Structure and Expression of Class I Genes of the Mouse Major Histocompatibility Complex

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The major histocompatibility complex (MHC) plays an important role in the regulation of the immune response in vertebrates. The MHC in man (*HLA*) and the mouse (*H-2*) is the most thoroughly studied case of a complex of linked genes (see [1] for a review). The *H-2* locus maps on the murine chromosome 17 (Fig. 1). Three different types of proteins are encoded by the *H-2* region. The class I molecules regulate the killing of virus-infected cells. The virus antigen is recognized in association with class I proteins. This is known as MHC restriction of T-cell recognition [2]. The best-characterized class I molecules are the classical transpantation antigens found on virtually all cells, encoded by the *H-2K*, *H-2D*, and *H-2L* loci. Class I antigens are also encoded by the *TL* complex, adjacent to the classical *H-2* loci. The Qa and Tla proteins are lymphoid differentiation antigens. Class I molecules are intrinsic membrane proteins with molecular weights (MW) of 40,000–45,000. They are associated with a smaller polypeptide, β_2 microglobulin, MW 12,000, not encoded by chromosome 17. The class II genes were originally discovered as immune-response genes. They regulate the interaction of T-helper cells and B cells to induce antibody production [3]. The class III molecules encoded by the *H-2* region are complement components. One of the most remarkable properties of the H-2 antigens is their genetic polymorphism. In fact, about 50 alleles at both the *H-2K* and *H-2D* loci have been detected. In this aspect, they differ sharply from almost all other genes. We are particularly interested in how the H-2 polymorphism is generated. We think it likely that the analysis of the class I genes by recombinant DNA techniques will provide some clues to the nature of the polymorphism.
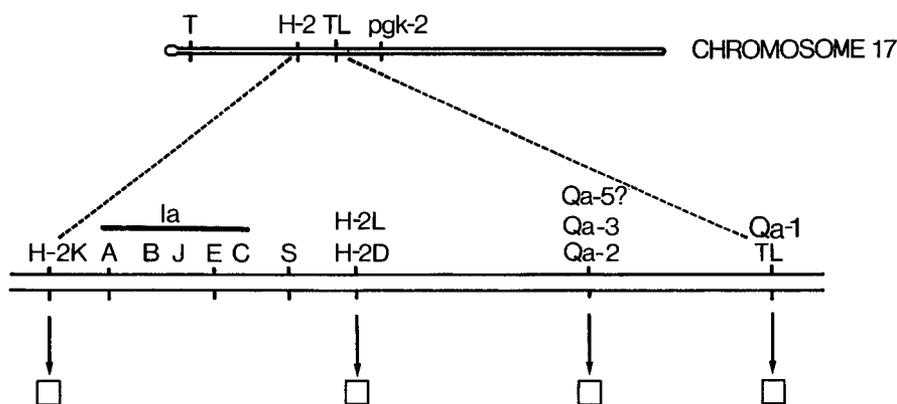

