The EMBO Journal vol.3 no.12 pp.2995–3003, 1984

Tracts of high or low sequence divergence in the mouse major histocompatibility complex

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Communicated by F.Melchers

The K, I and S regions of the mouse major histocompatibility complex (MHC) are composed of long tracts of DNA which differ in sequence divergence. A correlation exists between the location of an MHC gene in a variable or conserved chromosomal tract and the degree of polymorphism and diversity of the proteins encoded by its alleles. Variable tracts appear to be the result of mechanisms which mutate certain coding and non-coding sequences to the same extent and selective pressures operating on the genes.

Key words: major histocompatibility complex/polymorphism/evolution

Introduction

The major histocompatibility complex (MHC or H-2 complex in the mouse) is a large genetic region encoding molecules which play an important role in the immune response (Klein, 1975; Snell *et al.*, 1976). Two of the three classes of genes located in the MHC code for cell surface molecules. These are the classical transplantation antigens (class I) and the immune-response associated antigens (Ia antigens, class II), which serve as recognition elements for the T cell receptor in the detection of foreign antigens. Genes of the third class code for components of the complement cascade.

A genetic map of the MHC of the BALB/c mouse is shown in Figure 1. At least four transplantation antigens are encoded in the K and the D regions and two Ia antigens, both composed of two polypeptide chains, are encoded in the I region. The S region encoding certain complement proteins is located between the I region and the D region. To the right of the D region an unknown number of molecules is encoded in the MHC; these are structurally closely related to the classical transplantation antigens and are therefore also called class I molecules. Unlike the transplantation antigens, which are expressed on virtually all somatic cells, these molecules appear to be restricted in their expression to nucleated blood cells (Qa) or to thymocytes and certain leukemias (TL) (Michaelson *et al.*, 1983).

Serological, biochemical and molecular analyses of the molecules and genes of the MHC have revealed a surprising degree of polymorphism and sequence divergence between alleles (Klein and Figueroa, 1981; Steinmetz and Hood, 1983; Kaufman *et al.*, 1984). More than 50 alleles exist for the K and the D loci, and the A_α, A_β and E_β loci seem to be as polymorphic as the K and D loci. The other class I loci and the E_α locus exhibit a limited degree of polymorphism. Little is known about the polymorphism of the class III loci in the mouse. Because of the extensive polymorphism it has become convenient to call the set of H-2 alleles of a given inbred line its H-2 haplotype. Individual alleles are denoted by a capital letter for the gene and a superscript for the haplotype (e.g., the K^d, D^d, E^d_α, E^d_β alleles of the BALB/c mouse).

In general, the degree of polymorphism correlates with the amount of sequence divergence found between individual alleles (Steinmetz and Hood, 1983). Comparison of amino acid sequences has shown that the variability between molecules encoded by alleles is mainly confined to the amino terminus, namely to the $\alpha 1$ and $\alpha 2$ domains of class I molecules and to the $\alpha 1$ and to the $\beta 1$ domains of class II polypeptides. In these domains 10–20% of the amino acid residues vary between molecules encoded by alleles. In contrast, the amino acid sequences of the membrane-proximal extracellular domains are conserved. These conserved domains show sequence homology to immunoglobulin (Hood *et al.*, 1983) and T cell receptor molecules (Hedrick *et al.*, 1984), indicating evolutionary relationship between these different multigene families.

Over the last three years we and others have been concerned with the characterization of the MHC at the molecular level (Hood *et al.*, 1983; Steinmetz and Hood, 1983). Our studies have revealed that the BALB/c mouse contains two class I genes in the K region, three in the D region and, quite surprisingly, 31 class I genes in the Qa and Tla regions (Steinmetz *et al.*, 1982a). Except for the Qa-1 gene, all of the serologically defined class I genes have been identified among the 36 genes (Goodenow *et al.*, 1982). Furthermore, chromosomal walking procedures have allowed us to isolate 200 kb of contiguous DNA from the I region of the BALB/c mouse (Steinmetz *et al.*, 1982b). The cloned portion of the I region contains all four known class II genes and spans a hot spot for recombination in the middle of the E_β gene (Steinmetz and Hood, 1983).

To study the molecular basis of the extreme polymorphism and variability of certain MHC alleles, we have now cloned large regions of the MHC from different mouse strains. Comparison of the restriction maps obtained for three strains

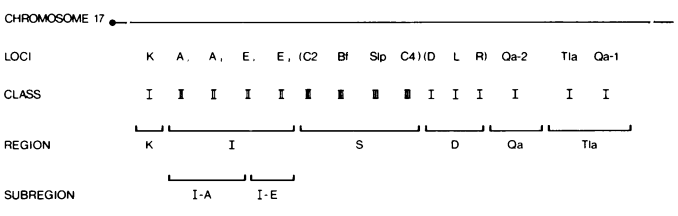

Fig. 1. Genetic map of the major histocompatibility complex of the BALB/c mouse. Genetic regions and subregions are not drawn to scale. The orientation of the four complement loci with respect to the class I and class II loci is not known. The order of the four complement loci has been determined in human DNA (Carroll *et al.*, 1984) and is the same in the mouse (M.Steinmetz, unpublished data). The order and orientation of the three D region loci D, L and R (Hansen *et al.*, 1981) is not known. The centromere is located to the left.

