Organization and evolution of the class I gene family in the major histocompatibility complex of the C57BL/10 mouse

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The major histocompatibility complex (MHC) encodes several classes of protein vital to the regulation of the immune response. We have isolated 26 class I genes that map to this region in the C57BL/10 mouse and linked these into three gene clusters. The number of genes differs from the number found in the BALB/c strain and comparison of the organization of the class I genes in these two strains shows conserved regions and polymorphic regions which probably result from deletions, insertions and translocations within the MHC.

CLASS I proteins of the murine major histocompatibility complex (MHC; *H-2*) consist of the classical transplantation antigens (K, D and L) found on virtually all cells (Fig. 1*a*) and appear to be involved in the presentation of foreign antigens, such as viral glycoproteins, to cytotoxic T lymphocytes. Class II antigens are believed to be involved in the presentation of foreign antigens to helper T lymphocytes and are found on the surface of a limited number of cell types including certain lymphocytes and macrophages[1].

Adjacent to the loci encoding these molecules on chromosome 17 is the *Tla* region which contains genes encoding the Qa and TL lymphoid differentiation antigens. These antigens, like the class I antigens, are integral membrane proteins with molecular weights of 40–45,000 and are expressed on the cell surface in association with β_2-microglobulin[2]. In addition, the genes encoding these antigens in both the *H-2* and *Tla* complexes are homologous; therefore these *Tla* complex genes are also referred to as class I genes.

One of the most intriguing aspects of the H-2 class I antigens is their extreme polymorphism. More than 50 alleles at both the *H-2K* and *H-2D* loci have been observed (for a review see ref. 1). *H-2* haplotypes have been defined serologically and consist of a particular set of alleles found at each *H-2* locus, for example the C57BL/10 (B10) mouse is $H\text{-}2^b$ at each *H-2* locus, thus expressing the $H\text{-}2K^b$ and $H\text{-}2D^b$ class I alleles. Many inbred mouse strains of different *H-2* haplotypes exist and a variety of recombinant lines have been generated from these strains, thereby providing a convenient system in which to study the evolution and function of this gene family.

Steinmetz *et al.*[3] identified 36 class I genes in the BALB/c mouse ($H\text{-}2^d$), which they grouped into 13 clusters. Winoto *et al.*[4] reported the location of these clusters within the MHC and corrected this gene number to 35. A comparison of the MHC of the B10 and BALB/c mice at the DNA level may help to explain the differential expression of certain class I genes (for example, *H-2L* and *TL*) in these mice.

Isolation of the class I genes

A total of 144 clones containing class I gene sequences was isolated from six cosmid libraries. We have arranged overlapping cosmids into three clusters containing a total of 26 different class I genes. One of these cosmid clusters spans the *H-2D* and *Qa2,3* regions; the other two clusters map to the *H-2K* and *TL* regions, respectively. The 5′ to 3′ orientations of the genes described here were determined by hybridization to the probes shown in Fig. 1*b*.

The gene clusters have been localized within the MHC using single or low-copy number probes from cosmids within each cluster. Each probe was hybridized to spleen DNA of B10 ($H\text{-}2^b$), DBA/2 ($H\text{-}2^a$) or AKR ($H\text{-}2^k$) mice that had been digested with a variety of restriction enzymes and Southern[5]-blotted. Probes that detected differences in the size of DNA restriction fragments among the inbred stains were then used to localize the polymorphic restriction fragment on the MHC map. This was done using congeneic strains that have undergone recombination in the MHC. Serological studies have previously identified the haplotype that donated the DNA at each locus in these strains. Thus, an appropriate panel of recombinant strains allows the correlation of a particular haplotype of DNA at a given locus with the appearance of a particular polymorphic restriction fragment, thereby mapping the probe, and hence the gene cluster from which the probe was isolated, to that region.

We have used a nomenclature for the class I genes which reflects the genetic location of the gene cluster and the position of a gene within the cluster. Where a gene product has been identified, we use the established nomenclature. Thus, the two genes in the *H-2K* region are termed *K1* and $H\text{-}2K^b$; the 10 genes in the *Qa2,3* region are termed *Q1* to *Q10*, and the 13 genes in the *TL* region are termed *T1* to *T13*.

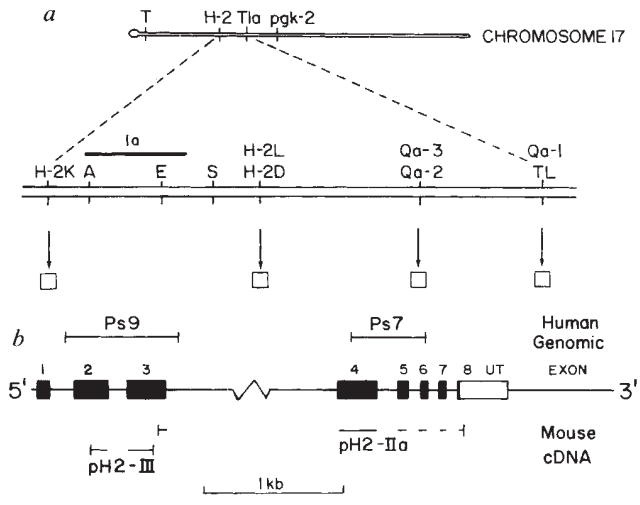

Fig. 1 *a*, Genetic map of the murine MHC. The centromere of chromosome 17 is located to the left. Class I molecules (□) are expressed from at least four separate loci as shown by the arrows below the line. The Ia region encodes the class II antigens. The S region encodes components of the complement system. *b*, Intron–exon organization of an MHC class I gene as described elsewhere[18]. Exons are shown as thick boxes (numbered 1–8). UT refers to the 3′ untranslated region. Relative positions of human genomic[21] and mouse cDNA[22] clones used in the initial stages of this study are shown.

