Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6245–6249, September 1985
Immunology

Expression of hybrid class I genes of the major histocompatibility complex in mouse L cells

(transplantation antigens/exon shuffling)

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Contributed by Leroy Hood, May 15, 1985

ABSTRACT **The class I genes of the major histocompatibility complex of the mouse can be divided into two categories: those encoding the transplantation antigens and those encoding the Qa and Tla antigens. The inbred BALB/c mouse has 28 potential *Qa/Tla* genes. The sites of tissue expression, developmental regulation, and functions of these genes are virtually unknown. We have used the technique of exon shuffling to construct hybrid genes between each of three *Qa* region genes (*Q5*, *Q7*, and *Q8*) and two other class I genes (*H-2L^d* and *Q6*). The hybrid genes have been transfected into mouse L cells, in which intact transplantation antigen genes generally are expressed and in which intact *Qa* genes generally are not expressed. Analysis of expression of the hybrid gene constructs indicates that the 5′ half of two of the *Qa* genes (*Q5* and *Q8*) can readily be expressed in the context of a hybrid molecule, whereas the 3′ half prevents cell-surface expression. The exon shuffling approach described here will be useful in characterizing *Qa/Tla* genes and in identifying or producing new reagents to study the *Qa/Tla* gene products, their tissue distribution, their developmental stages of expression, and, ultimately, their functions.**

The major histocompatibility complex (MHC) of the mouse is a multigene complex on chromosome 17 that encodes a number of cell-surface proteins that play an important role in the regulation of the immune responses (for recent review, see ref. 1). MHC products include class I molecules encoded by the *H-2* and *Qa/Tla* regions. The H-2 class I proteins, or transplantation antigens, are extremely polymorphic molecules expressed on most somatic cells. The H-2 molecules participate in recognition and destruction of virus-infected or neoplastically transformed cells by cytotoxic T lymphocytes. The class I molecules encoded by the *Qa/Tla* complex are much less polymorphic and have a limited tissue distribution, usually restricted to hematopoietic cells (2–4). They have been identified through alloreactive cytotoxic reactions and by biochemical and serological methods. The functions of Qa/Tla molecules are not known. The two types of class I proteins are structurally similar. Both have heavy chains of 38,000–45,000 daltons and are associated noncovalently with a 12,000-dalton light chain, β_2-microglobulin (β_2m), encoded outside the MHC (1, 5, 6). Inbred strains of mice have distinct constellations of MHC alleles denoted by their *H-2* haplotypes.

Genetic analysis of the MHC of BALB/c (*H-2^d* haplotype) mice has indicated that there are 33 genes per haploid chromosome set that might potentially encode class I antigens (7–11). Only 5 of these genes map in the *H-2* region. The remaining 28 have been assigned to the *Qa/Tla* region, and 10 of these genes map in the *Qa* subregion and are denoted *Q1–Q10*.

Most of the class I genes consist of eight exons. Exon 1 encodes the leader sequence; exons 2–4 encode the α_1, α_2, and α_3 external regions; exon 5 encodes the transmembrane region; and exons 6–8 encode the cytoplasmic region. The α_1 and α_2 regions fold to form one external domain and the α_3 and β_2m peptides generate a second membrane proximal external domain (12–15).

When the cloned H-2 genes are transfected into L cells, they are expressed and can be identified by serological, functional, and biochemical methods. In contrast, the majority of the *Qa/Tla* genes introduced into L cells do not express cell-surface products detectable with available reagents (10). We have recently developed a method that makes it possible to study the serological and T-cell recognition properties of class I molecules that are not ordinarily expressed on the surface of the transfected L cells (13). The approach involves fusion of the specific exons (1–3) of the *Qa* genes to complementary exons (4–8) of the *H-2* transplantation antigen genes (exon shuffling) followed by the transfection of the hybrid class I genes into L cells. We have demonstrated that the hybrid Qa/H-2 molecules can be expressed on the cell surface of the L-cell transfectants and that the *Qa* gene-encoded properties can be characterized in this system (13). Based on the serological studies of *Q6* hybrid products, it was proposed that the *Q6* gene encodes the CR (H-2 crossreactive) antigen (16) expressed mainly on peripheral T cells (13). We showed that the *Q6* gene carries a promoter and leader peptide that can function in L cells. The apparent lack of expression of the intact *Q6* gene in the transfected L cells was mapped to a trait(s) encoded by the 3′ portion of the *Q6* gene (13).

