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Duplicated gene pairs and alleles of class I genes in the Qa2 region of the murine major histocompatibility complex: a comparison

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DNA restriction maps of the major histocompatibility complex and hybridization with low copy probes have previously revealed strong homology between the Q6-Q7 and the Q8-Q9 class I gene pairs in the Qa2 region of the C57BL/10 mouse. After DNA sequence analysis of the Q7, Q8 and Q9 genes, we have compared the Q7 gene with its apparent allele, 27.1, from the BALB/c mouse; the 99% homology between Q7 and 27.1 indicates that this is a non-polymorphic gene. Comparison of Q7 with Q9, its homologue in the Q8-Q9 gene pair, revealed >99% homology, thus supporting our proposal that the Qa2 region has evolved by the duplication of gene pairs. Q7 was also found to be homologous (93%) to Q8, the second member of the Q8-Q9 pair. However, the first exon (encoding the leader sequence) as well as the first intron of Q7 and Q8, which are presumably not subject to strong selective pressure, are essentially identical in nucleotide sequence (having only one mismatch), which suggests that >200 bp of DNA may have been exchanged by gene conversion. Furthermore, transcripts of both Q7 and Q8 would have termination codons derived from the exon that normally encodes the transmembrane domain, thus these genes could encode either membrane-bound class I proteins that lack a cytoplasmic protein domain or class I proteins that are secreted.

Key words: Qa2 region/gene conversion/major histocompatibility complex/gene duplication

Introduction

The class I genes in the major histocompatibility complex (MHC) of the mouse encode 37 000–45 000 dalton glycoproteins that are non-covalently associated with β_2 microglobulin. The 3–5 class I genes mapped to the H-2 complex encode the polymorphic classical transplantation antigens (H-2K^b and H-2D^b in the C57BL/10 mouse) that are expressed on nearly all cells (Steinmetz *et al.*, 1982; Weiss *et al.*, 1984). Cytotoxic T lymphocytes recognize these antigens either in association with viral antigens or by themselves, as in the case of allograft rejection. The genes encoding these antigens comprise eight exons, the first five exons encoding respectively a leader sequence, three external protein domains (N, C1 and C2) and a transmembrane domain; the last three exons encode a cytoplasmic protein domain (Steinmetz *et al.*, 1981; Weiss *et al.*, 1983).

More than 20 additional murine class I genes have been cloned and mapped to the Qa2 and TL regions immediately telomeric to the H-2 complex (Steinmetz *et al.*, 1982; Weiss *et al.*, 1984). Class I gene products from these regions are expressed in only a limited number of tissues and their functions are not understood. For example, the Q10 gene has only been shown to be expressed in liver (Cosman *et al.*, 1982; Mellor *et al.*, 1984) and the Qa2 antigen is apparently expressed only on lymphoid cells (Flaherty, 1976).

Figure 1 shows the 10 class I genes we have mapped to the Qa2 region of the C57BL/10 mouse (Weiss *et al.*, 1984). By DNA restriction mapping and by hybridization with probes from the DNA flanking these genes we have shown that the Q4-Q10 genes are more closely related to each other than to either the H-2D^b gene, the Q1-Q3 genes or to the TL region genes. Within the Q4 to Q10 group of genes, alternating genes are more similar to each other than to adjacent genes, suggesting that this group of genes evolved first by the duplication of the ancestral gene and then by the duplication of a gene pair. The homology is strongest between the Q6-Q7 gene pair and the Q8-Q9 gene pair. Q5 also shows strong homology to Q7 and Q9. The Q4 and Q10 genes have substantially different restriction maps than either Q6, Q8 or the odd numbered gene groups; however, hybridization with low copy probes to the DNA flanking these genes suggest that Q4 and Q10 are more closely related to the Q5-Q9 genes than to other class I genes in the TL complex.

We have determined the DNA sequence of genes Q7 and Q8, thus allowing us to compare the odd numbered Q7 gene with both its allele in the BALB/c mouse, 27.1 (Steinmetz *et al.*, 1981; Weiss *et al.*, 1984), and with the Q8 gene, a member of the even-numbered gene group. To compare Q7 with another member of the odd-numbered group of genes, we determined the DNA sequence of the region containing exons 1, 2 and 3 of Q9; this region was chosen since these are generally the most polymorphic exons in class I genes. In this paper we use these comparisons as a basis for discussing the role of gene duplication and gene conversion in the evolution of the class I genes of the Qa2 region.

