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Immunology

The *I* region of the C57BL/10 mouse: Characterization and physical linkage to *H-2K* of an SBβ-like class II pseudogene, *ψAβ3*

(class II β-chain genes/mouse SBβ analog/major histocompatibility complex evolution/chromosome walking)

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ABSTRACT **In the C57BL/10 mouse, 140 kilobases (kb) of the *I* region (*I-A*^{b}, *I-E*^{b}) were isolated as recombinant cosmids. The class II genes *Aβ2*, *Aβ1*, *Aα*, *Eβ1*, *Eβ2*, and *Eα* are located from centromere to telomere in a region of ≈110 kb, which shows that the *I* region in the *b* haplotype has a similar overall organization to those described for the *d*, *k*, and *wr7* haplotypes. In addition to these genes, we have also isolated a class II gene, *ψAβ3*, which is physically linked to the class I *H-2K* region, 75 kb telomeric to the *H-2K*^{b} gene. This orients the *H-2K* region on the genetic map with the *H-2K*^{b} gene being located toward the *I* region. The sequence of the β2 domain of *ψAβ3* is similar to the immunoglobulin-like domain of other class II genes. Interestingly, it shows 83% nucleotide homology to the human *SBβ* gene, the same homology that was seen previously between the immunoglobulin-like exons of *Aβ1* and *DCβ* and between *Eβ1* and *DRβ*, respectively. It is likely, therefore, that *ψAβ3* represents a member of a third SB-like class II gene family present in addition to *I-A* and *I-E* genes and that the divergence of the SB family predates the speciation of rodents and primates. Comparison of the DNA sequence of the exon encoding the β2 domain of *ψAβ3* in the *b* or *k* haplotypes with functional class II genes shows that a deletion of eight nucleotides has occurred, such that the *ψAβ3* sequence cannot be translated into a functional class II protein. This suggests that *ψAβ3* is a pseudogene.**

The I region of the murine major histocompatibility complex (MHC) encodes two well characterized class II glycoproteins, the I-A and I-E immune response (Ia) antigens. These polymorphic cell-surface proteins are involved in the regulation of the T-cell-dependent immune response and are expressed mainly on B cells and antigen presenting cells, such as macrophages and dendritic cells. Each of these two murine Ia antigen complexes (A and E) consists of an α chain of ≈35,000 Da and a β chain of ≈29,000 Da. The Aβ, Aα, and Eβ polypeptides are encoded in the *I-A* subregion, while the Eα chain maps to the *I-E* subregion of the murine MHC (H-2) class II region. In addition to the genes encoding these four polypeptides, other class II genes, *Eβ2* (1) and *Aβ2* (2), were revealed in the *I-E* and *I-A* subregions of the *H-2 I* region, respectively.

In contrast to these four class II β-chain genes, in two subregions described previously in the mouse, the class II region of the human MHC (HLA-D) contains at least seven β-chain genes in three subregions (*DR*, *DC*, and *SB*) (3). Protein and DNA sequence analysis made it possible to associate the human *DR* gene family with the *I-E* gene family in the mouse and the *DC* gene family with the *I-A* gene family. No corresponding class II gene family to the human *SB* subregion could be found in the mouse MHC until now, making it possible that either the evolution of class II subregions in mouse and man happened in a very different way or the separation of the third subregion in the human class II region is a recent event having occurred after the separation of the rodent and mammal lineages. The identification of an SBβ-like class II gene in the MHC of the mouse makes it likely that the organization and evolution of the class II regions in these two species might not be as different as previously thought.

