Immunologic Research

A Selective Reference to Current Research and Practice

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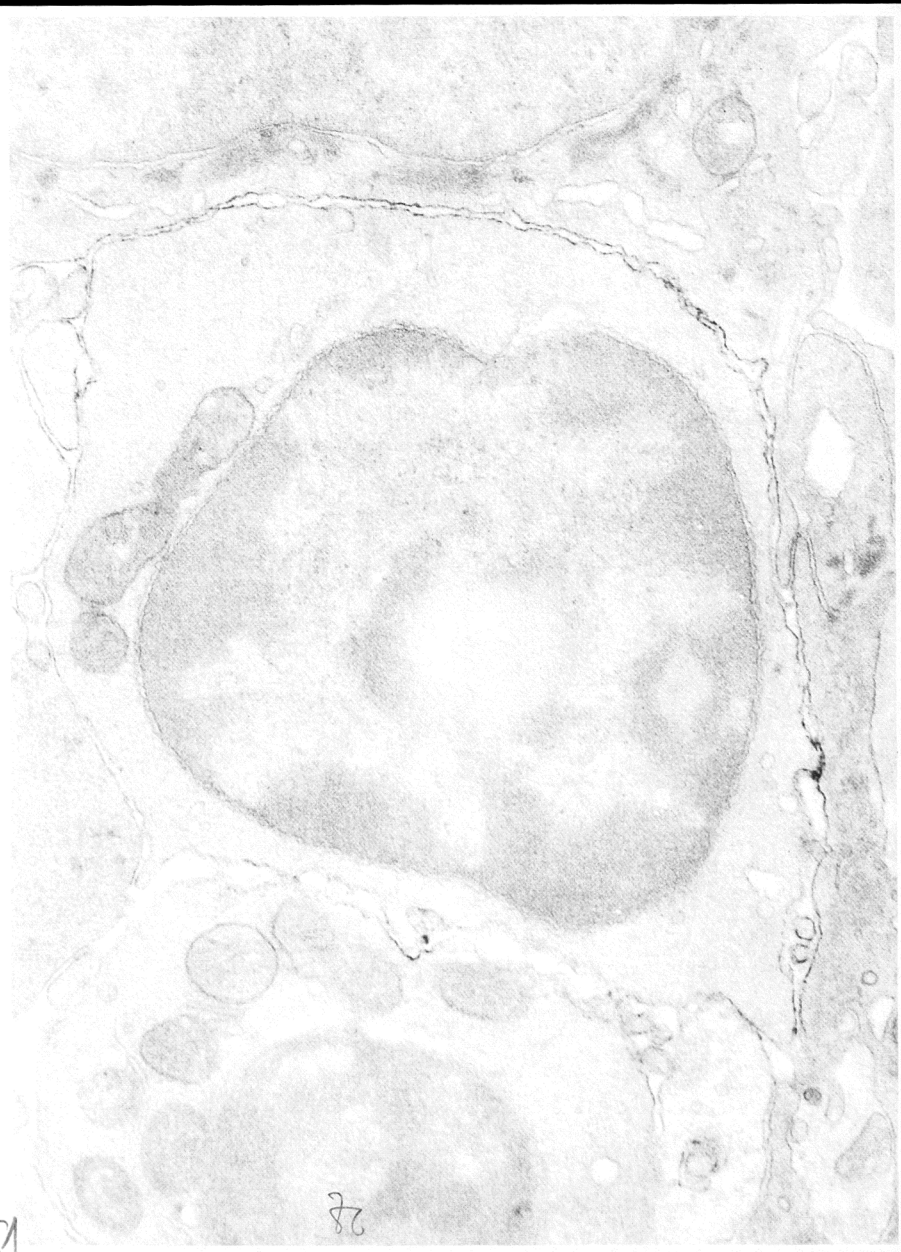

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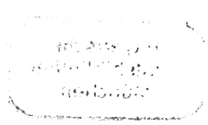

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Molecular Biology of the Mouse Q Region

