982), the renin gene copy number can be estimated by Southern blot analysis of genomic DNA using a short cDNA probe (pSM479) that corresponds to the last exon of the *Ren-2* structural gene. Most restriction enzymes that have a six-base recognition sequence produce only one fragment from each copy of the gene that contains sequences complementary to pSM479. Therefore, one-gene strains give only one band on a Southern blot. Two-gene strains give either a double intensity band or two single intensity bands of different size if there are restriction site differences between the two copies of the renin gene. In particular, the restriction endonuclease *Pvu*II produces different size fragments from *Ren-1* carried by one-gene strains, *Ren-1* carried by two-gene strains and *Ren-2*, permitting all three genes to be distinguished in a heterozygote. Detailed analysis of genomic DNA and genomic clones from inbred strains of mice has led to the fragment assignment shown in Figure 1 and in Figure 2, lanes a and b. It should be noted that the tentative *Pvu*II fragment assignment described by PICCINI, KNOPF and GROSS (1982) for two-gene strains has subsequently been revised.

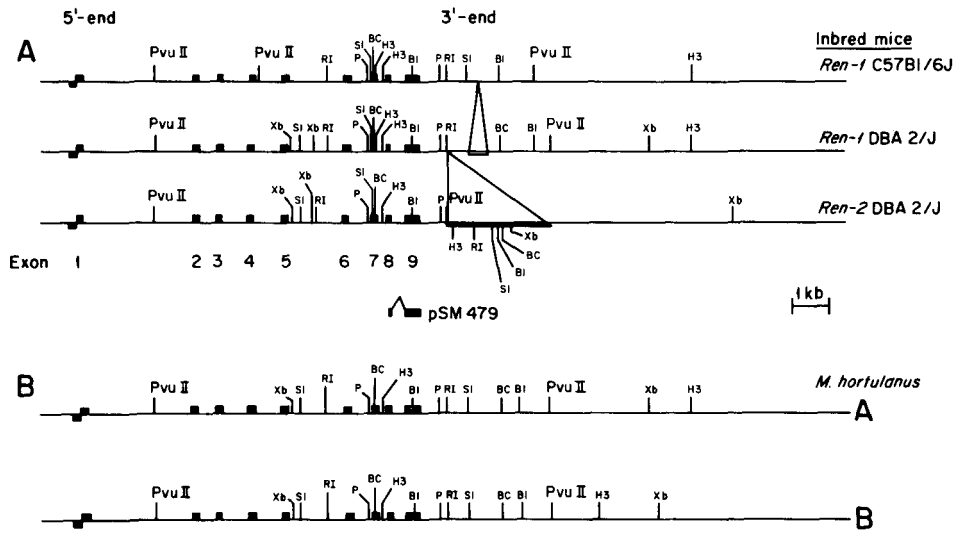

FIGURE 1.—A, Partial restriction site map for the renin genes of C57BL/6J and DBA/2J inbred strains. Restriction sites are derived from Southern analysis of genomic DNA and genomic clones (P. HOWLES and W. PHILBRICK, personal communication). The position of exons (solid blocks above lines) are derived from our own data and those of HOLM *et al.* (1984). The putative promoter region is shown as a solid block below the lines. Open blocks below the line indicate apparent insertions identified by MULLINS *et al.* (*Ren-2*) (1982) and ourselves (*Ren-1*, based on restriction fragment length polymorphism). The *Pvu*II sites that give rise to fragments detected by pSM479 probe are shown in bold face. B, Partial restriction site map for *M. hortulanus*. A similar exon structure is assumed for comparison. Bl, *Bgl*I; BC, *Bcl*I; H3, *Hin*dIII; P, *Pst*I; RI, *Eco*RI; S1, *Sac*I; XB, *Xba*I.

Individual animals were selected to represent either an apparently homogenous stock, or potential variants indicated by renin activity, thermolability or *Pep-3* heterozygosity as discussed before. Southern blot analysis of *Pvu*II-restricted genomic DNA from representative animals are shown in Figure 2. The stocks analyzed clearly divided into two groups, based upon the number and intensity of *Pvu*II bands detected.

