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-2Ld 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 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 ~2-microglobulin (~m), 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-2d 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 a1, a2, and a3 external regions; exon 5 encodes the transmembrane region; and exons 6-8 encode the cytoplasmic region. The a1 and a2 regions fold to form one external domain and the a3 and ~m 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 promotor 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-Ld), 20-8-4 (anti-Q6, -Kd, -Kb, -r, -s), 34-1-2 (anti-Q6, -Dd, -Kd, -Ld, -Kb, -r, -s, -p, -q), 30-5-7 (anti-Ld, -Dd, -Ld), 34-5-8 (anti-Dd), 34-2-12 (anti-Dd), 81-L (anti-Dd, -s, -u), 81.R (anti-Dd, -p, -u), 97.H (anti-Dd, -Kd, -p, -u, -v), 97.G (anti-Dd, -Kd, -p, -u, -v), and 11-4 (anti-Kb). 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, Ld, Dd, and Kb genes is given in refs. 8 and 10.

Abbreviations: MHC, major histocompatibility complex; ~m, ~2-microglobulin; bp, base pair(s); kb, kilobase(s).

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Construction of Hybrid Class I Genes. For construction of the hybrid Q5/Ld, Q7/Ld, and Q8/Ld 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 conserved region from the 3' end of exon 3. The procedure used for constructing these hybrid genes has been described for the Q6/Ld gene (Fig. 2b) (13). Briefly, a 3-kilobase (kb) Bgl II/BamHI 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 BamHI site of pBR322. The resultant plasmid 1104-4 was linearized with BamHI, and a 2.4-kb BamHI fragment carrying the 3' end of H-2Ld gene, including exons 4, 5, 6, 7, and 8, was inserted to make the Q6/Ld hybrid gene. For constructing the Q5/Ld, Q7/Ld, and Q8/Ld hybrid genes, the 1.8-kb Xba I fragment containing the 5' end of Q6 was deleted from the Q6/Ld hybrid construct, and it was replaced with the equivalent 1.8-kb Xba I fragment from the Q5, Q7, or Q8 genes (Fig. 2).

For construction of the Q6/Q7 hybrid gene, plasmid 1104-4 was linearized with BamHI and the 2.7-kb BamHI 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 BamHI 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 BamHI and HindIII sites of plasmid 1104-4, and inserting in its place the 3.7-kb BamHI/HindIII 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-2b haplotype) were transfected with plasmid DNAs as described (12-14, 17). Radioimmunoassays were performed as described (12-14, 17). Radioimmunoassays were performed as described (12-14, 17).

Identification of the H-2b Q10 Gene in BALB/c Mice. An 18-base oligodeoxyribonucleotide probe, 3' AGTGTTGTAACGACTAGAC 5', complementary to the DNA sequence encoding amino acids 285–290 of the H-2b Q10 protein (18, 19) and corresponding region of the homologous H-2d 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/Ld, Q7/Ld, and Q8/Ld. 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-2Ld 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, α2 and α3) was fused to the 3' portion (exons 4–8 encoding the α2 external, transmembrane, and cytoplasmic regions) of H-2Dd, H-2Kd, and H-2Ld genes were expressed. To test the
is likely that these two antibodies react with the 
Kd, and Q6 antigens (13, 20). Previous results have indicated that 
the 97.G antibody reacts with the Q81Ld and Q61Ld hybrid antigens, 
however, unambiguous in showing that 34-1-2 and 97.G 
dilution curves obtained with the Dd antigen. The results are, 
however, unambiguous in showing that 34-1-2 and 97.G 
products bound monoclonal antibody 34-1-2 in addition to 
monoclonal antibody 28-14-8, and that the Q81Ld antigen 
crossreacted weakly with monoclonal antibody 97.G (23).