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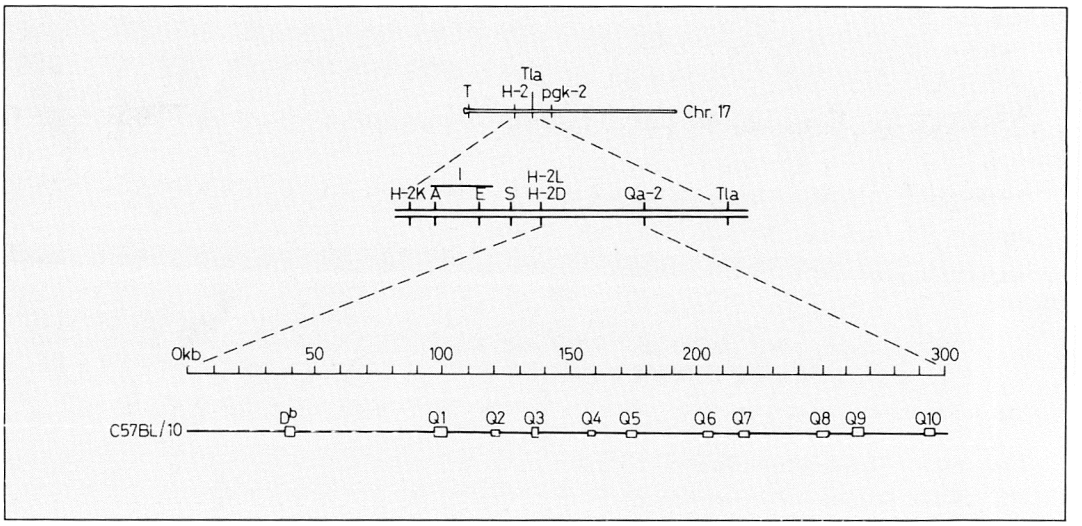
The major histocompatibility complex (MHC) of the mouse can be subdivided into three regions encoding class I genes. The H-2 region contains the genes for the classical transplantation antigens H-2K, H-2D and H-2L, which are expressed on virtually all cell types. Telomeric to the H-2 complex is the Qa-2 and Tl region, which encodes the Qa and the Tla antigens, respectively (fig. 1). In addition to the genes coding for these well-characterized polypeptides, the majority of all other class I genes are localized in this portion of the MHC. Their products are not expressed on all cells and are therefore named class I differentiation antigens.

The Qa-2 region is characterized by a gene product, the Qa-2 antigen, and is defined by the recombinant mouse strains B6.K1 and B6.K2 [1]. These recombinant mouse strains are identical at the Tla and H-2 loci (fig. 2), but differ in their expression of class I differentiation antigens, the Qa-2 molecules. It was shown that several mouse strains are not able to express these determinants [2] as, e.g., the H-2^k strain. Several groups took advantage of the existence of such a null allele for the genetic localization of a large number of genes. Thus, a cluster of ten linked class I genes spanning 250 kb was shown to be located in the Qa-2 region of the C57BL/10 strain by mapping polymorphic DNA restriction fragments as outlined before [3].

Localization of a Gene Cluster to the Qa-2 Region

The first gene to be localized in the Qa-2 region was the Q7 (27.1) gene by using a genomic 2.7 kb Bam HI fragment containing the 3′ half of the 27.1 gene as restriction fragment length polymorphism, which is absent in the genome of the AKR mouse and other strains which are negative for the expression of the Qa-2 antigen [4]. This polymorphic 2.7 kb Bam HI fragment is also present in the isolated genes Q5 and Q9 of the C57BL/10(B10) mouse, thus these genes can be mapped to the Qa-2 region as well. The genes Q5, Q7 and Q9 have been previously located in the Q region by employing a 0.64 kb 5′ flanking Bam HI fragment derived from the H-2K locus as polymorphic DNA probe on a Hind III digest or by using the 3′ flanking K region probe which hybridizes to polymorphic Bgl II fragments [3]. The Q1 gene was shown to be encoded in this region



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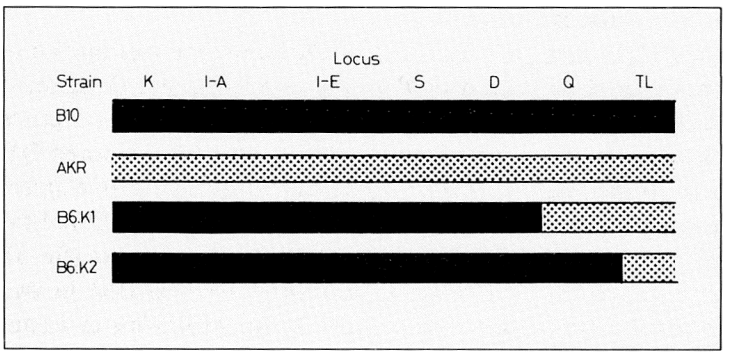

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Fig. 1. Map of the murine MHC on chromosome 17. The organization of the H2-D and Qa-2 regions in the C57BL/10 mouse is shown on the lower line; all genes are in the same 5′ to 3′ orientation.

Fig. 2. Genetic maps of the mouse strains used to localize cosmids to the Qa-2 region.

with a 4 kb Pvu II fragment just 5′ of the gene. In this way it was possible to localize the site of the recombination between the Qa-2 region and the H-2D region in the B6.K1 mouse to a 40 kb DNA segment upstream of the Q1 gene. The Q10 gene maps to this region through its physical linkage to the Q9 gene. Within the Q region it was easy to identify polymorphic restriction fragments, demonstrating that this region is rather variable among inbred mouse strains. It could be shown that the polymorphism is due to deletions of variable extent in some of the mouse strains. In contrast both ends of this segment, the region between H-2D^b and the Q1 gene and the part containing the Q10 gene, are nonpolymorphic.