Results

Comparison of alleles: Q7 and 27.1

Q7 and 27.1 have been cloned from the C57BL/10 (H-2^b) and BALB/c (H-2^d) mice, respectively. The similarity between the restriction maps of the DNA surrounding these genes strongly suggests that they are alleles (Weiss *et al.*, 1984; Steinmetz *et*

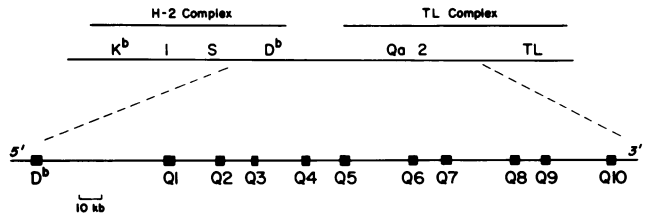

Fig. 1. Map of the murine MHC; the centromere is to the left of the H-2 complex. The H-2K^b and H-2D^b antigens are encoded in the H-2 complex as are class II antigens in the I region and complement components in the S region. The TL complex is subdivided into the Qa2 and TL regions. The gene organization of the D^b and Qa2 regions in the C57BL/10 mouse is shown on the lower line; all genes are in the same 5′ to 3′ orientation.

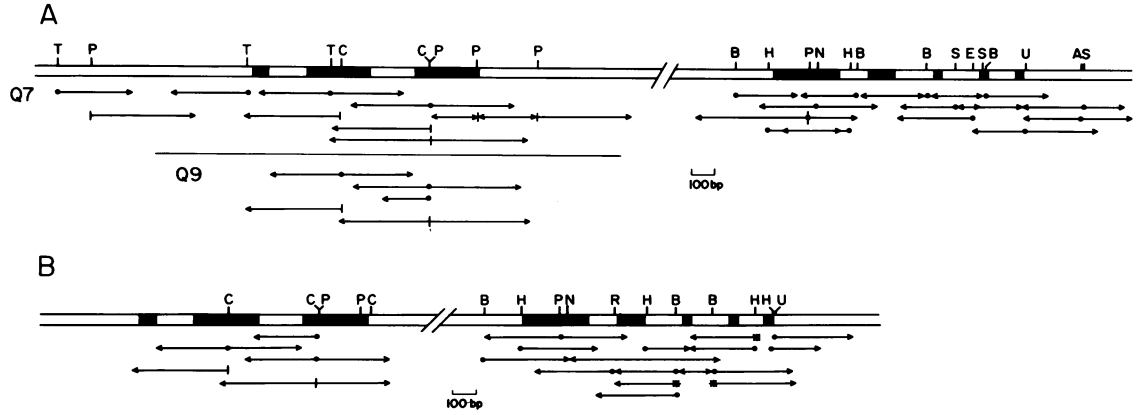

Fig. 2. Sequencing strategy for the Q7 and Q9 genes (**A**) and the Q8 gene (**B**). In this figure the dark boxes correspond to the regions homologous to exons 1–8 of H-2K^b. Only those restriction sites used in sequencing are shown (B = *Bgl*II, C = *Sac*II, H = *Hinf*I, K = *Kpn*I, E = *Nde*I, A = *Ava*I, P = *Pst*I, R = *Rsa*I, S = *Sac*I, T = *Taq*I and U = *Pvu*II). The arrows, indicating the individual sequences obtained by chemical degradation sequencing, begin with either a dot or a star to indicate either 3′ or 5′ labeling, respectively. Those arrows beginning with vertical lines indicate sequence that was obtained by dideoxy-sequencing.

al., 1981). We have, therefore, determined the DNA sequence of Q7 by the strategy shown in Figure 2; the DNA and predicted protein sequence is shown in Figure 3.