Fig. 1. Genetic map of the murine *H-2* and associated genetic loci. The *top line* shows a diagram of chromosome 17 with the centromere on the *left*. The *bottom line* shows an expanded diagram of the regions between the *H-2K* locus on the *left* and the *TL* locus on the *right*. H-2 class I molecules (□) are expressed from at least four separate genetic loci as shown by the *arrows* below the *line*
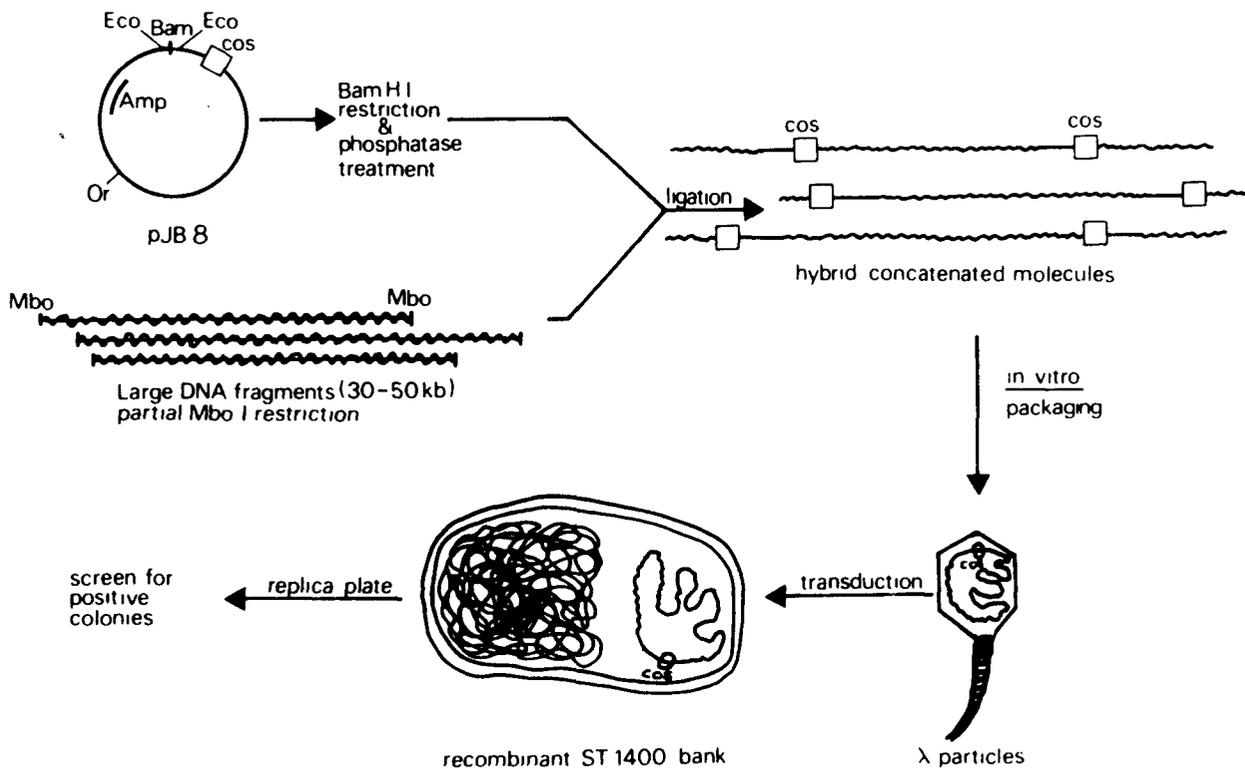
Fig. 2. Scheme for the cosmid cloning techniques

We isolated genes from the mouse major histocompatibility complex by cloning the genome of the B10 mouse (C57/BL10) H-2^b haplotype, in a cosmid library [4, 5]. The cosmid cloning technique has the advantage that large fragments of DNA (ca. 40,000 bps) are cloned and it is easy to obtain large amounts of recombinant DNA. The principle of the cosmid cloning technique [6] is shown in Fig. 2. Chromosomal spleen DNA was partially digested with the restriction enzyme MboI and the digested DNA size-fractionated on a density gradient. The MboI recognizes a four-bp-restriction site (GATC) and cuts the genome frequently. Specific DNA sequences are, therefore, randomly distributed over the gradient. The MboI-restricted fragments can be cloned into the Bam HI restriction site (G GATC C). Fragments of ca. 40,000 bps are ligated into the Bam site of a vector. A minimal requirement for a cosmid vector is that it contains the cohesive ends of phage λ (cos) and an antibiotic resistance marker for selection in bacteria. The vectors we used to construct the libraries [5] also contained another enzyme gene for selection in eukaryotes. The ligated DNA is packaged in vitro via the cos sequences into λ-particles and transfected in-

to recA$^-$ Escherichia coli cells. Once inside the bacterium, the DNA propagates as a plasmid. The complexity of the library was such that the sum of the bacterial colonies covered the murine genome at least four times. Two B10 libraries were constructed, one with the vector pOPF, the other with pTM. pOPF contains the thymidine kinase gene of herpes virus. Cosmids with this vector can be directly transfected into mouse L cells (tk$^-$) and transformants selected in hypoxanthine-aminopterin-thymidine medium. pTM contains the aminoglycoside phosphotransferase gene which makes eukaryotic cells resistant to the drug G418.

We screened the libraries with a human genomic class I gene probe (pHLA 12.4 [7])

Table 1.

Region	No. cosmids	kb	No. genes	Location
1	7	95	2	H-2K
2	2	70	1	H-2D
3	15	120	5	Qa2, 3
4	1	40	1.5	Qa2, 3
5	2	50	2	?
6	39	90	5	TL

or with mouse H-2 cDNA clones (pH2-III, pH2-IIa [8]). We isolated 82 cosmids containing class I related genes and began a detailed analysis of the structure, sequence organization, and expression of the *H-2*-related genes. By restriction enzyme and southern blotting analysis, the cosmid clones can be grouped into five clusters. We localized each cluster and some single cosmids by polymorphic restriction site mapping to distinct regions on the *H-2* map. The result of these experiments is shown in Table 1. Figure 3 shows in detail how we mapped region *6*, which contains the largest number of cosmids, to the *TL* locus. When a single copy 2.0-kb EcoRI fragment that maps 5' to the gene *6A* in the region [5] is hybridized to PstI-digested DNA, the fragment detects bands of different size in all three haplotypes (*b, d, k*). AKR DNA (*H-2^k*) shows a 3.7-kb band, whereas in B10 DNA (*H-2^b*) the 2.0-kb fragment lights up. The H-2 recombinant mouse strain B6K2 DNA contains a 3.7-kb band and our fragment, therefore, detects the *H-2^k* haplotype portion of the recombinant, that is the *TL* locus in B6K2. Region *6* maps to the *TL* locus.

