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SPECIAL LECTURES

Molecular Cloning and Analysis of H-2 Class I Genes of H-2^b Haplotype Mice

A. L. Mellor, L. Golden, E. Weiss, H. Bullman, H. Bud, and R. A. Flavell

WE HAVE cloned H-2 class I genes of the C57BL/10 (B10, H-2^b haplotype) mouse using cosmid cloning techniques developed in our laboratory.[1] Cosmids, which contain 40–45 kilobasepairs (Kbp) of mouse DNA per clone, were chosen for this project, since there are a large number of class I genes (20–35 per haploid genome) spread over a large region (about 1.5 cM or 1000 Kbp of DNA) mapping between the H-2K and TL loci on chromosome 17 (see Klein[2] for a review of H-2 genetics). Cosmids containing class I genes were isolated from cosmid libraries using human (HLA) genomic or mouse cDNA gene probes. In total, we have isolated 82 cosmids containing class I genes. Most, but not all, of these cosmids have been organized into 5 distinct clusters of cosmids containing overlapping mouse DNA on the basis of restriction enzyme mapping and Southern blot hybridization (Table 1). Together, these clusters define 15 unique class I gene regions. The remaining nonclustered cosmids, for which no overlaps have been detected, may account for a further 3 or 4 class I gene regions. Clearly then, there are many more H-2 class I gene regions than there are serologically characterized H-2 class I gene products. It remains to be seen whether these additional gene regions are silent pseudogenes or whether there are more H-2 class I proteins that remain undetected.

The DNA regions cloned in each cosmid cluster have been mapped to one of the four genetic loci known, from immunological analysis, to control the expression of class I cell surface antigens in the B10 mouse (H-2K, H-2D, Qa-2/3 and TL). To assign specific DNA regions to specific genetic loci, we exploited the extensive restriction enzyme polymorphisms that are detected between different H-2 inbred strains of mice using a Southern blotting procedure. Unique mouse DNA fragments (which therefore do not contain class I gene sequences or mouse repetitive DNA elements) were isolated from each cosmid cluster and used as hybridization probes on total digested DNA from H-2^b (B10 or B6), H-2^d (BALB/c), H-2^k (AKR), and H-2 recombinant inbred mouse strains. Strain-specific restriction enzyme polymorphisms can thus be used to map hybridizing bands to specific genetic loci defined by recombinant inbred mouse strains. As shown in Table 1, most of the 15 genes defined in the clusters map to the Qa or TL loci; only 3 genes map to the "classical" H-2K and H-2D loci. This result is surprising, given the extreme antigenic polymorphism that is detected at these later two loci[2] and the relatively low antigenic polymorphism at the Qa and TL loci.[3,4] This observation has led to speculation that Qa and/or the TL gene regions are able to modify the genes at the H-2K and H-2D loci by donating short segments of new DNA sequence into these genes, resulting in polymorphism. This phenomenon, which is

Table 1.

Cluster	No. of Cosmids	Kbp	No. of Genes	Location
1	7	95	2	H-2K
2	2	80	1	H-2D
3	20	120	5	Qa-2/3
4	10	80	2	Qa-2/3 or TL
5	39	90	5	TL
Totals	78	465	15	

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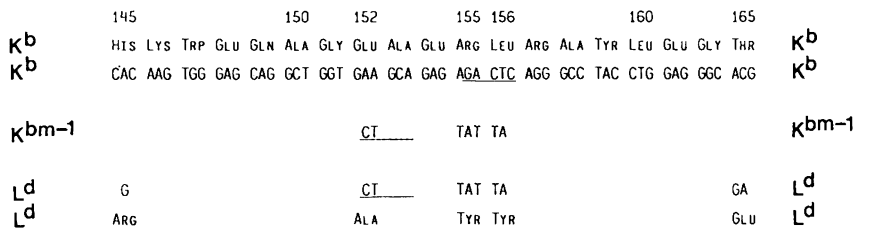

Fig. 1. DNA and amino acid sequences of the H-2K^b and H-2K^{bm1} gene in exon 3. The K^{bm1} sequence has 7 changed bases that result in 3 amino acid changes at positions 152, 155, and 156. These changes appear in the H-2L^d gene at this position. The base changes also cause the loss of a Hinf1 and the gain of a Pst I restriction enzyme site (underlined bases) in the K^{bm1} gene.

brought about by an as yet undefined mechanism, has been loosely termed "gene conversion"[5] and has precedents in fungal genetics, and more recently, has been proposed to explain one case of partial homology in the human embryonic Aγ and Gγ globin genes.[6] A suitable model system to study such gene conversion events in the H-2 class I genes is apparently provided by the bm series of mutants,[7] which have multiple amino acid changes in the H-2K^b polypeptide sequence arising out of a single genetic event. To study this phenomenon further, we have isolated cosmid clones from the H-2K region (corresponding to cluster 1 in Table 1) of the bm1 mutant, which has two amino acid changes (155, Arg → Tyr; 156, Leu → Tyr) compared to the H-2K^b polypeptide. Although the organization and gross restriction maps of the H-2K cluster in the B10 and bm1 mutant are identical, DNA sequence analysis of the third exon of the H-2K^b and H-2K^{bm1} genes shows that there are 7 nucleotide changes in the mutant gene (Fig. 1). No other nucleotide changes have been detected. The changes, as well as accounting for the amino acid changes predicted at positions 155 and 156, also cause an amino acid change at position 152 (Glu → Ala), which, presumably, went undetected in the protein analysis. The clustered nature of the nucleotide changes is consistent with the view that a highly localized gene conversion event took place in the H-2K^b gene to generate the mutant allele. In this case, we noted that the "donor" sequence for such an event is identical to the sequence of the H-2L^d gene[8,9] in this region. This raises the possibility that a gene with an identical H-2L^d-like sequence in this region is present in the B10 genome and acts as a donor gene for the generation of the bm1 mutant gene. This donor gene is probably located in the Qa or TL regions, since neither of the other two genes in the H-2K (unpublished results) or H-2D[10] loci contain the correct donor sequence.

Pertinent to this assumption is our finding that all of the genes in cluster 3 (which maps at the Qa-2/3 locus) have 5′ and 3′ flanking sequences that cross-hybridize with the equivalent flanking sequences of the H-2K^b and a closely linked gene in the same cluster. Indeed, we have noticed from this and restriction site similarities that the pair of linked genes at the H-2K locus are remarkably similar to two pairs of linked genes in cluster 3, suggesting that duplications of the gene pair occurred in mouse evolution. This is intriguing, since the genetic organization of the mouse-H2 complex is unique among mammals, as the H-2K locus is centromeric to the I region (class II). Perhaps then, the class I genes at the H-2K locus in mice were duplicated from genes at the Qa-2/3 locus and translocated to their present position early in mouse evolution.

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