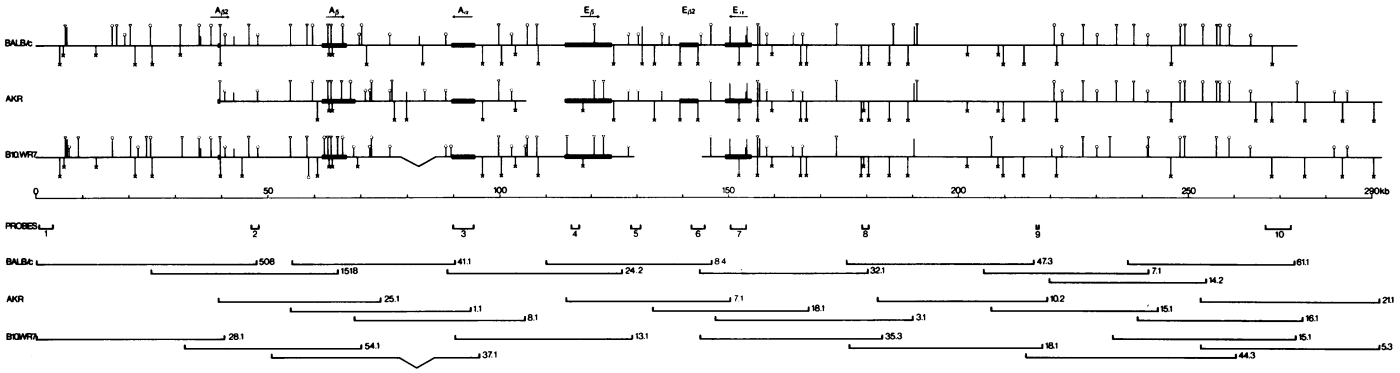

Fig. 2. Restriction map comparison of I region DNA from BALB/c, AKR and B10.WR7 mice. Restriction sites are indicated by symbols: | *Sal*I, ı *Cla*I, ⚸ *Kpn*I, ⚹ *Sac*II, ⚲ *Xho*I, ⚲ *Hpa*I, ⚳ *Sma*I, ⚲ *Nru*I. Class II genes, identified with class II cDNA clones (Steinmetz *et al.*, 1982b; Davis *et al.*, 1984), are shown as filled boxes and drawn to scale except for A_α and $E_{\beta 2}$ which have not been sequenced. Arrows indicate 5′ to 3′ orientation. A deletion was observed in B10.WR7 DNA presumably located between the two *Hpa*I sites at positions 76 and 88 kb and confirmed by Southern blotting of genomic DNA. Gaps not covered by cosmid clones are present in the AKR map from position 106 to 114 kb and in the B10.WR7 map from position 129 to 144 kb. Southern blot analyses of B10.WR7 DNA with probe 6 showed the presence of the $E_{\beta 2}$ gene on *Kpn*I and *Hind*III fragments identical in size to those found with BALB/c and AKR DNAs. Additional *Hpa*I sites present between positions 19 and 41 kb have not been mapped. Comparisons of restriction fragments from BALB/c, AKR and B10.WR7 cosmid clones run in parallel on the same agarose gel have revealed the presence of small deletions (100–500 bp in size) in three *Kpn*I fragments of BALB/c DNA (positions 23, 137 and 141 kb, respectively) and in a *Kpn*I-*Xho*I fragment in B10.WR7 DNA (position 31 kb). An insertion of ~300 bp in size was found in AKR DNA in the *Hpa*I fragment at position 44 kb. Restriction fragments used as probes in Southern blot hybridizations are labeled and have been described previously [probes 2–4 and 5–9 correspond to probes 1–3 and 5–9, respectively (Steinmetz *et al.*, 1982b)] except probes 1 (3-kb *Eco*RI-*Bam*HI fragment from clone 508) and 10 (5.6-kb *Eco*RI fragment from clone 61.1). Only 10, 10 and 9 of 23, 29 and 15 cosmid clones, respectively, isolated from the BALB/c, AKR and B10.WR7 cosmid libraries are shown. The polymorphic *Kpn*I site in AKR DNA present at position 264 kb still maps into the I–E subregion in strain A/J, as revealed by Southern blot analysis of genomic DNA with probe 10. This indicates that the I–E subregion is >140 kb of DNA (compare Steinmetz *et al.*, 1982b).

reveals that the I region falls into two tracts which have accumulated sequence changes to a different extent. The variable tract contains the A_β, A_α and E_β genes, whereas the conserved tract contains the E_α gene. Extensive Southern blot analyses of DNAs from four laboratory and five wild mouse strains, using ten probes derived from the I region, confirm the above results. Together with restriction map comparisons of two mouse strains in the K and the S region, these studies reveal that the chromosomal regions encoding highly polymorphic class I and class II molecules are hypermutable.

Results and Discussion

Cloning of I region DNA sequences from three mouse strains

Single copy probes isolated from the I region of the BALB/c mouse (H-2^d haplotype) (Steinmetz *et al.*, 1982b) were used to screen cosmid libraries constructed with AKR (H-2^k haplotype) and B10.WR7 (H-2^{wr7} haplotype) liver DNAs. The isolated clones were mapped with different restriction enzymes and ordered according to their overlapping restriction maps. Figure 2 shows the molecular maps obtained for the I regions of the three mouse strains. Although there are gaps in the AKR (upstream of the E_β gene) and the B10.WR7 maps (downstream of the E_β gene), it is clear that the number and organization of the class II genes is the same in the three mouse strains compared. There are no major deletions or insertions in the cloned region, except for a deletion of ~7.5 kb of DNA between the A_β and A_α genes in the B10.WR7 mouse. We do not know whether major insertions or deletions occur in the regions not yet cloned. These clones confirm our previous finding (Steinmetz *et al.*, 1982b) that the E_β gene, which might control the expression of I–J determinants (Steinmetz *et al.*, 1982b; Kronenberg *et al.*, 1983; Kobori *et al.*, 1984; Hayes *et al.*, 1984), spans about the same amount of DNA in different mouse strains.

A variable and a conserved tract in the I region

The comparison of the restriction maps shown in Figure 2 reveals that, in the three mouse strains, the left half of the I region is more variable than the right half. The boundary runs through the E_β gene, close to and perhaps overlapping with the hot spot for recombination in the middle of the E_β gene, separating the I–A and I–E subregions (Steinmetz *et al.*, 1982b). The three class II genes (A_β, A_α and E_β) which show extensive polymorphism and allelic variability, are all located in the variable tract, whereas the E_α gene, which shows little polymorphism and minor allelic variability, is located in the conserved tract of the I region.

These differences in sequence divergence show no correlation with coding or non-coding sequences. Figure 2 shows that most of the restriction sites mapped are located in non-coding sequences. Thus the surprising finding is that in the I–A subregion non-coding sequences are divergent whereas in the I–E subregion they are conserved. Hybridization studies using the cloned cosmids to search for mRNA transcripts in various tissues (unpublished data) and cell type-specific cDNAs to screen the cosmid clones (Davis *et al.*, 1984), have so far failed to reveal additional genes in the cloned portion of the I region. The variable tract of the I region appears to be at least 120 kb in length whereas the conserved portion spans at least 170 kb of DNA.

The K gene is located in a small variable tract

To find out whether the polymorphic and highly divergent K gene is also located in an area of extensive restriction site variability, we cloned part of the K region from BALB/c and AKR mouse DNA (Figure 3a). Ten overlapping cosmid clones were isolated from the AKR library with a K-specific probe (Kvist *et al.*, 1983), derived from the 3′ untranslated region of the K^d gene. The clones were mapped to the K region with two single copy sequences located 22 kb upstream