Table 1 shows that from the 82 cosmids containing *H-2*-like genes, two regions map to the loci controlling the expression of the classical transplantation antigens, H-2K and H-2D. The majority of the genes are localized outside the *H-2* locus proper, six genes mapping to the *Qa* locus and five genes to the *TL* locus. It is interesting that we found more class I related gene sequences than there are class I products on the genetic map. A similar distribution of class I genes in the BALB/C mouse (*H-2^d*) was described by Steinmetz et al. [9]. There are not enough class I genes in the *H-2* region to account for all different alleles.

To examine which of the cosmid clones contains a functional gene we introduced all the cosmids into mouse L cells [10] and selected for stable, thymidine-kinase-positive transformants. We tested for expression of new H-2^b cell surface antigens on the transformed cells using the following assays: (a) direct monoclonal and alloantibody binding; (b) antibody-dependant complement-mediated lysis; (c) alloreactive anti-H-2K^b and anti-H-2D^b T-cell mediated lysis (CML); (d) H-2K^b-
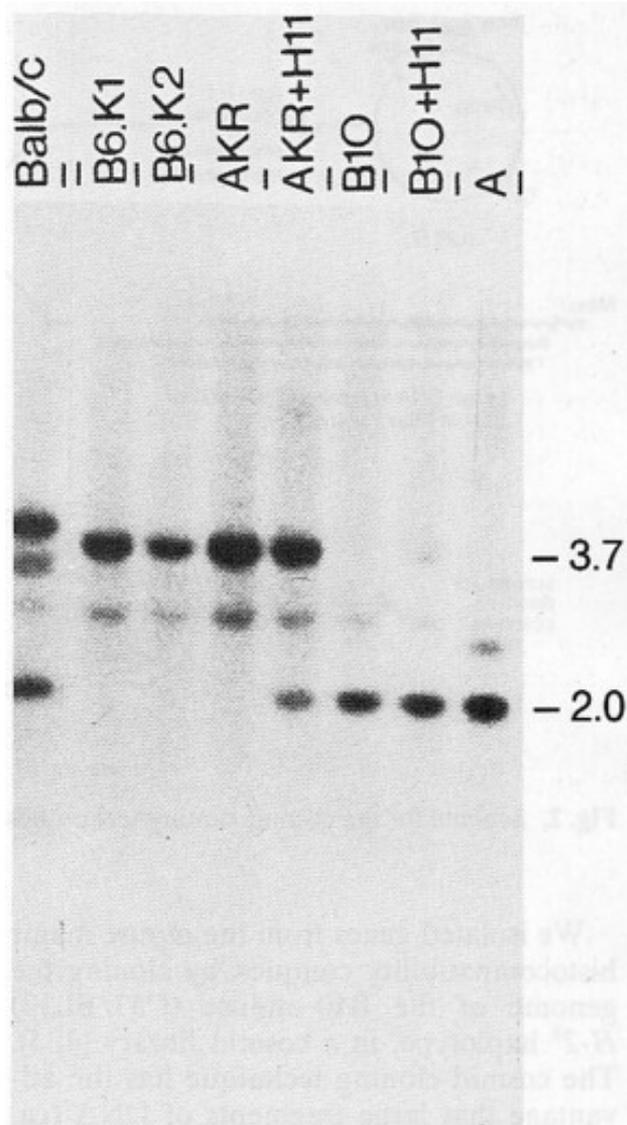

Fig. 3a. Chromosomal blot localizing region *6;* DNA was digested with PstI and probed with the 2.0-kb Eco-fragment from region *6*. In lanes indicated, 100 pg cosmid HII and 5 μg chromosomal DNA was digested with Pst I
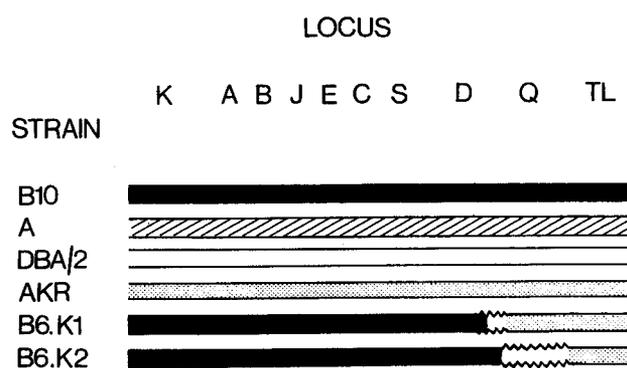