The Q Region of the C57BL/10 Mouse

In the B10 mouse the Q region is represented by 10 genes which were isolated in overlapping cosmid clones. This region is physically linked to the H-2D^b region, thus spanning 320 kb. All genes are arranged in a

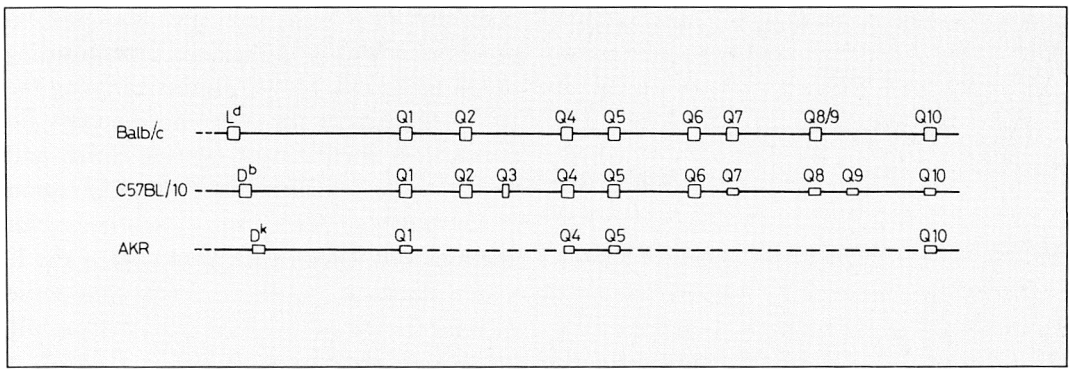

Fig. 3. Schematic comparison of the Q regions in the Balb/c, C57BL/10 and the AKR mouse.

head-to-tail configuration and by DNA sequence cross-reactivity fall into two groups. The genes Q1 to Q3 (the Q3 gene hybridizes only to a 5′ class I gene probe: exon 1–3) show homology among themselves in the gene flanking regions and also to several other genes which are localized in the Tla region. In the genes Q4 to Q10 a duplication unit in pairs is evident. Interestingly, this part of the Q region exhibits in its entire length strong sequence homology to the gene pair at the H-2K locus. Related genes are identified by their hybridization pattern with several flanking probes and also by similar restriction maps. By these criteria the odd-numbered genes Q5, Q7 and Q9 are as closely related to each other as are the even-numbered counterparts Q4, Q6, Q8 and Q10. Thus, alternating gene segments are more similar to one another than adjacent gene regions. However, it is not clear whether this region arose by a series of duplications of a prototype gene pair Q5 + Q6 or Q6 + Q7. For the two terminal genes of this duplication unit, Q4 and Q10, the restriction maps have diverged from their even-numbered counterparts.

The Q Region of Other Mouse Strains

In the Balb/c mouse a region of 500 kb of cloned DNA links the H-2D and Q region at the molecular level [5]. Eight class I genes map to the Q region which are denoted Q1, Q2, Q4, Q5, Q6, Q7, Q8/9 and Q10 in agreement with the nomenclature established for the C57BL/10 Q region class I genes (fig. 3). This region was originally isolated in two separate clusters, 1 and 9 [6]. Cluster 1 contains seven genes corresponding to the Q1 and Q9 region of B10 and the single gene on cluster 9 corresponds to Q10 of the B10 mouse. Rogers [7] has shown independently that the Balb/c clusters 1 and 9 are linked. Thus, the genes located on the two clusters are very similar to the Q region of C57BL/10. It appears that the Q3 gene is lost from the Balb/c mouse. Q7 corresponds to the previously sequenced gene 27.1 [4]. A comparison with the C57BL/10 mouse reveals that the genes corresponding to Q8 and Q9 of the B10 genome may have undergone an unequal crossing-over in the Balb/c mouse to produce a fusion Q8/Q9 hybrid gene [3] in a manner similar to the Hb-

Lepore gene from the human δ- and β-globin genes. Thus, the restriction map of this region in the Balb/c mouse is very similar to that of the C57BL/10 strain. The maps of the genes Q1, Q4, Q6 and Q7 are almost identical to the equivalent region of the Balb/c MHC. Therefore, it follows that gene Q7 of the B10 mouse is the allele of the 27.1 gene in the Balb/c genome. The maps of the Q10 genes differ slightly whereas the maps of the Q2, Q3 and Q5 genes show significant variance between the two haplotypes. Cluster 6 of the Balb/c mouse, previously mapped to the Qa-2 region, cannot be aligned with any B10 cluster. This cluster has now been localized between H-2D^d and H-2L^d, the genes being named D2^d and D3^d [5]. The recombinants used for allocating genes to the Qa-2 region are derived from inbred mouse strains lacking the D2 and D3 genes. The probe employed by Winoto et al. [8] was obtained from the D3^d gene and cross-hybridizes strongly to the 3′ flanking sequence of Q1, which explains the original localization data of cluster 6. At the DNA level no linkages of genes mapping to the Q region have been reported for other mouse strains besides B10 and Balb/c, although individual Q genes have been isolated from the AKR [9], C3H [10] and H-2^p [11] mice.

