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Immunology

Genetic mapping in the major histocompatibility complex by restriction enzyme site polymorphisms: Most mouse class I genes map to the *Tla* complex

(Southern blotting)

ASTAR WINOTO, MICHAEL STEINMETZ, AND LEROY HOOD

Division of Biology, California Institute of Technology, Pasadena, California 91125

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ABSTRACT **From a genomic library constructed from sperm DNA of the inbred BALB/c mouse, we previously isolated 54 cosmid clones that contain 36 class I genes and can be divided by restriction map analyses into 13 gene clusters. We have isolated single- and low-copy DNA probes from each of these clusters to visualize restriction enzyme site polymorphisms in the DNAs from various congeneic and recombinant congeneic mice. These polymorphisms permit us to map each of the 13 cosmid clusters to a precise location in the major histocompatibility complex of the mouse. Thirty-one of 36 class I genes map into the *Tla* complex of the major histocompatibility complex whereas the remaining 5 genes map to the *H-2* complex. Thus, all 36 class I genes are located in the major histocompatibility complex. Analysis of the number of restriction enzyme fragments visualized by the single- and low-copy DNA probes suggests that the class I genes in different inbred strains of mice probably undergo gene duplications and deletions, presumably by homologous but unequal crossing-over.**

The major histocompatibility complex (MHC) of the mouse contains at least three gene families, denoted class I, class II, and class III (1–4). The molecules encoded by the class I genes fall into two categories by virtue of difference in cellular distribution, the extent of their serological polymorphisms, and their functions. The class I genes designated *K*, *D*, and *L* encode transplantation antigens that are found on the cell surface of most nucleated cells and are highly polymorphic (5). These integral membrane proteins serve as restricting elements that permit cytotoxic T cells to recognize viral or tumor antigens on the surface of infected or transformed cells (6). The class I genes denoted *Qa-2,3*, *Tla*, and *Qa-1* encode antigens that are present on certain hematopoietic cells. They are far less polymorphic than their transplantation antigen counterparts and their functions are unknown (7).

The availability of inbred strains of mice as well as congeneic mice, which are genetically identical except for the genes of the MHC, has permitted immunogeneticists to use serologic polymorphisms and recombinational analyses to identify at least six class I genes that map to two distinct complexes of MHC. The *H-2* complex comprises the proximal part of the MHC on chromosome 17 and includes the *K* gene as its left-hand boundary and the *D* and *L* genes as its right-hand boundary. The class II and III gene families lie between the *K* and *D* regions of the *H-2* complex. The *Tla* complex comprises the distal part of the MHC and includes the *Qa-2,3*, *Tla*, and *Qa-1* genes. Various inbred strains of mice are distinguished by having different alleles at the six serologically defined loci and these constellations of alleles are denoted haplotypes. The MHC encompasses about 2 centimorgans of DNA and this may be equivalent to as much as 2,000–4,000 kilobases of DNA (1, 2, 8).

We have recently isolated 54 genomic clones from a cosmid library constructed from BALB/c sperm DNA. These 54 cosmid clones contain 36 distinct class I genes, which map into 13 gene clusters (9). In this paper, we report the use of the technique of genetic mapping by restriction enzyme site polymorphisms to locate the positions of all 13 of the class I gene clusters within the MHC. These results are in complete agreement with those provided by the serological identification of class I genes by gene transfer that identified six (*K*, *D*, *L*, *Qa-2,3*, and two *TL*) of the 36 class I genes (10). Our results show that 31 of the 36 class I genes are located in the *Tla* complex.

MATERIALS AND METHODS

Materials. The sources of the restriction enzymes, T4 DNA ligase, [α-^{32}P]dNTPs, and nitrocellulose filters have been described (9).