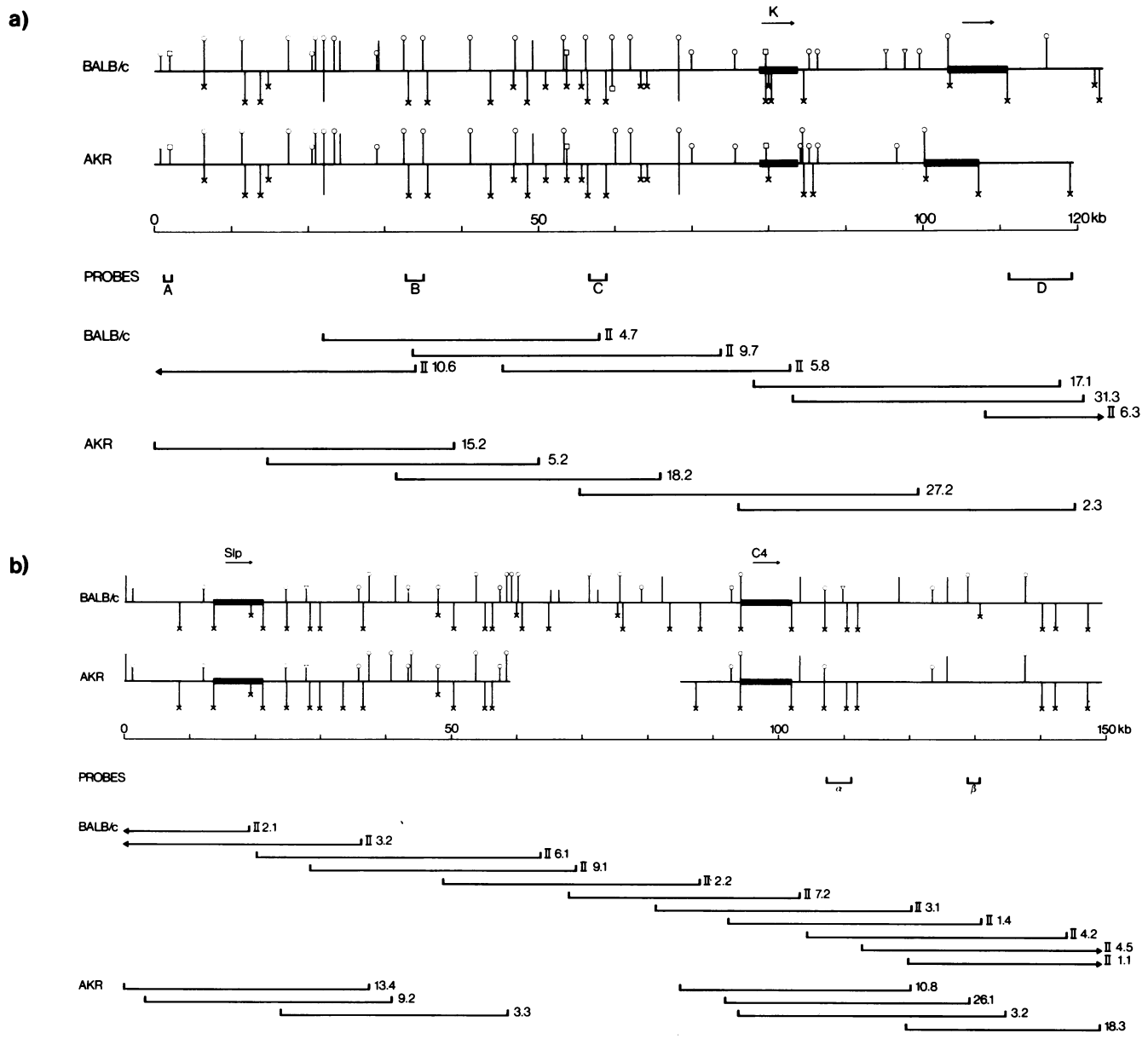

Fig. 3. Restriction map comparisons of the K and S regions of BALB/c and AKR DNAs. Symbols for restriction sites are the same as in Figure 2. Recognition sites for *Sma*I were not mapped, sites for *Mlu*I and *BssH*II are indicated by the small symbols ▽ and ▽, respectively. **(a)** The area covered by the K^d gene is known from sequence determination (Kvist *et al.*, 1983) and is assumed to be similar for the K^k gene. The extent and orientation of the second class I gene present has been determined with class I 5′ and 3′ probes (Steinmetz *et al.*, 1982a). Probes used for hybridization of Southern blots and cosmid libraries are labeled: (A) 1-kb *BssH*II-*Hpa*I fragment isolated from cosmid clone 15.2, (B) 2.6-kb *Xho*I fragment from cosmid 18.2, (C) 2.6-kb *Kpn*I fragment from cosmid 27.2, (D) 8.2-kb *Bam*HI-*Kpn*I fragment from cosmid 2.3. Probe C hybridizing to polymorphic *Eco*RI, *Kpn*I and *Bam*HI fragments mapped to the K region in B10.MBR and A.TL, probe D hybridizing to polymorphic *Eco*RI, *Kpn*I and *Hind*III fragments mapped to the K region in B10.MBR (see Klein *et al.*, 1983 for H-2 haplotypes of mouse strains used). Only seven out of 12 cosmid clones and five out of 16 cosmid clones isolated from the BALB/c and AKR cosmid libraries, respectively, are shown. **(b)** Orientation and location of the Slp and C4 genes were determined with 5′ and 3′ probes from class III cDNA clones described elsewhere (Tosi *et al.*, 1984; Lévi-Strauss *et al.*, 1984). Probes isolated from AKR cosmid clones are indicated: (α) 3.8-kb *Bam*HI fragment isolated from cosmid 10.8, (β) 1.8-kb *Eco*RI fragment isolated from cosmid 3.2. These two probes showed cross-hybridization to positions 27 and 52 kb, respectively, indicating that a duplication of a large genetic region gave rise to the two class III genes. This is in agreement with previous reports (Chaplin *et al.*, 1983). Only 11 out of 44 cosmid clones isolated from the BALB/c library are shown.

and 32 kb downstream of the K^k gene (see legend to Figure 3). The K^k was identified by gene transfer experiments (Arnold *et al.*, 1984). Chromosomal walking was then used to isolate additional overlapping cosmid clones both from the AKR and the BALB/c DNA libraries (see legend to Figure 3), providing the molecular maps which cover ~120 kb of the K region (Figure 3a).

The K region of the AKR mouse contains two class I genes, like the K regions of BALB/c (Steinmetz *et al.*, 1982a and Figure 3a) and C57BL/10 mice (Mellor *et al.*, 1982). In all three haplotypes the expressed K gene is located on the 5′ side of the second class I gene which appears to be inactive at least in BALB/c DNA (Goodenow *et al.*, 1982). The two class I genes are located closer to each other in AKR than in BALB/c DNA. Since the two genes are also found more closely spaced in C57BL/10 DNA (Mellor *et al.*, 1982), it appears that an insertion of ~4 kb of DNA occurred in BALB/c DNA leading to the appearance of two *Mlu*I sites

