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Genetic and molecular mapping of the *Hmt* region of mouse

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We have mapped a new region of the mouse major histocompatibility complex (MHC) that contains the nuclear gene, *Hmt*, for the maternally transmitted antigen, Mta. The *Hmt* region of chromosome 17 lies between a recombinational breakpoint distal to *Tla* and another proximal to *Tpx-1*, thus including *Pgk-2*. A novel MHC class I gene fragment, *R4B2*, was cloned and mapped to this region as was another new class I gene, *Thy19.4*. Both lie proximal to *Pgk-2*, within the distal inversion in *t*-haplotypes. The presence of several other MHC class I genes in the *Hmt* region is predicted from analysis of the recombinants that define the region.

Key words: genome organization/maternally transmitted antigen (Mta)/MHC class I genes/mouse chromosome 17/ *t*-complex

Introduction

Mta, the maternally transmitted antigen of the mouse, is an unusual transplantation antigen (Chan and Fischer Lindahl, 1985): it shows maternal inheritance, and is detected by cytotoxic T lymphocytes (CTL) that are not H-2 restricted (Fischer Lindahl *et al.*, 1980). Since CTL remains the only reagent available for the characterization of Mta, the antigen has proved difficult to isolate. We have chosen to analyze its components by molecular methods after they were defined by classical genetics.

Three genes are required for the expression of Mta: *Mtf*, the maternally transmitted factor (Fischer Lindahl and Bürki, 1982; Fischer Lindahl and Hausmann, 1983); *Hmt*, a chromosomal gene closely linked to *H-2* (Fischer Lindahl *et al.*, 1983); and *B2m*, the gene encoding β_2 microglobulin (β2m) (Fischer Lindahl and Langhorne, 1981; Fischer Lindahl *et al.*, 1988). The epitopes of Mta are determined jointly by *Hmt* and *Mtf* (Fischer Lindahl *et al.*, 1986). Our working hypothesis is that *Hmt* encodes a class I MHC molecule which, associated with β2m, binds the small peptide encoded by *Mtf*, and presents it on the cell surface to the cytotoxic T cells (Fischer Lindahl *et al.*, 1983). *Hmt* is considered a class I gene because Mta-specific killer T cells are not H-2 restricted; an active *B2m* gene is required for Mta expression; and *Hmt* maps at the end of a string of class I genes on chromosome 17 distal to *H-2* (Fischer Lindahl *et al.*, 1983).

Mtf has long been thought to be a mitochondrial gene (Ferris *et al.*, 1983; Smith *et al.*, 1983; Huston *et al.*, 1985; Hirama and Fischer Lindahl, 1985). By sequencing three mitochondrial genomes, representing allelic forms of *Mtf*, we could identify a candidate for the gene (H.Yonekawa, E.Hermel and K.Fischer Lindahl, in preparation; Wang *et al.*, to be published). We have recently shown that a peptide of 17 amino acids, when added to cells, can recreate the epitopes normally determined by *Mtf* (B.E.Loveland, C.-R.Wang, E.Hermel, H.Yonekawa and K.Fischer Lindahl, in preparation).

Our strategy for the isolation of *Hmt* has been first to define by genetic recombination, and as narrowly as possible, the region encompassing *Hmt*, then find DNA probes from this region to be used as starting points for chromosome walking and clone any new class I gene located in this region. Candidates for *Hmt* can be tested by transfection into a cell line that carries a null allele of *Hmt*: an active *Hmt* gene is expected to make this cell line susceptible to cytotoxic T cells specific for Mta. Here we report success in all three stages of the strategy.

Results

Castaneus recombinants

Hmt was first mapped by CTL typing for Mta of F2 hybrids produced in a cross of strains which differed at *Hmt*: C3H/HeJ (C3H) and *Mus musculus castaneus* (Fischer Lindahl *et al.*, 1983). C3H is $H\text{-}2^k$, $Qa\text{-}2^b$, Tla^b, $Qa\text{-}1^b$, Hmt^a, and the *M.m.castaneus* parent, CAS3, is $H\text{-}2^{c3}$, $Qa\text{-}2^a$, Tla^{c3}, $Qa\text{-}1^{c3}$, Hmt^b. Three recombinants, R1, R4-e, and R4-1, between the CAS3 and C3H haplotypes have been particularly informative for the mapping of *Hmt* (Figure 1).

The crossover in R1 occurred between *Qa-2* and *Tla*. The CAS3 parent donated the proximal end of chromosome 17 including *H-2* and *Qa-2*, and the C3H parent, *Tla*, *Hmt*, and the more distal regions. The R1 haplotype has since been backcrossed onto C57BL/10SnJ (B10), and, after 10 generations of backcrossing, it is now established in a congenic strain.

In the original, 'early' R4 (R4-e) recombinant, the *H-2* through *Tla* region of chromosome 17 was of the C3H parent, while the CAS3 parent donated the *Hmt* and more distal regions. During four subsequent backcrosses to C3H.SW ($H\text{-}2^b$, Tla^e, Hmt^a), a second recombination occurred between *Hmt* and *Tpx-1* with the C3H.SW parent

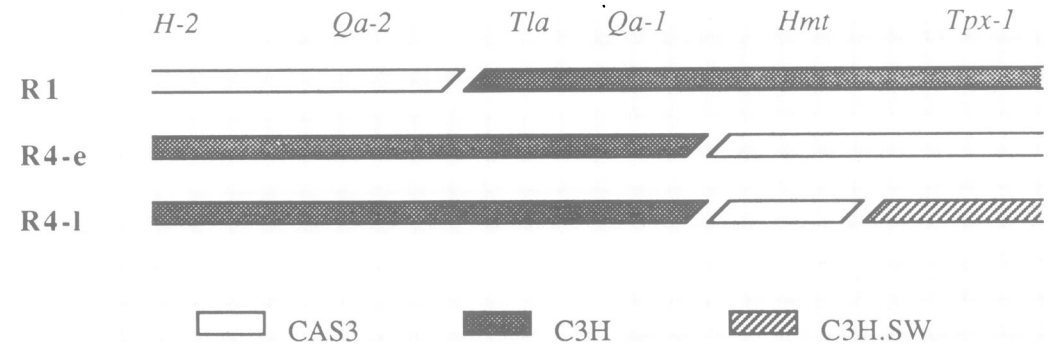

Fig. 1. The major histocompatibility complex in the *castaneus* recombinants. The origin of the *H-2*, *Qa-2*, and *Tla* genes as determined by serotyping, *Hmt* and *Qa-1* by CTL assay and *Tpx-1* by RFLP analysis.

