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Mep-1 gene controlling a kidney metalloendopeptidase is linked to the major histocompatibility complex in mice

(proteinase/proteinase deficiency/*H-2* locus/immunogenetics)

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ABSTRACT **Meprin, a glycoprotein with potent metalloendopeptidase activity, is an integral component of the brush border membrane of mouse kidney. Previously we reported that genealogically related inbred mouse strains (C3H and CBA) are markedly deficient in the activity of this enzyme. We report here that meprin deficiency is inherited as an autosomal recessive trait and that several other inbred strains also express low levels of meprin activity. All of the inbred strains deficient in meprin activity are of the *H-2*^{k} haplotype; however, two strains of this haplotype (C58 and C57BR/cd) expressed normal levels of the proteinase. Congeneic and recombinant mouse strains were examined to determine whether the deficiency was linked to the H-2 complex. The gene controlling the activity of meprin (*Mep-1*) maps on chromosome 17 to the right of the D end of the major histocompatibility complex. The *Mep-1* gene is closely linked to a gene that controls isoenzyme patterns of phosphoglycerate kinase (*Pgk-2*). This work represents the localization of a gene that determines the activity of an integral cellular endopeptidase in mammalian tissues. In addition, the *Mep-1* gene is the only identified gene linked to the major histocompatibility complex that regulates a proteinase activity.**

Previous work showing that a restricted group of genealogically related mouse strains (CBA/J, CBA/CaJ, C3H/J, C3H/HeJ, C3H/HeN) are deficient in meprin (metalloendopeptidase from renal tissue) activity documented the first-discovered heritable deficiency of a mammalian cellular proteinase activity (1–3). The enzyme deficiency (specific activity <2% of that of random bred or other inbred strains) appeared to be restricted to those strains that were derived from the Strong Stock C established from a cross between a D Stock mouse and an A mouse in 1922 (4). The meprin-deficient strains that we originally examined (3) were observed to be of the *k* haplotype (5), indicating that there may be a relationship between the major histocompatibility complex (H-2 region) and the regulation of the expression of meprin. Furthermore, meprin is a glycoprotein associated with the mouse renal brush border (a specialized plasma membrane), and the major histocompatibility complex regulates the expression of several cell-surface glycoprotein molecules, providing additional impetus to examine the genetic relationship between the *H-2* locus and the enzyme activity. Analysis of meprin activity in additional inbred strains, as well as congeneic and recombinant strains, has led to the localization of the gene(s) responsible for regulating meprin activity to the telomeric end of the *D* locus of the H-2 complex.

MATERIALS AND METHODS

Animals. Inbred strains were obtained from The Jackson Laboratory (J) or from Olac Ltd. (Ola; Bicester, Oxfordshire, U.K.); CHI mice were from S. J. Mann (Temple University, Philadelphia). Recombinant and congeneic strains were from the Mayo Medical School (Rochester, MN).

Preparation of Kidney Homogenates. Mice were killed by cervical dislocation. Kidneys were excised, dissected free of fat, decapsulated, and homogenized in 5 vol of water (wt/vol) in a glass tissue grinder fitted with a Teflon pestle. Homogenates were assayed for proteolytic activity within 2 hr; however, there was no evidence of loss of activity in homogenates after 24 hr at 4°C or after freezing samples for a week.

Assay for Proteolytic Activity. Meprin activity was determined by monitoring the digestion of azocasein (10 mg/ml) at pH 9.5, 37°C (1, 3). One unit of activity was equal to the solubilization of ≈1.3 μg of azocasein per min. Protein in homogenates was measured according to the method of Lowry *et al.* (6), using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Reciprocal F_1 hybrids of either sex were produced by crosses between a normal strain (BALB/c Ola) and a meprin-deficient strain (CBA/Ca Ola). All F_1 hybrids were meprin positive (Table 1). These data indicate that the sex of the offspring was without influence on the activity of the metalloendopeptidase and that meprin deficiency is inherited as an autosomal recessive trait. When meprin-positive F_1 hybrids (BALB/c × CBA) were backcrossed to CBA parents (of either sex), 12 of the offspring were meprin positive and 9 were meprin deficient. These data corroborate the proposal that meprin deficiency is an autosomal recessive trait.