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MATERIALS AND METHODS

Chromosome Walking. The construction of cosmid libraries, screening, and hybridization were done as described (4). The cloning of the *I* region was started from cosmid Iβ-101 (2), using the indicated gene probes to isolate cosmids Aβ22, Aβ9, and Aβ19 (Fig. 1*a*) as well as cosmids LS5, LS6, and LS11 (Fig. 1*b*). A 5.8-kilobase (kb) *Bam*HI fragment of cosmid Aβ19 (w) was used to isolate cosmid LS8. A 5′ gene probe of Eβ1^b (8), was used to isolate cosmids LS4/15 and LS3/15. Cosmids Eα1 and Eα2 were isolated by using 3′ and 5′ gene probes of the *Eα*^{d} gene from cosmid I^d-1 (5). The identification of the genes was done by sequence analysis for *Aβ2*, *Aβ1* (2), and *Eβ1* (8). *Eβ2* was identified by hybridization using a 3′ *Eβ1* gene probe; *Eα* was identified by hybridization using gene probes from the *Eα*^{d} gene (5); *Aα* was identified by hybridization using a DCα cDNA as probe (9). Chromosome walking in the *H-2K* region was performed toward the centromere from cosmid bm1-7, using probe *w2* to isolate cosmids LS1/1 and LS2/1; cosmids LB1 and LB2 were isolated by using probe w1. For walking toward the telomere, probe *w3* was used to isolate cosmids bm1-1/1 and bm1-3/1, which overlaps to the groups of cosmids isolated by using a 3′ probe of Aβ1 (LS5, LS6, LS11). The described cosmids are available upon request.

DNA Sequence Determination. Sequencing in *H-2*^{b} was done according to Maxam and Gilbert (10). The sequence around the eight-nucleotide deletion for *H-2*^{k} was determined directly from cosmid K1 (Fig. 1*b*) by using an oligomeric primer (21-mer) synthesized according to the sequence 5′ from this deletion in *H-2*^{b}. Computer analyses were done using programs described by Devereux *et al.* (11).

RESULTS AND DISCUSSION.

The *I* Region in the *b* Haplotype Mouse. The cloning of the *I* region of the C57BL/10 mouse revealed an organization of the class II genes in this haplotype similar to that described for the *H-2*^{d} and *H-2*^{k} haplotypes (1, 12)—i.e., *Aβ2*, *Aβ1*, *Aα*, *Eβ1*, *Eβ2*, and *Eα* map from centromere to telomere within ≈110 kb. In the *b* haplotype, the distance between *Aβ1* and *Aα* is ≈10 kb compared with 15 kb in the *H-2*^{d} and *H-2*^{k} haplotypes. A deletion might have occurred around the *Aβ1* gene because the *I-A* region is highly polymorphic (13, 14). A

Abbreviations: MHC, major histocompatibility complex; kb, kilobase(s).