Table 1 Summary of the mapping of restriction fragments

Probe:	5′ Flanking *K* region						3′ Flanking *K* region									D^b gene mapping probe	*Q1* gene mapping probe	*TL* mapping probe
Restriction enzyme:	*Hin*dIII						*Bgl*II									*Bgl*II	*Hin*dIII	*Pst*I
Size (kb):	6.6	4.4	3.4	3.0	2.7	2.4	9.0	8.5	6.0	4.8	3.5	2.3	2.0	1.8	1.5	2.75	4.7	2.0
Gene:	*K1*		K^b	*Q6, Q8 Q10*	*Q7 Q9*	*Q5*	*Q2*	D^b	*T1*	*Q5, Q7 Q9*	*Q1*	K^b	*Q6, Q8 Q10, T7*	*K1*	*T5*	D^b	*Q1*	*T1*
Strain																		
B10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A	−	+	−	+	−	+	+	+	+	+	+	−	+	−	'+	−(2.95)	+	+
DBA/2	−	+	−	+	−	+	+	+	+	+	+	−	+	−	+	−(2.95)	+	+
AKR	−	+	−	+	−	−	−	−	+	−	+	−	+	−	+	−(2.95)	−(3.3)	−(3.7)
B6.K1	+	+	+	+	−	−	−	+	+	−	+	+	+	+	+	+	−(3.3)	−(3.7)
B6.K2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	−(3.7)
D2.R107	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−(2.95)	+	+
D2.GD	−	+	−	+	+	+	+	+	+	+	+	−	+	−	+		+	+
B10.A(5R)	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+			+
B10.A(2R)	−	+	−	+	+	+	+	+	+	+	+	−	+	−	+			+
A.AL							+	+	+	+	+	−	+	−	+			
C3H.OH							−	−	+	−	+	−	+	−	+			
B10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Location	*H–2K*		*H–2K*			*Qa2,3*	*Qa2,3*	*H–2D*		*Qa2,3*		*H–2K*		*H–2K*		*H–2D*	*Qa2,3*	*TL*

The 5′ flanking *K*-region probe is the 0.6-kb *Bam*HI fragment 2.6-kb 5′ to the $H–2K^b$ gene (Fig. 2*a*). The 3′ flanking *K*-region probe is the 1.8-kb *Bgl*II fragment of H24 as indicated in Fig. 2*a*. As a D^b gene mapping probe, we isolated a 2.75-kb *Bgl*II fragment shown in Fig. 2*b*. The *Q1* mapping probe is a 4-kb *Pvu*II fragment, derived from the 7.2-kb *Kpn*I fragment of B1.24 indicated in Fig. 2*b*. To localize gene *T1* we used a 2.0-kb *Eco*RI fragment of H11 shown in Fig. 2*c*. + Indicates that the mapping probe detects a fragment of the same size in the B10 DNA and in the DNA of the respective haplotype; − indicates the absence of a band of the same size. If a B10 is absent and its corresponding fragment in another haplotype is known, its size is given in parentheses.

The *H–2K* region

This region is defined by 13 cosmid clones (five of which are shown in Fig. 2*a*) spanning 95 kilobases (kb) and contains two class I genes. The two genes are spaced about 15 kb apart and are arranged in a head-to-tail configuration with no other class I genes for at least 30 kb on either side.

We have established the genetic location of this cluster by showing that one of the genes in this region is the $H–2K^b$ gene, both by DNA sequence analysis[6] and by showing that it directs the expression of $H–2K^b$ antigens in L-cells transformed by cosmids or subclones containing this gene[7]. This result has been confirmed by polymorphic restriction site mapping using a 1.8-kb *Bgl*II fragment (the 3′ flanking *K*-region probe) isolated from cosmid H24 which contains exons 6 to 8 and 3′ flanking sequences of gene *K1* (Figs 2*a*, 3*a*, Table 1). When used as a hybridization probe on genomic blots of B10 DNA digested with *Bgl*II, this fragment hybridizes strongly with nine fragments (Fig. 3*a*, Table 1). As shown by the hybridization patterns in Fig. 3*a* and the genetic maps of the recombinant inbred strains in Fig. 3*e*, this fragment maps to the *H–2K* side of the *I–E* locus (see, for example, B10A.5R). Of the remaining eight DNA fragments detected, one flanks the $H–2K^b$ gene, one flanks the $H–2D^b$ gene and the remainder are found flanking class I genes in the *Tla* complex.

The *H–2D* and *Qa2,3* region

This region is defined by 67 cosmid clones (21 of which are shown in Fig. 2*b*) spanning 320 kb and contains 11 class I genes. One of these genes was identified as the $H–2D^b$ gene by transformation of L-cells with cosmids or subclones containing this gene and analysis with $H–2D^b$-specific antibodies[8] and cytotoxic T cells[9]. The location of this gene was confirmed using two mapping probes. A 2.7-kb *Bgl*II fragment isolated from the region shown in Fig. 2*b* maps to the *H–2D* region (Table 1). In addition, the 3′ *K*-region flanking probe hybridizes to a polymorphic 8.5-kb *Bgl*II fragment starting in this gene, which also maps to the *H–2D* region (Fig. 3*a*, Table 1).