Most data on the organization of the Q region in other inbred strains are derived from genomic Southern blot investigations. Analyses of Balb/cBy, a spontaneously occurring Qa2-Balb/cY subline, indicated that at least part of the structural or regulatory gene controlling the Qa-2 antigen family resides on a 3.7 kb XbaI DNA fragment which is absent in all Qa-2-negative strains tested so far [12]. Mellor et al. [13] could show that in this subline an unequal crossing-over had occurred between genes 6 and 7 (27.1) of Balb/cBy. This event correlates the Qa-2 expression in B10 to the region corresponding to Q6 up to Q9. Mouse strains carrying the H-2^k haplotype or the t chromosome [14] do not express the Qa-2 polypeptide family and lack a number of genes found in the Q region of the B10 and Balb/c mice. Southern blot analyses with class I gene [3, 4, 14] or the K region flanking probes [3] reveal that these strains lack the genes Q6 to Q9. An example of such an analysis is shown in figure 4: a genomic Bam HI digest hybridized with a 5′ class I gene and a K region flanking probe. The 5.8 kb Bam HI fragment containing the 5′ portion of genes Q5 to Q9 of the B10 mouse is absent in the AKR strain. That the AKR genome does encode a Q5 gene with a 5.5 Bam HI fragment was postulated by Eastman O'Neill et al. [15], interpreting the genomic hybridization pattern with 3′ flanking probes derived from the Qa-2 region. More surprisingly they could demonstrate that the H-2^f haplotype appears to possess a large deletion, where the Q1 through Q9 genes appear to be missing.

The Structure of Q Region Genes

The majority of the Q genes has been sequenced now: Q4 [16], Q7 [4, 17], Q8 and Q9 [17], and Q10 [18]. It was not surprising to learn that these genes display the same organization as the H-2K and H-2D genes. All the exon and intron structures of a H-2 class I gene can be identified in the Q genes. Even at the DNA sequence level the H-2 and Q region genes exhibit high homology which includes the 5′ gene flanking region, a result to be expected from the cross-hybridization pattern. The Q region genes do not exhibit increased homology to the Tla genes [19, 20].

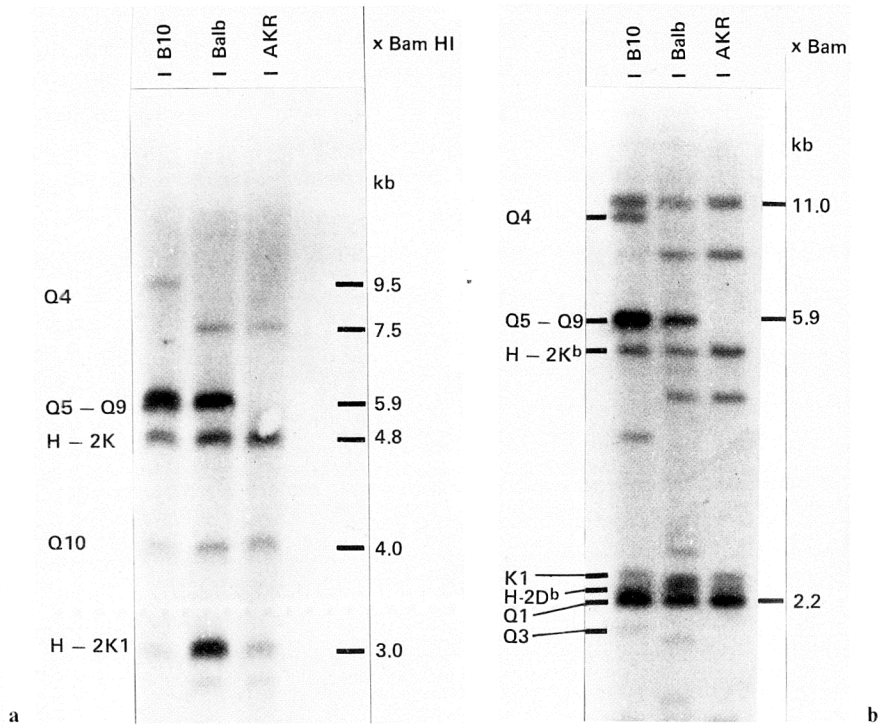

Fig. 4. Southern blot analysis of a Bam HI digest of murine DNA hybridized with 1.7 kb H-2K 5′ flanking Pst I fragment (**a**) and 2.2 kb 5′ gene Bam HI fragment of Q1 (**b**).