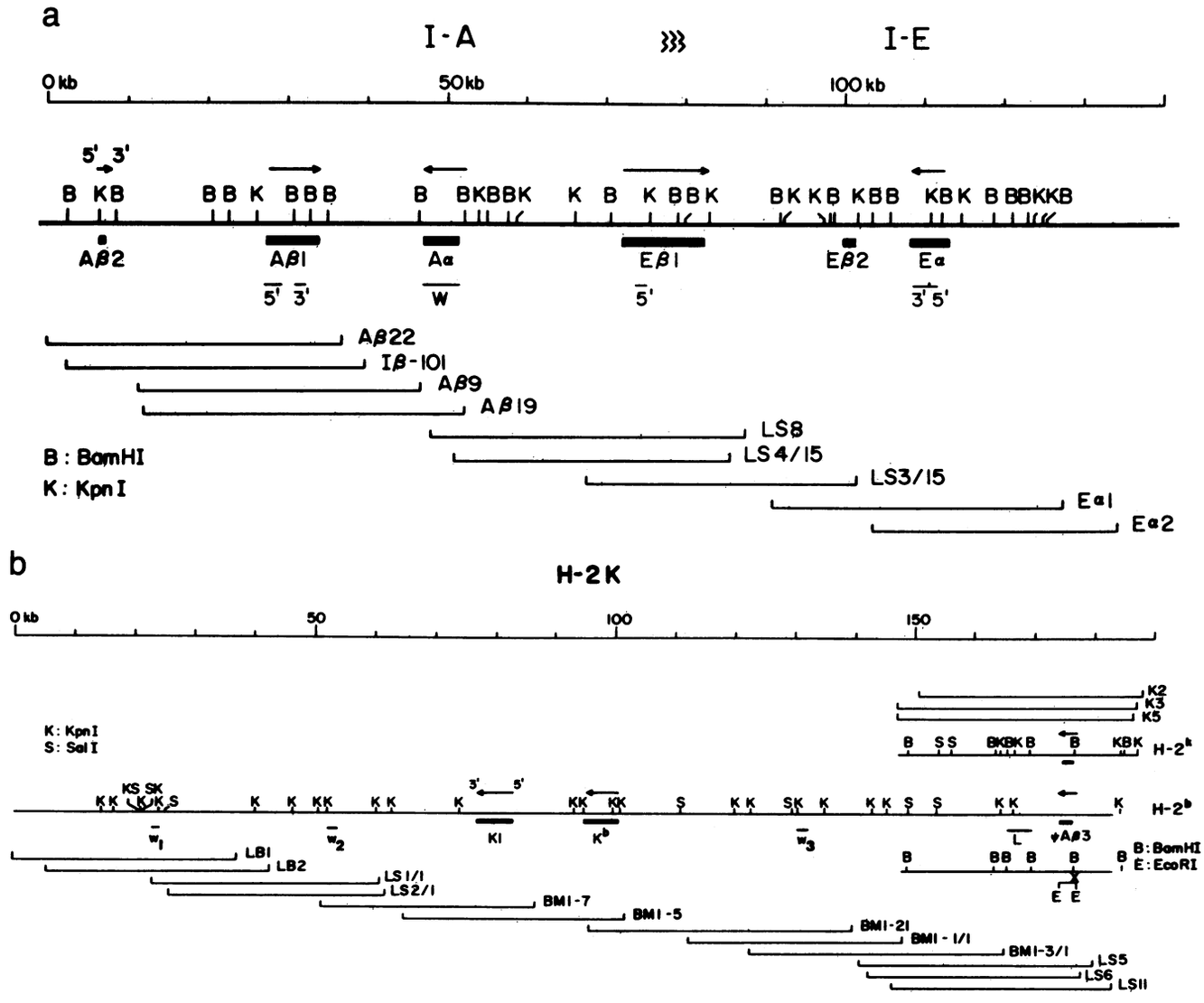


FIG. 1. Molecular maps of the *I* region and of the *H-2K* region of the C57BL/10 mouse. Class I and class II gene sequences are shown as solid bars; arrows indicate the direction of transcription, 5′ → 3′. The set of overlapping cosmids defining each region is given either above (*H-2*^{k}) or below (*H-2*^{b}) the restriction maps. The size scale in kb is drawn on the top lines. (*a*) *I* region, subdivided into the *I-A* and *I-E* subregions. Probes used in the genomic cloning are shown below the genes centromere to telomere, 5′ and 3′ gene probes from Aβ1^b from cosmid Iβ101 (2); w, 5.8-kb *Bam*HI fragment isolated from cosmid Aβ19; 5′ *Eβ*^{b} gene probe, isolated from cosmid LS8; 3′ and 5′ gene probes from *Eα*^{d}, isolated from cosmid I^d-1 (5). (*b*) *H-2K* region. Single-copy probes used for chromosome walking (w) are shown below the restriction map. w1 is a 0.6-kb *Kpn* I/*Sal* I border fragment of cosmid LS1/1; w2, a 0.3-kb *Pvu* II fragment, was isolated from an 8.5-kb *Kpn* I fragment of cosmid bm1-7 (6) and could be mapped to the left side of this fragment; w3 is a 2-kb *Kpn* I/*Rsa* I subfragment of a 4-kb *Kpn* I fragment from cosmid bm1-21 (6). L, a 4-kb *Bam*HI fragment of cosmid LS11, indicates the probe used to determine the genetic location of *ψAβ3* in recombinant inbred strains. *Bam*HI sites around ψAβ3 are shown below the gene as well as the 2.8-kb *Eco*RI fragment, which was used for sequence analysis after subcloning in pUC8 (7) (see Fig. 3). X indicates the 0.8-kb *Bam*HI/*Eco*RI fragment used to isolate the group of cosmids in *H-2*^{k}, which is shown with the restriction map above the map of *H-2*^{b}.

deletion between the *Aβ1* and *Aα* genes has also been observed in the *I* region of the B10.WR7 mouse (*H-2*^{wr7} haplotype) (14).