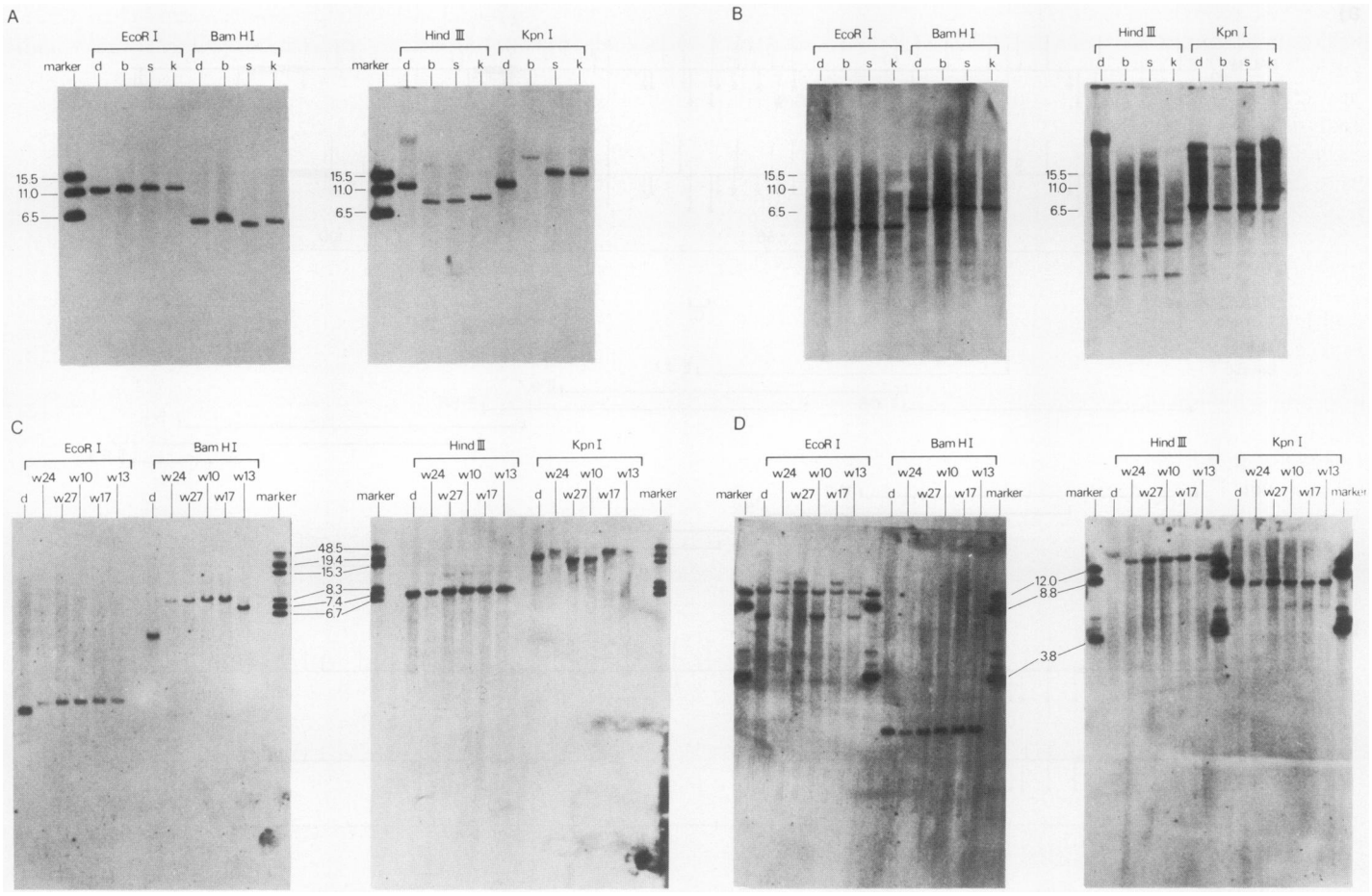

Fig. 4. Southern blot analysis of nine different laboratory and wild-derived mouse strains with probes from the I–A and I–E subregions. 10 μg of mouse liver DNAs were digested to completion with the enzymes indicated, electrophoretically separated, transferred to nitrocellulose filters, and hybridized with nick-translated probes as described (Steinmetz *et al.*, 1982b). Probes used were derived from BALB/c cosmid clones of the I region (Figure 2). **(A)** probe 3, **(B)** probe 10, **(C)** probe 4, **(D)** probe 9. Sizes of DNA marker fragments are indicated in kilobase pairs. H-2 haplotypes of the different mouse strains are listed (cf. Table I).

around position 97 kb (Figure 3a).

The two K regions compared in Figure 3a show little restriction site variability with the exception of a stretch of ~10 kb of DNA in length around the K gene. Again, as for the I region, the polymorphic restriction sites are not confined to coding sequences. In fact, all but one of the polymorphic restriction sites mapped occur in non-coding sequences (compare Kvist *et al.*, 1982).

The C4 and Slp genes are located in a conserved tract

A human cDNA clone encoding a portion of the C4 molecule (Carroll and Porter, 1983) was used to isolate cosmid clones from the AKR DNA library covering two distinct C4-related genes. The two regions were increased in length to the 3′ side by chromosomal walking (see legend to Figure 3). Probes derived from the AKR cosmid clones together with a mouse C4 cDNA clone (Tosi *et al.*, 1984) were then used to isolate a stretch of 150 kb of DNA containing the two C4-related genes of the BALB/c mouse (Figure 3b). Our data are in agreement with a previous report (Chaplin *et al.*, 1983) describing the isolation and mapping of the two C4-related genes from the BALB/c mouse to the S region. Southern blotting data indicate that BALB/c and AKR mice contain only the two C4-related genes isolated (Lévi-Strauss *et al.*, 1984). Restriction site polymorphism allowed the identification of the leftward gene as the Slp gene (Lévi-Strauss *et al.*, 1984) whereas the rightward gene encodes the C4 polypeptide (Chaplin *et al.*, 1984). It is interesting to note that the Slp allele is also found in the AKR mouse which does not express the Slp molecule (reviewed in Shreffler, 1981).

Figure 3b shows a comparison of the cloned portion of the S region between BALB/c and AKR mouse DNA. Relatively little restriction site variability is observed between the two haplotypes. Thus this part of the S region appears to be as conserved as the right half of the I region.

Quantitation of the sequence divergence in the K, I and S regions

From the restriction maps a statistical estimate of the nucleotide diversity was obtained (Table II). By this method (Nei and Li, 1979) the evolutionary change between two mouse strains is calculated from the proportion of shared restriction sites. For the relatively conserved portions to the left and to the right of the K gene we obtained 0.8% and 1.6% nucleotide diversity, respectively, whereas 6.7% diversity was obtained for the variable stretch which includes the K gene. Similarly, 2.1% nucleotide diversity was found for the conserved portion of the I region, whereas the variable portion showed 6.7% diversity on average. Around the Slp and C4 genes 1.3 and 2.4% nucleotide diversity, respectively, was observed.

In addition to the laboratory mouse strains discussed

above, two more laboratory (C57BL/10 and A.SW) and five wild-derived mouse strains (B10.KPA44, B10.CAS2, B10.DRB62, B10.STA10 and B10.STA62) were analyzed for restriction site polymorphism in the I region by Southern blot analysis, using four different restriction enzymes (*Eco*RI, *Bam*HI, *Hind*III and *Kpn*I). The hybridization probes used were derived from the I−A subregion (probes 1−4) and the I−E subregion (probes 5−10) (cf. Figure 2).

Figure 4 shows the results obtained for probes 3 and 10 with DNA from laboratory and for probes 4 and 9 with DNA from wild-derived mouse strains. With probe 3 (Figure 4a) all four laboratory strains can be distinguished according to their restriction fragment patterns, whereas with probe 10 (Figure 4b) all of them are alike. Similarly the five wild-derived mouse strains are more different when tested with probe 4 (Figure 4c), than with probe 9 (Figure 4d).

The results of the hybridization experiments are summarized in Table I and statistically evaluated (Nei and Li, 1979) in Table II. On average, 3.5% nucleotide diversity was calculated for the region tested with probes 1−4, whereas only 1.2% diversity was found for the region analyzed with probes 5−10. Of the nine H-2 haplotypes tested, H-2^b and H-2^s are most closely related, while H-2^d and H-2^{w27} are the most different. It is important to note that the wild-derived mouse strains [four of which were captured around Ann Arbor, Michigan, and one (B10.CAS2) originates from Asia (Klein *et al.*, 1983)] show the same differential sequence divergence in the I region as the laboratory inbred strains whose origin is not well known (Festing and Lovell, 1981). Thus, with regard to the differential sequence divergence in the I region the laboratory inbred strains are representative of wild mice.

The values obtained for the sequence divergence in the I region by Southern blot analysis are in agreement with those obtained by restriction map comparison. These values correlate also with those obtained by direct DNA sequence comparisons of H-2 alleles (Table II). Alleles located in the variable tracts (K, A_β, A_α, E_β) show 1.7−6.3% nucleotide diversity. By contrast, the E_α gene, which is located in the conserved tract of the I region, shows only 0.9% nucleotide diversity. In agreement with the restriction map comparisons, these sequence comparisons reveal that, at least for some alleles, the variability occurs throughout coding and noncoding sequences (Figure 5). Thus, all three approaches agree that the nucleotide diversity is ~2- to 4-fold higher in the variable than in the conserved chromosomal tracts. Furthermore, they all indicate that the transition from the variable to the conserved tract is located around the E_β gene.

Variable and conserved chromosomal tracts inside and outside of the MHC

In addition to the K region and the I−A subregion, the D region of the MHC encodes a highly polymorphic and variable class I molecule (Klein and Figueroa, 1981; Steinmetz and Hood, 1983). Serological and molecular data, however, indicate that mouse strains differ in the number of class I loci located in the D region (Steinmetz *et al.*, 1982a; Hansen *et al.*, 1981). It appears also that in mice carrying H-2^b haplotype genes the D^b gene is the allele of the L^d gene and not of the D^d gene (Maloy and Coligan, 1982). Thus, for the D region part of the polymorphism and the apparently high allelic variability of class I molecules may be due to the expression of different class I loci.

Human class II α and β probes also detect polymorphic restriction fragments with different frequencies. Whereas DR and DC β chain, as well as DC α chain, probes reveal a high level of restriction site polymorphism among related individuals, only little polymorphism is detected with DRα and SBα chain probes (Wake *et al.*, 1982; Trowsdale *et al.*, 1983; Spielman *et al.*, 1984). It will be interesting to find out whether the highly polymorphic human class II genes are also linked to a hypermutable chromosomal region.

The Qa and Tla region sequences so far analyzed represent conserved tracts, as judged from the low frequencies with which polymorphic restriction sites are detected (Winoto *et al.*, 1983). This is in agreement with a low degree of polymorphism and limited sequence variability of Qa and Tla alleles (Yokoyama *et al.*, 1983; Mellor *et al.*, 1984).