Sequencing part of the Q5 gene of the B10 mouse gave evidence that the Q5 gene might be a pseudogene in this mouse strain [16]. A 7 bp deletion in exon 3 of the Q5 gene of clone Bm1.2 [3] gives rise to an early translational termination codon at amino acid position 164. A transcript derived from this gene would lack the third and the following protein domains and thus be impaired in the binding of the light chain, β_2-microglobulin.

A unique feature of the Q genes sequenced so far is the presence of an early translational stop codon: TGA in exon 5, encoding the transmembrane domain (fig. 5). A T-to-A transition, ten nucleotides short of the end of exon 5 in the Q7 gene and presumably also in the Q9 gene, results in a stop codon. A translation product of Q7 (Q9) lacks the cytoplasmic tail and, containing only two basic amino acid residues following the hydrophobic peptide segment, might not be capable of a stable membrane positioning.

In the Q4 and Q8 genes, due to a frame shift caused by a G nucleotide deletion of the triplet GTG (amino acid 296), there is a termination codon 14 bp 5′ to the TGA codon of the Q7 gene, which shortens a translation product by additional five amino acids. The Q10 gene codes for a stop codon in the same position as in the even-numbered counter-

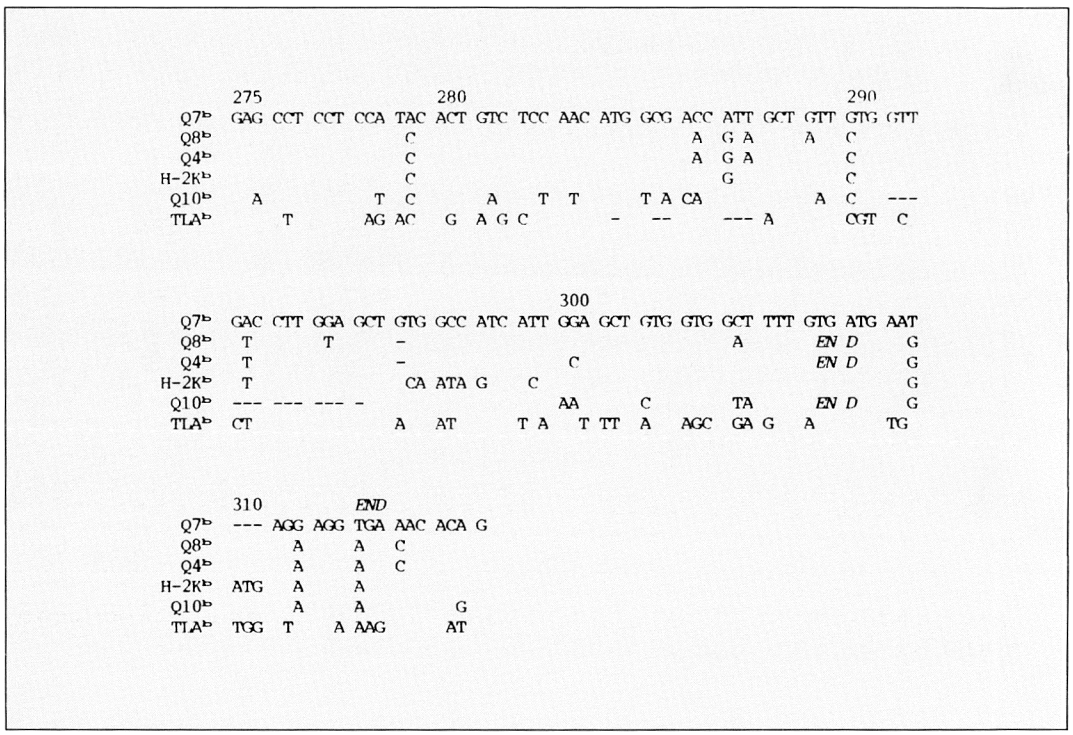

Fig. 5. Comparison of exon 5 sequences of several class I genes. The lack of a nucleotide due to deletions is indicated by a dash. The TLA^b sequence was determined by Obata et al. [20].

parts Q4 and Q8, which here is the result of a 13-base pair deletion encompassing the triplets 291 to 294 [18]. This deletion has a more profound effect on the encoded protein structure. It not only drastically reduces the transmembrane domain, but even abolishes the hydrophobic structure of this domain due to a frame shift. The unusual exon 5 sequence has suggested that the Q10 encodes a secreted class I antigen [18, 21].