Methods. Isolation of mouse DNA, plasmid DNA, and the procedure used for subcloning have been described (9, 11–13). Southern blot hybridization was carried out as follows. DNA blots were prepared and hybridized for 16 hr at 68°C in a rotisserie oven in 0.30 M NaCl/0.03 M Na citrate, pH 7.0/0.10% polyvinylpyrrolidone/0.10% Ficoll/0.1% bovine serum albumin/10% dextran sulfate/denatured salmon sperm DNA (100 μg/ml)/denatured BALB/c mouse DNA (100 μg/ml)/5 mM EDTA/0.1% $NaDodSO_4$ with nick-translated probes at 1×10^6 cpm/ml. Filters were washed twice with 0.30 M NaCl/0.03 M Na citrate, pH 7.0/0.1% $NaDodSO_4$ and twice with 15 mM NaCl/1.5 mM Na citrate, pH 7.0/0.1% $NaDodSO_4$ at 68°C for 20 min each. Dextran sulfate and denatured mouse DNA were omitted when nick-translated mouse DNA was used as a probe.

Two procedures have been used for the isolation of single- or low-copy probes. First, labeled mouse DNA was hybridized to electrophoresed restriction fragments of individual cosmid clones that were blotted onto nitrocellulose under conditions in which hybridization occurs only to fragments containing repetitive sequence elements (14). Those fragments failing to hybridize were then subcloned into phage M13 mp8 or plasmid pBR322. A second approach was used for those cases in which we were unable to identify single- or low-copy restriction fragments by this procedure. DNA fragments from cosmid clones were cleaved with frequent-cutting restriction enzymes such as *Sau*3A and the resulting small fragments were cloned into phage M13 mp8. Phage clones were then analyzed by a plaque hybridization procedure with total mouse DNA and the cosmid fragment as probes.

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

Table 1. Comparison between MHC alleles and polymorphic restriction fragments used for mapping in congeneic and

MHC allele*										
K	*D*	*Qa-2,3*	*Tla*	Strain	1 (*Kpn* I)	2 (*Bam*HI)	3 (*Eco*RI)	4 (*Kpn* I)	5 (*Kpn* I)	6 (*Kpn* I)
d	*d*	*a*	*c*	BALB/c	15.4	8	2.2	14	13	10.5
k	*k*	*b*	*b*	C3H	15.4		MB	14		16
k	*k*	*b*	*b*	AKR		10		14	13	
b	*b*	*a*	*b*	C57BL/6	MB	8	MB	14	10	13
b	*b*	*a*	*b*	C57BL/10						
k	*d*	*a*	*a*	A/WySn			2.2	—†	13	
s	*d*	*a*	*c*	A.TL						
k	*d*	*a*	*c*	A.AL		8				
q	*d*	*a*	*a*	A.QR						
b	*b*	*a*	*b*	A.BY			MB			
k	*d*	*a*	*a*	B10.A			2.2	—		
k	*b*	*a*	*b*	B10.AM		8	2.2			
k	*b*	*a*	*b*	B10.A(1R)				14		13
b	*d*	*a*	*a*	B10.A(3R)				—	13	
d	*b*		(*b*)	B10.BDR-1					10	
d	*d*	*a*	*c*	B10.D2						
q	*q*	*a*	*b*	B10.Q						
b	*b*	*a*	*a*	B6-Tla^a	MB	8	2.2	—	13	13
k	*k*		*b*	B6-H-2^k	15.4	10	MB		13	16
b	*b*	*b*	*b*	B6.K1	15.4	8	MB	14	13	16 and 13
b	*b*	*a*	*b*	B6.K2	MB	8	MB	14	13	13
Cluster locations					*Qa*	*D*	*Tla*	*Tla*	*Qa/Tla*	*D–Qa*

The probes used for mapping the clusters were cluster 1, 2.1-kb *Eco*RI/*Nru* I fragment of cosmid 65.1 subcloned into the *Eco*RI/*Nru* I sites of pBR322; cluster 2, *Sau*3A fragment of 8-kb *Bam*HI fragment from cosmid 59.2 subcloned into the *Bam*HI site of M13 mp8; cluster 3, 2.2-kb *Eco*RI fragment of cosmid 12.2 subcloned into the *Eco*RI site of M13 mp8; cluster 4, 2-kb *Kpn* I/*Sma* I fragment of cosmid 66.1 subcloned into the *Hin*cII site of M13 mp8; cluster 5, 2.6-kb *Xho* I/*Hpa* I fragment of cosmid 47.1; cluster 6, 2-kb *Sma* I fragment of cosmid 50.2 subcloned into the *Hin*cII site of M13 mp8; cluster 7, 3-kb *Xho* I fragment of cosmid 20.1 subcloned into the *Sma* I site of M13 mp8; cluster 8, 3-kb *Hpa* I fragment of cosmid 49.1 subcloned into the *Hin*cII site of M13 mp8; cluster 9, *Sau*3A fragment from 6.4-kb *Kpn*I fragment of cosmid 36.2 subcloned into the *Bam*HI