Animals that carry one renin gene: One group of animals gave only one *Pvu*II band, equal in intensity to the band observed with C57BL/6J (lane a, Figure 2), consistent with the presence in these animals of a single copy of the renin structural gene. Almost all of these animals produced low amounts of thermostable renin, and they are, therefore, analogous to inbred laboratory strains that carry only *Ren-1*.

The *M. caroli* and *M. cervicolor cervicolor* putative activity variants just described also produced a single *Pvu*II fragment of a similar intensity to this group. This result is consistent with elevated levels of gene expression from a single structural gene producing the increased levels of SMG renin activity seen in these animals, and it rules out the possibility of a gene dosage effect.

Virtually all of the wild-derived single gene animals gave an 11.5-kb *Pvu*II fragment, similar in size to the *Pvu*II fragment derived from the *Ren-1* gene of inbred laboratory strains that carry both *Ren-1* and *Ren-2*. In the case of

one *M. castaneus* mouse (no. 2, Figure 2A, lane g) the 11.5-kb fragment was accompanied by an 8.0-kb band, identical in size with that produced by one-gene inbred strains such as C57BL/6J. This animal was a *Pep-3*$^{a/b}$ heterozygote and produced low amounts of thermostable renin (Table 1). Subsequent breeding experiments demonstrated that the single-copy gene giving the 11.5-kb fragment was linked to *Pep-3*^{b} and the single-copy gene giving the 8.0-kb band to *Pep-3*^{a}. Both genes produced low amounts of thermostable renin (data not shown).

The inbred strain-like combination of *Pep-3*^{a} linked to a single renin gene producing an 8.0-kb *Pvu*II fragment was rare in the stocks we examined and occurred only in *M. castaneus* animals. We did detect this combination in a stock derived from animals trapped in California, but since we could not exclude the possibility of contamination of this stock, we have not included it in our survey.

*Pvu*II restriction of rat genomic DNA gave a single 4.0-kb fragment similar in intensity to the C57BL/6 band (Figure 2B, lane i) suggesting a single copy of the renin gene. It is possible that this result would also be obtained if the rat carried two renin genes with identical *Pvu*II sites that are sufficiently different from the mouse in coding sequence to give a weaker hybridization signal that would mimic a single copy. Therefore, we restricted rat DNA with other enzymes (*Hin*dIII, *Pst*I and *Bgl*II, data not shown). In each case, we obtained a single fragment, consistent with the presence of a single copy of the renin gene.

Animals that carry two renin genes: The second group of animals gave two or more *Pvu*II fragments. All produced high levels of SMG renin activity. Most of them gave two bands identical in size with the bands seen in inbred mouse strains that carry both *Ren-1* and *Ren-2*. With the exception of *M. spretus* (discussed before), SMG renin activity was thermolabile. These animals were, therefore, indistinguishable from two-gene inbred strains. Most animals were homozygous *Pep-3*^{b}, paralleling the distribution of alleles in inbred strains.

Restriction site variants in two-gene mice: M. spretus (Morocco) appears to carry a restriction site polymorphism. Animal 2 (Figure 2A, lane k) gave an 8.5-kb *Pvu*II fragment, presumably from a copy of the *Ren-2* gene. However, instead of an 11.5-kb *Ren-1* fragment, *Pvu*II digestion produced a 15.0-kb fragment. The simplest explanation for this result is a restriction site polymorphism in the *Ren-1* copy of the gene, although without additional restriction site mapping we cannot exclude other possibilities, such as the insertion of an additional 2 kb of DNA between the normal *Ren-1 Pvu*II sites. *M. spretus* (Morocco) no. 1 (Figure 2A, lane j) appears to be an F_1-type animal resulting from the mating of an animal with the 8.5-, 15.0-kb restriction pattern with an animal that gives the normal 11.5-, 8.5-kb two-gene inbred-type pattern. *M. spretus* (Morocco) no. 1 was, therefore, probably homozygous at the *Ren-2* locus and heterozygous at the *Ren-1* locus.