As all the Q gene products terminate in the transmembrane domain, exons coding for the cytoplasmic polypeptide tail in so far have no importance. By the criterium of homology, sequences can be identified corresponding to exon 6, 7 and 8 in the H-2 class I genes. But the sequences at the 3′ end of these genes, starting with intron 6, diverge significantly and the homology is rather low comparing all known class I sequences in this region. Restriction mapping and hybridization using gene flanking probes gave evidence for a pairwise organization in the genes Q4 to Q10. This characteristic is only partially reflected in the gene sequences. All the genes exhibit the same level of homology using a 1.5 kb 5′ flanking probe (fig. 4). The 3′ flanking K region probe, in contrast, hybridizes strongly to the odd-numbered genes Q5 to Q9 and only weakly to the even-numbered genes Q4 to Q10 [3].

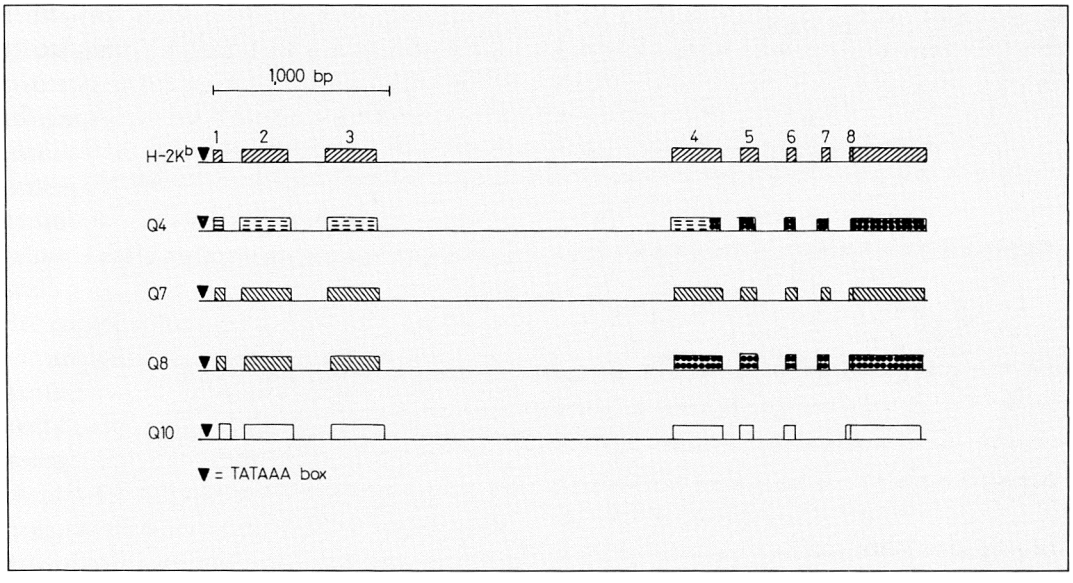

Fig. 6. Schematic comparison of class I gene sequences of the C57BL/10 mouse showing sequence homology between the various genes.

The finding that alternating genes are more similar to each other than to the neighboring genes is supported by a sequence comparison of the Q4 and Q8 genes (fig. 6). At the 3′ end, the genes are virtually identical and display a similar degree of homology as the H-2K alleles [23]. A more impressive example is found in the Q7 and Q9 genes. In a comparison of the exon 2 and 3 region, which is the most polymorphic among alleles of the H-2 complex class I genes, the two genes revealed only a single base change destroying a PstI restriction site out of 959 bases. This mismatch in exon 3 predicts the presence of a polymorphic amino acid residue in Q7 and in Q9, and introduces a charge difference in the two possible translation products. This result is confirmed by the expression experiments discussed by Robinson [24]. Interestingly, the Q8 gene is in the part of the gene which is transcribed into protein very similar to the genes Q7 and Q9 [17] and the percentage of sequence divergency is less than 5%. It therefore can be concluded that the genes Q6 to Q9 encode closely related polypeptides, which all end after the transmembrane segment, the Q7 and Q9 proteins being slightly larger. At the DNA sequence level comparing the genes Q4 to Q10, the evolution through a pairwise duplication is evident (fig. 6). The proteins derived from the Q7 to Q9 genes are very similar. The Q4 gene in the 5′ gene segment has diverged significantly from the other Q genes. Interestingly, the Q4 gene displays a significantly higher homology to the H-2K^b gene in this region than to any other class I gene (90%) and is within a short segment encompassing the end of intron 2 and two thirds of exon 3, identical to H-2K^b with the

exception of three single-base changes [25]. The Q10 gene differs from all the other Q region or H-2 genes in its entire length. Even the nontranslated 3′ part is not more closely related to the even-numbered counterparts than to the Q7 gene.