The other 10 genes in this cosmid cluster are located in the *Qa2,3* region. The *Q1* gene was mapped to the *Qa2,3* region using the 4-kb *Pvu*II fragment just 5′ of the gene (Fig. 2*b*). This probe hybridizes to a 4.5-kb *Hin*dIII fragment in B10 and B6.K2 DNA, but to a 3.3-kb fragment in AKR and B6.K1 DNA (Fig. 3*b*, Table 1). Thus, the site of recombination between the *Qa2,3* region and the *H–2D* region in the B6.K1 mouse must be in the 40-kb DNA between the 8.5-kb *Bgl*II fragment of the $H–2D^b$ gene and 4-kb *Pvu*II fragment adjacent to the *Q1* gene (see Fig. 2*b*). Genes *Q2*, *Q5*, *Q7* and *Q9* were mapped to the *Qa2,3* region independently using the 3′ flanking *K*-region probe which hybridizes to polymorphic *Bgl*II fragments (Fig. 3*a*, Table 1).

Additional information on the location and relationships among the class I genes was obtained using a 0.64-kb *Bam*HI fragment (the 5′ flanking *K*-region probe) isolated from a position 2.6-kb to the 5′ side of the $H–2K^b$ gene (Fig. 2*a*). This probe hybridizes to six fragments of *Hin*dIII-digested B10 DNA (Fig. 3*c*). Two polymorphic *Hin*dIII fragments (6.6 and 3.4-kb; Fig. 3*c*, Table 1) map to the *H–2K* region of the B10 mouse (Fig. 2*a*). The other polymorphic fragments (2.7 and 2.4-kb) map to the *Qa2,3* region (Fig. 3*c*, Table 1) and are found in the 5′ flanking regions of genes *Q7* and *Q9* (2.7-kb fragment) and gene *Q5* (2.4-kb fragment). Two non-polymorphic *Hin*dIII fragments are also contained in cosmids from this cluster: the 3-kb fragment is found in the 5′ flanking region of the *Q6*, *Q8* and *Q10* genes; the 4.4-kb fragment is located between the $H–2D^b$ and *Q1* genes (Fig. 2*b*). Thus, except for this last case, the 5′ flanking *K*-region probe always hybridizes to regions which are 2.6–3.8-kb to the 5′ side of class I genes in the *H–2K* and *Qa2,3* regions.

We were able to link the *H–2D* and *Qa2,3* regions after screening cosmid libraries with the 5′ flanking *K*-region probe and isolating cosmids containing the 4.4-kb *Hin*dIII fragment described above. This fragment does not flank a class I gene; however, the 2.3-kb of DNA adjacent to it is homologous to the DNA sequences immediately flanking the $H–2K^b$ gene on its 5′ side; this indicates that a gene may once have been present at this site. Inspection of the restriction map of cosmid LS-AW1, which contains the $H–2D^b$ gene, showed significant overlap with cosmid Bm1-25. Moreover, cosmid LS-AW15 overlaps with cosmid B1.24, which contains gene *Q1* (Fig. 2*b*); this overlap was confirmed by another series of 12 cosmids (not shown).