We have used Southern blot analysis to examine five individuals from the colony of *M. hortulanus* (Pancevo). All five animals gave the same restriction fragments and carried a gene duplication, although the restriction pattern

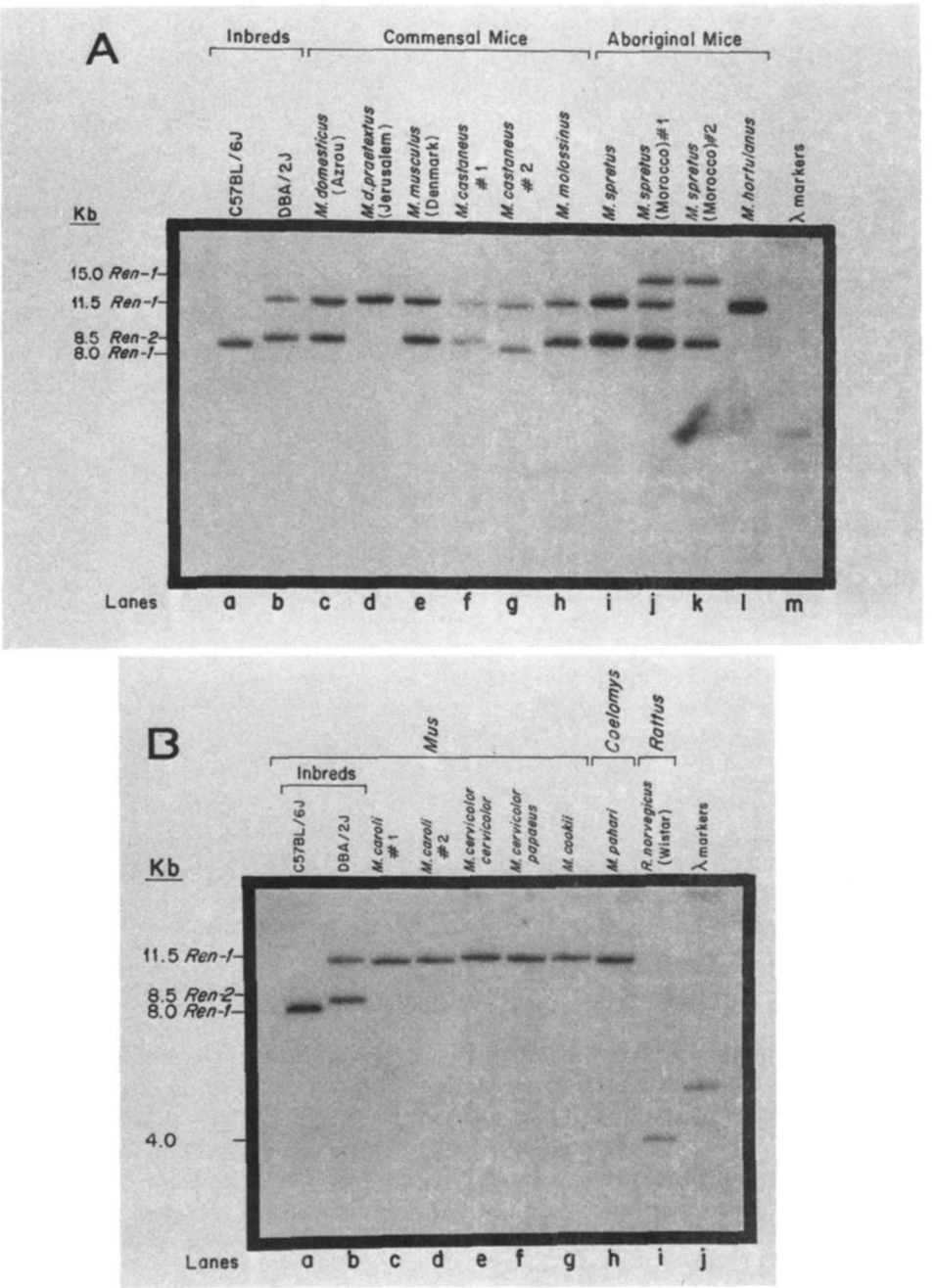

FIGURE 2.—A, Southern blot of *Pvu*II-digested DNA from inbred, commensal and aboriginal mice. C57BL/6J is the type strain for inbred mice that produces low levels of thermostable SMG Renin-1 and carries a single renin gene. DBA/2J is the type strain for inbred mice that carries two renin genes and produces high levels of thermolabile SMG Renin-2. *M.d. praetextus* (Jerusalem) mice (*Pep-3*^{b}, *Ren-1*, *Ren-2*) and *M. musculus* from Skive, Belgrade and Brno (not shown) are all indistinguishable from DBA/2J by *Pvu*II restriction analysis. *M.d. praetextus* (Egypt) mice (*Pep-3*^{b},