Allelic Polymorphism Does Not Occur in the Q Region

With respect to the H-2K and H-2D alleles, the high conservation of Q region genes is unusual. A comparison of the Q genes between two mouse strains revealed that allelic sequences are remarkably conserved [16, 18]. Even the unusual structures in exon 5 are identical. Q7 and its allele 27.1 show a 99% homology at the DNA level and predict amino acid sequences with only three differences [4, 17]. Mellor et al. [18] have shown that there is a 99% homology between the Q10 genes of the C57BL/10 (H-2^b) and SW R/J (H-2^q) mouse. The H-2D28.5 partial genomic sequence published by Jaulin et al. [26] derived from the Balb/c mouse is, except for three base pair changes, identical to the Q4 gene of the B10 mouse [16], thus including the Q4 sequence among the nonpolymorphic Q region genes. The lack of polymorphism may indicate that these Qa-2 region genes have an important function and that their conservation is due to selective pressure.

Q Region Genes as Donor Genes Generating Polymorphism in the H-2K Gene

The large diversity among alleles of the classical transplantation antigens (H-2K, H-2D) is a unique phenomenon of these antigens/genes and is not observed at any other genetic loci. It was therefore very important to find out which mechanisms are involved in the generation of genetic diversity. Analyses of the available data showed that allelic amino acid differences, e.g., in H-2K genes, are generally clustered and are also found at homologous positions in other class I molecules. This finding and the presence of a class I multigene family in the genome led to the speculation that a gene conversion-like mechanism may be responsible for the generation of diversity in H-2 genes. It was proposed that blocks of nucleotides circulate within genes by this nonreciprocal process [23, 27–31]. It seemed reasonable that polymorphism is a direct result of the mechanism introducing mutations in the H-2K gene.

Gene conversion was shown first to be indeed a mechanism generating H-$2K^b$ mutations by the genetic analyses of the H-$2K^{bm1}$ mutant [30, 31]. It was shown that the Q10 gene encodes the sequence which, donated into the H-$2K^b$ gene, causes the H-$2K^{bm1}$ mutation [34]. Therefore, the nonpolymorphic gene Q10 could be the donor gene for this H-$2K^b$ variant. The length of converted sequences is a minimum of 13 and a maximum of 51 base pairs long encompassing the seven substituted nucleotides found in the Bml gene. This approach was extended to other H-$2K^b$ mutants, in particular to five mutants of the 'bg series' [25, 35]. The Q4 gene was identified as the single donor gene for the 'bg' mutants. Interestingly, the amino acid substitutions are localized in a 96 bp segment of exon 3, where H-$2K^b$ and Q4 are otherwise identical. Excluding the single-base, silent nucleotide difference in the triplet coding for amino acid 108, the segment of sequence identity can be extended into intron 2.

It is of interest that the two donor genes identified so far reside in a part of the Q region which also displays a high homology to the H-2K locus in the gene flanking regions and which is separated from the H-2K^b gene by more than 500 kb of DNA [3]. In this context, it would not be surprising to find other Q genes capable of interacting with and donating sequences to the H-2K gene. The relatively large DNA segment of identical sequences between H-2K^b and Q4 may explain the enhanced frequency of interactions resulting in the mutants of the 'bg series'. However, in comparison with studies on other mammalian systems which show genetic transfers of several hundred nucleotides, the length of donated DNA between the Q region donor gene and H-2K^b is relatively short. Gene conversions of this type thus lead to the generation of polymorphism in the H-2K gene but do not result in a homogenization of the involved class I genes. Interestingly, these micro-gene conversion events are unidirectional; the donor genes are conserved among the mouse strains. So far, only two potential donor genes have been identified and, as the detailed mechanism of DNA transfer is still unknown, it is speculative whether the relative orientation of donor to acceptor genes on one chromosome is of importance. Q4 and Q10, the genes under discussion, are localized in the opposite direction to H-2K.

The B10 Q region also provides evidence for large-scale gene conversions acting on genes in this portion of the MHC. The almost complete homology between the exon encoding the leader sequence and the first intron of genes Q7 and Q8, which are not generally subject to selective pressure, points to a gene conversion event having occurred. Such a process may also explain the similarities observed between the Q7 and Q9 genes as well as the above-described similarity among members of both the even-numbered gene group (Q4, Q6, Q8 and Q10) and the odd-numbered one (Q5, Q7 and Q9). 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.

Expression of the Q Region Genes

A large number of class I cDNA clones have been isolated from various tissues and cell lines facilitating the analyses of the genes which can be expressed [22, 28]. The first gene in the Qa-2 region found to be expressed exclusively in the liver was the Q10 gene [21, 22, 36, 37]. Several groups succeeded in identifying and characterizing the secreted class I-related molecule encoded by the Q10 gene [38, 39]. Lalanne et al. [22] could demonstrate through the isolation of cDNA clones that the Q7 gene is expressed in the liver of the Balb/c mouse. Using gene-specific oligonucleotide probes, Geliebter et al. [25] showed that Q4-specific mRNA is present in the spleen of B6 mice but absent in liver tissue. Flavell et al. [40] reported on the expression of Q genes. Differentiating between the homologous genes Q7 and Q9 by employing specific oligomer probes, they showed that only the Q7 gene is expressed in a cytotoxic T cell clone, which was confirmed by isolating Q7-derived cDNA clones. Using a similar approach Hunt et al. [41] identified transcripts of the genes Q4, Q6, Q7 and Q8/9 in the thymus of the Balb/c mouse. A Q9 transcript was detected in an embryonic cDNA library at day 8.5 [42].