The exon boundaries have been assigned to the Q7 gene based on the presence of consensus splice donor and acceptor sequences (Cech, 1983), and by comparison with the H-2K^b gene (Weiss *et al.*, 1983). The region corresponding to exon 7 in the H-2K^b gene is presumably spliced out in the transcript of the Q7 gene since the AG dinucleotide, normally present immediately 5′ to the beginning of an exon, is not present 5′ of the region in the Q7 gene corresponding to the H-2K^b exon 7 (Figure 3). A similar situation exists in this region of the Q10 gene, whose transcript also apparently splices out the segment corresponding to H-2K^b exon (Mellor *et al.*, 1984; Kress *et al.*, 1983). In addition, a cDNA clone from the DBA/2 (H-2^d) mouse has recently been isolated that lacks the region homologous to exon 7 of H-2K^b. This cDNA clone is homologous to the odd-numbered gene group (Q5, Q7, Q9) in the C57BL/10 (H-2^b) mouse (Lalanne *et al.*, 1985).

Unlike the highly polymorphic genes of the H-2 complex which commonly display homology between alleles of only ~90%, Q7 and 27.1 are 99% homologous (Table I). Furthermore, in contrast to the H-2K^b and H-2K^d alleles (Weiss *et al.*, 1983), most of the differences are in the introns. Of the five base changes that occur in the coding region, three predict an amino acid difference between the Q7 and 27.1 gene products. Both 27.1 and Q7 would encode a protein with a potential glycosylation site (Asn-X-Thr) in the N and C2 domains as well as four conserved Cys residues that may be involved in disulfide bond formation within the C1 and C2 domains. The C2 domain of class I proteins is the most conserved of the three large protein domains, presumably because it must associate with β_2 microglobulin. However, Q7 and 27.1 have a 9-bp insertion relative to other class I genes, which encodes three additional amino acids at the C-terminal end of the C2 domain. Both genes have a termination codon in exon 5; this termination codon in the exon that normally encodes the transmembrane domain originally led to the suggestion that 27.1 was a pseudogene (Steinmetz *et al.*, 1981). However, it has since been shown that the 27.1 promoter is functional in L cells transformed with a hybrid 27.1 gene (Stroynowski *et al.*, 1985).

Comparison of duplicated Qa2 region genes: Q7 and Q9

Exons 2 and 3 are the most polymorphic among alleles of H-2 complex class I genes; for example, H-2K^b and K^d are only 80% homologous in these exons and >95% homologous in the other six exons (Weiss *et al.*, 1983). We therefore determined the DNA sequence of the region containing the first three exons of Q9 using the strategy shown in Figure 2 and found that Q7 and Q9 are highly conserved. Comparison of this region of Q7 and Q9 revealed only a single base change out of 959 bases. This mismatch in exon 3 predicts the presence of a Gln residue in Q7 and a Glu residue in Q9 (Figure 3). In addition, the *Pst*I site in Q7 at this location is not found in Q9 due to this change.

Comparison of adjacent Qa2 region genes: Q7 and Q8

While we found Q7 to be very similar to Q9, its homologue in the Q8-Q9 gene pair, it was less similar to the Q8 gene, whose DNA sequence was determined using the strategy shown in Figure 2. The first exon of Q8 is identical to that of the 27.1 gene. Stroynowski *et al.* (1985) have shown that hybrid gene constructs consisting of the first three exons of the 27.1 gene and the last five exons of the H-2L^d gene are expressed on the surface of transformed L cells, thus indicating that the first exon of the 27.1 gene, and hence also the Q7 and Q8 gene, encodes a functional leader sequence.

Like Q7 and Q10, there is a termination codon in exon 5 of Q8. This termination codon in the exon that normally encodes the transmembrane domain of class I genes is 14 bp 5′ of the termination codon in the Q7 gene. The Q8 gene predicts an amino acid sequence that contains the same conserved Cys residues and potential glycosylation sites as the Q7 gene (Figure 3).

Like Q7, the Q8 gene is missing the AG dinucleotide that signals the beginning of exon 7 in the H-2K^b gene. However, there is an AG dinucleotide in a splice consensus sequence 9 bp upstream of this point. This corresponds to the splice site used at the 5′ end of exon 7 in several human class I genes (Strachan *et al.*, 1984). Thus, Q8 probably consists of eight exons, exon 7 being 9 bp longer than the H-2K^b exon 7. In addition, the splice acceptor sequence in the region homologous to H-2K^b exon 8 is not present in the Q8 gene; thus, exon 7 of Q8 may splice to the region which is homologous to the beginning of the H-2D^b exon 8, which is 27 bp 3′ to the beginning of the H-2K^b exon 8.