differed from that of other two-gene strains. *Pvu*II does not distinguish the two copies and gives a double intensity 11.5-kb band. Detailed restriction analysis of one individual (Figure 1B) demonstrated that *M. hortulanus* appears to carry two copies of the renin gene that are both very similar to the *Ren-1* gene of inbred mice and differ from the *Ren-2* copy of inbred mice in the absence of a 3-kb insert-type fragment at the 3′-end of the *Ren-2* gene.

Phylogenetic relationship of species examined

Our initial survey of the wild-derived stocks of mice, based on SMG renin activity and thermostability, led us to postulate that animals producing low levels of thermostable SMG renin carried a single renin gene and that animals producing relatively high levels of more thermolabile SMG renin carried two genes. The results from Southern blot analysis of genomic DNA clearly support this conclusion. If restriction site polymorphisms are taken into account, the only exceptions to these two classes were found in *M. caroli* and *M. cervicolor cervicolor* stocks. Three animals carried only one renin gene but produced intermediate levels of SMG renin. They may represent gene expression variants.

The phylogenetic relationship between the species we examined is summarized in Figure 3, taken from the work of FERRIS *et al.* (1983) (commensal mice, aboriginal mice, *M. cervicolor*) and CALLAHAN and TODARO (1978) (*M. cervicolor, M. caroli, M. cookii, M. pahari, Rattus norvegicus*). Since different techniques were used by each group to determine relationships, no attempt has been made to depict genetic distances.

Of the species we examined, the presence of two renin genes was only observed in commensal and aboriginal mice. More distantly related members of the *Mus* genus, and the rat, appeared to carry only one renin structural gene.

On the basis of electrophoretic mobility, three alleles of Peptidase-3 have been recognized in laboratory strains of mice, designated *a*, *b* and *c*. In all of the two-gene animals examined thus far, the *Ren* loci are linked to one of these alleles, predominantly to *Pep-3*^{b}. We found no animals in which two renin

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Ren-1) (not shown) are indistinguishable from the *M.d. praetextus* (Jerusalem) mice carrying a single renin gene that we bred for *Pep-3*^{c} homozygosity (lane d). *M. castaneus* no. 1 (lane f) was one of the *Pep-3*^{b} homozygotes listed in Table 1 that produced high levels of thermolabile SMG renin, and *M. castaneus* no. 2 (lane g) was the *Pep-3*^{c}/*Pep-3*^{b} heterozygote that produced low levels of thermostable SMG renin. Five different *M. spretus* (France/Spain) animals gave the restriction pattern shown in lane i. *M. spretus* (Morocco) animals (lanes j and k) are those listed in Table 1. Five different *M. hortulanus* (Pancevo) animals all gave a double intensity 11.5-kb *Pvu*II fragment as shown in lane l. B, Southern blot of *Pvu*II-digested DNA from inbred mice that carry one renin gene (C57BL/6J) or two renin genes (DBA/2J), distantly related wild-derived mice and rat. *M. caroli* no. 1 and no. 2 are the same animals listed in Table 2C. *M. caroli* no. 1 produced 20-fold higher levels of thermostable SMG renin than no. 2 and is a putative *Ren-1* gene expression variant. The *M. cervicolor cervicolor*, *M. cervicolor popaeus*, *M. cookii* and *M. pahari* animals shown were the males obtained from M. POTTER. The females of these species gave identical results (data not shown).