In the present study, we have analyzed three other *Qa* genes (*Q5*, *Q7*, and Q8) immediately adjacent to *Q6* on chromosome 17 (Fig. 1). These genes were chosen for study because of their close linkage to the previously studied *Q6* gene. In addition, the similarity in restriction maps between the *Q5*, *Q6*, and *Q7* genes suggested that this cluster may represent a family of closely related genes (8).

MATERIALS AND METHODS

Materials. Monoclonal antibodies used in this study included 28-14-8 (anti-L^d), 20-8-4 (anti-Q6, -K^d, -K^b, -r, -s), 34-1,2 (anti-Q6, -D^d, -K^d, -L^d, -K^b, -r, -s, -p, -q), 30-5-7 (anti-L^d, -D^q, -L^q), 34-5-8 (anti-D^d), 34-2-12 (anti-D^d), 81.L (anti-D^d, -s, -u), 81.R (anti-D^d, -p, -u), 97.H (anti-D^d, -K^d, -p, -u, -v), 97.G (anti-D^d, -K^d, -p, -u, -v), and 11-4 (anti-K^k). The sources and reactivities of the monoclonal antibodies are described in detail in refs. 10–14 and 17. The origin of the cloned *Q5*, *Q6*, *Q7*, *Q8*, *L^d*, *D^d*, and *K^d* genes is given in refs. 8 and 10.

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Abbreviations: MHC, major histocompatibility complex; β_2m, β_2-microglobulin; bp, base pair(s); kb, kilobase(s).

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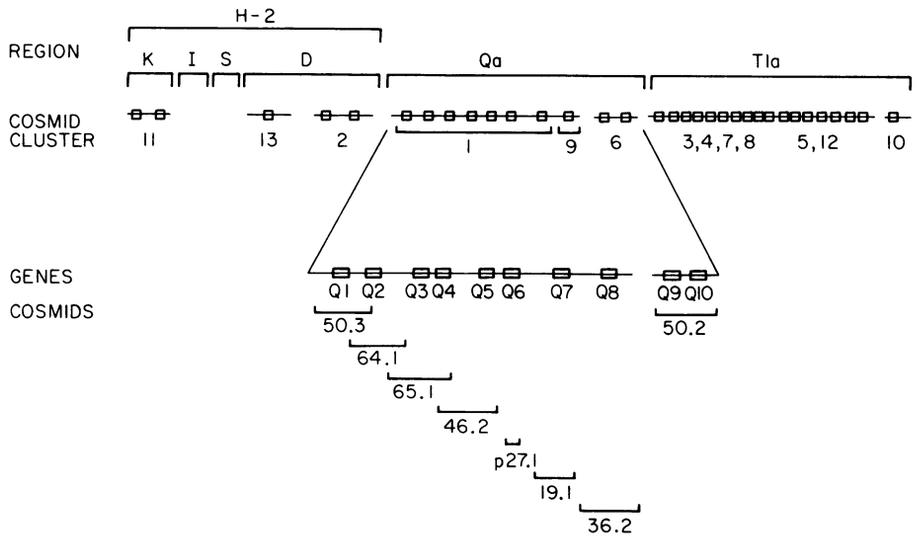

FIG. 1. Map of *H-2* and *Qa/Tla* complex of BALB/c mice. Cosmid designations are from Steinmetz *et al.* (8).

Construction of Hybrid Class I Genes. For construction of the hybrid $Q5/L^d$, $Q7/L^d$, and $Q8/L^d$ genes, two *Xba* I sites conserved in *Q5*, *Q6*, *Q7*, and *Q8* were utilized (Fig. 2). One of these sites is 414 base pairs (bp) upstream from the protein initiation codon in exon 1, and the other is within the large intervening sequence between exons 3 and 4, 409 bp downstream from the 3′ end of exon 3. The procedure used for constructing these hybrid genes has been described for the $Q6/L^d$ gene (Fig. 2*b*) (13). Briefly, a 3-kilobase (kb) *Bgl* II/*Bam*HI fragment of the *Q6* gene derived from clone λ 27.1 (7), which carries exons 1–3 and 926 bp of 5′ flanking region, was cloned into the *Bam*HI site of pBR322. The resultant plasmid 1104-4 was linearized with *Bam*HI, and a 2.4-kb *Bam*HI fragment carrying the 3′ end of $H\text{-}2L^d$ gene, including exons 4, 5, 6, 7, and 8, was inserted to make the $Q6/L^d$ hybrid gene. For constructing the $Q5/L^d$, $Q7/L^d$, and $Q8/L^d$ hybrid genes, the 1.8-kb *Xba* I fragment containing the 5′ end of *Q6* was deleted from the $Q6/L^d$ hybrid construct, and it was replaced with the equivalent 1.8-kb *Xba* I fragment from the *Q5*, *Q7*, or *Q8* genes (Fig. 2).