Overall, the Q7 and Q8 genes are 93% homologous (Table I); however, in the 220 bp starting at the ATG encoding the first Met residue, there is only one difference between these two genes, suggesting that there has been an exchange of information between Q7 and Q8. Exons 2, 3 and 4 of Q7 and Q8, which nor-

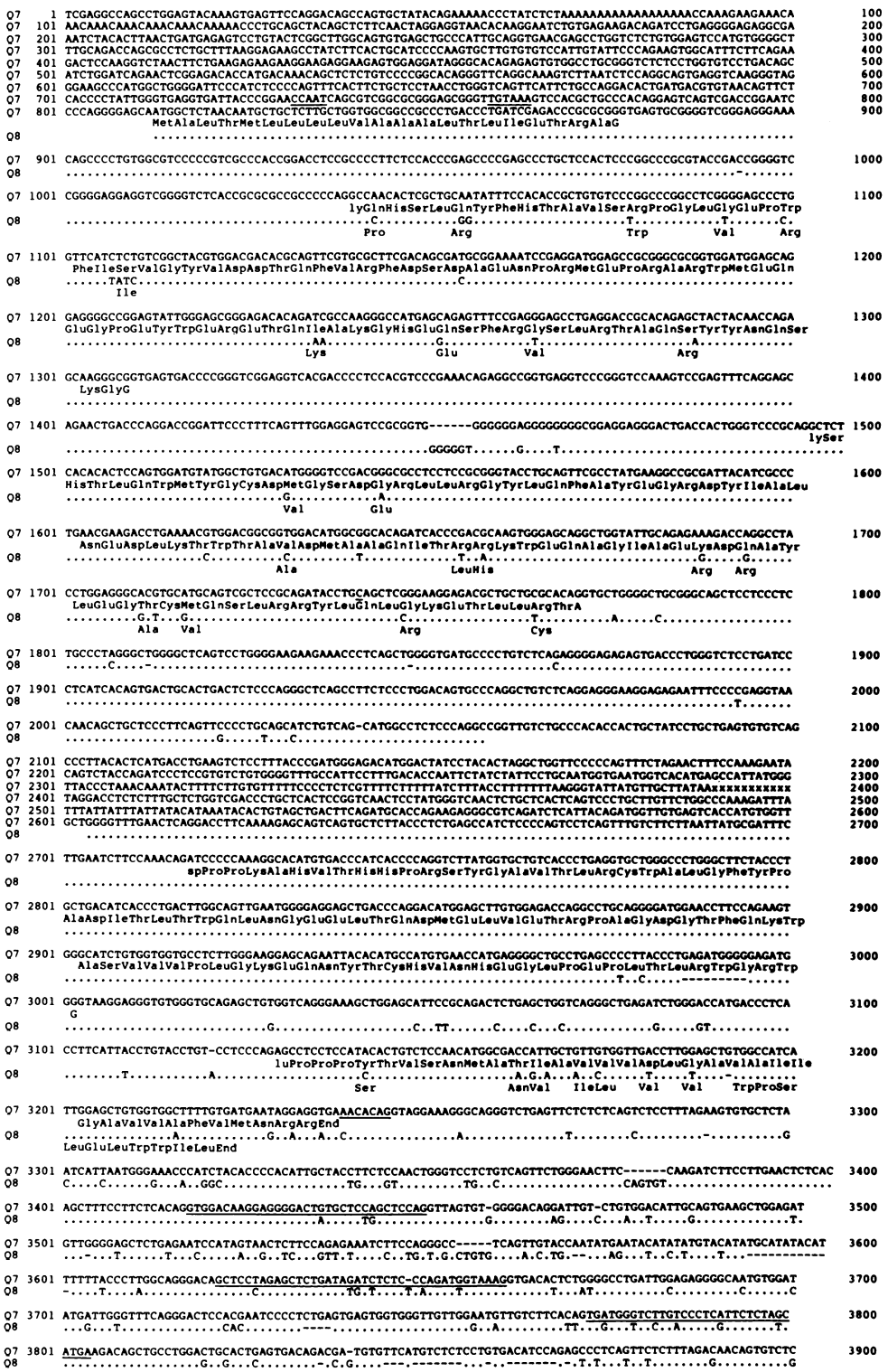

Fig. 3. Comparison of the Q7, Q8 and Q9 genes. The DNA sequence of Q7 is shown with its predicted amino acid sequence on the line below. The Xs in intron 3 represent an ~1100 bp gap in the sequence that has been estimated from restriction maps. The Q8 sequence is shown below the Q7 sequence; only differences are shown. Dots in the Q8 sequence indicate that Q7 and Q8 are identical at that position as well as delimiting the extent of the Q8 sequence that has been determined. The lack of a nucleotide in one sequence relative to the other sequence due to insertions or deletions is indicated by a dash. The sequence of Q9 that has been determined begins at the first nucleotide of exon 1 and ends at the last nucleotide of exon 3. The difference between Q7 and Q9 is in exon 3, the underlined C in the Q7 sequence is a G in the Q9 sequence. Those regions homologous to the 3′ end of exon 5, and to exon 6, 7 and 8 of the K^b gene have been underlined. Those regions homologous to sequences associated with eukaryotic promoters have been underlined (the CAT and TATA boxes in the 5′-untranslated region); the polyadenylation signal sequence (AATAAA) in the 3′-untranslated region has also been underlined.

Table I. DNA sequence homology

		Length[a]		Changes[b]					
		Q7	Q8	Q7/Q8	Q7/Q10	Q7/27.1	Q7/K^b	Q8/Q10	Q8/K^b
Leader	exon 1	64	64	0 (0)	17 (27)	0 (0)	16 (25)	17 (50)	16 (47)