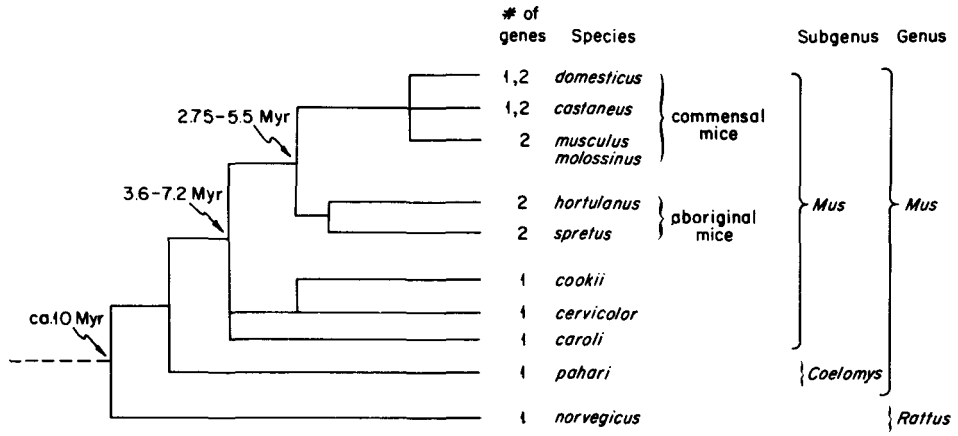

FIGURE 3.—The phylogenetic relationship of species examined in this survey is shown. Data is taken from FERRIS *et al.* (1983) and CALLAHAN and TODARO (1978). The distance between branch points is not intended to represent genetic distance.

genes were linked to a noninbred-type Peptidase-3 allele, such as those found in more distantly related species.

DISCUSSION

Inbred strains of mice carry either one or two copies of the renin structural gene. Strains with one copy produce low levels of a thermostable SMG renin encoded by *Ren-1*. Strains with a second copy, designated *Ren-2*, produce on average 150-fold higher levels of thermolabile SMG renin activity. The results of our survey of wild-derived stocks of mice indicate that, with the possible exception of relatively rare gene expression variants, even distantly related species can be classified in a similar manner.

The phylogenetic distribution of two-gene animals is rather limited. First, we detected two renin genes only in those species closely related to the inbred strains, that is, the commensal and aboriginal mice. More distantly related species, including the rat, all appeared to carry only one gene. Preliminary results from the distantly related genus *Peromyscus*, a member of the Cricetidae family, indicate that *Peromyscus* also produces low levels of SMG renin and carries a single copy of the gene (data not shown). Second, the gene duplication was only found in animals with the *Pep-3* alleles *a*, *b* or *c*.

The simplest explanation for these observations is that a gene duplication arose in the ancestors of the subgenus *Mus*, in a population of animals carrying the inbred-like *Pep-3* alleles, predominantly *Pep-3*^{b}. Since the gene duplication is present in both commensal and aboriginal mice, the duplication event very probably occurred prior to their separation, estimated from mitochondrial DNA restriction site polymorphism at 2.75–5.5 million yr ago (FERRIS *et al.* 1983). The observations that the *Pep-3 a*, *b*, and *c* alleles appear to be confined to the subgenus *Mus*, and the presence of two copies of the renin gene to aboriginal and commensal mice, are consistent with a gene duplication event occurring after the speciation of *M. cervicolor*, estimated at 3.6–7.2 million yr

ago. This estimate is consistent with a value of 3 million yr determined from comparative sequence analysis of the *Ren-1* and *Ren-2* genes of inbred mice (HOLM *et al.* 1984). However, the phylogenetic approach that we used cannot strictly be used to determine the absence of a second renin gene within a species, since we could have fortuitously chosen individuals to examine that only carried one gene, whereas other individuals of the same species might carry two. Therefore, we cannot discount the possibility that the gene duplication occurred earlier than 3.6–7.2 million yr ago, but the duplication was not fixed in the ancestral population.

This approach assumes that only one change at the *Ren* locus, a duplication, led to the existing distribution of genes in different species. However, we cannot completely exclude the existence of an ancestral duplication, followed by a deletion event that was not fixed in the population. In addition, no allowance is made for the possibility of gene conversion. The occurrence of gene conversion at the *Ren* locus during the evolution of the mouse would seriously affect estimates of the time of the postulated gene duplication. Interestingly, none of the species we examined showed evidence of pseudogenes, even when a full length cDNA probe was used in the Southern analysis (data not shown).