Fig. 3b. Genetic map of the recombinant strains. *Jagged section* indicates area where chromosome is derived from either B10 or A

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Table 2. Immunoselection assays for L cells transformed with cosmids containing class I genes

Assay	Ltk⁻	B10	LH8	LH8EcoB	LH25	LH11	LH1.1
Antibody binding							
Anti-H-2^k	+	−	+	+	+	+	+
Anti-H-2K^b	−	+	+	+	−	−	−
Anti-H-2D^b	−	+	−	−	−	−	+
Antibody-dependant cytotoxicity							
Anti-H-2^k	+		+		+		
Anti-H-2^b	−		+		−		
Anti-H-2K^b	−		+		−		
Anti-H-2D^b	−		−		−		
Allogenic cytotoxic T-cell killing							
Anti-H-2^k	+	−	+	+	+	+	
Anti H-2^b	−	+	+	+	−	−	
T$_c$-cell-mediated killing of transformed L cells infected with influenza virus							
H-2K^b-restricted	−	+	+				−
H-2D^b-restricted	−	+	−				+

Ltk, mouse L cells (H-2^k) used for transfection experiments; B10, spleen cells of B10 mouse, H-2^b control cells; LH8, L cells transformed with cosmid H8 containing the *A* and *B* gene of region *1*; LH8EcoB, L cells transformed with an Eco subclone of H8, containing only the *B* gene of region *1*; LH25, L cells transformed with cosmid H25 containing the *A* gene of region *1*; LH11, L cells transformed with cosmid H11, containing the *A, B,* and *C* genes of region *6*; LH1.1, L cells transformed with cosmid H1.1 coding for the *H-2D^b* gene of region *2*

and H-2D^b-restricted influenza-virus-specific T-cell killing.

To date we have obtained positive expression data only for the *H-2K^b* [10] and *H-2D^b* [11] genes. The results are shown in Table 2. The newly expressed H-2 antigens are not only recognized by allo- and monoclonal antibodies, they also retain their in vivo function. They are recognized by alloreactive cytotoxic T cells and act as restriction elements for T$_c$-cell-mediated killing of influenza-virus-infected cells. The

Fig. 4. Comparison of the DNA and protein sequence of the *H-2K^b* gene, *H-2K^{bml}* gene, and *H-2L^d* gene [19, 20] starting from amino acid 145 to 163. The recognition sequences for the enzyme PstI and HinfI are *underlined.* Only the nucleotides and amino acid differences from *H-2K^b* are shown in the *H-2K^{bml}* and *H-2L^d* gene

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gene *B* of region *1* codes for the H-$2K^b$ antigen and the single gene of region *2* is the H-$2D^b$ gene. The fact that the full complement of epitopes normally associated with the H-2K^b and the H-2D^b molecule is present on the surface of transformed cells makes it possible to analyse which determinants are recognized by antibodies and the T-cell receptor. Specifically, it should now be possible to transform L cells with hybrid *H-2* genes constructed in vitro.

We [12] and others [13] proposed gene conversion as a mechanism of generating different *H-2* alleles. Gene conversion is a mechanism by which genetic information can be transferred from one gene to another related gene; it was first described in fungi [14, 15]. One way to obtain information about the mechanism that generates polymorphism is to compare the DNA sequences of allelic genes. As the alleles are presumably the result of many gene conversion events, it might be difficult to trace individual sequences in the mosaic genes. We therefore decided to analyse a very recent allele, which was detected in one of the Bm- mutants [16]. These mutant mice (*H-2^b* haplotype background) express a changed H-2K antigen.

We cloned the H-$2K^{bml}$ gene [17], the antigen it codes for was shown by peptide analysis to contain Tyr-Tyr at position *155* and *156* instead of Arg-Leu. We sequenced the H-$2K^b$ gene [18] and the translated regions of the H-$2K^{bml}$ gene. We found in total seven bp changes (Fig. 4) in a short region of 13 nucleotides, two result in another amino acid substitution as well as the two amino acid changes at positions *155* and *156*. Our data show that these changes occurred in the H-$2K^b$ gene. The same sequences are found in the H-$2L^d$ gene [19, 20]. We consider that a gene conversion event is the most likely cause for this phenomenon. In this case, there must be a donor gene that contains this novel nucleotide sequence. We are currently searching for a donor gene. We think it likely that gene conversion will turn out to be a major force if not *the* major force in the generation of new alleles of *H-2* genes.

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