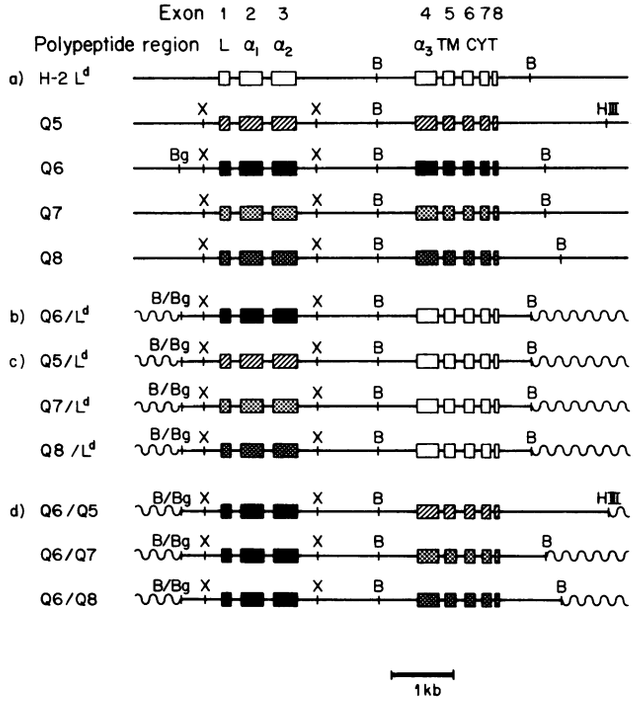

FIG. 2. Exon–intron structure of class I and hybrid class I genes. Wavy lines denote pBR322 DNA of the cloning vector. Restriction sites: B, *Bam*HI; Bg, *Bgl* II, H, *Hin*dIII; X, *Xba* I; B/Bg, hybrid site created by ligation of *Bam*HI and *Bgl* II sticky ends, no longer recognized by either enzyme.

For construction of the *Q6/Q7* hybrid gene, plasmid 1104-4 was linearized with *Bam*HI and the 2.7-kb *Bam*HI fragment derived from cosmid 19.1 (8) containing the 3′ portion of the *Q7* gene was inserted. For construction of the *Q6/Q8* hybrid gene, plasmid 1104-4 was linearized and the 3.0-kb *Bam*HI fragment derived from cosmid 36.2 (8) containing the 3′ portion of the *Q8* gene was inserted. The *Q6/Q5* hybrid gene was constructed by deleting the 0.3-kb fragment of pBR322 DNA between the *Bam*HI and *Hin*dIII sites of plasmid 1104-4, and inserting in its place the 3.7-kb *Bam*HI/*Hin*dIII fragment derived from cosmid 46.2 (8) and containing the 3′ end of the *Q5* gene. The identity of each hybrid gene construct was checked by restriction enzyme analysis.

Transfection of Mouse L Cells and Radioimmunoassays. Mouse thymidine kinase-negative cells (Ltk^-) derived from C3H mice ($H\text{-}2^k$ haplotype) were transfected with plasmid DNAs as described (12–14, 17). Radioimmunoassays were performed as described (12–14, 17).

Identification of the $H\text{-}2^b$ *Q10* Gene in BALB/c Mice. An 18-base oligodeoxyribonucleotide probe, 3′ AGTGTGTAA-CGACTAGAC 5′, complementary to the DNA sequence encoding amino acids 285–290 of the $H\text{-}2^b$ Q10 protein (18, 19) and corresponding region of the homologous $H\text{-}2^q$ class I protein (20), was synthesized, radiolabeled, and hybridized to 2-μg amounts of cosmid DNA spotted on nitrocellulose filters.