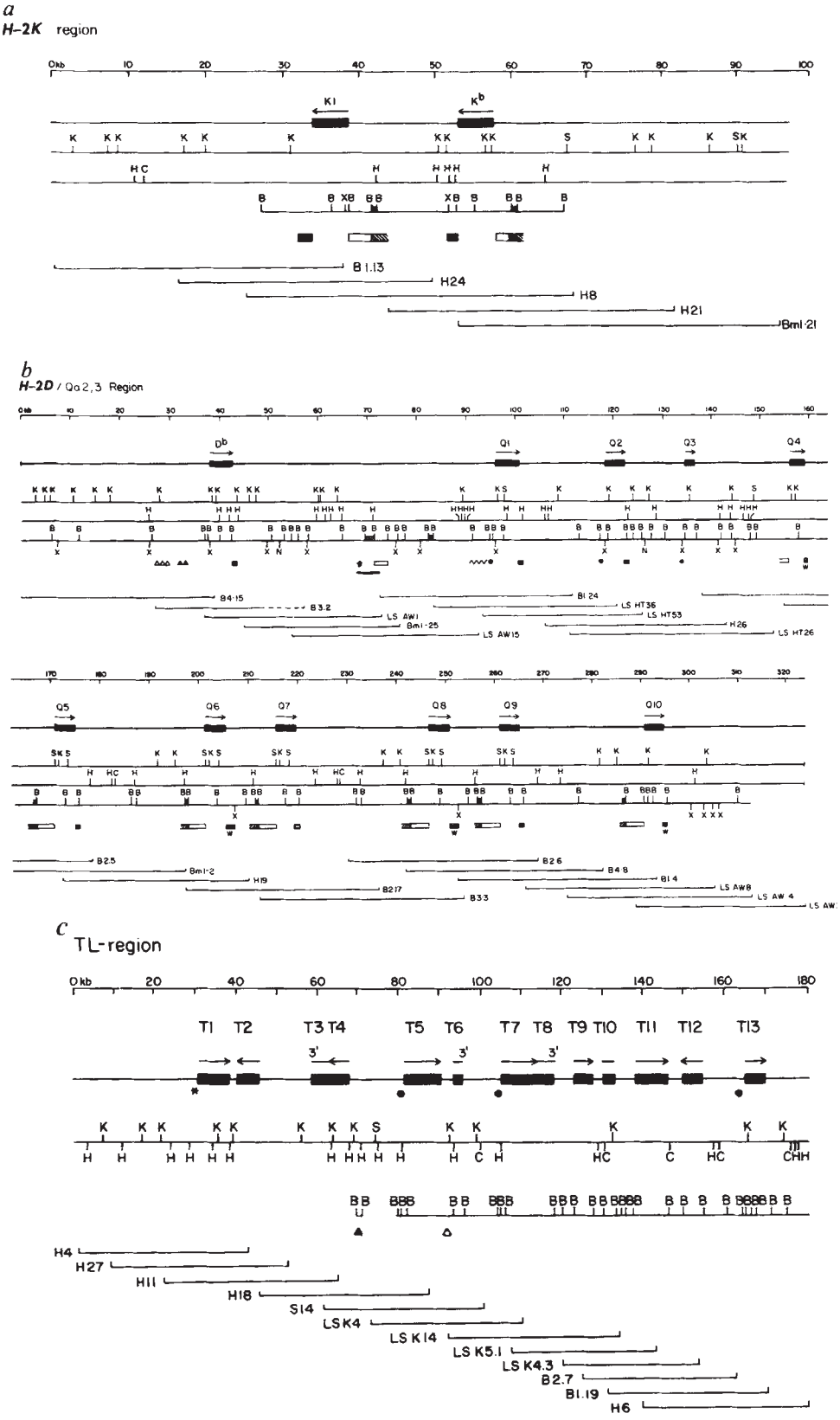

Fig. 2 Restriction maps of the three cloned regions. The length of each region (kb) is shown on the top line. The following sites are indicated: B, *Bam*HI; C, *Cla*I; H, *Hpa*I; K, *Kpn*I; N, *Nru*I; S, *Sal*I; X, *Xho*I. Class I genes are shown as solid boxes with arrows indicating the direction of transcription, 5′→3′. A representative set of overlapping cosmids defining each region is given below the restriction map. *a*, *H-2K* region. ▤, Regions hybridizing to the 3′ *K*-region flanking probe (1.8-kb *Bgl*II fragment) isolated from cosmid H24 at map position 32–34. Regions hybridizing to four different 5′ flanking region probes are indicated: ■ on the line showing the *Bam*HI restriction sites indicates *Bam*HI fragments that hybridize to the 5′ *K*-region flanking probe (0.64-kb *Bam*HI fragment 5′ to the *H-2K*^{b} gene). ▧, Regions which cross-hybridize to the 1.8-kb *Bam*HI fragment isolated from cosmids spanning the region between the *Q1* and *H-2D*^{b} genes (see *b* below). □, Regions which hybridize to two probes spanning the 2.45-kb region immediately 5′ to the coding sequence of the *H-2K*^{b} gene (1.75-kb *Pst*I fragment and 0.7-kb *Pst*I–*Kpn*I fragment; see ref. 6). *Bam*HI sites are shown only for the region between 25 and 68-kb. *b*, *H-2D/Qa2,3* region. The *Q3* gene contains only exons 1–3 of a class I gene. Most of the overlapping cosmid clones shown were isolated by screening libraries with class I gene probes. In some cases overlaps were confirmed by screening cosmid libraries with low-copy number gene flanking probes or walking probes. The region between the *Q1* and *H-2D*^{b} genes is defined by cosmids which were isolated using either the 5′ *K*-region flanking probe (Bm1-25) or the *Qa* walking probe (LS-AW1 and LS-AW15). The first probe hybridizes to a 4.4-kb *Hin*dIII fragment (see text and Fig. 3*c*) shown as the solid bar at 70-kb on the map, and the second probe (1.8-kb *Bam*HI fragment indicated by ▨ on the *Bam*HI restriction site line) was isolated from this same region. Note that this probe hybridizes twice in this region (at 70 and 82-kb on the