A Class II Sequence: *ψAβ3*. In addition to the gene cluster described above, a second group of cosmids was isolated that gave weaker hybridization to our *Aβ1* 3′ gene probe. Because these cosmids are overlapping (cosmids LS5, LS6, and LS11 in Fig. 1*b*), we assumed that a weak but specifically hybridizing class II sequence, which we call *ψAβ3*, was present.

To determine the genetic location of *ψAβ3*, we mapped polymorphic restriction sites by using two different intra-MHC recombinant inbred strains in which the recombination had occurred between the *H-2K* and *I-A* region. Strain B10.AQR carries the *H-2K* region of the q haplotype and the *I* region of the *k* haplotype; strain A.TL carries the *H-2K* region of the *s* haplotype and the *I-A*^{k} subregion. We used as a probe a single copy fragment located ≈5 kb to the 3′ side of *ψAβ3* (Fig. 1*b*). As shown by the hybridization patterns in Fig. 2*a*, the probe (and probably ψAβ3) maps to the *H-2K* region in B10.AQR and to the *I-A* subregion in A.TL. The recombination events between *H-2K* and *I-A* have therefore occurred at different sites in these two strains.

These data map *ψAβ3* between *Aβ2* in the class II region and the class I *K* gene. Chromosome-walking experiments from the *K* region were therefore performed in both directions (for details, see Fig. 1*b*) until the class II ψAβ3 cosmid cluster was linked to the class I *K* region. The distance between the *H-2K*^{b} gene and the *ψAβ3* sequence is ≈75 kb (Fig. 1*b*).

Orientation of the *H-2K* Region. The linkage of ψAβ3 to

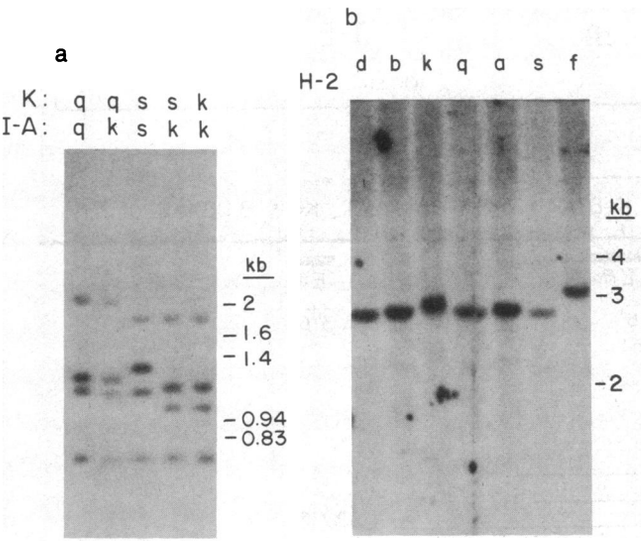


FIG. 2. Southern blot (15) analysis of different digests of murine DNA with single copy probes 3′ and 5′ from $\psi A\beta 3$. Scale is indicated on the right side. (*a*) *Pvu* II digest of DNA (10 μg per lane) of the following strains (from left to right): B10.G; B10.AQR; A.SW; A.TL; A. The *H-2* alleles for the *H-2K* regions and the *I-A* subregion in these strains are shown on top of each lane. The probe used, a 4-kb *Bam*HI fragment centromeric of ψAβ3, is indicated in Fig. 1*b* (L). (*b*) *Eco*RI digest of DNA of the following mouse strains (from left to right): BALB/c; B10; C3H; B10.G; B10.A; A.SW; B10.M. Ten micrograms of DNA was applied per lane (C3H, slightly more; A.SW, slightly less) and probed with a 0.8-kb *Eco*RI/*Bam*HI fragment, which is indicated (X) in Fig. 1*b*. The *H-2* haplotypes (16) are shown on top of each lane.