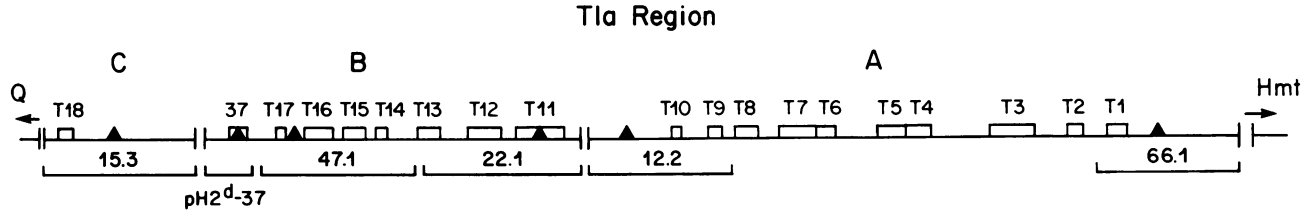

Fig. 2. The *Tla* region in the BALB/c mouse. A, B and C are clusters of genes represented in contiguous cosmid clones (Steinmetz *et al.*, 1982; Fisher *et al.*, 1985; Transy *et al.*, 1987). In this figure, the orientation and location of A is based on data from Passmore and Romano (1988), B is oriented the same way to reflect the extensive homologies with A that presumably arose by gene duplication and C could have been located anywhere. *Tla* lies between the proximal *Qa* (Q) and the distal *Hmt* regions. The genes of the *Tla* region are indicated by boxes. The cosmid clones used in this study are shown below the genes, and the probes made from them are indicated by black triangles.

donating the region distal to *Hmt* to the 'late' R4 (R4-l) haplotype (Figure 1). The R4-l haplotype was subsequently backcrossed to both C3H.SW (mice designated CSW.R4-l) and to C57BL/6J (B6) (mice designated B6.R4-l).

Mapping Hmt with Tla region probes

The *Tla* region encompasses at least 19 genes in BALB/c (Tla^c) (Figure 2) and 15 genes in B6 and B10 (Tla^b) (Brown *et al.*, 1988). It was therefore possible that *Hmt* mapped within the *Tla* region but distal to the gene encoding the TL antigen in CAS3 (corresponding to gene $T13^c$). The organization and orientation of the *Tla* region is not completely established. At least three gene clusters (A, B and C), separated by gaps of unknown length, have been reported. Figure 2 represents one construct on current data.

To map *Hmt* with reference to the *Tla* region, we used DNA probes that hybridize to the ends of each *Tla* cluster as shown in Figure 2. The *T17* probe also hybridizes to *T9* (S.W.Hunt, K.A.Brorson, H.Cheroutre, A.Matsuura, F.-W. Shen and L.Hood, in preparation) as seen by doublet bands in Table I. The gene *37* probe cross-hybridizes with *T10* (Transy *et al.*, 1987). The sizes (or the deletion) of restriction fragments detected by these probes show that R1 and R4-e share the C3H, rather than CAS3, equivalents of the BALB/c genes *T1, T9, T10, T11, T17* and *T18* and *37* (Table I). If the orientation and order is unchanged for those class I genes common to *Tla* haplotypes, *Hmt* must be distal to all known class I genes within the *Tla* region of BALB/c. This result is consistent with our failure to render a B10.CAS2 (Hmt^b) cell line Mta positive by transfection with any cosmid from the BALB/c *Tla* region (K.Fischer Lindahl and M.Steinmetz, unpublished).

The distal limit of the Hmt region

Among (C3H × CAS3)F2 progeny we found several recombinants between *Hmt* and *Upg-1* (Fischer Lindahl

Table I. Mapping Hmt by *Tla* region probes

Probe:		T18	37	T17	T11	T10	T1
Enzyme:		*Hin*dIII	*Bgl*I	*Bam*HI	*Hin*dIII	*Bam*HI	*Bam*HI
Strain	*Tla*	Estimated fragment size (kb)					
BALB/c	*c*	3.8	14.2, 3.0	4.5, 4.2	3.4	9.8, 4.3	10.5
CAS3	*c3*	1.9, 1.6	8.8, 6.6	6.2, 5.1	3.4	7.3, 6.9	d
C3H	*b*	3.8	14.2, 7.6	4.5, 3.8	d	d	10.5
R1	*b*	3.8	14.2, 7.6	4.5, 3.8	d	d	10.5
R4-e	*b*	3.8	14.2, 7.6	4.5, 3.8	d	d	10.5

d = deleted.

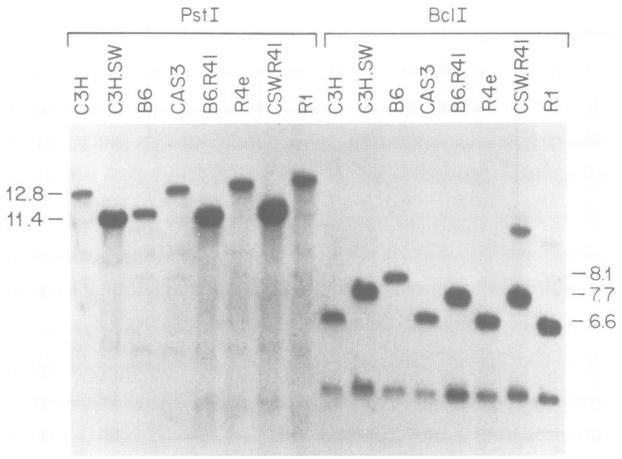

Fig. 3. Demonstration of the 'late' R4 recombination. The origin of the R1 and R4 haplotypes is described in the text. Liver DNA from the strains indicated was digested with *Pst*I or *Bcl*I and hybridized to probe 1-1-1H4 (Kasahara *et al.*, 1987). The highest mol. wt band in CSW.R4-1 DNA cut with *Bcl*I represents a partial digestion.

et al., unpublished), showing that *Hmt* lies between *H-2D* and *Upg-1*. However, this range is too wide as *Upg-1* lies ≃6 cM from *H-2D* (Szymura and Klein, 1981). But the 1-1-1H4 probe for the *Tpx-1* gene, which maps between *Pgk-2* and *Mep-1*, proximal to *Ce-2* (Kasahara *et al.*, 1989), revealed a recombinant that enabled us to define the *Hmt* region more precisely.