	intron 1	163	162	1 (1)	48 (11)	6 (3)	46 (13)	49 (12)	47 (14)
N	exon 2	270	270	16 (6)	39 (14)	2 (1)	34 (13)	34 (13)	29 (11)
	intron 2	186	192	10 (3)	27 (12)	6 (3)	32 (17)	32 (10)	41 (19)
C1	exon 3	276	276	14 (5)	34 (12)	1 (1)	37 (13)	37 (13)	37 (13)
	intron 3	2060	2060	11 (3)	34 (6)	18 (2)	58 (8)	10 (4)	18 (5)
C2	exon 4	285	276	11 (1)	22 (5)	2 (1)	27 (7)	13 (5)	18 (6)
	intron 4	126	127	13 (10)	12 (10)	9 (6)	12 (10)	9 (7)	6 (5)
TM	exon 5	117	116	14 (12)	34 (19)	0 (0)	17 (13)	33 (19)	16 (14)
	intron 5	172	177	25 (12)	29 (13)	9 (1)	26 (12)	17 (10)	5 (3)
	exon 6	33	33	3 (9)	4 (12)	0 (0)	4 (12)	5 (15)	1 (3)
	intron 6	171	163	56 (23)	35 (13)	1 (1)	33 (12)	37 (23)	62 (22)
	exon 7	39	40	7 (18)	6 (15)	0 (0)	6 (15)	6 (15)	5 (12)
	intron 7	112	108	19 (14)	20 (18)	0 (0)	12 (11)	29 (24)	20 (16)
	exon 8	32	32	6 (19)	6 (19)	0 (0)	4 (12)	7 (21)	5 (16)
	3′ UT	410	184	43 (14)	38 (14)	1 (1)	91 (6)	61 (17)	49 (16)
Total				(7)	(10)	(1)	(12)	(13)	(11)

[a]All lengths are given in base pairs; for the sake of comparison the exon-intron boundaries used were based on homology to H-2K^b, even though Q7 and Q8 presumably splice differently (see text). The 3′-untranslated region used for comparison begins after the termination TGA used by H-2K^b, even though, in the case of Q7 and Q8, the entire region after exon 5 is untranslated. The length of intron 3 is approximate and is based on restriction mapping. Due to sequence gaps in intron 3, the number of bases available for comparison of Q7 to Q8, Q10, 27.1 and H-2K^b were 339, 406, 935, and 453 respectively; the number of bases available for comparison of Q8 to Q10 and H-2K^b were 201 and 326 respectively. A total of 200 and 270 nucleotides, respectively, were available for the comparison of the 3′ UT region of Q7 to Q8 and Q10.