H-2K orients the *H-2K* cluster. The cloned *H-2K* regions from both the *b* haplotype (4, 6, 17) and the *d* haplotype (18) contain two class I genes in a head to tail configuration. Since the $\psi A\beta 3$ gene of the H-2K/ψAβ3 cluster maps in *I-A*, the *H-2K* gene must be proximal to the *I* region, as can be seen in Fig. 1*b*. In addition, this linkage of class I and class II regions makes it possible to look for other genes that have been previously mapped genetically between *K* and *I-A* (19).

The H-2K/ψAβ3 cluster has not yet been physically linked to the I-A/I-E cluster. The *I*-region cluster from the *d* haplotype extends ≈30 kb beyond our cluster in the centromeric direction (12, 13). These 30 kb from $H\text{-}2^d$ do not appear to overlap with our $\psi A\beta 3$ cluster from $H\text{-}2^b$ based on restriction maps (14). The distance between $\psi A\beta 3$ and $A\beta 2$ is therefore at least 50 kb.

$\psi A\beta 3$ Represents the Mouse Analog to the Human $SB\beta$ Gene. The nucleotide sequence of part of the $\psi A\beta 3$ gene was determined by sequencing regions of a 2.8-kb *Eco*RI subclone of the cosmid LS11. A murine $A\beta 1$ genomic probe containing the β2 and transmembrane exons hybridized to a segment identified from its sequence as the β2 domain of $\psi A\beta 3$. The region containing the β1 exon was detected by hybridization with a probe from a human DCβ cDNA. In fact, sequence analysis showed that the class II gene with the highest homology to $\psi A\beta 3$ is the $SB\beta$ gene (see below).

In Fig. 3*a* we compare the nucleotide sequence of $\psi A\beta 3$ to that of the human $SB\beta$ gene by using the dot-matrix method. There is an obvious homology in the exon encoding the β2 domain. The gap in the dot matrix is caused by a deletion of eight nucleotides in $\psi A\beta 3$ (Fig. 3*b*). This deletion causes a shift in reading frame that precludes the synthesis of a functional class II protein. Terminaton codons in all frames follow this deletion and hence make it likely that $\psi A\beta 3$ is a pseudogene.

Homology can clearly be detected between the regions of the $\psi A\beta 3$ and $SB\beta$ genes that include the exon encoding the β1 domain (Fig. 3*a*). However, all three reading frames in this region of $\psi A\beta 3$ contain stop codons, and we could not detect convincing putative promoter or signal sequences ("CAT" and "TATA") in the appropriate area indicated by the dot-matrix (data not shown).

Table 1 shows the degree of homology of the β2 domains among the murine and human class II β-chain genes. Previous sequence comparisons have shown that the murine *I-E* genes are most homologous to the human *DR* genes and the murine *I-A* genes are most homologous to the human *DC* genes (3, 24). No gene family has previously been found in other species that is homologous to the *SB* gene family. Interestingly, $\psi A\beta 3$ is most homologous to the human $SB\beta$ gene at both the nucleotide level (83%) and the amino acid level (75%). The nucleotide homology between the β2 exons of $\psi A\beta 3$ and $SB\beta$ is the same as that found between $A\beta 1$ and $DC\beta$ and between $E\beta 1$ and $DR\beta$, respectively.

It was previously reported that the human $SB\beta$ gene was most homologous to the murine $E\beta 2$ gene, based on the hybridization of an $SB\beta$ gene probe to a single 2.9-kb genomic *Eco*RI fragment and to a 2.9-kb *Eco*RI fragment of an Eβ2-containing cosmid clone from $H\text{-}2^d$ mice (24). Since the nucleotide sequence of $E\beta 2$ shows that $SB\beta$ is about equally related to $E\beta$, $A\beta$, and $E\beta 2$ (25), and since we show here that $\psi A\beta 3$ is most homologous to $SB\beta$, we assume that the 2.9-kb genomic *Eco*RI fragment obtained after hybridization with $SB\beta$ is in fact the 2.8-kb $\psi A\beta 3$-containing *Eco*RI fragment and not the $E\beta 2$ as proposed (24).