As shown in Figure 3, 1-1-1H4 hybridizes with a fragment of the same size in DNA from the CAS3 parent and from R4 of the first intercross generation (R4-e); in samples from the later intercrosses (R4-l from CSW.R4-1 and B6.R4-1), the 1-1-1H4 reactive fragment is the same size as that of the C3H.SW backcross parent. As R4-e and R4-l mice are both Hmt^b, a second crossover must have occurred during the backcrossing of R4 to C3H.SW, separating *Hmt* from *Tpx-1* (Figure 1). Table II shows that the *Pgk-2* gene in both R4-e and R4-l is derived from CAS3. Therefore, this second crossover occurred between *Pgk-2* and *Tpx-1*. *Hmt* can thus be mapped to the stretch between the recombinational breakpoints in R4-e (distal to *Tla*) and R4-l (proximal to *Tpx-1*), and this stretch includes *Pgk-2*.

Table II. Size (in kb) of restriction enzyme fragments detected by *Pgk-2* probe

Strain	*Tla*	*Hmt*	*Tpx-1*	*Bgl*II	*Kpn*I	*Bam*HI
C3H	C3H	C3H	C3H	7.2	6.0	10.9
R4-e	C3H |	CAS	CAS	10.9	10.9	18.8
CAS3	CAS	CAS	CAS	10.9	10.9	18.8
R4-1	C3H	CAS |	SW	10.9	10.9	18.8
C3H.SW	SW	SW	SW	7.2	6.0	10.9

| marks position of recombinational breakpoint.

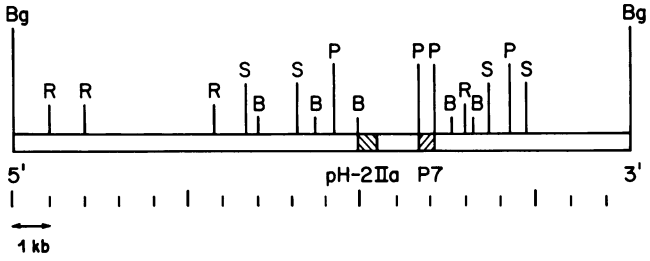

Fig. 4. Restriction map of *R4B2*. The recognition sites for *Eco*RI (R), *Sac*I (S), *Pst*I (P), *Bam*HI (B) and *Bgl*II (Bg) are shown. The fragments that hybridize to the exon 4 probe (pH-2IIa) and to the single copy probe (P7.500) are cross-hatched. The orientation of the gene was deduced from the sequence of the exon 4 fragment. *Cla*I, *Hin*dIII, *Kpn*I, *Not*I, *Sac*II, *Sal*I and *Xho*I did not cut the clone.

Cloning of new class I genes within Hmt

A new class I gene, *Thy19.4*, was recently discovered in a BALB/c thymus cDNA library screened with a probe for exon 4 of MHC class I genes. Using the R4 recombinants, we could map *Thy19.4* to the *Hmt* region (Brorson *et al.*, 1989). The gene has been sequenced, and it closely resembles a class I gene. It has five exons, consensus splice sites, a promoter region and poly(A) addition signals, as required of a functional gene, and spliced mRNA can be detected at very low levels in the thymus.

A more systematic approach is to use an exon 4 probe to search for restriction fragment length polymorphisms (RFLPs) that can be mapped to the *Hmt* region. In addition to the known 30–40 class I genes, the pH-2IIa probe detects several bands that were present in C3H (Hmt^a) and absent in R4 and CAS3 (Hmt^b), or vice versa (see Figure 2 in Fischer Lindahl *et al.*, 1988). These bands can be enriched and cloned in a band library to be screened with the same probe.

R4B2, a 17 kb *Bgl*II fragment from R4-l, was the first locus to be cloned and restriction mapped (Figure 4). The sequence hybridizing to the exon 4 probe is located in the middle of the fragment, making it likely that the clone contains a complete class I gene, as they range from 3.4 to 7 kb, with an average of 5 kb. The putative exon 4 was sequenced and compared to several class I genes (Figure 5).

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          1                    *                                  50
R4B2      DPPKAHVAHH PRPKGDVTLR CWALGFYPAD ITLTWQKDEE DLTQDMELVE
Thy19.4   E----Y-T-- ---E------ --------S- -IMI--R-G- -Q----DVI-
Mbl       -T--I-MT-K I--DRKT--- ---FN---PE ------R-GS NQ-----MI-
T13c      ----T--T-- A--E------ ---------H ------LNG- E-I--T----
37        -------T-- --SEDE---- ---------- ------LNG- E---------
Q7d       -------T-- --SY-A---- ---------- ------LNG- E----T----
pH-2II    -------T-- R--E------ ---------- ------LNG- E---------
Ld        -S-----T-- --S--E---- ---------- ------LNG- E---------