Are tracts of differential sequence divergence found also outside of the MHC? We tested the chromosomal regions around the genes encoding β_2-microglobulin (β_2m) (Parnes and Seidman, 1982) and the invariant chain (Ii) (Singer *et al.*, 1984 and M.Steinmetz, unpublished data). For both genes two probes were used, one derived from the coding region and one located ~30 kb upstream of the two genes (M.Steinmetz, unpublished data). No restriction fragment polymorphism was found for β_2m in four inbred strains (BALB/c, C57BL/10, AKR, A.SW), analyzed with four different restriction enzymes (*Eco*RI, *Bam*HI, *Hind*III, *Kpn*I). Only two restriction fragment length polymorphisms were found for the Ii probes when the same four laboratory strains were analyzed with the same four enzymes and two more laboratory strains (DBA/2 and C57BL/6) were analyzed with 19 different restriction enzymes (not shown). Clearly, these two genes are located in regions which are conserved in different subspecies of mice. This is in agreement with a previous report that the β_2m gene, as well as the immunoglobulin C_k gene, are located in chromosomal regions which show much less restriction polymorphism than the region around the A_β gene (Robinson *et al.*, 1983). Also, the mouse immunoglobulin heavy chain constant region genes, the T cell receptor, β chain genes and the actin genes are located in conserved chromosomal areas (Ben-Neriah *et al.*, 1981; Shimizu *et al.*, 1982; Caccia *et al.*, 1984). On the other hand, probes derived from immunoglobulin V_H genes reveal a high degree of restriction fragment length polymorphism in different mouse strains (Ben-Neriah *et al.*, 1981; Kataoka *et al.*, 1982). Moreover, the immunoglobulin γ2a heavy chain gene shows extensive sequence divergence in BALB/c and C57BL/6 mice whereas the γ2b gene, located just upstream of γ2a, is conserved (Schreier *et al.*, 1981; Ollo and Rougeon, 1983).

Origin of variable chromosomal tracts

From the DNA sequence comparison shown in Figure 5 for the A^b_β and A^d_β alleles it is clear that the mutations are evenly distributed over exons and introns with no hypervariable regions apparent. However, amino acid sequence comparisons have shown that the β1 domain of the alleles is highly variable whereas the β2 domain is conserved (Steinmetz and Hood, 1983; Gustafsson *et al.*, 1984). Selection obviously operated at the protein level and resulted in A_β alleles with multiple productive nucleotide changes in the β1 exon (leading to amino acid replacements) whereas only silent nucleotide substitutions were tolerated in the β2 exon (Gustafsson *et al.*, 1984). The same appears to be true for A_α alleles (Benoist *et al.*, 1983). In the case of the E_β gene and the K gene, strong positive selection for variability in the amino-terminal part of

Table I. Lengths of restriction fragments detected with probes from the I region in nine different laboratory and wild-derived mouse strains

Probe		1				2			
Strain	H-2 Haplotype	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I
BALB/c	d	4.6	3.9	5.1	6.3	7.0	13.0	6.7+2.9	32
C57BL/10	b	4.6	3.9	5.1	6.3	8.2	13.0	6.7+2.9	23
AKR	k	4.6	3.9	5.1	6.3	15.3	13.8	6.7+2.9	23
A.SW	s	4.6	3.9	5.1	6.3	8.2	13.0	6.7+2.9	23
B10.DRB62	w10	4.4	3.9	4.9	6.3	15.3	13.8	6.7+2.9	23
B10.STA10	w13	4.4	3.9	4.9	6.3	7.0	13.8	6.7+4.5	23
B10.CAS2	w17	4.6	4.1	5.1	6.6	8.2	13.8	6.7+3.0	32
B10.KPA44	w24	4.4	3.9	4.9	6.3	7.0	13.0	6.7+2.9	32
B10.STA62	w27	4.6	4.1	5.1	6.6	8.2	13.8	6.7+2.9	23

Probe		3				4				5			
Strain	H-2 Haplotype	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I
BALB/c	d	10.8	5.6	11.0	13	2.0	4.8	7.4	17	12.2+6.0	12.5	2.3	6.5
C57BL/10	b	10.8[a]	6.0[a]	8.2+1.5[a]	22[a]	2.2	8.2	7.4	9.6	6.0+6.0[a]	12.5[a]	2.3[a]	9.0[a]
AKR	k	10.8[b]	6.0[b]	8.5[b]	17[b]	2.2	6.2	7.4	17	12.2+6.0[b]	12.5[b]	2.3[b]	9.0[b]
A.SW	s	10.8	5.6	8.2+2.5	17	2.2	8.2	7.4	25	12.2+6.0	12.5	2.3	25.0
B10.DRB62	w10	10.8	5.6	8.2+2.5	20	2.2	8.2	7.4	N.D.	6.0+6.0	12.5	2.3	9.0
B10.STA10	w13	10.8	6.0	8.2+2.5	17	2.2	6.2	7.4	N.D.	12.2+6.0	12.5	2.3	25.0
B10.CAS2	w17	10.8	5.6	8.5+2.5	22	2.2	8.2	7.4	N.D.	12.2+6.0	12.5	2.3	25.0
B10.KPA44	w24	10.8	9.5	8.5+2.5	20	2.2	8.2	7.4	25	12.2+6.0	12.5	2.3	25.0
B10.STA62	w27	10.8	6.0	8.5+1.5	22	2.2	8.2	7.4	17	6.0+6.0	12.5	2.3	9.0

Probe		6				7			
Strain	H-2 Haplotype	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I
BALB/c	d	3.2	4.6	9	8.8+4.0	8.2+3.0	6.6+6.6	9.0	8.8+4.2
C57BL/10	b	3.2[a]	4.6[a]	9[a]	8.8+4.0[a]	8.2+3.0[a]	6.6+6.6[a]	9.0[a]	8.8+3.5[a]
AKR	k	3.2[b]	4.6[b]	9[b]	8.8+4.2[b]	8.6+3.0[b]	6.6+6.6[b]	9.0[b]	8.8+4.2[b]
A.SW	s	3.2	4.6	9	8.8+4.0	8.2+3.0	6.6+6.6	9.0	8.8+3.5
B10.DRB62	w10	3.2	4.6	9	8.8+4.0	8.2+3.0	6.6+6.6	8.6	8.8+4.2
B10.STA10	w13	3.2	4.6	9	8.8+4.0	7.5+3.0	6.6+6.6	9.0	8.8+4.2
B10.CAS2	w17	3.2	4.6	9	8.8+4.0	7.8+3.0	6.6+5.8	8.0	8.8+3.5
B10.KPA44	w24	3.2	4.6	9	8.8+4.0	8.2+3.0	6.6+6.6	9.0	8.8+4.2
B10.STA62	w27	3.2	4.6	9	8.8+4.0	8.2+3.0	6.6+6.6	9.0	8.8+4.2

Probe		8				9				10			
Strain	H-2 Haplotype	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I	*Eco*RI	*Bam*HI	*Hind*III	*Kpn*I
BALB/c	d	22.0	16	10.5	1.6	12.3+ 7.3	2	18.0	7.1	6	8	4+2.6	8
C57BL/10	b	19.5	8	10.5	1.6	12.3+ 7.3	2	14.8	7.1	6[a]	8	4+2.6	8[a]
AKR	k	22.0	16	10.5	1.6	12.3+ 7.3	2	14.8	7.1	6[b]	8[b]	4+2.6[b]	8[b]
A.SW	s	19.5	8	10.5	1.6	12.3+ 7.3	2	14.8	7.1	6	8	4+2.6	8
B10.DRB62	w10	22.0	8	10.5	1.6	12.3+ 7.3	2	14.8	7.1	6	5	4+2.6	15
B10.STA10	w13	19.5	16	10.5	1.6	12.3+ 7.3	2	14.8	7.1	6	8	4+2.6	8
B10.CAS2	w17	19.5	8	10.5	1.6	17.2+12.3	2	14.8	7.1	6	8	4+2.6	8
B10.KPA44	w24	22.0	16	10.5	1.6	17.2+12.3	2	14.8	7.1	6	5	4+2.6	15
B10.STA62	w27	22.0	16	10.5	1.6	17.2+12.3	2	14.8	7.1	6	8	4+2.6	8

Probes are described in Figure 2. Lengths of restriction fragments (in kilobase pairs) detected by Southern blot hybridization were determined with standard fragments run in parallel. Note that mouse strains C57BL/10 and B10.STA62 which are serologically indistinguishable at the A_β and A_α loci (Klein *et al.*, 1983) differ from another when analyzed with probes 2 and 3.