map) and to the 5′ flanking region of genes *Q5* to *Q10*, *K1* and *H-2K*^{b}. Other symbols: △△△, the location of the 2.75-kb *Bgl*II fragment used as an *H-2D* region mapping probe; ▲▲, the region which cross-hybridizes to a DNA fragment isolated from the region flanking the *H-2L*^{d} gene[4]; ⁓, the 4.0-kb *Pvu*II fragment used as *Qa2,3* region mapping probe; ●, regions which cross-hybridize to a 0.5-kb *Bam*HI fragment isolated from the 5′ flanking region of the *T7* gene (*c*). Note that this probe also hybridizes to the previous (4.0-kb *Pvu*II) probe. For other symbols see *a*. W indicates weak hybridization to the 3′ flanking *K*-region probe. The dotted line at the right end of cosmid B3.2 indicates that this segment is rearranged. *c*, *TL* region. Cosmids defining this region were isolated by screening cosmid libraries with class I gene probes or using low-copy probes. ▲, A 0.7-kb *Bam*HI probe isolated from cosmid H18 which also hybridizes to a region between genes *T5* and *T6*. *, Location of the 2.0-kb *Eco*RI fragment obtained from clone H11 which was used as a *TL* region mapping probe. ●, A probe isolated from the 5′ flanking region of gene *T7* (0.54-kb *Bam*HI fragment) cross-hybridizes to the 5′ flanking regions of genes *T5* and *T13* as well as to genes in the *Qa2,3* region (Fig. 2*b*). The orientation of gene *T10* has not been determined. Genes *T3*, *T6* and *T8* may be 3′ gene fragments since they do not have adjacent sequences which hybridize to 5′ class I gene probes. Construction of cosmid libraries: libraries were screened and clones picked using procedures developed previously[23] incorporating the modifications of Ish-Horowicz[24] and using cosmid vectors constructed in our laboratory[25]. Five libraries were used. Cosmids with prefix H are from a library[7] made with B10 spleen DNA and vector pOF1; library B, from B10 spleen DNA using vector pTM; library LS, from B10 liver DNA using vector pTCF; library S was made with B10 spleen DNA using pTCF as vector. Finally, one library (Bml) was made using liver DNA from the B6 mouse strain which carries the *H-2K*^{bml} mutation[26] and vector pTM.