RESULTS AND DISCUSSION

Strategy of Genetic Mapping by Restriction Enzyme Site Polymorphisms. This approach has three basic steps. First, single- or low-copy probes are isolated from each of the 13 class I gene clusters. Second, each of these probes is used to analyze the DNAs of various mouse inbred and recombinant strains for restriction enzyme site polymorphisms. Third, these restriction enzyme site polymorphisms are correlated with the serologic class I polymorphisms that define the four regions (*K*, *D*, *Qa*, and *Tla*) in the MHC by recombinational analysis. Thus, the cosmid cluster positions are identified by the correlation of the polymorphisms in single- or low-copy probes and class I serological markers.

Single- and low-copy probes isolated from the 13 cosmid clusters are listed in Table 1. These probes fell into two categories. Three probes were single copy—those from clusters 4, 7, and 10—and the 10 low-copy probes from the remaining clusters each hybridized with two to eight restriction fragments under stringent conditions. In those cases in which the low-copy probes hybridized to multiple bands from BALB/c DNA, the parental band corresponding to the probe was identified by virtue of its comigration with cloned BALB/cDNA, because it was the most intense band in the autoradiogram, or both.

In theory, an analysis of the single-copy probes against BALB/c DNA and the genomic DNAs from various mouse inbred strains might show one of four types of hybridization patterns. These patterns are (*i*) no change in band size (no restriction enzyme site polymorphism), (*ii*) a change in band size (mutation of a restriction enzyme site), (*iii*) loss of the band (deletion of the probe sequence), and (*iv*) multiple bands (duplication of the probe sequence). The last three patterns can be used as genetic markers to map the corresponding sequences to positions in the MHC. Each of these patterns was observed in analyzing various mouse DNAs with our single- and low-copy probes.

The final step in mapping by this approach is to correlate the restriction enzyme site polymorphisms of various DNAs with the serologically defined recombinational maps of the corresponding congeneic and recombinant congeneic mouse strains.

Five Class I Genes from Three Clusters Map to the *K* and *D* Regions of the *H-2* Complex. Because a useful combination of restriction enzyme site polymorphisms and recombinant congeneic strains of mice is not always available, it often is necessary to go through a multistep analysis to map the cosmid clusters. The mapping of cluster 13 to the *D* region required three independent analyses. First, the 3.4-kilobase (kb) fragment used as a low-copy probe detects five restriction enzyme fragments in *Eco*RI-digested BALB/c DNA. The 25-kb fragment is the most intense and therefore presumably contains the probe sequence (Fig. 1). This band is missing in AKR DNA and in the DNA of the congeneic mouse strain B6-H-2^k. These initial observations map cluster 13 to the MHC of the mouse, because B6-H-2^k mice contain the MHC from AKR mice on the B6 background and the DNA from B6 mice contains the 25-kb fragment. Second, an analysis of *Hin*dIII-digested genomic DNA using the same probe shows that the 13.5-kb fragment detected in BALB/c, A/WySn, and B10.A(3R) DNAs is replaced by a 10.6-kb fragment in C57BL/6 DNA. These observations map the probe for cluster 13 distal in the recombination point in the B10.A(3R), which is located in the class II region between the *K* and *D* loci (Table 1). Finally, because the 25-kb *Eco*RI fragment that is absent from the DNA from B6-H-2^k is present in