Our original studies of meprin activity in nine inbred strains of mice (C57BL/6J, C57BR/cdJ, DBA/1J, A/J, BALB/cJ, CBA/Ca, CBA/J, C3H/HeJ, C3H/HeN) had led us to conclude that meprin deficiency was associated with the Strong Stock C lineage (1). Analysis of another strain derived from the C Stock, CHI, which also is meprin deficient, supported this conclusion (Table 2). However, more extensive analyses of inbred strains yielded several other meprin-deficient lines that could not be related directly to the C Stock lineage. For example, mice of the AKR/J,

Table 1. Inheritance of the *Mep-1* gene

Parents		Sex of	Meprin,	Phenotype
Female	Male	progeny	specific activity	Mep-1
BALB/c	BALB/c	F	2.04 ± 0.50	a
BALB/c	BALB/c	M	3.53 ± 0.40	a
CBA/Ca	CBA/Ca	F	0.07 ± 0.06	b
CBA/Ca	CBA/Ca	M	0.07 ± 0.09	b
CBA/Ca	BALB/c	F	2.33 ± 0.15	a
CBA/Ca	BALB/c	M	2.86 ± 0.24	a
BALB/c	CBA/Ca	F	2.46 ± 0.21	a
BALB/c	CBA/Ca	M	3.13 ± 0.27	a

Meprin specific activity (units per mg of protein) was determined as described (1, 3) and is expressed as the mean ± SD (n = 5) for each group of animals. Mice were purchased from Olac Ltd.

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Table 2. Meprin phenotype in inbred mice: Relationship to H-2 haplotype and genotype at closely linked genes

Strain	*H-2* haplotype	Qa2	Tla	Qa1	Pgk-2	Ce-2	Upg-1	Meprin, specific activity	Mep-1
CBA/J	k	b	b	b	b	b	f	0.09 ± 0.06	b
C3H/HeJ	k	b	b	b	b	b	f	0.05 ± 0.04	b
CHI	k	b	b	b	—	—	—	0.02 ± 0.03	b
AKR/J	k	b	b	b	b	b	f	0.20 ± 0.26	b
CE/J	k	b	b	b	b	b	—	0.16 ± 0.18	b
MaMy/J	k	b	b	b	b	b	s	0.19 ± 0.06	b
RF/J	k	b	b	b	b	b	s	0.03 ± 0.01	b
C58/J	k	—	a	—	a	a	s	4.51 ± 0.54	a
C57BR/cdJ	k	—	a	—	a	a	s	6.46 ± 0.23	a
DBA/1J	q	a	b	b	a	b	s	3.28 ± 0.29	a
DBA/2 Ola	d	a	c	b	a	a	f	2.86 ± 0.11	a
A/Ola	a	a	a	a	a	a	f	3.02 ± 0.43	a
Au/SsJ	q	a	b	b	a	b	s	3.66 ± 0.20	a
C57BL/10 Ola	b	—	b	—	a	a	—	3.54 ± 0.23	a
C57L/J	b	a	b	b	c	a	s	2.84 ± 0.17	a
129/J	b	a	c	b	c	b	s	2.67 ± 0.08	a
LP/J	bc	—	c	—	c	b	—	3.18 ± 0.09	a

Values for meprin specific activity (units per mg of protein) are presented as mean ± SD (n = 4). Phenotypic expressions at defined loci are derived from published data (5, 7–10); a dash indicates that the phenotype is not published. All mice were male.

CE/J, MaMy/J, and RF/J strains were also markedly deficient in meprin activity.

One common feature of all the meprin-deficient strains was that they were all of the *H-2*^{k} haplotype (Table 2). In contrast, most of the meprin-positive strains were not *k* haplotype. There were two strains, however, that did not fit these generalizations: C58/J and C57BR/cdJ mice were of the *H-2*^{k} haplotype but had normal levels of meprin activity. The latter two strains differed from the meprin-deficient strains in the phenotype of several genes located to the right of the H-2 complex (Tla, Pgk-2, Ce-2).