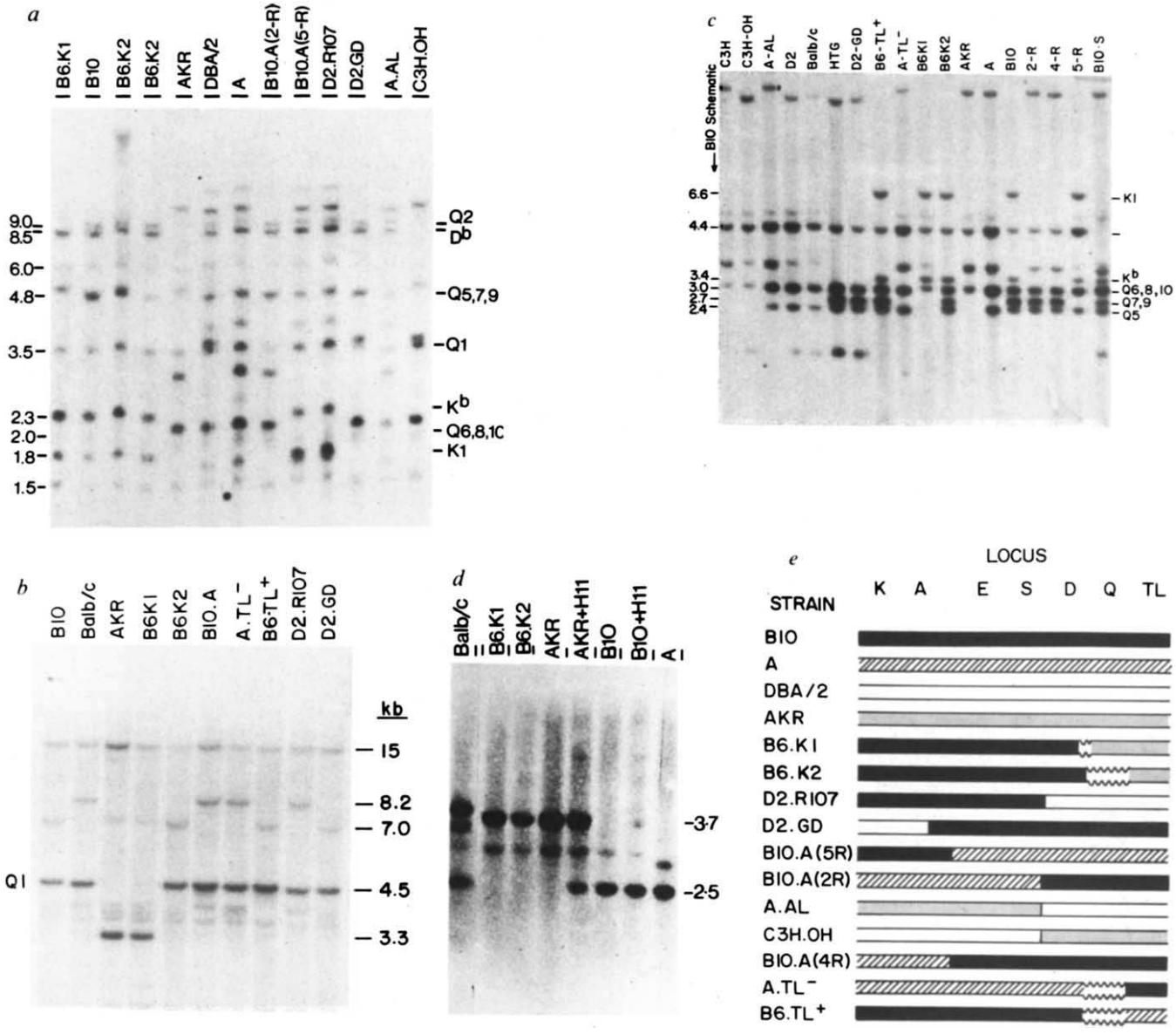

Fig. 3 Southern[5] blot analysis of different digests of murine DNA with single or low-copy probes from the *H–2K*, *Qa2,3* and *TL* regions of the B10 mouse. Southern blots were hybridized at 65 °C in 3× SSC, 10× Denhardt's solution containing 0.1% SDS, 0.1% sodium pyrophosphate, 10% dextran sulphate[7,23,27]; 20 μg ml^{-1} denatured murine DNA was added to compete with the hybridization of repeated sequences. The filters were given a final wash with 0.1× SSC, 0.1% SDS at 65 °C. DNA fragments were isolated for subcloning and used as hybridization probes by the method of Weislander[28]. ***a***, *Bgl*II digest, probed with the 3′ flanking *K*-region probe (1.8-kb *Bgl*II fragment from clone H24; Fig. 2*a*). The sizes (kb) of the nine strongest B10 bands are indicated on the left. The class I genes most closely linked to each of the nine bands are given on the right. The weakly hybridizing 6.0- and 1.5-kb *Bgl*II fragments lie in the 3′ ends of the *T1* and *T5* genes, respectively. The 4.5-kb *Hin*dIII fragment lies 5′ to the *Q1* gene. ***b***, *Hin*dIII digest, probed with the *Q1* mapping probe (4-kb *Pvu*II fragment, 5′ to the *Q1* gene; see Fig. 2*b*). The sizes of the bands (kb) are indicated on the right. ***c***, *Hin*dIII digest, probed with the 5′ flanking *K*-region probe (0.64-kb *Bam*HI fragment; Fig. 2*a*). The sizes of the six B10 bands are given (kb) on the left and the closest linked gene(s) on the right. The non-polymorphic 4.4-kb *Hin*dIII fragment is located between the *H–2D^b* and *Q1* genes (Fig. 2*b*). D2, DBA/2 strain mice. ***d***, *Pst*I digest, probed with the 2-kb *Eco*RI mapping probe from cosmid H11 (Fig. 2*c*). The sizes of the strongest bands (kb) are given on the right. Two tracks (+H11) contain H11 cosmid DNA digested with *Pst*I mixed with AKR- or B10-restricted DNA. ***e***, Genetic maps of mouse strains used to localize cosmids to MHC loci. HTG (not shown) is *H–2K^d*, *I–A^d* and *H–2D^b*. ≈, DNA in these regions could be derived from B6 or A.

The *H–2D^b* gene is in the same 5′ to 3′ orientation as the genes in the *Qa2,3* region (Fig. 2*b*).

The *Qa2,3* region map suggests that this region has evolved by a series of sequential (or possibly simultaneous) gene duplications. Related class I genes can be identified by the hybridization pattern of four different 5′ flanking probes and a single 3′ flanking *K*-region probe (Fig. 2*a*, *b*). Each probe hybridizes to the respective flanking sequences of genes *Q5* to *Q10* (Fig. 2*b*) as well as genes *K1* and *H–2K^b* (Fig. 2*a*). The hybridization of two of the 5′ flanking probes to gene *Q4* suggests a more distant similarity between gene *Q4* and genes *Q5* to *Q10*. Furthermore, the restriction maps of DNA flanking the *Q5*, *Q7* and *Q9* genes are similar as they are for the *Q6*, *Q8* and *Q10* genes. Thus, alternating gene regions are more similar to one another than to adjacent gene regions, suggesting that after the initial gene duplication, subsequent duplications involved gene pairs. It is not clear whether the duplicated pairs are *Q4* + *Q5*, *Q6* + *Q7*, *Q8* + *Q9* and *Q10* + ? or ? + *Q4*, *Q5* + *Q6*, *Q7* + *Q8* and *Q9* +