                                                          Amino acid
          51                   *                   91     homology
R4B2      TRPSGDGTFQ KWAAVVVPSG EEQRYTCYVH HEGLTEPLAL KW     100%
Thy19.4   ---A------ --V------- K--N---H-A ----P---L- R-      75%
Mbl       ---------- -------ST- --HI---H-N ----S--ITI R-      65%
T13c      ---A------ ---------- ---K---H-Y ----P---T- R-      80%
37        ---A------ -T------L- K--Y---H-Y ----P---T- R-      79%
Q7d       ---A-V---- ---S----L- K--N---H-N ----P---T- R-      78%
pH-2II    ---A------ ---S----L- K--N---R-Y ----P---T- R-      81%
Ld        ---A------ ---S----L- K--N---R-Y ----P---T- R-      80%
```

Fig. 5. Comparison of the exon 4 sequence of *R4B2* to that of other class I genes. The conserved cysteines are marked (*), and dashes indicate identities with *R4B2*. The sequences were taken from: *Thy19.4*, Brorson *et al.* (1989); *Mbl*, Singer *et al.* (1988); *T13*^{c}, Fisher *et al.* (1985); *37*, Transy *et al.* (1987); *Q7*^{d}, Steinmetz *et al.* (1981b), pH-2II, Steinmetz *et al.* (1981a) and *L*^{d}, Moore *et al.*, (1982).

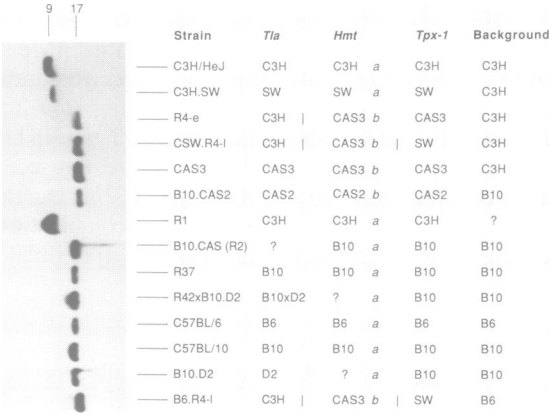

Fig. 6. Mapping of probe P7.500 to the *Hmt* region. Liver DNA from the strains indicated was digested with *Bgl*II and the Southern blot was probed with P7.500, the probe for the 3′ end of R4B2. Fragment sizes are given in kb.

Table III. Size (in kb) of restriction enzyme fragments of class I genes in the *Hmt* region of strains C3H (*a*) and R4 (*b*)

Probes	*Pst*I		*Bgl*II		*Hin*dIII		*Eco*RI		*Kpn*I	
	C3H	R4	C3H	R4	C3H	R4	C3H	R4	C3H	R4
Multi copy										
pH-2IIa (exon 4)	9*	7	?	17*	?	?	5	7*	?	6
Single copy										
P7.500 (R4B2 3′ end)	ns	ns	9	17*	23	23	8	7*	23	ns
Thy19.4 3′ end	9*	3*	ns	ns	13*	9*	3	3	9*	9*
Thy19.4 5′ end	3	3*	1	1	13*	9*	1	1	9*	9*

? = fragment not identifiable.
ns = no signal (small fragments run off the gel).
*In each column, pairs of fragments known (*Pst*I 9 kb and *Bgl*II 17 kb) or presumed to be identical are marked.

R4B2 is more similar at the amino acid level (79−81%) to exon 4 of genes from the *H-2*, *Qa-2* and *Tla* region than to exon 4 of *Thy19.4* (75%), the other class I gene in the *Hmt* region, and *Mb1* (65%), a newly described class I gene distal to *Qa-2* (Singer *et al.*, 1988). The two cysteines at positions 21 and 77 are conserved as expected of a functional class I gene. The exon is bounded 5′ by AG and 3′ by GT, suggesting functional splice sites. There is no glycosylation site in exon 4 of *R4B2*.

A single-copy probe, P7.500, was subcloned from *R4B2* as a 500 bp *Pst*I fragment. It is downstream of the gene (Figure 4), and its sequence shows no similarity to any class I gene. This region appears conserved with limited restriction site polymorphism, but using *Bgl*II we could confirm the mapping of P7.500 and *R4B2* to the *Hmt* region (Figure 6). As expected of an *Hmt* gene, the R4-e and R4-l recombinants have the 17 kb fragment of the CAS3 parent, while R1 has the 9 kb fragment of the C3H parent.

More class I genes from the *Hmt* region are currently being cloned using the RFLP approach. By comparing the fragment sizes detected by pH-2IIa and by P7.500 or the Thy19.4 5′-end and 3′-end probes, we find at least one additional class I gene in both Hmt^a (*Eco*RI 5 kb from C3H) and Hmt^b strains (*Pst*I 7 kb from R4) (Table III).

Table IV. Mapping *Thy19.4* and *R4B2* by Passmore's recombinants

Strain	*Tla*		*Hmt*		
	12.21	pTLA.5	*R4B2* and *Thy19.4*	*Pgk-2*	*Ce-2*
MA.R1	D2	D2	D2	MA	MA
MA.R2	D2	D2	D2	D2	MA
MA.R5	MA	MA	MA	MA	D2
B10.DR1	D2	D2	D2	D2	K

Vertical lines indicate crossover points.

Chromosomal origins (D2 = B10.D2, MA = MA/MyJ, K = B10.K) were taken from Passmore and Romano (1988) or determined in this study for *R4B2* and *Thy19.4*. *R4B2*: *Bgl*II RFLP detected by P7.500, where D2 = 11.2 kb, MA and K = 8.4 kb. *Thy19.4*: *Hin*dIII RFLP detected by Thy19.4 5′-end probe, where D2 = 6.6 kb, MA and K = 11.2 kb.

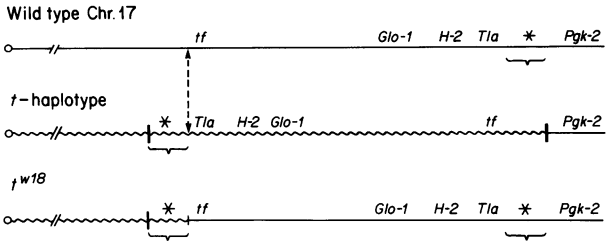

Fig. 7. Schematic comparison of wild-type and *t* complex chromosomes. Straight and wavy lines indicate wild-type and *t* haplotype chromosomes respectively, and circles a centromere. The breakpoints of the distal inversion in the *t* haplotypes are represented by vertical bars. The asterisk refers to the loci *D17Leh89*, *D17Leh467* and *D17Leh525*. The brackets mark the region duplicated as a result of the crossover (indicated by dashed arrows).

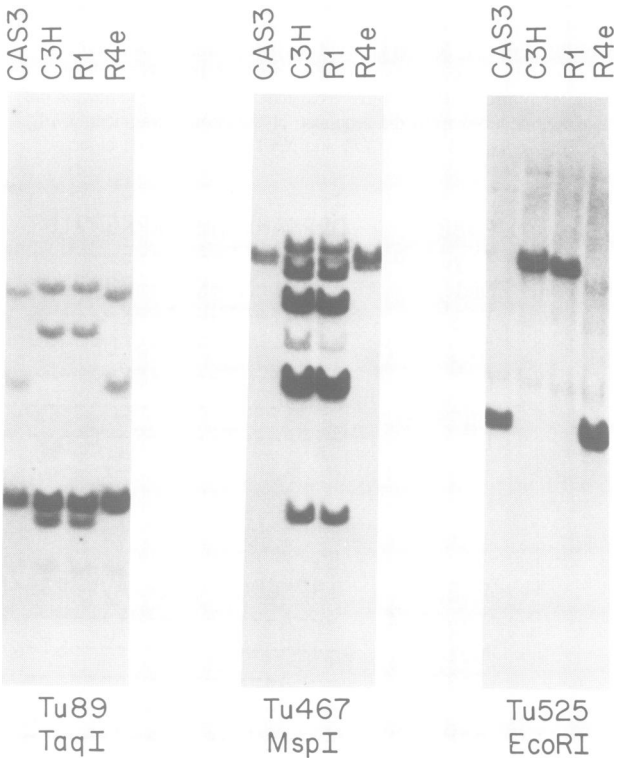

Fig. 8. Mapping of the loci *D17Leh89*, *D17Leh467* and *D17Leh525* to the *Hmt* region. Liver DNA from the six strains was digested with the enzymes shown, and Southern blots were hybridized with the probes listed.

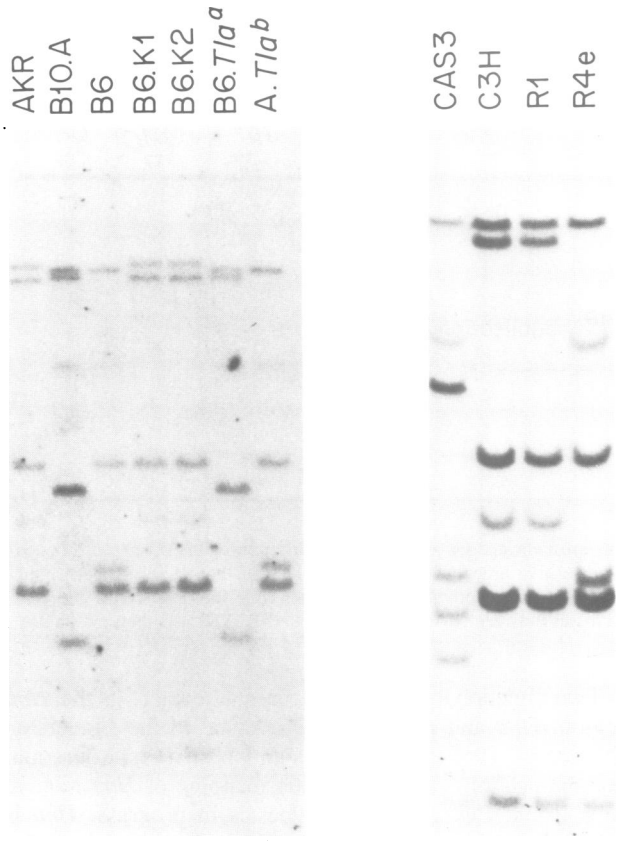

Fig. 9. Probe Tu108 detects DNA fragments on both sides of the R4-e crossover. Liver DNA from the strains indicated was digested with *Taq*I and the Southern blots were probed with Tu108.