recombinant congeneic mouse strains

Clusters, kb							
						13	
7 (*Pvu* II)	8 (*Hin*dIII)	9 (*Hin*dIII)	10 (*Bam*HI)	11 (*Hin*dIII)	12 (*Hin*dIII)	*Eco*RI	*Hin*dIII
2.8	3.2	12.3	9	10.6	3.4	25	13.5
1.6	7.7	8.8	9	10.6	—		10.6
				10.6		12.3	
2.8	3.2	8.4	9	5.6	—	25	10.6
2.8			9				
1.6	—		11		—		13.5
			9				
			9		3.4		13.5
			11				
1.6	—		9				
2.8	—				—		
	3.2		9	10.6	—		
	—	12.3	11	5.6	—		13.5
				10.6			10.6
	3.2				3.4		
			9		—		
2.8	—	8.4	11		—	25	
2.8	7.7	8.8			—	12.3	
2.8	7.7	8.8			—	25	
2.8	7.7	8.4			—	25	
Tla	*Tla*	*Qa*	*Tla*	*K*	*Qa/Tla*	*D*	

site of M13 mp8; cluster 10, 3-kb *Xho* I fragment of cosmid 15.3; cluster 11, 2.6-kb *Xho* I/*Nru* I fragment of cosmid 17.1 subcloned into the *Hinc*II site of M13 mp8; cluster 12, 2-kb *Bam*HI fragment of cosmid 22.1 subcloned into the *Bam*HI site of M13 mp8; cluster 13, 3.4-kb *Sma* I/*Nru* I fragment of cosmid 18.1. MB, Because of multiple bands, the corresponding fragment could not be identified.

*Taken from refs. 15 and 16.

†The corresponding fragment could not be identified because of deletion or comigration with other bands.

the recombinant congeneic strain B6.K1, which is identical with the B6-H-2^k strain at both the *Tla* and *Qa-2,3* loci but differs at the *K* and *D* loci, we can conclude cluster 13 must map proximal to the *Tla* complex. Together, these restriction enzyme site polymorphisms suggest that cluster 13 maps to the *D* region. Similar approaches have mapped cosmid clusters 11 and 2, respectively, to the *K* and *D* regions (Table 1).

Ten Class I Genes from Three Clusters Map to the *Qa* Region. Clusters 1, 6, and 9, which contain seven, two, and one class I genes, respectively (9), map to the *Qa* region (Table 1). We will discuss the data mapping cluster 6 as an example. The low-copy probe isolated from cluster 6 detected a 10.5-kb *Kpn* I fragment in BALB/c (*d* haplotype) DNA whereas a 16-kb and a 13-kb *Kpn* I fragment were detected in C3H (*k* haplotype) and in C57BL/6 (*b* haplotype) DNAs, respectively. Since the recombinant congeneic strain B10.A(1R) has the *b* haplotype-specific fragment, we can localize this cluster distal to the *S* region. Analyses of the strains B6-Tla^a (13-kb fragment), B6-H-2^k (16-kb fragment), and their recombinant B6.K2 (13-kb fragment) allow us to place cluster 6 proximal to the *Tla* region because B6.K2 DNA contains the *Tla* region genes from the B6-H-2^k mouse. The recombinant congeneic strain B6.K1, also derived by recombination from B6-Tla^a and B6-H-2^k, however, showed an interesting result in that both the 16- and the 13-kb *Kpn* I fragments were found. The simplest explanation of this finding is that the B6.K1 haplotype was generated by an unequal crossing-over event between B6-Tla^a and B6-H-2^k chromosomes, leading to a duplication of DNA at the site of recombination. Cluster 6 would map close to this recombination point and has therefore been duplicated in strain B6.K1. Cluster 9 can be mapped only tentatively to the *Qa* region because multiple restriction fragments hybridized with the probe from this cluster.

Fourteen Class I Genes from Five Clusters Map to the *Tla* Region. Clusters 3, 4, 7, 8, and 10, which have six, three, two, two, and one class I genes, respectively (9), were mapped to the *Tla* region (Table 1). Fig. 1 shows the results obtained with a single-copy probe isolated from cluster 4. This probe identifies a 14-kb *Kpn* I fragment in BALB/c and C57BL/6 DNA whereas this sequence is absent from strain A/WySn DNA. Analysis of the congeneic strain B6-Tla^a (*Tla* region from A on a C57BL/6 background) shows that this sequence is absent from this strain also and therefore maps cluster 4 to the *Tla* region. As a control, Fig. 1 shows that the 14-kb fragment is present in strains B6.K1, B6.K2, and B10.A(1R) (*Tla* regions from AKR and C57BL/10) but absent from strains B10.A(3R) and B10.A (Tla region from A).