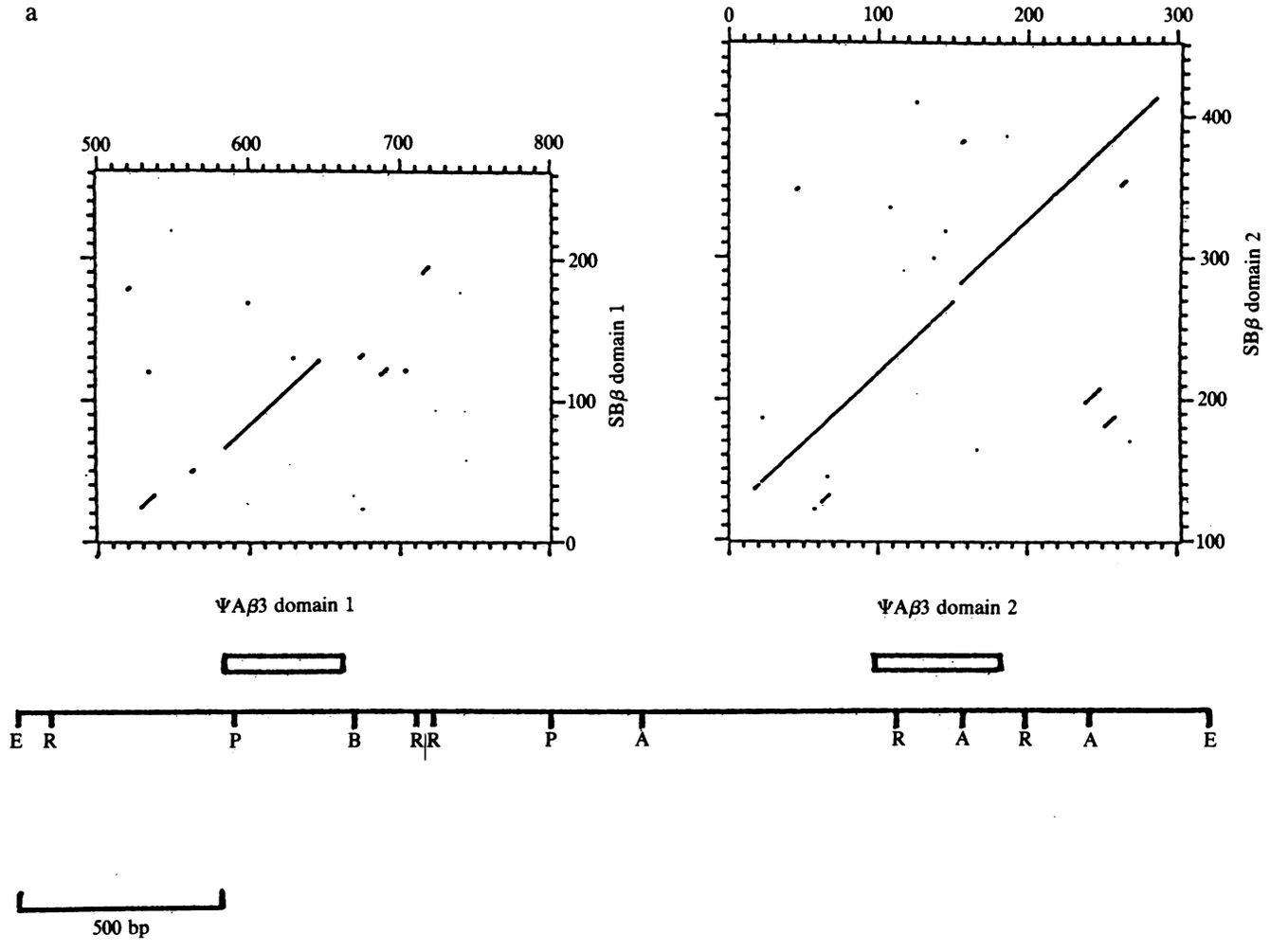
Evolution of Class II Genes in Man and Mouse. The evolution of class II genes of the MHC might have occurred by duplication events of an ancestral unit coding for the α- and β-polypeptide chains. In man, two duplications or a triplication would have been necessary to give rise to the *DR*, *DC*, and *SB* subgroups. Until now, only two class II subgroups, *I-A* and *I-E*, were identified in the mouse. The discovery of $\psi A\beta 3$ and its strong homology to $SB\beta$ suggests that the same two duplication steps have occurred in mouse and therefore that these events predate the separation of the rodent and primate lineages. To determine whether $\psi A\beta 3$ is present in murine haplotypes other than $H\text{-}2^b$ we hybridized genomic DNA of *d*, *k*, *q*, *f*, and *s* haplotypes with a 5′ $\psi A\beta 3$ probe. Fig. 2*b* shows that $\psi A\beta 3$ is present in all haplotypes tested.