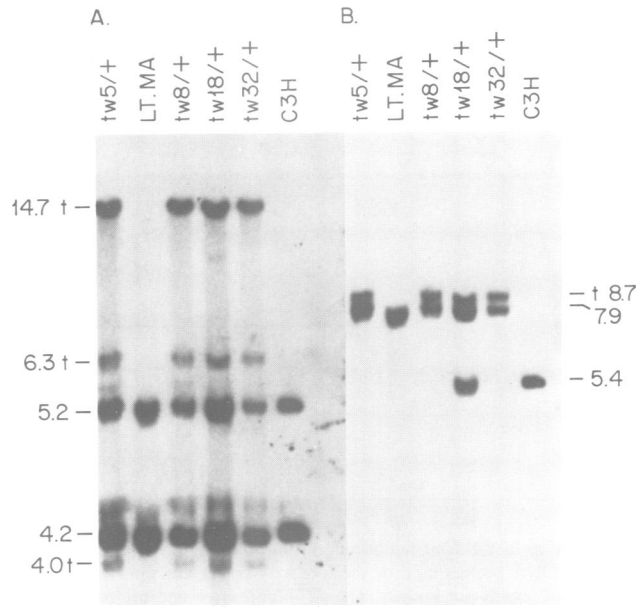

Fig. 10. Mapping of *R4B2* and *Thy19.4* within the distal *t* inversion. DNA was prepared from homozygous, wild-type strains C3H/HeJ (C3H) and LT.MA-*Glo-1*^{b} (LT.MA) and from heterozygous mice with the *t* haplotypes *t*^{w5}, *t*^{w8}, *t*^{w18} or *t*^{w32} and the LT.MA-*Glo-1*^{b} wild-type (+) chromosome 17. *Stu*I digests were probed with P7.500 (**A**) and the Thy19.4 5′ end probe (**B**). Specific *t* (t) and wild-type bands and their size in kb are indicated.

Thy19.4 and R4B2 are proximal to Pgk-2

Passmore has produced a set of recombinants in the *Tla* to *Ce-2* interval between strains B10.D2 and MA/MyJ or B10.K (Passmore and Romano, 1988). As the parental strains are all *Hmt*^{a}, *Hmt* could not be mapped with these recombinants, but RFLPs have allowed us to map *Thy19.4* and *R4B2* (Table IV).

The recombinant MA.R1 was particularly informative. Its *H-2* through *Tla* regions are derived from B10.D2, MA donating *Pgk-2* and the distal part of chromosome 17. Since the probes for *Thy19.4* and *R4B2* revealed fragments of the B10.D2 size, these genes can be placed proximal to *Pgk-2*. The other recombinants are consistent with this interpretation.

The Hmt region includes part of the t-complex

Among the characteristics of *t* haplotypes of chromosome 17 are two large inversions, the distal one including *tf* and *H-2:Tla* (Figure 7), and the proximal one including *T* and *qk*. *Pgk-2* is believed to lie outside the inverted region (Nadeau, 1983; J.-Y.Tsai and L.Silver, personal communication). Rare crossovers between wild-type and *t* chromosomes produce partial *t* haplotypes. If the recombination occurs within an inverted region, as shown in Figure 7 for *t*^{w18}, it will result in a duplication of one end of the inverted region and a deletion of the other end.

Several DNA clones isolated from the proximal portion of mouse chromosome 17 by microdissection and microcloning (Röhme *et al.*, 1984) detect loci that are duplicated in *t*^{w18} with both wild-type and *t* haplotype alleles present (Bucan *et al.*, 1987). Thus, the loci *D17Leh89, D17Leh467,* and *D17Leh525* could be mapped to the end of the distal inversion between *Tla* (not duplicated) and the inversion breakpoint. These same probes detect bands that are shared by R4-e and CAS3 on the one hand and by R1 and C3H on the other, mapping them distal to *Tla* (Figure 8). Accordingly, the end of the distal inversion in *t* haplotypes lies in the *Hmt* region, and that part of the *Hmt* region is duplicated in *t*^{w18}.

Another probe, Tu108, detects several bands, all of which can be mapped distal to the *Qa-2* region and the recombinational breakpoint in strain B6.K2 (Figure 9). B6.K1 and B6.K2 display the AKR pattern, B6.*Tla*^{a} and B10.A have a different one, and A.*Tla*^{b} and C57BL/6 yet another. Since the polymorphic bands belong to the differential segment in the *Tla* congenic strains, they must be proximal to *Upg-1*, but could be distal to *Pgk-2* (Klein *et al.*, 1982). The loci detected by Tu108 are not included in the *t*^{w18} duplication (M.Bucan and H.Lehrach, unpublished).

When used to probe DNA from the CAS3 recombinants, Tu108 identifies the R1 bands with C3H, not CAS3, confirming their location distal to *Qa-2* (Figure 9). R4-e gives a composite pattern: it shares two polymorphic bands with C3H and two with CAS3, and lacks two C3H and three CAS3 bands. Similar results were obtained with *Eco*RI, *Msp*I and *Bam*HI. We conclude that some of the bands detected by Tu108 are located proximal to the R4-e crossover, in the *Tla* region, and others are distal, possibly inside the *Hmt* region.

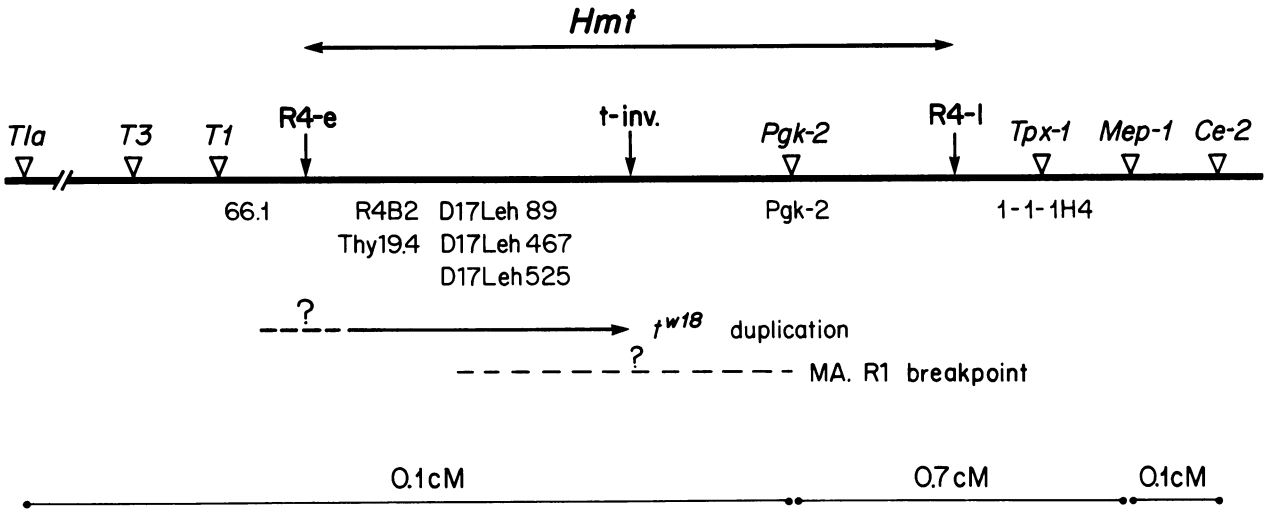

Fig. 11. Map of the *Hmt* region on chromosome 17. Recombinational breakpoints are indicated by arrows and the location of known genes by open triangles. DNA probes used or the loci they detect are shown below the map. The relative order within the *Hmt* region of the new class I genes and the *D17Leh* loci is not known. The t^{w18} duplication and the possible position of the MA.R1 recombinational breakpoint are indicated below the probes they cover. The genetic distances from Reckelhoff *et al.* (1988) refer to crosses between MA/MyJ, B10.D2 and B10.K, and they may not hold generally (Steinmetz *et al.*, 1986).

Mapping R4B2 and Thy19.4 in the t complex

With the aim of mapping *Thy19.4* and *R4B2* in recombinants between the *t* haplotypes t^{w5}, t^{12} and t^{w12}, we used the Thy19.4 5'-end and P7.500 probes to search for polymorphisms but found none with more than 24 restriction enzymes. Such strong conservation of these genes suggests that they are located within the *t* complex.