[b]Under changes, the number of nucleotide differences is listed for each region (e.g., a 5-nucleotide deletion was counted as 5). When the percentage changes were calculated (shown in parentheses), each deletion or insertion was counted as a single event regardless of the number of nucleotides involved.

mally encode the extracellular protein domains, are greater than ~96% homologous, while these genes show only 85% homology 3′ of exon 4.

Comparison of Q7 and Q8 with the other class I genes

Soloski *et al.* (1982) have reported the partial amino acid sequence of the Qa2 antigen. Two amino acids must be deleted from the amino-terminal end of the N domain of this sequence in order to obtain maximum homology when aligned with other class I sequences. Neither Q7, Q8 or Q9 would encode the two additional amino acids reported to be present in the Qa2 antigen; however, Stroynowski *et al.* (1982) have shown that the N and C1 domains of 27.1, the allele of Q7, are recognized by antisera and cytotoxic T cells that recognize the Qa2 antigen. Thus, those antigenic determinants that currently define the Qa2 may be expressed on a number of different class I molecules.

In earlier work we used probes to the DNA flanking class I genes to show that the Q10 gene was more closely related to the Q4 to Q9 genes than to the D^b to Q3 genes or to the TL region genes (Weiss *et al.*, 1984). While we have noticed some additional features that the Q10 gene shares with Q7 and Q8 (e.g., a B2 repeat at the end of the third intron), the DNA sequence of Q10 is not obviously more closely related to the Q8 than to the Q7 gene.

We have isolated a probe that hybridizes to a 640-bp *Bam*HI fragment located 2.6 kb 5′ of the H-2K^b, Q6 and Q8 genes as well as to a 680-bp *Bam*HI fragment located 2.6 kb 5′ of the K1, Q7 and Q9 genes. This relationship between gene pairs – the K1-K^b pair in the H-2K region, and the Q6-Q7 and Q7-Q8 gene pairs in the Qa2 region – suggested that the H-2K region may have been generated by the translocation of a gene pair from the Qa2 region (Weiss *et al.*, 1984). The presence of the 640-bp fragment suggested that the H-2K^b gene would be the homologue of the Q8 gene; however, the DNA sequence of the H-2K^b gene is no more closely related to the Q8 gene than to the Q7 gene. In fact, the Q8 gene seems to be a mosaic of other class I genes; for example, the 33 bp at the 5′ end of exon 5 are 100% homologous to the K^b gene but only 85% homologous to the D^b gene, while a block of 97 bp near the 3′ end of exon 5 are 98% homologous to the D^b gene but only 81% homologous to the K^b gene (not shown). This relationship of Q8 to H-2K^b and H-2D^b again suggests that sequence information may be transferred between genes >1000 kbp apart by gene conversion.

Discussion

Although the H-2 class I antigens are among the most polymorphic proteins known, the class I genes in the Qa2 region display a surprisingly low level of polymorphism. Q7 and its allele 27.1 are 99% homologous at the DNA level and predict amino acid sequences with only three differences (not shown). Mellor *et al.* (1984) have shown that there is >99% homology between the Q10 gene of the C57BL/10 (H-2^b) and SWR/J (H-2^q) mice. The lack of polymorphism in this region may indicate that these Qa2 region genes have an important function and that their conservation is due to selective pressure. However, the near identity of the region of the Q7 and Q9 genes that we compared might suggest that this region of the chromosome may be subject to a lower than normal frequency of mutation since it seems unlikely that there would be a selection for the conservation of two nearly identical copies of a gene. We believe a more likely alternative is that gene conversion may act to homogenize genes in this region.

Mellor *et al.* (1983) have previously presented evidence that the Q10 gene may have been the donor of the DNA sequence that generated the H-2K^{bml} mutation by gene conversion. In that

case, a short segment of only tens of nucleotides may have been transferred over a distance of >1000 kb from the Q10 gene to the H-2K gene. The lack of polymorphism between alleles of the Q10 gene (Mellor *et al.*, 1984) suggested to us that this gene had been the source of the DNA sequence to generate the *bml* mutation but that the Q10 gene itself had not been altered by similar events.