The mutation of $\psi A\beta 3$ to a pseudogene might be a recent event and therefore may not have occurred in all strains of mice. For example, the $E\alpha$ gene is nonfunctional in the $H\text{-}2^b$ haplotype but is expressed in several others such as $H\text{-}2^d$ and $H\text{-}2^k$. To determine whether $\psi A\beta 3$ is a pseudogene in the *k* haplotype, we cloned this region of the chromosome of the *k*

Table 1. Nucleotide and amino acid homologies between human and murine class II β-chain second domain exons

% homology	$A\beta 3^b$	$A\beta 2^b$	$A\beta 1^b$	$E\beta^b$	DCβ	DRβ	SBβ
$A\beta 3^b$	—	66	74	67	73	70	83
$A\beta 2^b$	54	—	66	71	71	73	69
$A\beta 1^b$	65	59	—	71	83	71	75
$E\beta 1^b$	58	64	64	—	75	83	78
DCβ	68	63	81	70	—	72	78
DRβ	60	65	67	83	67	—	77
SBβ	75	62	70	69	74	73	—
			Amino acids				

Numbers below the diagonal show the amino acid homology, numbers above the diagonal indicate the homology on the nucleotide level. The compared sequences derive from the following sources: ψAβ3, this paper; $A\beta 2^b$ and $A\beta 1^b$, Larhammar *et al.* (2); $E\beta 1^b$, Widera and Flavell (8); DCβ, Larhammar *et al.* (22); DRβ, Long *et al.* (23); SBβ, Roux-Dosseto *et al.* (21).



b

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                                    5                  10                  15                  20                  25                  30
                          Arg Gly Arg Val His Val Ser Pro Ser Arg Lys Val Thr Leu Lys His His Asp Leu Leu Val Cys His Val Thr Asn Phe Tyr Pro
ψAβ3b  CCTTTTCAACTCCAGG TC CGG GGT AGG GTA CAT GTA TCA CCC TCC AGG AAG GTC ACC CTG AAG CAC CAT GAC CTG CTG GTC TGC CAT GTG ACA AAT TTC TAC CCA
                         !! ! !   ! !!! !!  !! !!  !!! !!! ! ! !!! !   !!  !!  !!  !!! !!  !!  !!! !!  !!! !!! !!  !!! !!  !!  !!! !!! !!!
SB8                      TC CAG CCT AGG GTG AAT GTT TCC CCC TCC AAG AAG GGG CCC TTG CAG CAC CAC AAC CTG CTT GTC TGC CAC GTG ACG GAT TTC TAC CCA
                            Gln Pro --- --- Asn --- --- --- --- Lys --- Gly Pro --- Gln --- --- Asn --- --- --- --- --- --- --- Asp --- --- ---

                    35                  40                  45                  50                  55                  60
                Gly Val Ile Gln Val Gln Trp Phe Trp Asn Ser Gln Glu Glu Thr          Leu Ser Thr Ser Leu Ile TER Asn Gly Asp Trp Thr Phe Gln Ile Leu
ψAβ3b           GGC GTC ATT CAA GTC CAA TGG TTC TGG AAT AGC CAG GAG GAA AC           TC CTG TCC ACC AGC CTG ATC TGA AAT GGG GAC TGG ACC TTC CAG ATT CTG
                !!!  !  !!! !!! !!! ! ! !!! !!!  ! !!! !  !!! !!! !!! !!        !! !! !!! !!! ! ! !!! !!!  !  !!! !!  !!! !!! !!! !!! !!! !!  !!!
SB8             GGC AGC ATT CAA GTC CGA TGG TTC CTG AAT GGA CAG GAG GAA ACA GCT GGG GTC GTG TCC ACC AAC CTG ATC CGT AAT GGA GAC TGG ACC TTC CAG ATC CTG
                --- Ser --- --- --- Arg --- --- Leu --- Gly --- --- --- --- Ala Gly Val Val --- --- Asn --- --- Arg --- --- --- --- --- --- --- --- ---