If *Thy19.4* or *R4B2* lies within the part of the *t* inversion that is duplicated in t^{w18}, then the appropriate probe should detect both a *t*-specific and a wild-type band. If no *t* band were detected in t^{w18}, then the locus must lie outside the duplication. Figure 10 shows a *Stu*I digest of DNA from mice representing four *t* haplotypes (all heterozygous with LT.MA-*Glo-1*^{b}) and two wild-types (homozygous) probed with P7.500 for *R4B2* and the 5'-end probe for *Thy19.4*. Both probes detect bands peculiar to the *t* haplotypes, and these bands are indeed found in t^{w18}. The t^{w18} lane in panel B also displays two wild-type bands, one from the LT.MA chromosome and one from the wild-type parent of the recombinant chromosome. The same result was obtained with the enzymes *Kpn*I and *Eco*RV. Microdensitometry of the wild-type bands, with the *t* specific band as standard, confirmed that *Thy19.4* and *R4B2* are duplicated in t^{w18}. *Thy19.4* and *R4B2* can therefore be mapped to the very end of the distal inversion in the *t* haplotypes, between the breakpoint and the crossover in t^{w18}.

Discussion

The Hmt region

A map of the *Hmt* region is shown in Figure 11. We relied on two types of landmarks: recombinational breakpoints and DNA probes (or functional genes). The recombinational breakpoints R4-e and R4-I define the *Hmt* region, and it includes the *t* inversion breakpoint. We do not yet know the location of the t^{w18} crossover relative to the proximal boundary. The MA.R1 recombinational breakpoint is also located in the *Hmt* region between *Pgk-2* and the class I genes *R4B2* and *Thy19.4*.

Two functional genes have been allocated to the *Hmt* region, *Hmt* and *Pgk-2*. *Pgk-2* is distal to the *t* inversion (Nadeau, 1983; J.-Y.Tsai and L.Silver, personal communication), and the genetic mapping of *Hmt* relative to *H-2* and *Pgk-2* in *t* haplotypes is in progress. *Hmt* is generally highly conserved, and only by screening *Mus spretus* and various subspecies of *Mus musculus* did we find new alleles (Fischer Lindahl *et al.*, 1983; Fischer Lindahl *et al.*, 1986). However, testing of a few *t* haplotypes revealed several (new?) alleles (K.Fischer Lindahl and H.Winking, to be published). Given the overall conservation of *t* markers, this polymorphism was unexpected. It could be accounted for by *Hmt* being very close to the inversion breakpoint, perhaps outside the *t* complex, and the region rendered genetically unstable by the inversion.

Several DNA probes hybridize inside the *Hmt* region, most of them to the proximal part included in the *t* complex. *D17Leh89*, *D17Leh67* and *D17Leh525* are located on separable *Sfi*I DNA fragments of ~200 kb (Bucan and Lehrach, unpublished); their relation to each other and to the new class I genes *Thy19.4* and *R4B2* remains to be determined. The Tu108 probe sees fragments on both sides of the R4-e breakpoint, some of which are clearly in the *Tla* region.

Our first marker distal to the *Hmt* region is the 1-1-1H4 probe for the *Tpx-1* gene, which in turn is proximal to *Mep-1*, *Ce-2*, and *Upg-1*. The genetic distance from *Tla* to *Mep-1* has been estimated as ≃0.7 cM (Passmore and Romano, 1988; Reckelhoff *et al.*, 1988), which is then an upper limit for the length of the *Hmt* region. The DNA probes now available will allow us to estimate the molecular distance by pulsed-field gel electrophoresis.

The Tla region

Using molecular probes derived from BALB/c (*Tla*^{c}), we have mapped the *Hmt* region distal to the *Tla* region in a C3H:CAS3 recombinant. The molecular map of the *Tla* region of C3H and CAS3 is completely unknown, and our results did show that some of the genes found in BALB/c

are deleted in C3H or CAS3. Since C3H is TL negative (Tla^b), one could argue that it should be mapped relative to B10, which is also Tla^b. Therefore, BALB/c probes which also detect regions of homology in B10 were used.

A recent comparison of the Tla^c (BALB/c) and Tla^b (B10/B6) regions revealed both gene duplications and deletions. Despite extensive reorganization, these haplotypes share the following homologous regions: $T3^c-T7^c$ and $T5^b-T8^b$; $T9^{b/c}-T10^{b/c}$; $T14^c-T17^c$ and $T7^b-T10^b$ (Brown *et al.*, 1988). The genes $T11^b-T15^b$ (>35 kb) have no counterpart in BALB/c. As probes for these genes were not available, we were unable to determine whether the *Hmt* region included $T11^b-T15^b$. Since *Hmt* is distal to all the *Tla* probes tested, we conclude that it must be distal to all genes in that region of BALB/c, whatever its orientation.

New class I genes in the Hmt region

Several class I genes, found in genomic and cDNA libraries, were missed in the original screening of BALB/c (Steinmetz *et al.*, 1982) and B10 (Weiss *et al.*, 1984) cosmid libraries. Some have lower homology to other class I genes (*Mb1*) and may have been missed because the screening stringency was too high. Others may have been overlooked because the DNA is not easily cloned into cosmids (*Thy19.4*). Genes *37* and $T11^b-T15^b$ have been mapped to the *Tla* region, proximal of *Hmt*; *Mb1* is known to be distal to *Qa-2*, but it remains to be mapped with respect to the *Hmt* region.