RESULTS

Expression of Hybrid Genes $Q5/L^d$, $Q7/L^d$, and $Q8/L^d$. The strategy for testing the expression capacity of the Qa genes is based on the pilot study of exon shuffling between the *Q6* and $H\text{-}2L^d$ genes (13). Although the intact *Q6* gene is not expressed on the surface of mouse L cells after transfection, a hybrid gene in which the 5′ portion of *Q6* (exons 1–3 encoding the leader peptide and two external regions, α_1 and α_2) was fused to the 3′ portion (exons 4–8 encoding the α_3 external, transmembrane, and cytoplasmic regions) of $H\text{-}2D^d$, $H\text{-}2K^d$, and $H\text{-}2L^d$ genes were expressed. To test the

Table 1. Reactivity of transfected L cells with monoclonal antibodies directed against transplantation antigens

Transfectant	Antibody/reactivity										
	28-14-8/ αL^d	20-8-4/ αK^d	34-1-2/ $\alpha K^d D^d L^d$	30-5-7/ αL^d	34-5-8/ αD^d	34-2-12/ αD^d	81.L/ αD^d	81.R/ $\alpha D^d L^d K^d$	97.H/ $\alpha D^d K^d L^d$	97.G/ $\alpha D^d K^d L^d$	11-4/ αK^k
$Q5/L^d$	14,556	524	15,276	366	230	328	332	179	144	239	10,768
$Q6/L^d$	24,698	35,736	31,944	330	570	504	402	211	152	177	11,426
$Q7/L^d$	724	546	718	510	378	456	296	222	141	210	12,840
$Q8/L^d$	18,960	356	478	350	230	270	424	540	514	1,317	10,320
$Q6/Q5$	808	820	934	476	344	404	358	242	322	259	14,230
$Q6/Q7$	662	662	498	562	268	368	385	283	204	175	11,872
$Q6/Q8$	506	686	480	358	166	422	414	288	151	182	12,816
D^d	728	332	34,244	296	30,366	6736	16,067	19,354	17,557	16,820	12,796
L^d	37,948	602	20,672	29,900	296	602	306	5,484	3,655	10,876	6,624
K^d	886	29,822	30,714	426	304	412	212	1,603	6,888	15,966	12,438
Ltk^+ cells	660	392	384	406	208	452	273	127	122	131	12,888

Radioimmunoassays on mass cultures of transfected L cells were performed as described (17). Results are expressed as ^{125}I-labeled protein A bound (cpm per 5×10^5 cells). The numbers shown are an average of two experiments, with experimental error being <10% of the values shown.

expression potential of the *Q5*, *Q7*, and *Q8* genes, two sets of hybrid genes were constructed (Fig. 2).

The $Q5/L^d$, $Q6/L^d$, $Q7/L^d$, and $Q8/L^d$ hybrid genes were cotransfected into thymidine kinase negative (tk^-) mouse L cells with the thymidine kinase gene of herpes simplex virus. Initially, each transfected culture was analyzed for expression of determinants reacting with monoclonal antibody 28-14-8, which recognizes determinants on the α_3–β_2m domain of the H-2L^d antigen (12, 13, 20). The L cells transfected with the $Q5/L^d$, $Q6/L^d$, and $Q8/L^d$ hybrid constructs bound the 28-14-8 antibody, while L cells transfected with $Q7/L^d$ constructs did not (Table 1). These data suggest that L cells transfected with $Q5/L^d$, $Q6/L^d$, and $Q8/L^d$ hybrid genes expressed membrane-bound hybrid protein products, while cells transfected with $Q7/L^d$ gene did not. Formally, we cannot exclude the possibility that fusion of the *Q7* α_1–α_2 domain to the L^d carboxyl terminus has distorted polymorphic determinants on the α_3–β_2m domain and rendered it undetectable to the 28-14-8 antibody. This interpretation, however, appears unlikely in view of the apparent conformational independence of α_1–α_2 and α_3–β_2m domains of class I molecules (12, 13, 20–22). We also screened mass cultures of the $Q5/L^d$, $Q6/L^d$, $Q7/L^d$, and $Q8/L^d$ transfectants with a panel of monoclonal antibodies directed against H-2^d transplantation antigens (Table 1). The results of these experiments and the results obtained with cloned lines of these transfectants (Fig. 3) revealed that $Q5/L^d$ hybrid gene products bound monoclonal antibody 34-1-2 in addition to monoclonal antibody 28-14-8, and that the Q8/L^d antigen crossreacted weakly with monoclonal antibody 97.G (23). Since the stability of the expression of hybrid genes in the transfectants is not known, we cannot derive a quantitative estimate of the degree to which the different molecules crossreacted with the antibodies tested. Typically, cells transfected with the $Q5/L^d$ construct bound antibodies 28-14-8 and 34-1-2 at a lower saturation level than cells transfected with the L^d and D^d genes. In addition, the dilution curves for reaction of the 97.G antibody with the Q8/L^d hybrid gene product were shifted approximately one order of magnitude toward higher antibody concentrations relative to dilution curves obtained with the D^d antigen. The results are, however, unambiguous in showing that 34-1-2 and 97.G antibodies react with the Q5/L^d product and the Q8/L^d product, respectively. Previous results have indicated that the 34-1-2 antibody reacts with the α_1–α_2 domain of L^d, D^d, K^d, and Q6 antigens (13, 20) and that the 97.G antibody reacts with the α_1–α_2 domain of the D^d molecule (14). Therefore, it is likely that these two antibodies react with the α_1–α_2 domain of the Q5/L^d and Q8/L^d hybrid antigens.