Transfection experiments with Q region genes into L cells failed in the detection of gene products on the cell surface [13, 43]. The lack of cell surface expression of the polypeptides encoded by the Q genes after transfection of the genes into L cells can be explained by the early translational termination codon at the end of the transmembrane exon. Other approaches to the study of Qa-2 region gene expression have involved the transfection of hybrid genes [44–46]. The constructs contained exon 1 to 3 of Q genes ligated to the 3′ portion (exon 4 to the 3′ untranslated region) of an H-2 class I gene. These studies showed the cell surface expression of Qa-2,3, serologic determinants with some Q gene constructs, only when the 3′ segment was derived from H-2K, H-2D or H-2L. These results suggest that the 3′ segment of Q genes may be important in the tissue-specific expression of Qa-2 molecules. The use of different recipient cell lines for expression studies finally enabled the detection of expressed Q gene products on the cell surface. Stroynowski et al. [47] transfected Q region genes into a murine hepatoma cell line. They showed surface-bound Qa-2 antigen reactivity with the Q7 gene of the Balb/c mouse and the Q7 and Q9 genes isolated from the B10 strain. Using a thymoma as a recipient cell line, Waneck et al. [42] also succeeded in cell surface expression of the gene products. The genes Q7 and Q9 of the C57BL/10 mouse (the Q7 gene in Balb/c) encode the Qa-2 antigens. They are and can be expressed in the appropriate tissues.

Analysis of the transcripts derived from the Q genes provided evidence for alternatively spliced mRNAs, thus increasing the repertoire of potentially expressed class I-related polypeptides. Alternative spliced transcripts of the Q10 gene have been reported [22]. A major Q10-derived mRNA is found in the liver, which lacks exon 3. A transcript without exon 3 might also be synthesized in liver and spleen from the Q7 gene [22]. Smaller mRNA species derived from the Q7 gene have been reported [42]. The Q9 transcript, detected during early embryonic development, does not contain the sequences of exon 4 and 5. No protein products encoded by these alternative Q gene messages have been described so far.

The Evolution of the Q Region

As discussed before, part of the Q region of the C57BL/10 mouse was evidently constructed by the duplication of a pair of genes, resulting in the present constellation: Q4/Q5, Q6/Q7, Q8/Q9 and Q10. Thus, these genes arose from a primordial Q-even and Q-odd class I gene pair, which later duplicated and diverged. The H-2K region was generated by the translocation of such a Q region gene pair to its present site, centromeric to the class II genes. The presence of the 640 bp flanking fragment suggested that the H-2K gene would be the homologue of the even-numbered Q genes [3]. This is supported by the strong homology of the 5′ and 3′ ends of the Q4/Q8 gene exon 5 to H-2K (fig. 5). The most striking similarity between a Q gene and the H-2K^b gene is observed in the Q4 gene. Its 5′ gene segment is highly homologous to H-2K^b. The Q4 gene, therefore, might be the true homologue of H-2K. Another explanation would be that the two genes retained their similarities or have become similar through gene conversion events. Interrelationship between the genes Q1 to Q3 and TL genes has been detected [3]. The similarity of these genes again might

be due to a gene conversion-like process or a translocation of genes Q1 to Q3 from the TL region. When comparing the genes Q5 to Q9, which display a very high homology throughout most of the gene sequences, evidence is found that gene conversion may act to homogenize genes in this region.

The in vivo role of proteins that are encoded by the Q genes is not known, and it therefore remains questionable if the Q region is essential to the survival of the mouse [48]. Mapping studies have uncovered large deletions in the Q region of some haplotypes, involving mostly the H-2K region homologous genes Q5 to Q9. It was postulated that in these strains the Q4 gene product might fulfill the Q region function [12]. The extensive deletion found in the H-2^f strain makes it far more difficult to assign any essential role to the Q region [15]. This haplotype does not appear to have any functional Q genes that are homologous to the genes found in the Q region of the B10 or Balb/c mouse. The Q10 gene, present in this strain, has been reported to be untranscribed and untranslated [49].

If one assumes that the Q region has a function, two hypotheses remain to account for the large deletion in the H-2^f haplotype. Genes within the H-2K, H-2D or TL regions may serve the hypothetical functional role of the deleted Q region genes [15]. Alternatively, it is also conceivable that additional class I genes are located in the Q region of these strains, which have not been isolated so far.

Acknowledgements

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