We have mapped *Thy19.4*, a complete class I gene (Brorson *et al.*, 1989), and *R4B2*, which may be a full-length class I gene, to the *Hmt* region. Based on comparison of their exon 4 sequences, both of these genes show greater similarity to expressed class I genes of the *H-2*, *Qa* and *Tla* region than to *Mb1*. They were mapped proximal of *Pgk-2*, both by their inclusion in the distal *t* inversion and by Passmore's recombinant MA.R1.

We are continuing the cloning of class I genes from the *Hmt* region using the RFLP approach. The Hmt^a candidates include an *Eco*RI 5 kb fragment, which differs in size from both *Thy19.4* and *R4B2* (Table III). The Hmt^b candidates include the *Pst*I 7 kb and *Kpn*I 6 kb fragments.

Could any of the new class I genes be Hmt?

Mta, and hence *Hmt*, is expressed on all nucleated cells tested (including fibroblasts and lymphoid, myeloid, epithelial and endothelial cells) (Rodgers *et al.*, 1986). *Mb1* is unlikely to be *Hmt*, because there is no evidence of expression at the RNA level. The same argument rules out *Thy19.4*, which is expressed only at extremely low levels, predominantly in the thymus. Furthermore, transfection with the *Thy19.4* genomic clone from an Hmt^a strain failed to cause expression of Mta on an Hmt^b cell line (Loveland *et al.*, unpublished).

Since the epitopes of Mta are highly conserved among inbred strains, the gene must be present in all Hmt^a strains; yet *Mb1* is missing in Hmt^a mice derived from the $H\text{-}2^k$ haplotype. Similarly, the $T11^b-T15^b$ genes can be ruled out because they have no parallel in Hmt^a BALB/c mice. Gene *37*, with its wide tissue distribution and high degree of conservation (Lalanne *et al.*, 1985; Transy *et al.*, 1987), seemed a promising candidate for *Hmt*, but it clearly lies in the *Tla* rather than the *Hmt* region.

Our probes for *R4B2* and *Thy19.4* react with all strains we have tested, including Hmt^b. Since Hmt^b is immunologically a null allele, we cannot test *R4B2* or any other class I gene cloned from Hmt^b mice by transfection followed by a killer assay for a new Mta antigen. Rather, we must first use a probe from the Hmt^b gene to clone the Hmt^a homolog. An Hmt^a counterpart of *R4B2*, which we cloned from C3H as a 9 kb *Bgl*II fragment binding P7.500 and exon 4 probes, has turned out to contain only the 3′ end of a class I gene. Neither could we obtain a full-length clone when screening an A/J genomic library with P7.500. The genetic divergence of the *a* and *b* alleles, which allowed us to detect *Hmt* in the first place, is reflected in the difference in their restriction maps, and makes it more difficult to clone corresponding *Hmt* region genes.

The Hmt gene

Finally, the case for our working hypothesis that *Hmt* is an MHC class I gene. Although *Hmt* has not yet been located, we have shown that there are indeed several new class I genes in the *Hmt* region. Hmt^b mice do not express a detectable Mta antigen, yet the number of class I genes found in the *Hmt* region of *b* does not differ from that of *a* mice. It is quite possible that the Hmt^b gene product is only immunologically silent.

Immunization of the CSW.R4-l mice with C3H gives rise to two kinds of H-2 unrestricted CTL (B.E.Loveland and K.Fischer Lindahl, to be published). One kind reacts only with Hmt^a:Mtf^α mice, as do standard anti-Mta CTL raised in combinations that differ for the mitochondrial factor *Mtf*. But the second kind of CTL reacts with all Hmt^a mice, irrespective of their *Mtf* type, and thus appears to recognize Hmt as an alloantigen, as one would expect for an MHC class I antigen.

A short peptide that mimics the *Mtf* product and a single amino acid difference that accounts for the allelic forms of *Mtf* (Loveland *et al.*, in preparation) strongly support our view of *Hmt* as a restriction element that presents a peptidic ligand to the CTL. At the same time , this finding rules out several more fanciful models of Mta (Rodgers *et al.*, 1986; Han *et al.*, 1987). It remains to be seen whether Mtf is the only ligand for Hmt, and why Hmt rather than H-2K or H-2D presents Mtf.

Materials and methods

Mice

Standard inbred strains. Mice were bred at the Basel Institute for Immunology or they were purchased from the Jackson Laboratory (Bar Harbor, ME). The particular sublines used were BALB/cJ, B10.D2/nSnJ, C3H/HeJ, C3H.SW/SnJ, C57BL/6J and C57BL/10SnJ.

Castaneus haplotypes. These mice were all bred in the colony of KFL at the Basel Institute for Immunology or at the University of Texas Southwestern Medical School.

Passmore's recombinants. Genomic DNA from the strains MA/MyJ, B10.K, MA.R1, MA.R2, MA.R5, and B10.DR1 was a gift from H.C.Passmore (Rutgers University, Piscataway, NJ).