As a control for these experiments, three hybrid genes were constructed in which the *Xba* I fragments covering the 5′ end of the *Q5*, *Q7*, and *Q8* genes were cloned in the inverted

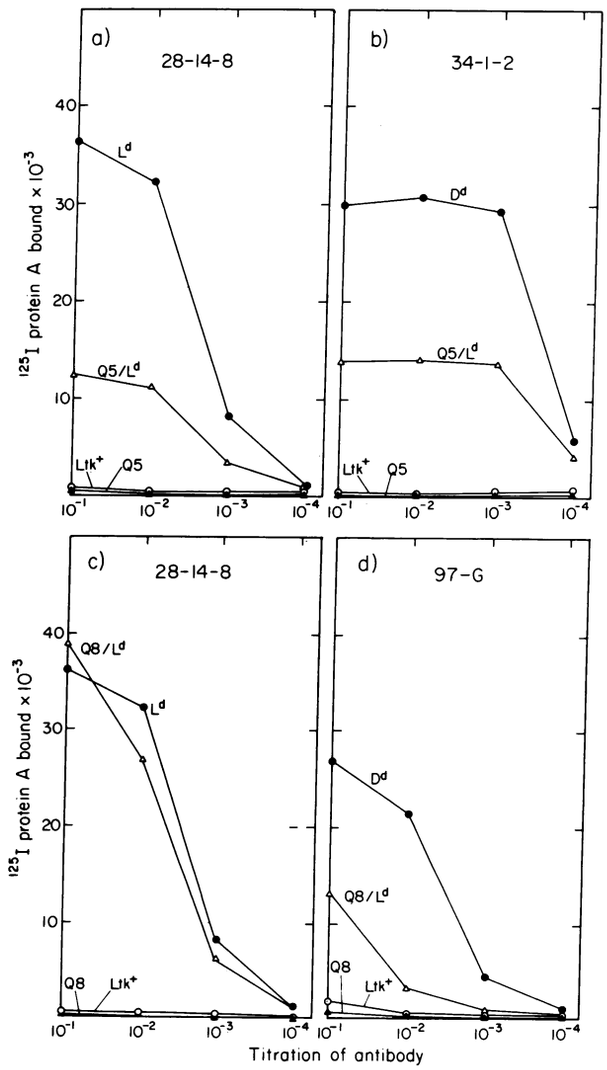

FIG. 3. Results of radioimmunoassays (average of two experiments) on cloned cell lines transfected with hybrid genes, transplantation antigen genes, and intact *Qa* genes. Monoclonal antibodies used in titration experiments were 28-14-8 (*a* and *c*), 34-1-2 (*b*), and 97.G (*d*).

orientation in combination with the 3′ end of the L^d gene. These control hybrid genes would not be predicted to code for a class I protein product because they have an inversion including the promoter region and the first three exons. Mouse L cells transfected with these control hybrid genes did not in any case bind the 28-14-8 antibody (results not shown). Therefore, expression of the L^d antigenic determinant by the $Q5/L^d$, $Q6/L^d$, and $Q8/L^d$ hybrid genes was dependent on the introduction of a complete gene with all coding regions in the proper order and orientation. Expression of the transfected gene was thus apparently not attributable to recombination with class I genes of the recipient mouse L cells (17). Our overall results are consistent with the interpretation that the *Q5* and *Q8* genes, like the *Q6* gene (13), encode promoters, leader peptides, and α_1–α_2 domains that are functional in L cells, although we cannot absolutely exclude the possibility that the *Q6* gene contributed an upstream control element distal to the first *Xba* I site at position −414.