[a]Instead of C57BL/10 DNA, C57BL/6 DNA was analyzed.

[b]C3H DNA was analyzed instead of AKR DNA.

N.D., not determined.

Table II. Nucleotide diversity in the K, I and S regions (%)

H-2 Haplotypes compared	Restriction map comparisons											
		K region			I region				S region			
	Tracts[a] (kb)	0–77	77–87	87–120	0–120[c]	120–290[d]			0–60	85–150		
d,k		0.8	6.7	1.6	8.7	1.3			1.3	2.4		
d,wr7					4.7	2.8						
k,wr7					6.8	2.2						
d,k,wr7					6.7	2.1						
	Southern blot comparisons											
		I region										
	Probes[b]	1	2	3	4	5	6	7	8	9	10	1 to 4	5 to 10
b,d,k,s,w10, w13,w17,w24,w27		3.4	3.5	5.1	2.1	1.5	0.3	1.6	1.8	0.9	1.0	3.5	1.2
d,w27												6.5	0.8
b,s												1.3	0.4
	Sequence comparisons												
	Genes	K	A_β	A_α	E_β	E_α							
b,k			5.2		1.7								
b,d		4.0	4.4		2.4								
d,k			6.3		4.0	0.9							
b,d,f,k,q,u				3.5									

Nucleotide diversity was calculated as described in Materials and methods.
[a]Tracts are given in map positions in kilobase pairs (compare Figures 2 and 3).
[b]Probes used are indicated in Figure 2.
[c]For the H-2 haplotype comparisons d, k and k, wr7 the region from 40 to 120 kb was analyzed.
[d]For the H-2 haplotype comparisons d, k and d, wr7 the region from 120 to 270 kb was analyzed.

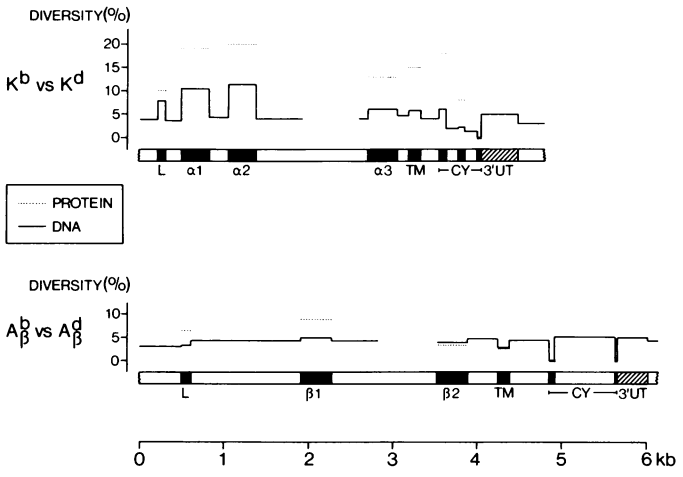

Fig. 5. Sequence divergence of two K and two A_β alleles. Nucleotide and amino acid sequence diversities between the alleles shown (in percent) have been calculated separately for each exon (filled boxes) and intron (open boxes) counting deletions or insertions as single events irrespective of length. Sequence information: K^d (Kvist *et al.*, 1983), K^b (Weiss *et al.*, 1983), A^b_β (Larhammar *et al.*, 1983), A^d_β (Malissen *et al.*, 1983). Gaps are due to a portion not sequenced (K^b) and to a region of non-homology (A^b_β *versus* A^d_β). L, leader exon; α1, α2, α3, β1, β2, exons encoding external domains; TM, transmembrane exon; CY, exons encoding cytoplasmic regions; 3′ UT, 3′ untranslated region.

the molecules might be responsible for the hypervariability of the β1 and the α1 and α2 exons (Widera and Flavell, 1984; Figure 5).

What might be the molecular mechanism generating tracts of differential sequence divergence in the MHC? Gene conversion events seem to be an important mechanism generating hypervariability in the external domains of the K molecule, probably making use of the many class I genes in the Qa and Tla regions as donor sequences (Mellor *et al.*, 1983). Recently some evidence has been obtained that gene conversion events may also contribute to the diversity of class II genes, both in mice and man (Widera and Flavell, 1984; Auffray *et al.*, 1984).

Variable chromosomal tracts could arise if mutational events (e.g., through gene conversion) in coding regions occur simultaneously with distinct mutational events in non-coding regions over a certain distance, and if allelic variability of a given locus is of advantage for the survival of the species. From the size of the variable tract in the K region and the distance of the polymorphic genes in the I–A subregion one would predict that linked mutational events occur over some 10–20 kb in length. Indeed, in the case of the sickle cell anaemia β globin gene, the mutation in the coding region has been found to be linked to a second mutation 5 kb downstream of the gene (Kan and Dozy, 1978).

The proximity of the boundary between the variable and conserved tracts and the recombinational hot spot in the I region (but not in the K region) may indicate that the same structural element, which focuses recombinational events to a certain area, will also terminate mutational events arising in the I–A subregion. That genes found in variable tracts are members of multigene families is in agreement with the possibility that at least some of their variability is due to gene conversion events and selective pressures. An apparent exception are the class II α genes of which only two have been identified

in the mouse (Steinmetz *et al.*, 1982b; Davis *et al.*, 1984). Whereas the E_α gene is conserved, the A_α gene appears to be polymorphic. That the conservation of the E_α gene is not due to selective pressures is best documented by the frequent occurrence of inactive alleles (Dembić *et al.*, 1984). Perhaps some of the diversity of the A_α gene is acquired in a passive way, due to its location between the A_β and E_β genes which are actively diversified.

Alternatively, it is possible that mutations are introduced into coding and non-coding sequences independently of one another. In this model it is assumed that distinct structural elements (e.g., repetitive DNA sequences, differences in base composition, chromatin organization) will distinguish variable from conserved chromosomal tracts. Mutations would occur more frequently both in coding and non-coding sequences of variable tracts as opposed to conserved tracts, and functional alleles would be selected by the same pressures as above. Such a mechanism is attractive because it would allow genes which have moved into variable tracts to mutate at a higher rate than others in their conserved chromosomal neighborbood.

Materials and methods

Construction and screening of cosmid libraries

Cosmid clones were isolated from three different BALB/c DNA libraries, constructed using the vectors pTL5 (Lund *et al.*, 1982), pJB8 (Ish-Horowicz and Burke, 1981) and pNNL (Grosveld *et al.*, 1982), one AKR library and one B10.WR7 library (both constructed with pTL5). Cosmid libraries were constructed from BALB/c sperm DNA (in pTL5) or liver DNA (all other libraries) as described (Steinmetz *et al.*, 1982a; Steinmetz *et al.*, 1984). Restriction mapping of cosmid clones, isolation of DNA fragments used as hybridization probes for Southern blots and cosmid libraries and screening of cosmid libraries were as described previously (Steinmetz *et al.*, 1982a; Steinmetz *et al.*, 1984).