expression potential of the Q5, Q7, and Q8 genes, two sets of 
hybrid genes were constructed (Fig. 2).
The Q5/Ld, Q6/Ld, Q7/Ld, and Q8/Ld 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 α1−β2m 
domain of the H-2Ld antigen (12, 13, 20). The L cells transfected 
with the Q5/Ld, Q6/Ld, and Q8/Ld hybrid constructs bound the 
28-14-8 antibody, while L cells transfected with Q7/Ld constructs did not (Table 1). These data suggest that L cells 
transfected with Q5/Ld, Q6/Ld, and Q8/Ld hybrid genes expressed membrane-bound hybrid protein products, while cells 
transfected with Q7/Ld gene did not. Formally, we 
cannot exclude the possibility that fusion of the Q7 α1−α2 
domain to the Ld carboxyl terminus has distorted poly­ 
morphic determinants on the α1−β2m domain and rendered it 
detectable to the 28-14-8 antibody. This interpretation, 
however, appears unlikely in view of the apparent 
conformational independence of α1−α2 and α1−β2m domains of 
class I molecules (12, 13, 20–22). We also screened mass 
cultures of the Q5/Ld, Q6/Ld, Q7/Ld, and Q8/Ld transfec­ 
tants with a panel of monoclonal antibodies directed against 
H-2d transplantation antigens (Table 1). The results of these 
expressions and the results obtained with cloned lines of 
these transfec­
tants (Fig. 3) revealed that Q5/Ld hybrid gene 
products bound monocular antibody 34-1-2 in addition to 
monocular antibody 28-14-8, and that the Q8/Ld antigen 
crossreacted weakly with monocular 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/Ld construct bound antibodies 
28-14-8 and 34-1-2 at a lower saturation level than cells 
transfected with the Ld and Dd genes. In addition, the dilution 
curves for reaction of the 97.G antibody with the Q8/Ld 
hybrid gene product were shifted approximately one order of 
magnitude toward higher antibody concentrations relative to 
dilution curves obtained with the Dd antigen. The results are, 
however, unambiguous in showing that 34-1-2 and 97.G 
antibodies react with the Q5/Ld product and the Q8/Ld product, respectively. Previous results have indicated that 
the 34-1-2 antibody reacts with the α1−α2 domain of Ld, Dd, 
Kd, and Q6 antigens (13, 20) and that the 97.G antibody reacts 
with the α1−α2 domain of the Dd molecule (14). Therefore, it 
is likely that these two antibodies react with the α1−α2 domain of 
the Q5/Ld and Q8/Ld hybrid antigens.

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

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

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Radioimmunoassays on mass cultures of transfected L cells were performed as described (17). Results are expressed as 125I-labeled protein A bound (cpm per 5 × 10^6 cells). The numbers shown are an average of two experiments, with experimental error being <10% of the values shown.

As a control for these experiments, three hybrid genes were constructed in which the Xba I fragments covering the S′ 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 Qα genes. Monoclonal antibodies used in titration experiments were 28-14-8 (a and c), 34-1-2 (b), and 97.G (d).](image-url)
orientation in combination with the 3' end of the Ld 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. Moreover, when infected with the complete Q5/Q6 hybrid, not in any case bind the 28-14-8 antibody (results not shown). Therefore, expression of the Ld antigenic determinant by the Q5/Ld, Q6/Ld, and Q8/Ld 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 \( \alpha_1-\alpha_2 \) 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 NH2-terminal portion of the Q6 molecule to the COOH terminus of the Kd, Ld, and Dd molecules did not distort determinants located on the \( \alpha_1-\alpha_2 \) 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 \( \alpha_1-\alpha_2 \) domain of Q6 appear to be fairly independent of interaction with other \( \alpha_1-\beta_2 \)-m domains.

The hybrid genes were transfected into L cells, and the transfectants were screened with the panel of monoclonal antibodies 34-1-2 and 34-1-2 antibodies, or any of the other anti-H-2d 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 Ld 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/Ld 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/Ld 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 genes transfectants with monoclonal antibodies 34-1-2 and 97-G (Fig. 3). These determinants, shown to be encoded by the \( \alpha_1-\alpha_2 \) 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-2\( ^d \) Q10 Equivalent in BALB/c Mice.**

Mellor _et al._ (24) have recently characterized a nonpolymorphic class I gene (H-2\( ^d \) Q10) from C57BL/10 (H-2\( ^d \) 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 antigen genes 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-2\( ^d \) Q10 gene may be similar to the gene in cosmid cluster 9 of the H-2\( ^d \) haplotype (H-2\( ^d \) Q8). To test whether the gene in cosmid cluster 9 is in fact the H-2\( ^d \) haplotype equivalent of the H-2\( ^b \) Q10 gene, an 18-base oligonucleotide probe specific for exon 5 of the H-2\( ^b \) Q10 gene was synthesized and hybridized with DNAs from cosmids isolated from H-2\( ^b \) 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-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 the Qa region genes carry promoter elements and leader peptides that are functional in L cells and that the \( \alpha_1-\alpha_2 \) 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-2\( ^d \) Q7 gene has been proposed to have arisen as a fusion product of two genes in the H-2\( ^d \) haplotype (H-2\( ^b \) Q8 and H-2\( ^d \) 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-2\( ^d \) haplotype equivalent of the H-2\( ^d \) 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-2\( ^b \) Q10 gene compared with a cDNA sequence derived from H-2\( ^b \) haplotype revealed only seven nucleotide substitutions out of 1368 bp (24). Therefore, one feature residing in the 3' portion of the homologous H-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 CD (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 α1-α2 domains encoded by these genes contain determinants recognized by antibodies directed against polymorphic regions of H-2d 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 α1-α2 domains of the Q5, Q6, and Q8 hybrid antigens exhibit different patterns of reaction with the panel of anti-H-2d monoclonal antibodies, indicating differences in antigenic determinants. It will be possible to use L cells transfected with the Qa/Ld hybrid genes to generate new monoclonal antibodies directed against the α1-α2 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. Kronenberger, 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.