The presence of single-copy genotypes in the commensal mice could be due to the gene duplication not being fixed in the population prior to their speciation, resulting in the single-copy genotype persisting through the radiation of commensal species. This would imply the absence of any strong selection pressure for or against the presence of a second copy of the renin structural gene. This appears to be true for animals raised in captivity. Polymorphism at the *Ren-2* locus has persisted in a colony of HA/ICR that has been randomly bred since 1954 (HAUSCHKA and MIRAND 1973). Eight of 16 animals tested produced high levels of Renin-2, and the remainder produced only Renin-1 (results not shown). In addition, two restriction site variants in a *Ren-1*-type gene have persisted in the random-breeding colony of *M. castaneus* we examined that also carries the *Ren-2* gene. Alternatively, single-copy genotypes in the commensal mice could also be due to a deletion not fixed prior to their speciation.

During the course of our survey we detected several potentially interesting variants. The *M. caroli* and *M. cervicolor cervicolor* colonies we examined appeared to carry single copies of a renin gene that is expressed in the SMG at levels substantially above those found in other single-gene strains. These variants may, therefore, carry alterations in the tissue-specific regulation of the renin gene. We are currently breeding animals for this trait for use in future studies.

The colonies of *M. spretus* and *M. hortulanus* that we examined appear to carry renin genes that encode variants in the structural protein. Preliminary results from *in vitro* translation of SMG RNA from both species, followed by two-dimensional gel electrophoresis (O'FARRELL 1975), indicate a pI difference in the predominant renin precursor polypeptide (data not shown). Confirmation of this will require purification and characterization of the renin isozymes.

The close similarity of the two *M. hortulanus* genes to one another, and to

Ren-1 (Figure 1), could be accounted for in three ways. The structure of the *Ren* locus in *M. hortulanus* could be an evolutionary relic, representing the gene duplication prior to subsequent modifications such as, perhaps, the insertion of the 3 kb of DNA at the 3′-end of the *Ren-2* gene. Alternatively, the 3-kb insert could have been present in the original duplication and was subsequently deleted in *M. hortulanus*. Finally, a gene conversion event might have occurred during the evolution of *M. hortulanus*, producing two very similar copies of the gene. The results obtained in this study do not resolve this issue, as properties of SMG renin from *M. hortulanus* cannot be explained simply on the basis of the properties of typical *Ren-1* and *Ren-2* genes found in laboratory strains. Since *M. hortulanus* produces high levels of SMG renin, absence of the 3-kb insert found at the 3′-end of the *Ren-2* gene in inbred strains would appear to preclude any straightforward role for this insert in affecting tissue-specific regulation of the *Ren-2* gene.

These variants represent interesting and potentially valuable candidates for future investigation. In particular, sequence analysis of coding and promoter regions of the genes could help resolve remaining questions about the evolution of the *Ren* locus and may provide insights into the control mechanisms involved in tissue-specific regulation of a gene.

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LITERATURE CITED

BARKA, T., 1980 Biologically active polypeptides in submandibular glands. J. Histochem. Cytochem. **28:** 836–859.

BING, J. and K. POULSEN, 1971 The renin system in mice: effects of removal of kidneys or (and) submaxillary glands in different strains. Acta Pathol. Microbiol. Immunol. Scand. (A) **79:** 134–138.

CALLAHAN, R. and G. J. TODARO, 1978 Four major endogenous retrovirus classes each genetically transmitted in various species of *Mus*. pp. 689–713. In: *Origins of Inbred Mice*, Edited by H. C. MORSE, III. Academic Press, New York.

COHEN, S., J. M. TAYLOR, K. MURAKAMI, A. M. MICHELAKIS and T. INAGAMI, 1972 Isolation and characterization of renin-like enzymes from mouse submaxillary glands. Biochemistry **11:** 4286–4293.

DICKINSON, D. P. and K. GROSS, 1982 *Pep-3* and *Rnr* gene alleles. Mouse News Lett. **67:** 35.

FERRIS, S. D., R. D. SAGE, E. M. PRAGER, U. RITTE and A. C. WILSON, 1983 Mitochondrial DNA evolution in mice. Genetics **105:** 681–721.