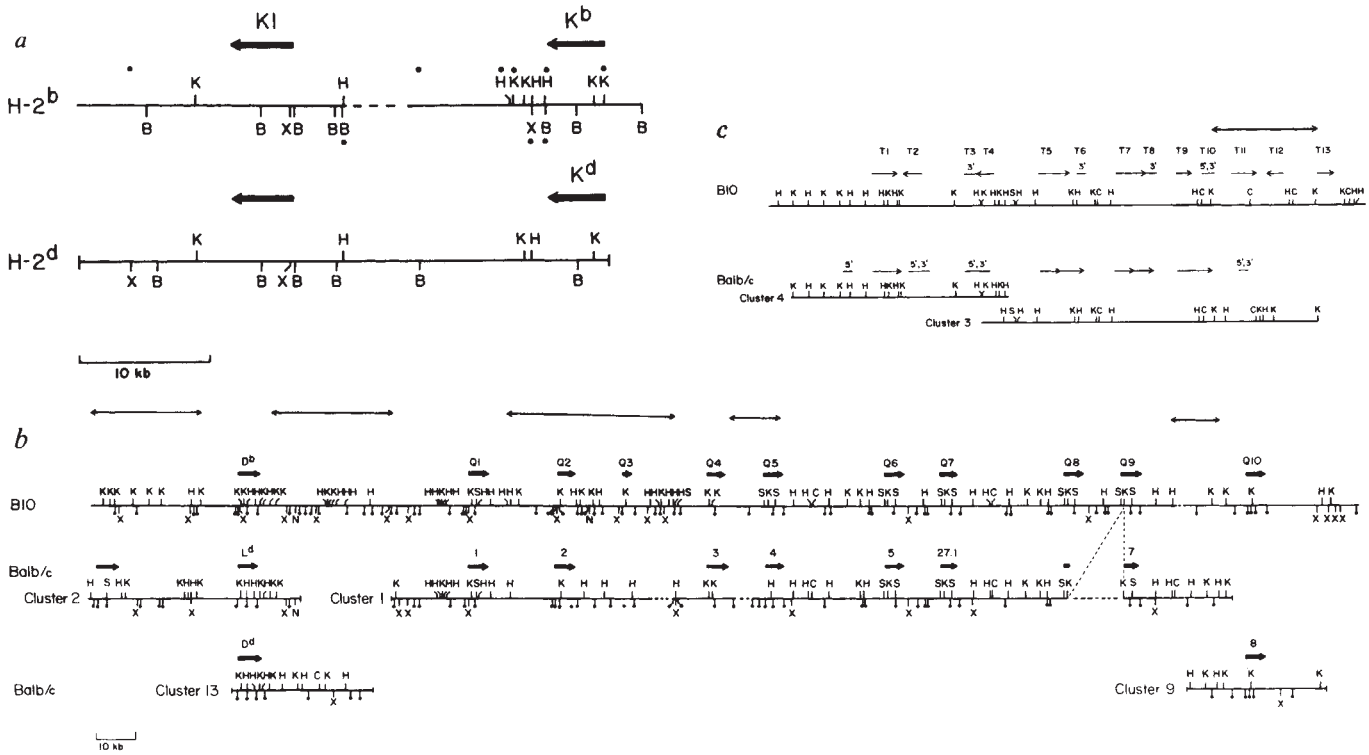

Fig. 4 Comparison of the three class I gene regions in the B10 mouse with the corresponding clusters in the BALB/c mouse[3]. ---, Deleted DNA sequence compared with the other haplotype. C, *Cla*I; H, *Hpa*I; K, *Kpn*I; N, *Nru*I; S, *Sal*I; X, *Xho*I and ↓ or B, *Bam*HI. *a*, *H-2K* region. Polymorphic restriction sites are marked with ●. *b*, *H-2D/Qa2,3* region. Polymorphic regions are indicated by a line above the restriction maps. *c*, *TL* region, polymorphic regions indicated as in *b*.

Q10. The question marks in the gene pairs indicate that one of the genes in the hypothetical duplicated gene pair is no longer present in the B10 genome.

The *TL* region

The *TL* region cluster contains 13 class I genes in 180-kb of DNA. It is described by 68 overlapping cosmids, and a representative subset of these is shown in Fig. 2*c*. These genes are not uniformly oriented as they are in the *Qa2,3* region. The cluster was localized using a 2.0-kb *Eco*RI probe located immediately 5 to gene *T1* (Fig. 2*c*). This probe detects bands of 2.5 and 3.7-kb in *Pst*I-digested B10 and AKR genomic DNA, respectively (Fig. 3*d*), and also hybridizes to a 3.7-kb *Pst*I fragment in B6.K2 DNA, thereby mapping it to the AKR portion of B6.K2 DNA, that is, to the *TL* region. Other recombinant mice analysed with this probe give results consistent with this conclusion (not shown).

We have detected similarity between the 5′ flanking regions of genes in the *TL* and *Qa2,3* region. The probe from the 5′ side of gene *T7* in Fig. 2*c* hybridizes to the 5′ flanking regions of genes *T5* and *T13* as well as to corresponding regions of genes *Q1*, *Q2* and *Q3*. In contrast, these regions display no similarity to any of the 5′ flanking *K*-region probes.

Comparison between B10 and BALB/c mice

We have identified 26 class I genes, whereas at least 35 class I genes have been reported in the BALB/c mouse[3,4], although the BALB/c number has recently been corrected to 32 (L. Hood, personal communication). The difference in gene number between the two haplotypes may reflect genuine strain differences, or over/underestimates from the two studies. However, the probe mapping to the *TL* region did hybridize to three fragments in *Pst*I digests of BALB/c DNA but only to a single fragment of *Pst*I-digested B10 DNA (Fig. 3*d*). This is consistent with the presence of more class I genes in the BALB/c genome. Most B10 class I genes are found in the *Qa2,3* and *TL* regions. Winoto *et al.* made similar observations in their study of BALB/c class I genes[4].

The *H-2K* region of both B10 and BALB/c mice contains two genes in a head-to-tail configuration (see Fig. 4*a*), the *H-2K* gene being in the same position in both clusters. The most significant difference between the *H-2K* regions is that the genes are further apart in the $H\text{-}2^d$ haplotype. Comparison of the $H\text{-}2K^b$ and $H\text{-}2K^d$ gene sequences has shown that polymorphism in these genes is generated by introducing clustered nucleotide changes over regions of limited extent (9–24 base pairs, bp) in exons 2 and 3 (refs 6, 10). Thus, at least in some cases, it seems that highly localized gene conversion[6] events are responsible for generating *H-2K* allelic polymorphism and do so without giving rise to extensive restriction site polymorphism within the *H-2K* region as a whole.

In the *H-2D* region a comparison of the restriction maps shows that the $H\text{-}2D^b$ and $H\text{-}2L^d$ genes are quite similar both at the 3′ and 5′ side of the genes, while the restriction maps of $H\text{-}2D^b$ and $H\text{-}2D^d$ regions are significantly different. In addition, a probe isolated from the 5′ side of the $H\text{-}2L^d$ gene[3] hybridizes 5′ to the $H\text{-}2D^b$ gene. It detects only one band in *Bam*HI and *Kpn*I digests of chromosomal B10 DNA (11 and 10 kb, respectively). These fragments map just 5′ to the $H\text{-}2D^b$ gene (Fig. 2*b*). Thus, $H\text{-}2D^b$ is more closely related to $H\text{-}2L^d$ than to $H\text{-}2D^d$.