I region cosmid clones

Clones 41.1, 24.2, 8.4, 32.1, 47.3, 7.1, 14.2 and 61.1 (Figure 2) were described previously (Steinmetz *et al.*, 1982b; Hood *et al.*, 1983). Clones 508 and 1518 (Figure 2) were isolated from the BALB/c library constructed with pJB8.

K region cosmid clones

Cosmid clones 27.2 and 2.3 (Figure 3a) were isolated from the AKR cosmid library with a K-specific probe described by Kvist *et al.* (1983). Two chromosomal walking steps, first with probe C and subsequently with probe B led to the isolation of clones 18.2, 5.2 and 15.2 (Figure 3a). BALB/c clones with a Roman numeral II were isolated from the library constructed in pNNL with probes A, B, C and D (Figure 3a). Clones 17.1 (published previously by Steinmetz *et al.*, 1981a) and 31.3 were isolated from the BALB/c library constructed in pTL5.

S region cosmid clones

Cosmid clones from the AKR library were isolated with a human C4 cDNA clone (Carroll and Porter, 1983) except for clones 3.3 and 18.3 which were obtained with probes α and β (Figure 3b). BALB/c cosmid clones were all isolated from the library constructed in pNNL by screening with probes α and β and the mouse class III cDNA clone pMC4/21 described by Tosi *et al.* (1984).

Nucleotide diversity calculated by restriction map comparisons

Proportions of shared resriction sites in the regions analyzed in Figures 2 and 3 were used to calculate nucleotide diversity as described by Nei and Li (1979) using equations 8 and 10. No attempt was made to correct for an over- or under-estimation of the polymorphism resulting from the presence of insertions or deletions of DNA ecept for the large deletion in B10.WR7 (in the I region) and the large insertion in BALB/c DNA (in the K region). Both events were counted as one mutation. The restriction map comparisons (Figures 2 and 3) reveal that insertions/deletions are not the major cause of the observed polymorphism. Small insertions/deletions revealed by parallel electrophoresis of restriction fragments are detailed in the legends to Figures 2 and 3.

Nucleotide diversity calculated by Southern blot comparisons

Proportions of shared restriction fragments between any two of the mouse strains analyzed were calculated for each probe from the data given in Table I. These were then used to estimate nucleotide diversities as described by Nei and Li (1979) using equations 20 and 21. In Table II the average value for the nucleotide diversity observed between the nine mouse strains is given for each probe. Also, the average divergence for the regions covered by probes 1–4 and 5–10 are given, as well as the highest and lowest divergence observed in the pairwise strain comparisons. Differences in nucleotide diversities obtained by restriction map and Southern blot comparisons are probably due to the differential use of restriction enzymes containing the dinucleotide CpG in their recognition sequence. Such restriction enzymes are known to reveal a higher degree of restriction site polymorphism (Barker *et al.*, 1984). Whereas all of the enzymes mapped (except *Kpn*I and *Hpa*I) recognize CpG containing sites, none of those used for the Southern blots do.

Nucleotide diversity calculated by sequence comparisons

The K^b (Weiss *et al.*, 1983) and the K^d alleles (Kvist *et al.*, 1983) were compared over 3.4 kb of DNA sequence available, excluding the hypervariable $\alpha 1$ and $\alpha 2$ exons. 5 kb of sequence were compared for the A^b_β (Larhammar *et al.*, 1983) and the A^d_β alleles (Malissen *et al.*, 1983), excluding a non-homologous stretch of 0.6 kb in A^b_β and 0.8 kb in A^d_β located in the large intervening sequence between the $\beta 1$ and $\beta 2$ exons (compare Figure 5). The A^b_β *versus* A^k_β and A^d_β *versus* A^k_β comparisons are for 688 bp of coding region only (Choi *et al.*, 1983). The comparison of the six A_α alleles over 873 bp of cDNA sequence has been published (Benoist *et al.*, 1983). Widera and Flavell (1984) compared E^b_β *versus* E^k_β and E^d_β *versus* E^k_β over 1037 bp of coding sequence and E^b_β *versus* E^d_β over 2448 bp of coding and non-coding sequence. For the E^d_α (McNicholas *et al.*, 1982; Hyldig-Nielsen *et al.*, 1983) and E^k_α alleles (Mathis *et al.*, 1983) 3.7 kb of coding and non-coding sequence were compared. In all comparisons, insertions or deletions were counted as single events irrespective of length.

Acknowledgements

We thank Michael Carroll, Rodney Porter, Mario Tosi and Tommasi Meo for the C4 cDNA clones, M.Zuniga for BALB/c cosmid clones containing the β_2m gene, S.Kvist for the K-gene specific probe, J.Klein and K.Fischer-Lindahl for the wild-derived mouse strains, L.Gisler, L.Moser and C.Tschachtli for preparation of the manuscript, H.Bider for graphic design and Stephen Fazekas de St.Groth and James Kaufmann for comments on the manuscript. M.M. acknowledges support by the Fondation pour la Recherche Médicale and CNRS (France). The Basel Institute for Immunology was founded and is supported by F.Hoffmann-La Roche Company Limited, Basel, Switzerland.

References

Arnold,B., Burgert,H.-G., Hamann,U., Hämmerling,G., Kees,U. and Kvist,S. (1984) *Cell*, **38**, 79-87.

Auffray,C., Lillie,J.W., Arnot,D., Grossberger,D., Kappes,D. and Strominger,J. (1984) *Nature*, **308**, 327-333.

Barker,D., Schafer,M. and White,R. (1984) *Cell*, **36**, 131-138.

Ben-Neriah,Y., Cohen,J.B., Rechavi,G., Zakut,R. and Givol,D. (1981) *Eur. J. Immunol.*, **11**, 1017-1022.

Benoist,C., Mathis,D.J., Kanter,M.R., Williams,V.E.,II and McDevitt, H.O. (1983) *Cell*, **34**, 169-177.

Caccia,N., Kronenberg,M., Saxe,D., Haars,R., Bruns,G.A.P., Goverman, J., Malissen,M., Willard,H., Yoshikai,Y., Simon,M., Hood,L. and Mak, T.W. (1984) *Cell*, **37**, 1091-1099.

Carroll,M.C. and Porter,R.R. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 264-267.

Carroll,M.C., Campbell,R.D., Bentley,D.R. and Porter,R.R. (1984) *Nature*, **307**, 237-241.

Chaplin,D.D., Woods,D.E., Whitehead,A.S., Goldberger,G., Colten,H.R. and Seidman,J.G. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 6947-6951.

Chaplin,D.E., Sackstein,R., Perlmutter,D.H., Weis,J.H., Kruse,T.A., Coligan,J., Colten,H.R. and Seidman,J.G. (1984) *Cell*, **37**, 569-576.

Choi,E., McIntyre,K., Germain,R.N. and Seidman,J.G. (1983) *Science (Wash.)*, **221**, 283-286.

Davis,M.M., Cohen,D.I., Nielsen,E.A., Steinmetz,M., Paul,W.E. and Hood,L. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2194-2198.

Dembić,Z., Singer,P.A. and Klein,J. (1984) *EMBO J.*, **3**, 1647-1654.

Festing,M.F.W. and Lovell,D.P. (1981) *Symp. Zool. Soc. Lond.*, **47**, 43-62.

Goodenow,R.S., McMillan,M., Nicolson,M., Sher,B.T., Eakle,K., Davidson,N. and Hood,L. (1982) *Nature*, **300**, 231-237.

Grosveld,F.G., Lund,T., Murray,E.J., Mellor,A.L., Dahl,H.H.M. and Flavell,R.A. (1982) *Nucleic Acids Res.*, **10**, 6715-6732.