GRESIK, E. W., 1980 Postnatal developmental changes in submandibular glands of rats and mice. J. Histochem. Cytochem. **28:** 860–870.

HAUSCHKA, T. S. and E. A. MIRAND, 1973 The "breeder:HA(ICR)" Swiss mouse, a multipurpose stock selected for fecundity. pp. 319–331. In: *Perspectives in Cancer Research and Treatment*, Edited by G. P. MURPHY. Alan R. Liss, New York.

HOATH, S. B., J. LAKSHMANAN, S. M. SCOTT and D. A. FISHER, 1983 Effect of thyroid hormones on epidermal growth factor concentration in neonatal mouse skin. Endocrinology **112:** 308–314.

HOLM, I., R. OLLO, J.-J. PANTHIER and F. ROUGEON, 1984 Evolution of aspartyl proteases by gene duplication: the mouse renin gene is organized in two homologous clusters of four exons. EMBO J. **3:** 557–562.

INAGAMI, T., K. MURAKAMI, H. YOKOSAWA, T. MATOBA, N. YOKOSAWA, Y. TAKII and K. MISONO, 1980 Purification of renin and precursors. pp. 7–30. In: *Enzymatic Release of Vasoactive Peptides*, Edited by F. GROSS and G. VOGEL. Raven Press, New York.

LEWIS, W. H. P. and G. M. TRUSLOVE, 1969 Electrophoretic heterogeneity of mouse erythrocyte peptidases. Biochem. Genet. **3:** 493–498.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK (Editors), 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MARSHALL, J. T. and R. D. SAGE, 1981 Taxonomy of the house mouse. Symp. Zool. Soc. Lond. **47:** 15–25.

MULLINS, J. J., D. W. BURT, J. D. WINDASS, P. MCTURK, H. GEORGE and W. J. BRAMMAR, 1982 Molecular cloning of two distinct renin genes from the DBA/2 mouse. EMBO J **1:** 1461–1466.

O'FARRELL, P. H. 1975 High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:** 4007–4021.

PANTHIER, J.-J., I. HOLM and F. ROUGEON, 1982 The mouse Rn locus: S allele of the renin regulator gene results from a single structural gene duplication. EMBO J. **1:** 1417–1421.

PANTHIER, J.-J. and F. ROUGEON, 1983 Kidney and submaxillary gland renins are encoded by two non-allelic genes in Swiss mice. EMBO J. **2:** 675–678.

PICCINI, N., J. L. KNOPF and K. W. GROSS, 1982 A DNA polymorphism, consistent with gene duplication, correlates with high renin levels in the mouse submaxillary gland. Cell **30:** 205–213.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

STAATS, J., 1980 Standardized nomenclature for inbred strains of mice: seventh listing. Cancer Res. **40:** 2083–2128.

THALER, L., F. BONHOMME and J. BRITTON-DAVIDIAN, 1981 Processes of speciation and semi-speciation in the house mouse. Symp. Zool. Soc. Lond. **47:** 27–41.

WILSON, C. M., M. CHERRY, B. A. TAYLOR and J. D. WILSON, 1981 Genetic and endocrine control of renin activity in the submaxillary gland of the mouse. Biochem. Genet. **19:** 509–523.

WILSON, C. M., E. G. ERDOS, J. F. DUNN and J. D. WILSON, 1977 Genetic control of renin activity in the submaxillary gland of the mouse. Proc. Natl. Acad. Sci. USA **74:** 1185–1189.

WILSON, C. M., E. G. ERDOS, J. D. WILSON and B. A. TAYLOR, 1978 Location on chromosome 1 of *Rnr*, a gene that regulates renin in the submaxillary gland of the mouse. Proc. Natl. Acad. Sci. USA **75:** 5623–5626.

WILSON, C. M., M. J. MYHRE, R. C. REYNOLDS and J. D. WILSON, 1982 Regulation of mouse submaxillary gland renin by thyroxine. Endocrinology **110:** 982–989.

WILSON, C. M. and B. A. TAYLOR, 1982 Genetic regulation of thermostability of mouse submaxillary gland renin. J. Biol. Chem. **257:** 217–223.

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