To investigate the possibility that the gene controlling meprin activity (*Mep-1*) is linked to the major histocompatibility complex, we extended our analyses to include several congeneic and recombinant strains that map this region of the genome (Table 3). Three sets of animals indicated a relationship between *Mep-1* and the H-2 complex. The strains (BALB/c, BALB.K; C57BL/10, B10.K; C57BL/6, B6-H-2^k) differ only at the major histocompatibility complex and closely linked loci. BALB/c, C57BL/10, and C57BL/6 expressed high activities of meprin, while their congeneic partners, BALB.K, B10.K, and B6-H-2^k all possessed low levels of this enzymic activity. In all three pairs, the congeneic line has been derived from a meprin-sufficient background and a meprin-deficient donor. Furthermore, the congeneic lines have all been produced by the substitution of a segment of chromosome 17 that includes the major histocompatibility complex. The background/donor strains for the three pairs are BALB/c/C3H, B10/CBA, and B6/AKR (5). Thus, the alteration of the genome has in each case resulted in the presentation of *k* haplotype, albeit from different origins, and the expression of meprin deficiency. These data further indicated that the segregation of the animals into high-meprin and low-meprin strains is controlled by a gene(s) close to or within the major histocompatibility complex region of chromosome 17. More precise positioning of the gene required the use of recombinant strains. Two such strains, C3H.OH and C3H.OL, were derived from parental strains C3H (meprin deficient, *Mep-1*^{b}) and DBA/2 (meprin sufficient, *Mep-1*^{a}). In both of these recombinants, the D end of the H-2 complex is derived from the C3H parent and the K end is derived from the DBA/2 parent. A third recombinant, C3H.A, differs in that the D end is derived from an *H-2*^{d} haplotype donor and the K end is derived from a donor of *H-2*^{k} haplotype. Table 3 shows that two of the recombinants, C3H.OH and C3H.OL, together with the donor strain C3H

Table 3. Distribution of meprin phenotype in inbred, congeneic, and recombinant mouse strains: Relationship to genes in the major histocompatibility complex and closely linked genes

Strain	*H-2* haplotype	K	A	E	S	D	Qa2	Tla	Pgk-2	Ce-2	Upg-1	Meprin, specific activity	Mep-1
BALB/c Ola	d	d	d	d	d	d	a	c	a	a	f	3.53 ± 0.40	a
BALB.K/Ola	k	k	k	k	k	k	b	b	b	—	f	0.26 ± 0.08	b
C57BL/10J	b	b	b	b	b	b	a	b	a	a	s	3.54 ± 0.23	a
B10.K/SgDV	k	k	k	k	k	k	b	b	—	—	—	0.03 ± 0.02	b
C57BL/6J	b	b	b	b	b	b	a	b	a	a	s	6.35 ± 0.16	a
B6-H-2^k/BeDv	k	k	k	k	k	k	b	b	b	—	f	0.03 ± 0.02	b
B6-AK1/F1Dv	oz1	b	b	b	b	k	b	b	b	—	f	0.35 ± 0.04	b
C3H/HeJ	k	k	k	k	k	k	b	b	b	b	f	0.05 ± 0.04	b
DBA/2J	d	d	d	d	d	d	a	c	a	a	f	2.86 ± 0.06	a
C3H.A/HaDv	a	k	k	k	d	d	a	a	a	—	f	3.44 ± 0.15	a
C3H.OH/SfDv	o2	d	d	d	d	k	b	b	b	b	—	0.09 ± 0.07	b
C3H.OL/SfDv	o1	d	d	d	k	k	b	b	—	b	—	0.01 ± 0.01	b
B10.BR	k	k	k	k	k	k	b	a	a	—	s	2.76 ± 0.29	a

Meprin activities and phenotypic expressions were determined as in Tables 1 and 2.

express low levels of meprin. Furthermore, DBA/2 and C3H.A mice are meprin positive. Collectively, these data demonstrate that a gene that is responsible for the expression of meprin activity (*Mep-1*) is located to the right of H-2S. Confirmation of this suggestion is provided by the recombinants B6-H-2^k and B6-AK1, which are both *Mep-1*^{b} and phenotypically identical at established loci between *H-2D* and *Upg-1*, the gene that codes for electrophoretic variation in urinary pepsinogen (7).

The *Mep-1* locus does not correlate with the *Upg-1* locus or the *Ce-2* locus. This is evident, for example, from the observation that C3H (*Mep-1*^{b}) and DBA/2 (*Mep-1*^{a}) mice are phenotypically identical at the *Upg-1* locus. Furthermore, DBA/1 is *Ce-2*^{b} and DBA/2 is *Ce-2*^{a}, while both of these strains are *Mep-1*^{a}. From the analyses of inbred strains, *Mep-1*^{a} appeared to segregate with *Pgk-2*^{a} or *Pgk-2*^{c} and *Qa2*^{a} and *Mep-1*^{b} segregated with *Pgk-2*^{b} and *Qa2*^{b}. However, B10.BR mice have the $Qa2^b$ phenotype and are *Mep-1*^{a} (Table 3). Also, analysis of B6-K1 (*Qa2*^{b}, *Pgk-2*^{b}) and B6-K2 (*Qa2*^{a}, *Pgk-2*^{b}) revealed that both are *Mep-1*^{b} (5). Thus, the Mep-1 phenotype correlates best with the Pgk-2 phenotype.