                65                  70                  75                  80                  85                  90                  94
                Phe Met Leu Glu Ile Thr Pro Gln Gln Gly Asp Val Tyr Ala Cys His Val Glu His Pro Ser Leu Glu Ser Pro Ile Thr Val Glu Trp
ψAβ3b           TTC ATG CTG GAG ATA ACT CCA CAG CAG GGA GAT GTG TAT GCC TGC CAC GTG GAG CAC CCC AGC CTG GAG AGC CCC ATC ACA GTA GAG TGG A GTGAGTCACCCC
                 !  !!! !!! !!  !!  !!  !!  !!! !!! !!! !!! !!  !!  !!  !!  !!! !!! !!  !!! !!! !!! !!  !!  !!  !!  !!! !!! !!! !
SB8             GTG ATG CTG GAA ATG ACC CCC CAG CAG GGA GAT GTC TAC ACC TGC CAA GTG GAG CAC ACC AGC CTG GAT AGT CCT GTC ACC GTG GAG TGG A
                Val --- --- --- Met --- --- --- --- --- --- --- --- Thr --- Gln --- --- --- Thr --- --- Asp --- --- Val --- --- --- ---
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FIG. 3. Nucleotide sequence comparison between *ψAβ3* and *SBβ*. (*a*) Diagonal dot-matrix comparison (11) of the nucleotide sequence of *ψAβ3* and the nucleotide sequence of domain 1 (20) and domain 2 (21) exons of *SBβ*. Window, 21; stringency, 14. Locations of the *ψAβ3* sequences used for the dot-matrix comparison on the 2.8-kb *Eco*RI fragment from cosmid LS11 (Fig. 1*b*) are shown below the dot plots; orientation of the *Eco*RI fragment is in 5′ → 3′ direction for *ψAβ3*. E, *Eco*RI; R, *Rsa* I; P, *Pst* I; B, *Bam*HI; A, *Ava* I. (*b*) Aligned nucleotide and translated amino acid sequences of the *ψAβ3* second domain exon and the *SBβ* second domain exon. Arrows indicate splice junctions deduced from different class II second domain exons (3). Matching nucleotides are indicated (!); differing amino acids are shown for *SBβ*. Matching nucleotides to $\psi A\beta 3^k$ are underlined, the only differing nucleotide is indicated (*H-2*^{b}, G; *H-2*^{k}, A).

haplotype by using a 5′ *ψAβ3* probe. The cosmids obtained show very similar DNA organization to the *H-2*^{b} cluster (Fig. 1*b*). Using an oligonucleotide primer and the dideoxy sequencing procedure (26), we revealed the same 8-base-pair deletion (Fig. 3*b*). Hence the *ψAβ3* gene of the *H-2*^{k} haplotype is probably also a pseudogene.

With the discovery of *ψAβ3*, there are now five class II β-chain sequences (including one pseudogene) defined in the MHC of the mouse compared with seven class II β-chain sequences [including at least one pseudogene (27)] described for the human MHC (3). Using a 3′ probe of the DRβ cDNA, we have also isolated two overlapping cosmids containing a putative class II gene that maps to the *S* region of the MHC (C. T. Wake, personal communication). This may mean that the MHC of the mouse might contain additional class II β-chain genes that have diverged more extensively than the

class II β-chain genes in the human MHC and that therefore might be more difficult to detect by using DNA·DNA hybridization. This suggests that the organization of the class II genes of the murine and human MHC may not be as different as had been supposed (3).

Note Added in Proof. According to the new nomenclature DC is now DQ and SB is DP.

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