Expression of Hybrid Genes *Q6/Q5*, *Q6/Q7*, and *Q6/Q8*. The second set of hybrid genes was constructed by recombination of the 5′ portion of the *Q6* gene with 3′ portions of the other *Qa* genes (Fig. 2). We chose these particular combinations because it has been demonstrated previously that fusion of the NH_2-terminal portion of the Q6 molecule to the COOH terminus of the K^d, L^d, and D^d molecule did not distort determinants located on the α_1–α_2 domain of Q6 and did not alter their recognition by monoclonal antibodies 20-8-4 and 34-1-2 (13). Therefore, determinants located in the α_1–α_2 domain of Q6 appear to be fairly independent of interaction with other α_3–β_2m domains.

The hybrid genes were transfected into L cells, and the transfectants were screened with the panel of monoclonal antibodies shown in Table 1. None of the three new constructs produced a protein product on the surface of the L cells that reacted with the 20-8-4 or 34-1-2 antibodies, or any of the other anti-$H\text{-}2^d$ monoclonal antibodies tested. To demonstrate that the p1104.4 vector used in construction of the hybrid genes was intact, a control experiment was performed in which the 3′ end of the L^d gene was cloned into the same DNA preparation of plasmid 1104-4 used for constructing the *Q6/Q5*, *Q6/Q7*, and *Q6/Q8* hybrids. The reconstructed $Q6/L^d$ hybrid gene was transfected into L cells. The resultant transfectants strongly bound the 20-8-4 and 34-1-2 antibodies, indicating that the reconstructed $Q6/L^d$ hybrid gene was functional. These results indicate that the 3′ portions of the *Q5*, *Q7*, and *Q8* genes, like the 3′ portion of the *Q6* gene, contain some feature(s) that prevent the display of their products on the surface of L cells. Consistent with this interpretation are the results of the screening of *Q5* and *Q8* gene transfectants with monoclonal antibodies 34-1-2 and 97.G (Fig. 3). These determinants, shown to be encoded by the α_1–α_2 domain of *Q5* and *Q8*, are not detectable on the cell surface of L cells transfected with the intact parental genes. This result is attributed to the presence of the 3′ portion of these genes.

Identification of the $H\text{-}2^b$ *Q10* Equivalent in BALB/c Mice. Mellor *et al.* (24) have recently characterized a nonpolymorphic class I gene ($H\text{-}2^b$ *Q10*) from C57BL/10 ($H\text{-}2^b$ haplotype) mice that maps to the *Qa* region. Its sequence is generally homologous to transplantation antigen genes but contains an unusual exon 5. This region, which in transplantation antigens encodes the transmembrane region, contains codons for three polar amino acids and a premature termination codon (24), which would interfere with its insertion into the plasma membrane. The product of this gene appears to be a secreted, truncated class I-like protein that is produced primarily in the liver (24–27). Based on restriction maps, Mellor *et al.* (24) suggested that the $H\text{-}2^b$ *Q10* gene may be similar to the gene in cosmid cluster 9 of the $H\text{-}2^d$ haplotype ($H\text{-}2^d$ *Q8*). To test whether the gene in cosmid cluster 9 is in fact the $H\text{-}2^d$ haplotype equivalent of the $H\text{-}2^b$ *Q10* gene, an 18-base oligonucleotide probe specific for exon 5 of the $H\text{-}2^b$ *Q10* gene was synthesized and hybridized with DNAs from cosmids isolated from $H\text{-}2^d$ mice covering the entire *Qa* region (ref. 8; Fig. 1). Very strong hybridization was obtained with the cluster 9 cosmid 36.2 but not with any of the other cosmids (results not shown). The result indicates that the *Q8* gene in cluster 9 corresponds to the $H\text{-}2^b$ *Q10* gene.

DISCUSSION

The MHC of BALB/c mouse encodes 33 class I genes (7–11). The functions of three of these, the *H-2K*, *-D*, and *-L* molecules, are known at least in part. Several interesting questions remain concerning the other 30 class I genes. First, how many can be expressed as polypeptides? Alternatively, how many are pseudogenes? Second, in what tissues and during which stages of development are these genes expressed? Finally, what functions are encoded by the non-*H-2* class I genes? The approach described here of exon shuffling between non-*H-2* class I genes and a class I *H-2* gene whose expression capacity is established will be extremely useful in exploring the coding capacities, tissue-specific regulatory elements, and ultimately the functions of these genes.