Both conserved and polymorphic segments of DNA map to the *Qa2,3* region of the B10 and BALB/c mouse. The restriction maps of the intergenic DNA downstream from *Q5* up to and including *Q10* of the B10 mouse are very similar to the corresponding sequences of cluster 1 from the BALB/c mouse[3]. Gene *Q10*, which has been sequenced in our laboratory[11] and may encode a non-polymorphic, liver-specific[12], secreted[13] class I antigen, corresponds to the gene in cluster 9 of Steinmetz *et al.*[3]. Rogers *et al.*[14] have shown independently that the BALB/c clusters 1 and 9 are linked. Comparison reveals that the equivalent of genes *Q8* and *Q9* of the B10 mouse may have undergone an unequal crossing-over in the BALB/c mouse (Fig. 4*b*) to produce a fusion gene in a manner similar to the Hb-Lepore gene from the human δ- and β-globin genes[15–17]. Thus, the restriction map of the DNA flanking gene 7 of cluster 1 of the BALB/c mouse is almost identical to the 5′ map of

gene *Q8* and the 3′ map of gene *Q9* from the B10 MHC (Fig. 4*b*). The map of genes *Q4*, *Q6* and *Q7* is almost identical to the corresponding region of the BALB/c MHC (Fig. 4*b*). It follows, therefore, that gene *Q7* of the B10 mouse is the allele of the 27.1 'pseudogene' (ref. 18) in the BALB/c mouse. The maps of the *Q2*, *Q3* and *Q5* genes differ significantly between the two haplotypes. We have not detected the equivalent of cluster 6 of BALB/c *Qa2,3* region[3,4] that contains a gene encoding the *Qa2,3* antigen[19].

The B10 *TL* region comprising genes *T1* to *T10* closely parallels clusters 3 and 4 of the BALB/c mouse (Fig. 4*c*). This suggests that these clusters in fact overlap; alternatively, a deletion may have occurred between genes *T4* and *T5* in the B10 mouse. Genes *T11–T13* do not seem to be present in any of the *TL*-mapped clusters of the BALB/c mouse, and we have not detected analogues of the BALB/c clusters 5, 7, 8, 10 and 12 in the B10 genome. The basis for the differential expression of the TL antigen in the two haplotypes may well be in these non-homologous regions.

Translocations during evolution

During these studies we have noted several interrelationships between genes located at distant sites that may indicate how the MHC class I gene family has evolved. The 5′ flanking *TL* region probe hybridizes to the 5′ flanking regions of genes *Q1* to *Q3* in the *Qa2,3* region genes as well as *T5*, *T7* and *T13* of the *TL* region. The similarity of these genes may be due to a gene conversion-like process or a translocation of genes *Q1* to *Q3* from the *TL* region.

A more intriguing relationship is the striking similarity of the two genes in the *H–2K* region to the *Q6* and *Q7* as well as the *Q8* and *Q9* gene pairs. The genes in each pair are in a head-to-tail configuration and are about the same distance apart. The *H–2K* and *Qa2,3* region gene pairs show similar hybridization patterns with the three 5′ flanking *K*-region probes and the 3′ flanking *K*-region probe. For example, the 0.64-kb *Bam*HI 5′ flanking *K*-region probe hybridizes to 0.64-kb *Bam*HI fragments flanking the $H–2K^b$, *Q6* and *Q8* genes, while it hybridizes to 0.68-kb fragments flanking the other genes in each pair (that is, *K1*, *Q7* and *Q9*). The 3′ flanking *K*-region probe also hybridizes to both the *K* region and *Qa2,3* region gene pairs. Together, these data suggest that the $H–2K^b$ gene may be related to the *Q6* and *Q8* genes and that the gene *K1* is related to the genes *Q7* and *Q9* and perhaps the *Q5* gene. This similarity probably reflects an evolutionary relationship betwen the genes. In some animals, all class I gene loci are telomeric to the class II genes. In the mouse, however, the *H–2K* region is centromeric to the class II genes. For this reason it has been suggested that the *H–2K* locus arose by translocation to its present site[20]. We propose that the *H–2K* region was generated by the translocation of a *Q6*- and *Q7*-like gene pair from the *Qa2,3* region.

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Functional role for c-*myc* in mitogenic response to platelet-derived growth factor

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In BALB/c-3T3 cells, expression of the c-myc *gene is stimulated by platelet-derived growth factor (PDGF). Using mouse mammary tumour virus promoter: c-*myc *recombinant plasmids, 3T3 sublines were constructed in which hydrocortisone was the primary determinant of* myc *mRNA content. The* c-myc *gene product is an intracellular mediator of the growth response to PDGF though probably not the only one. Both the human and the mouse* c-myc *genes stimulate clonal growth of 3T3 cells in PDGF-free medium suggesting new strategies for analysis of oncogenes which do not function in focus formation assays.*

PLATELET-derived growth factor (PDGF)[1–4] renders BALB/c-3T3 cells 'competent' to replicate their DNA and divide[5]. The PDGF-induced competent state consists of an acquired sensitivity to the mitogenic action of epidermal growth factor (EGF) and the insulin-like growth factors[6,7]. The induction of competence is a transcription-dependent event[8]. At least five independent members of a PDGF-inducible gene family which we term 'competence' have been isolated by molecular cloning techniques[9]; moreover, c-*myc* seems to be a member of the competence gene family in 3T3 cells[10]. The abundance level of c-*myc* mRNA increases more than 40-fold several hours after the addition of PDGF to the culture medium of density-arrested