Seven Class I Genes from Two Clusters Map to Either the *Qa* or the *Tla* Region. Clusters 5 and 12, which have four and three class I genes, respectively (9), map in the MHC distal to the *D* region (Table 1). Due to the lack of appropriate strains and restriction enzyme site polymorphisms, it is not possible to map these clusters more precisely with the probes used. From cluster 5, a 2.6-kb fragment was isolated and used to identify a 13-kb *Kpn* I fragment in BALB/c DNA from which this low-copy probe was derived. As shown in Fig. 1, this 13-kb *Kpn* I fragment is replaced by a 10-kb *Kpn* I fragment in DNA from C57BL/6 and B10.BDR-1 mice. All other strains analyzed show the 13-kb *Kpn* I fragment. Because the congeneic and recombinant congeneic strains B6-Tla^a, B6.K1, and B6.K2 do not show the C57BL/6-specific 10-kb band, we can conclude that cluster 5 maps to the right of the *D* locus on chromosome 17. A correlation with the *Qa-2,3* and *Tla* alleles present in these strains (Table 1) is not possible, indicating that cluster 5 maps to a locus in the *Tla* complex not yet defined by serological reagents.

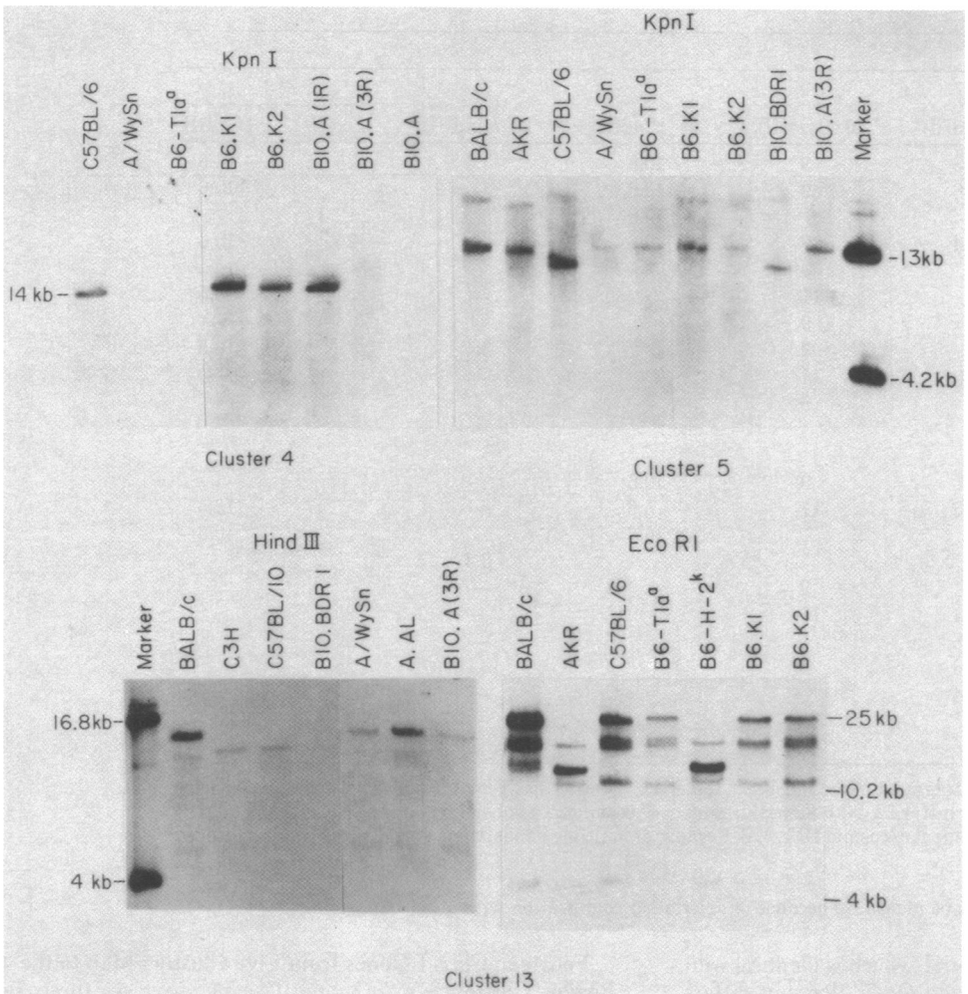

FIG. 1. Southern blot analyses of DNAs from inbred, congeneic, and recombinant congeneic mice with the single- or low-copy probes isolated from clusters 4, 5, and 13.

A Class I Gene Cluster Possibly Mapping to the Distal End of the *Tla* Region. Comparison of the restriction maps for clusters 7 and 8 shows that position 2 (27 kb) of cluster 7 overlaps position 12 (37 kb) of cluster 8 (9). In fact, these two clusters can be merged into one cluster of 70 kb containing three class I genes if it is assumed that part of cosmid 8.3 in cluster 8 [position 37 (47 kb) (9)] is derived from noncontiguous DNA ligated to the class I gene sequence during construction of the library. This possible interpretation escaped our notice earlier (9). The low-copy probe isolated from the overlapping portion between clusters 7 and 8 (3-kb *Hpa* I fragment) map these class I genes to the *Qa* and *Tla* regions (Table 1). A single-copy probe isolated further downstream from the nonoverlapping portion of cluster 7 (a 3-kb *Xho* I fragment), however, maps outside the MHC (Table 1). Assuming that this *Xho* I end of cluster 7 (which is defined only by a single cosmid clone) does not represent a cloning artifact, this would map cluster 7 to the distal end of the *Tla* region encompassing the