t haplotypes. Genomic DNA from strain LT.MA-*Glo-1*^{b} and its F1 hybrids with *t* haplotypes t^{w5}, t^{w8}, t^{w18}, and t^{w32} was a gift from J.H. Nadeau (Jackson Laboratory, Bar Harbor, ME). DNA from mice with *t* haplotypes t^{w5}, t^{12}, and t^{w12} was a gift from K.Arzt (Department of Zoology, University of Texas, Austin, TX).

Southern blot analysis

Mouse liver DNA was extracted using the methods of Blin and Stafford (1976). The DNA was digested for 4–16 h with various restriction enzymes (IBI), and 10 μg of the digested DNA loaded per lane on a 0.7% agarose gel, to be electrophoresed at 60 V for 17 h in TBE buffer (Maniatis *et al.*, 1982). The gel was then placed in 1.5 M NaCl, 0.5 M NaOH for 1 h. The transfer to Hybond membrane (Amersham) was done overnight, using the same buffer. After neutralization in 50 mM sodium phosphate buffer, pH 6.5, for 2 min, the membrane was baked for 2 h in a vacuum oven at 80°C. Prehybridization in 0.5 M sodium phosphate buffer, pH 7.2, 7% SDS, 1 mM EDTA, 0.1 mg/ml salmon sperm DNA at 65°C for 4 h was followed by hybridization with ^{32}P-labeled probes (10^9 c.p.m./μg) overnight in the same solution. Restriction fragments used as probes were isolated and labeled with [α-^{32}P]CTP (3000–4000 Ci/mmol, NEN) by the method of Feinberg & Vogelstein (1984). Following hybridization the filter was washed for 5 min at room temperature and for 2 × 40 min at 65°C in 40 mM sodium phosphate buffer, pH 7.2, 1% SDS. The filter was then exposed for 12–36 h to KODAK X-OMAT XAR 5 film, with two intensifying screens at −80°C.

In Table I the protocol of Steinmetz *et al.* (1986) was followed, except that the hybridization solution contained 1 M NaCl and 1.0% SDS and no mouse genomic DNA. For Figures 8 and 9, the protocol of Bucan *et al.* (1987) was followed.

Molecular probes

1-1-1H4. This probe, a gift from M.Kasahara and J.Klein (Department of Microbiology, University of Florida, Miami, FL), is a 950 bp *Hin*dIII fragment isolated from cosmid clone 1-1-1, derived from mouse chromosome 17 (Kasahara *et al.*, 1987), that contains the *Tpx-1* gene and maps between *Pgk-2* and *Mep-1* (Kasahara *et al.*, 1989).

Pgk-2. Probe pcAB12EH1.4, a gift from M.McBurney (Departments of Medicine and Biology, University of Ottawa, Ottawa, Ontario), is a 1.4 kb fragment just downstream of the *Pgk-2* gene, which was cloned from BALB/c (*Pgk-2*^{a}) testis cDNA (Boer *et al.*, 1987).

Tu89, Tu108, Tu467 and Tu525. These are 1.7, 0.4, 1.7 and 2.0 kb *Eco*RI clones derived by microdissection of chromosome 17 (Röhme *et al.*, 1984).

pH-2IIa. A 440 bp *Sac*I–*Hha*I fragment from a cDNA clone which detects exon 4 of all known class I genes (Steinmetz *et al.*, 1981a).

T1–T18. Single or low copy probes derived from cosmids of the BALB/c (*Tla*^{c}) mouse (Winoto *et al.*, 1983), were provided by K.Minard, A.Winoto, and L.Hood (Division of Biology, California Institute of Technology, Pasadena, CA). (i) *T1*: 2 kb *Kpn*I–*Sma*I fragment of cosmid 66.1 cut out with *Eco*RI, *Hin*dIII and subcloned in M13mp8; (ii) *T10*: 2.2 kb *Eco*RI fragment of cosmid 12.2 cut out with *Bam*HI and subcloned in M13mp8; (iii) *T11*: 2 kb *Bam*HI fragment of cosmid 22.1 cut out with *Bam*HI and subcloned with M13mp8; (iv) *T17*: 2.6 kb *Xho*–*Hpa*I fragment of cosmid 47.1 cut out with *Eco*RI, *Hin*dIII and subcloned in M13mp8; v) *T18*: 3 kb *Xho*I fragment of cosmid 15.3.

37. A 250 bp *Pst*I fragment from clone pH-2^d-37, isolated from a DBA/2 (*H-2*^{d}) liver cDNA library (Lalanne *et al.*, 1985), came from J.L.Lalanne (Institut Pasteur, Paris, France).

Thy19.4 3′ end. A 500 bp *Eco*RI–*Bgl*II fragment subcloned from a BALB/c thymus cDNA clone (Hunt *et al.*, in preparation). The probe was used to clone a complete and new class I gene as a 7.2 kb *Hin*dIII fragment from a BALB/c genomic band library, and it detects the 3′ untranslated region of this gene (Brorson *et al.*, 1989).

Thy19.4 5′ end. A 1.2 kb *Eco*RI fragment subcloned from the Thy19.4 genomic clone described above. This single-copy probe detects exons 1, 2 and 3 of the gene.

P7.500. A 500 bp *Pst*I subclone, located downstream of the *R4B2* gene (see below), acts as a single-copy probe in genomic blots.

Cloning of R4B2

Genomic DNA (500 μg) from strain CSW.R4-l was digested with *Bgl*II and separated on a 10–25% sucrose gradient (Maniatis *et al.*, 1982). The fraction containing the desired size range was cloned in the EMBL3 vector (Stratagene). The band library was screened using pH-2IIa and washed in 2 × SSC at 65°C to detect the class I gene, R4B2. The insert was cloned into the *Sal*I site of Bluescribe Vector (Strategene) for restriction mapping.

Two fragments were sequenced by the dideoxy method (Sanger *et al.*, 1977). The sequences were analyzed and compared to known class I genes with programs from the University of Wisconsin Genetics Computer Group.

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