Gustafsson,K., Wiman,K., Emmoth,E., Larhammar,D., Böhme,J., Hyldig-Nielsen,J.J., Ronne,H., Peterson,P.A. and Rask,L. (1984) *EMBO J.*, **3**, 1655-1661.

Hansen,T.H., Ozato,K., Melino,M.R., Coligan,J.E., Kindt,T.J., Jadinski, J.J. and Sachs,D.H. (1981) *J. Immunol.*, **126**, 1713-1716.

Hayes,C., Klyzeck,K.K., Krum,D.P., Whitcomb,R.M., Hullet,D.A. (1984) *Science (Wash.)*, **223**, 559-563.

Hedrick,S.M., Nielsen,E.A., Kavaler,J., Cohen,D.I. and Davis,M.M. (1984) *Nature,* **308**, 153-158.
Hood,L., Steinmetz,M. and Malissen,B. (1983) *Annu. Rev. Immunol.,* **1**, 529-568.
Hyldig-Nielsen,J.J., Schenning,L., Hammerling,U., Widmark,E., Heldin,E., Lind,P., Servenius,B., Lund,T., Flavell,R., Lee,J.S., Trowsdale,J., Schreier,P.H., Zablitsky,F., Larhammar,D., Peterson,P.A. and Rask,L. (1983) *Nucleic Acids Res.,* **11**, 5055-5071.
Ish-Horowicz,D. and Burke,J.F. (1981) *Nucleic Acids Res.,* **9**, 2989-2998.
Kan,Y.W. and Dozy,A.M. (1978) *Proc. Natl. Acad. Sci. USA,* **75**, 5631-5635.
Kataoka,T., Nikaido,T., Miyata,T., Moriwaki,K. and Honjo,T. (1982) *J. Biol. Chem.,* **257**, 277-285.
Kaufman,J.F., Auffray,C., Korman,A.J., Shackelford,D.A. and Strominger,J. (1984) *Cell,* **36**, 1-13.
Klein,J. (1975) *Biology of the Mouse Histocompatibility Complex,* published by Springer Verlag, Berlin, FRG.
Klein,J. and Figueroa,F. (1981) *Immunol. Rev.,* **60**, 23-57.
Klein,J.,Figueroa,F. and David,C.S. (1983) *Immunogenetics,* **17**, 553-596.
Kobori,J.A., Winoto,A., McNicholas,J. and Hood,L. (1984) *J. Mol. Cell. Immunol.,* **1**, 125-131.
Kronenberg,M., Steinmetz,M., Kobori,J., Kraig,E., Kapp,J.A., Pierce, C.W., Suzuki,G., Tada,T. and Hood,L. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 5704-5708.
Kvist,S., Roberts,L. and Dobberstein,B. (1983) *EMBO J.,* **2**, 245-254.
Larhammar,D., Hammerling,U., Denaro,M., Lund,T., Flavell,R.A., Rask, L. and Peterson,P.A. (1983) *Cell,* **34**, 179-188.
Lévi-Strauss,M., Tosi,M., Steinmetz,M., Klein,J. and Meo,T. (1984) *Proc. Natl. Acad. Sci. USA,* in press.
Lund,T., Grosveld,F.G. and Flavell,R.A. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 520-524.
Malissen,M., Hunkapiller,T. and Hood,L. (1983) *Science (Wash.),* **221**, 750-754.
Maloy,W.L. and Coligan,J.E. (1982) *Immunogenetics,* **16**, 11-22.
Mathis,D.J., Benoist,C.O., Williams,V.E., II, Kanter,M.R. and McDevitt, H.O. (1983) *Cell,* **32**, 745-754.
McNicholas,J., Steinmetz,M., Hunkapiller,T., Jones,P. and Hood,L. (1982) *Science (Wash.),* **218**, 1229-1232.
Mellor,A.L., Golden,L., Weiss,E., Bullmann,H., Hurst,J., Simpson,E., James,R., Townsend,A.R.M., Taylor,P.M., Schmidt,W., Ferluga,J., Leben,L., Santamaria,M., Atfield,G., Festenstein,H. and Flavell,R.A. (1982) *Nature,* **298**, 529-534.
Mellor,A.L., Weiss,E.H., Ramachandran,K. and Flavell,R.A. (1983) *Nature,* **306**, 792-795.
Mellor,A.L., Weiss,E.H., Kress,M., Jay,G. and Flavell,R.A. (1984) *Cell,* **36**, 139-144.
Michaelson,J., Boyse,E.A., Chorney,M., Flaherty,L., Fleisner,I., Hämmerling,U., Reinisch,C., Rosenson,R. and Shen,F.-W. (1983) *Transplantation Proc.,* **15**, 2033-2038.
Nei,M. and Li,W.-H. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 5269-5273.
Ollo,R. and Rougeon,F. (1983) *Cell,* **32**, 515-523.
Parnes,J.R. and Seidman,J.G. (1982) *Cell,* **29**, 661-669.
Robinson,R.R., Germain,R.N., McKean,D.J., Mescher,M. and Seidman, J.G. (1983) *J. Immunol.,* **131**, 2025-2031.
Schreier,P.H., Bothwell,A.L.M., Mueller-Hill,B. and Baltimore,D. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 4495-4499.
Shimizu,A., Takahashi,N., Yaoita,Y. and Honjo,T. (1982) *Cell,* **28**, 499-506.
Shreffler,D.C. (1981) in Hildemann,W.H. (ed.), *Frontiers in Immunogenetics,* Elsevier, Amsterdam, pp. 107-120.
Singer,P.A., Lauer,W., Dembić,Z., Mayer,W.E., Lipp,J., Koch,N., Hämmerling,G., Klein,J. and Dobberstein,B. (1984) *EMBO J.,* **3**, 873-877.
Snell,G.D., Dausset,J. and Nathenson,S. (1976) *Histocompatibility,* published by Academic Press, NY.
Spielman,R.S., Lee,J., Bodmer,W.F., Bodmer,J.G. and Trowsdale,J. (1984) *Proc. Natl. Acad. Sci. USA,* **81**, 3461-3465.
Steinmetz,M. and Hood,L. (1983) *Science (Wash.),* **222**, 727-733.
Steinmetz,M., Winoto,A., Minard,K. and Hood,L. (1982a) *Cell,* **28**, 489-498.
Steinmetz,M., Minard,K., Horvath,S., McNicholas,J., Frelinger,J., Wake, C., Long,E., Mach,B. and Hood,L. (1982b) *Nature,* **300**, 35-42.
Steinmetz,M., Stephan,D., Dastoornikoo,G.R., Gibb,E. and Romaniuk,R. (1985) in Lefkovits,I. and Pernis,B. (eds.), *Immunological Methods* Vol. **III**, in press.
Tosi,M., Lévi-Strauss,M., Duponchel,C. and Meo,T. (1984) *Phil. Trans. R. Soc. Lond. Ser. B,* in press.
Trowsdale,J., Lee,J., Carey,J., Grosveld,F., Bodmer,J. and Bodmer,W. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 1972-1976.
Wake,C.T., Long,E.O. and Mach,B. (1982) *Nature,* **300**, 372-374.
Weiss,E., Golden,L., Fahrner,K., Simpson,E., Hurst,J. and Flavell,R.A. (1983) *EMBO J.,* **2**, 453-462.
Winoto,A., Steinmetz,M. and Hood,L. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 3425-3429.
Widera,G. and Flavell,R.A. (1984) *EMBO J.,* **3**, 1221-1225.
Yokoyama,K., Stockert,E., Pease,L.R., Obata,Y., Old,L.J. and Nathenson, S.G. (1983) *Immunogenetics,* **18**, 445-451.

Received on 27 July 1984; revised on 29 August 1984