It is as yet unknown whether the structural gene for meprin is synonymous with the *Mep-1* gene. The alternative, that *Mep-1* is a regulatory gene, is an attractive proposition that has precedents. For example, it has been shown that the H-2 complex is closely linked to a gene that codes for a neuraminidase (11, 12). Strains that are deficient in this hydrolase exhibit excessive sialylation of a number of lysosomal hydrolases (acid phosphatase, aryl sulfatase B, β-glucosidase, and α-mannosidase) that can result in altered enzymatic activity (13). However, the strain distribution of the neuraminidase deficiency and the distribution of the Mep-1 phenotype do not correlate. It is not possible, therefore, to attribute differences in Mep-1^a and Mep-1^b phenotypes to altered sialylation elicited by this *Neu-1* gene. Altered glycosylation is probably responsible for the biochemical polymorphism coded at locus *Ce-2* (8). In this example, post-translational modification of kidney catalase elicits an altered isoenzyme distribution. Mep-1 phenotype and Ce-2 phenotype do not correlate, however, and therefore it is unlikely that the same modifications are responsible for polymorphism in meprin and catalase.

The Mep-1 phenotype correlates strongly with the Pgk-2 phenotype. The latter gene regulates phosphoglycerate kinase isoenzymes in mammalian testis and spermatozoa (9). The Pgk-2 isoenzyme variants are all monomers with a molecular weight of ≈47,000 but they have different electrophoretic mobilities. The Pgk-2c variant is a minor electrophoretic variant superimposed on Pgk-2a; however, the Pgk-2c isoenzyme has very low enzymatic activity (2%) compared to the other isoenzymes (14). It has been suggested that this form (Pgk-2c) may result from a structural gene mutation that affects the active site of this variant (15, 16). The relationship between *Mep-1* and *Pgk-2* genes and the molecular nature of the alterations found in their protein products warrants further investigation.

Several genes that code for enzymes or isoenzyme variants have been linked to the major histocompatibility complex (e.g., see refs. 17 and 18). At present, it is unclear whether this linkage is purely fortuitous or whether the enzymes are related functionally to the immune response. For example, the *Neu-1* gene may modulate the glycosylation of cell surface antigens. It is possible that the *Mep-1* gene functions in an analogous fashion by regulating proteolytic modification of similar antigens. It may be relevant that, while most mammalian metalloproteinases have a high degree of substrate specificity, meprin has little specificity and will hydrolyze small peptides as well as large proteins (3). The molecules encoded by the major histocompatibility complex genes play a critical role in processes such as the rejection of organ transplants and the control of the immune response. It is possible that the molecules encoded by the genes closely linked to the complex are also operative in these physiological processes and subject to regulation as the major histocompatibility complex gene products. There is a great deal of interest in the evolution, characterization, and polymorphism of the *H-2* linked loci (19, 20), and meprin represents an additional genetic marker available to study the structure, organization, and function of this important area of the genome.

The physiological role(s) of renal brush border peptide peptidohydrolases has not been established. The kidney is a major site of degradation of many circulating polypeptides and, for those that pass into the glomerular filtrate, this degradation may take place in the proximal tubule where the brush border is localized. The brush border peptidohydrolases are active in the inactivation and degradation of a number of peptide hormones such as angiotensin, bradykinin, and glucagon (21, 22). Furthermore, partial hydrolysis of other polypeptides in the tubular lumen may precede or enhance their uptake into the tubular cells where intralysosomal degradation can occur (23). The mouse strains that are deficient in meprin activity provide a unique experimental model to study the role of one renal brush border endopeptidase. The close linkage between the major histocompatibility complex and the *Mep-1* gene will facilitate the development of congeneic strains that are genetically distinguishable only at *Mep-1*. Finally, elucidation of the event that leads to meprin deficiency may add valuable information on membrane biogenesis and post-translational processing of membrane proteins.

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