This was concordant with the proposal of Brégégère (1983), suggesting that gene conversion could help generate polymorphism in the H-2 complex rather than homogenizing all class I genes only if the conversions were directional, that is, if there were a strong bias toward the non-polymorphic genes in the Qa2/TL regions donating sequence information to the polymorphic genes in the H-2 complex. However, the DNA sequences presented in this paper suggest that gene conversion also occurs within genes in the Qa2 region. The almost complete homology between the exon encoding the leader sequence and the first intron of genes Q7 and Q8, combined with the fact that leader sequences and introns are not generally subject to stringent selective pressure, suggests that a gene conversion, involving >200 bp, has occurred. Additional evidence for the involvement of the Q8 gene in gene conversions is the strong homology of the 5′ and 3′ ends of the Q8 gene exon 5 to H-2K^b and H-2D^b, respectively. The process of gene conversion may also explain the similarities between the Q7 and Q9 genes that we have presented in this paper as well as our previous observation of similarity among members of both the even-numbered gene group (Q4, Q6, Q8 and Q10) and the odd-numbered gene group (Q5, Q7 and Q9) at the level of restriction enzyme maps and hybridization with low copy DNA probes (Weiss *et al.*, 1984). It seems likely that these groups of genes have either retained their similarity or been made similar by gene conversion events involving hundreds or thousands of nucleotides rather than tens of nucleotides, as in the generation of mutant class I genes such as H-2K^{bml}. This type of large-scale gene conversion has been observed in other systems, e.g., the globin gene family (Slightom *et al.*, 1980).

Large-scale gene conversions may give a functionally but not mechanically directional character to conversions within the MHC. Thus, genes in the Qa region might accept small-scale gene conversion events such as that observed in the generation of the H-2K^{bml} mutant. However, these mutations may subsequently be eliminated by gene conversion involving a large segment of DNA from either its allele or another member of its group of homologous genes. The ultimate result of this would be to give gene conversion in the MHC the directional character predicted by Brégégère (1983). However, if this is true, then the question becomes why such large-scale gene conversion events do not occur in the H-2K and H-2D genes since they appear to occur in the Qa2 region.

The *in vivo* role of proteins that may be encoded by the Qa2 region is not known; however, there are several lines of evidence suggesting that the Q7, Q8, and Q9 genes are not simply pseudogenes. First, both gene transfer experiments involving a hybrid 27.1 − H-2L^d gene and the isolation of Q7-lie cDNA clones from the DBA/2 mouse demonstrate that Q7-like genes are transcribed (Stroynowski *et al.*, 1985; Lalanne *et al.*, 1985). Secondly, the strong homology between the Q7 and 27.1 alleles suggests that there has been selection for the conservation of the protein that they might encode. Finally, the termination codon in exon 5 of the Q7 and Q8 genes need not prevent the expression of functional class I genes. Inspection of exon 5 of the Q8 gene indicates that it would encode a hydrophobic domain followed by the hydrophilic sequence, Asn-Arg-Arg (Figure 3). This may well anchor the Q8 protein in the membrane to generate a membrane-bound protein like the H-2 and Qa2 antigens. The aspartic acid residue 19 residues from the carboxy terminus of the predicted Q7 protein makes it more difficult to predict whether the Q7 gene encodes a membrane-bound protein. However, the demonstration that the Q10 gene of the C57BL/10 mouse could encode a secreted class I protein (Maloy *et al.*, 1984; Devlin *et al.*, 1985) provides precedence for the existence of genes in this region that encode secreted class I proteins.

Materials and methods

Using standard techniques (Maniatis *et al.*, 1982), the Q7 gene was subcloned from cosmid B1.27, and the Q8, as well as the Q9 gene were subcloned from cosmid B4.8 (Weiss *et al.*, 1984). These genes were sequenced using published procedures (Maxam and Gilbert, 1977; Bencini *et al.*, 1984; Sanger *et al.*, 1980). Enzymes were purchased from New England Biolabs or Boehringer Mannheim. The dideoxy-sequencing kit was purchased from Amersham.

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