sequence containing the recombination point in a number of congeneic mouse strains. Indeed, a second low-copy probe from cluster 7 (a 1.5-kb *Bst*EII/*Nru* I fragment) together with the 3-kb *Xho* I fragment as probe allowed us to establish, by restriction enzyme analysis of the genomic DNA, that the organization of the cloned DNA in cluster 7 is colinear with genomic DNA (data not shown).

Duplication and Deletion of Class I Gene Clusters in Inbred Mice. Southern blot analyses with class I cDNA probes have shown that different inbred strains of mice contain similar numbers of class I genes (12, 17–20). With the probes that we have isolated from the 13 gene clusters, we are now in a position to analyze the duplication and deletion of class I gene clusters in a more precise way by counting the total numbers of bands that are generated by Southern blot analyses of DNAs from various inbred strains. The assumption made here is that the duplication or deletion of the probe sequences is roughly proportional to the duplication and deletion of adjacent DNA sequences such as the class I genes. We have previously reported an example of DNA deletion in that a sequence for the 5′ flanking part of the L^d gene was deleted in strains of the *b* and *k* haplotypes, indicating a possible deletion of the L^d coding sequence, which is not expressed in these two strains (9). Furthermore, as discussed above, a duplication of the sequence used as a probe for cluster 6 occurs in recombinant strain B6.K1. Using all 13 low- or single-copy probes, we have detected 124 bands in BALB/c DNA, 102 bands in C3H DNA, and 107 bands in C57BL/6 DNA. Thus, expansion and contraction of the probe sequences and presumably the class I gene does occur in these different inbred strains.

All 36 Class I Genes Map to the MHC. A striking finding is that all 36 of the isolated class I genes map to the MHC. In several other multigene families, including globin (21) and the antibody genes (22, 23), it appears that pseudogenes can map to other chromosomal locations than the functional genes. We cannot formally exclude the possibility that there are additional class I genes or distantly related pseudogenes not present among the cloned cosmid genes that lie outside the MHC.

Five of the 36 genes map to the classical *H-2* complex (Fig. 2). Three of these five genes have been shown by DNA-mediated gene transfer to express the *K*, *D*, and *L* gene products.