The present experiments extend previous expression studies on the *Q6* gene (13) to three genes closely linked to *Q6*: *Q5*, *Q7*, and *Q8*. Using the same approach of exon shuffling and L-cell transfection, we demonstrate that the strategy used to characterize the *Q6* gene product can be applied to other class I genes and allows a definition of their serological and T-cell recognition properties. Our results show that three of four of the *Qa* region genes carry promoter elements and leader peptides that are functional in L cells and that the α_1–α_2 external domains encoded by these genes can be expressed as structurally stable components of hybrid class I proteins. These data are consistent with the supposition that at least three of the *Qa* genes are functional. Constructs containing the 5′ or 3′ portions of the fourth *Qa* gene, *Q7*, are not expressed in L cells. The $H\text{-}2^d$ *Q7* gene has been proposed to have arisen as a fusion product of two genes in the $H\text{-}2^b$ haplotype ($H\text{-}2^b$ *Q8* and $H\text{-}2^b$ *Q9*) (18). The apparent lack of expression of this gene in transfection experiments suggests the possibility that the fusion may have generated a pseudogene.

In the present study, we have identified the gene in cosmid cluster 9 as the $H\text{-}2^d$ haplotype equivalent of the $H\text{-}2^b$ *Q10* gene (18, 19, 24–27). This gene appears to be very highly conserved among different strains of mice (24). The sequence analysis of the $H\text{-}2^b$ *Q10* gene compared with a cDNA sequence derived from $H\text{-}2^q$ haplotype revealed only seven nucleotide substitutions out of 1368 bp (24). Therefore, one feature residing in the 3′ portion of the homologous $H\text{-}2^d$ *Q8* gene that interferes with expression on the surface of L cells is very likely a transmembrane region that cannot insert into the plasma membrane.

On the other hand, the *Q6* gene was proposed to encode a cell-surface molecule whose lack of expression on L cells is caused by a requirement for tissue-specific factors missing in fibroblasts (13). This explanation is based on theoretical calculations of the hydrophobicity values for the transmembrane and cytoplasmic regions of the Q6 molecule, and on serological resemblance of Q6 hybrid proteins to the CR (H-2 crossreactive) antigen (16) found normally on the surface of some T cells and B cells. Thus, although all four *Qa* genes have some feature at the 3′ end that prevents expression on the surface of L cells, the molecular mechanisms for this phenomenon may differ. In analogy with the *Q6* and *Q8* genes, some of these genes might code for secreted proteins while others might be cell-surface antigens restricted in expression to certain tissues. DNA sequence analysis of

Q5 and *Q7* as well as other *Qa/Tla* genes is necessary in order to gain further insights into the expression potential and possible function(s) of this family of genes.

Serological characterization of the Q5 and Q8 antigens revealed that the α_1–α_2 domains encoded by these genes contain determinants recognized by antibodies directed against polymorphic regions of H-2^d transplantation antigens. These observations further support the hypothesis that the external domains of the Qa/Tla gene products are structurally similar to transplantation antigens. It remains to be determined whether the Q5 and Q8 molecules can carry out any of the known functions of the transplantation antigens (e.g., induction of T-cell proliferation or presentation of antigens to cytotoxic T cells), or if, as proposed by Kress *et al.* (26) for secreted class I-like molecules, they can block interactions of T-cell receptors with other target molecules. The α_1–α_2 domains of the *Q5*, *Q6*, and *Q8* hybrid antigens exhibit different patterns of reaction with the panel of anti-H-2^d monoclonal antibodies, indicating differences in antigenic determinants. It will be possible to use L cells transfected with the *Qa/*L^d hybrid genes to generate new monoclonal antibodies directed against the α_1–α_2 domains of the Qa antigens. These antibodies can then be used to screen different tissues and cells in various stages of embryogenesis and differentiation to find the cells expressing the Qa antigens. This approach may provide clues to the role of the *Qa/Tla* region genes in cell–cell interactions.

We thank Dr. S. J. Horvath for synthesis of the oligonucleotide probe; Drs. D. Sachs and Ted Hansen for monoclonal antibodies to class I antigens; Drs. S. Hunt, M. Kronenberg, and M. Zuniga for critical comments on the manuscript; and Ms. Kay Blackburn for excellent technical assistance. This research was supported by National Institutes of Health Grant AI19624.

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