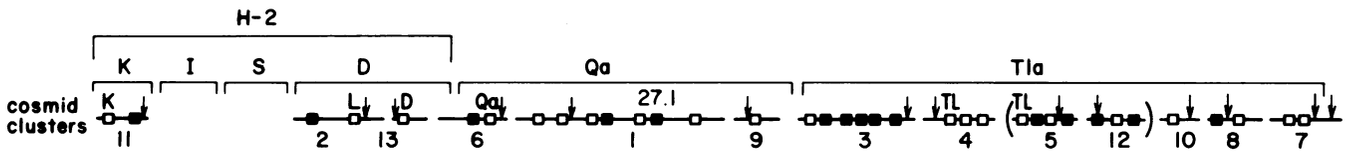

FIG. 2. Locations of the 13 class I gene clusters with respect to the genetic map of the major histocompatibility complex. Cluster 11 maps to the *K* region; clusters 2 and 13 map to the *D* region; clusters 1, 6, and 9 map to the *Qa* region; clusters 3, 4, 7, 8, and 10 map to the *Tla* region; and clusters 5 and 12 map to either the *Qa* or the *Tla* region. The order of clusters in separate regions is not known with respect to each other. The locations of the probes used for mapping are indicated by arrows. The genes in each cluster are denoted by boxes. Open boxes signify elevated β_2-microglobulin levels whereas genes given by closed boxes did not lead to elevated levels of surface β_2-microglobulin in gene-transfer experiments. The implication is that the former class I genes are expressed and the latter are not by this assay (10).

The other two genes fail to express gene products associated with β_2-microglobulin on the cell surface (10) and are provisionally assumed to be pseudogenes. Thus, the *H-2* complex encoding the classical transplantation antigens appears to contain a very modest number of class I genes as compared with the *Tla* complex encoding the structurally related hematopoietic differentiation antigens. Thirty-one of the 36 class I genes map to the *Qa* and *Tla* regions (Fig. 2). At least three of these class I genes express the serologically defined *Qa-2,3* and two *TL* gene products (10). In addition, at least 10 additional class I genes in the *Tla* complex express what we have termed as novel class I gene products (10). The novel gene products were detected by increased levels of β_2-microglobulin on the surface of mouse L cells that had been transformed with these class I genes.

Mapping the class I genes by restriction enzyme site polymorphisms assumes that the flanking sequences from which we isolate the probes are not rearranged with respect to the class I genes in the other mouse strains analyzed. Three observations support this assumption. First, cluster 1 has been mapped independently to the *Qa* region with two probes, gene *27.1* (9), and the 2.1-kb restriction fragment from the 3′ flanking sequence of gene 2 (this paper). Thus, two sequences linked by molecular cloning are also linked by genetic mapping. Second, a precise correlation exists between the results obtained by serological identification of class I genes and the mapping of the corresponding cluster by restriction enzyme polymorphism with flanking sequence probes. Third, probes isolated from the clusters mapping to the *Qa* and *Tla* regions detected the same sized restriction fragments in multiple mouse strains, indicating a general conservation of sequence organization in these regions.

Restriction Enzyme Site Polymorphisms Correlate with Serological Polymorphisms. In agreement with the extreme polymorphism of the transplantation antigens is our finding that restriction enzyme site polymorphisms were readily detected with probes from the cosmid cluster containing the *K*, *D*, and *L* genes. This indicates that the allelic variability of the coding regions of the *K*, *D*, and *L* molecules as defined serologically does extend into the flanking DNA sequences. For the class I gene clusters mapping to the *Tla* complex, it was much more difficult to detect restriction enzyme site polymorphisms. For example, 10 restriction enzymes were analyzed for cluster 10 before a polymorphism was found. Once again, the limited restriction enzyme site polymorphisms correlate with the limited serological polymorphism of the Qa-1, Qa-2,3, and TL antigens. Thus, certain regions of the major histocompatibility complex appear to exhibit extensive polymorphism (*K* and *D*) whereas others do not (*Qa* and *Tla*). The explanation for these striking differences is unknown. This difference in polymorphisms between the numerous class I genes of the *Tla* complex and the few class I genes of the classical *H-2* complex also explains the limited restriction enzyme polymorphism that is detected in Southern blot analyses of different mouse DNAs with class I gene sequences (12, 17–20).

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