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ORGANIZATION OF THE AKR Qa REGION: STRUCTURE OF A DIVERGENT CLASS I SEQUENCE, $Q5^k$

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SUMMARY

We established the organization of the AKR Qa region and determined the sequence of the Q4 and Q5 genes. Restriction mapping and genomic Southern blot analysis revealed that the AKR strain codes for only three H-2K homologous genes in this region. The AKR Q5 gene is not homologous to the Q5 gene of the C57BL strain, but is presumably allelic to the Q5 gene isolated from Balb/c. The organization and structure of the AKR Qa family is virtually identical to the Qa genes of the C3H mouse. The AKR Q5 gene, in contrast to other H-2K homologous Qa region genes, codes for a typical transmembrane region, and upon transfection into BHK cells, a 1·6 kb Q5 transcript is detected.

INTRODUCTION

The murine major histocompatibility complex (MHC) encodes cell surface glycoproteins which serve as receptors for viral or allogeneic peptides and this complex is essential for antigen specific immune responsiveness. Class I genes determine glycoproteins which are noncovalently associated with a β_2-microglobulin subcomponent. In mice, the H-2K, D and L antigens implicated in the immune recognition phenomena, are expressed on the surface of nearly all cells. In contrast, class I molecules encoded in the Qa and Tla regions are expressed in only a few tissues and do not appear to direct immune responses in the same way as H-2 (Flaherty, 1981). Some genes in the Qa region, Q4 to Q10, show a striking similarity both in the gene and in the gene flanking regions to the H-2K locus (Weiss *et al.*, 1984). But their gene products differ in one common feature drastically from classical transplantation antigens. Due to mutations in exon 5 coding for the transmembrane domain, the molecules are no longer integral membrane proteins anchored to the cell membrane by a hydrophobic polypeptide. They constitute either secreted molecules (Q10, Mellor *et al.*, 1984) or are

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expressed on the cell surface through a phosphatidyl-inositol (PI) linkage (Stroynowski *et al.*, 1987; Waneck *et al.*, 1988). It has been postulated that γ/δ TCR positive T lymphocytes might recognize nonconventional class I products presumably localized in the Qa or Tla region (Strominger, 1989). Neither secreted nor PI-linked class I antigens can be recognized by antigen-specific T cells (Mann *et al.*, 1989). It was thus of interest whether other mice strains have a similar organization and contain the same set of nonpolymorphic class I genes as the well characterized C57BL/10 and Balb/c strains (Weiss, 1987). We concentrated on the AKR mouse (H-2^k), as additional class I specificities expressed on AKR leukemic cells have been reported (Schmidt *et al.*, 1986; Labeta *et al.*, 1989).

MATERIALS AND METHODS

Characterization of the AKR Qa region

Class I genes isolated from an AKR cosmid library (Steinmetz *et al.*, 1984) were screened with the H-2K 5′ flanking probe (640 bp BamHI fragment, Weiss *et al.*, 1984). A comparison of the restriction maps and hybridization patterns with genes from the C57BL mouse allowed the allocation of cosmids 9.2 and 9.3. No similarities could be found between the Q5 gene of the C57BL and the AKR strains.

DNA sequence analysis

The DNA sequence analysis of M13 subclones was carried out by the dideoxy chain termination method (Sanger *et al.*, 1977).

Southern and northern blot analysis

Southern blot analysis of genomic DNA from various mouse strains was done according to standard protocols (Southern, 1975). Hybridizations were performed as described (Weiss *et al.*, 1984), except that the restriction fragments were labelled according to Feinberg & Vogelstein (1984). Purification of the oligonucleotide (synthesized by Dr Ronald Mertz, Genzentrum München, FRG) and hybridization was done as described (Cate *et al.*, 1986).

Gene transfer and mRNA detection

5 μg cosmid DNA containing 1 μg neo-plasmid was transfected into BHK cells using standard procedures (Chen & Okayama, 1987). Colonies resistant to 0·6 mg/ml G418 were either pooled or cloned, and expanded. RNA was prepared by cesium chloride step gradient centrifugation and electrophoresed through formaldehyde/1% agarose gels, and transferred to nylon membranes.

RESULTS

Linkage of Q4 to Q10 in the AKR MHC

When we isolated the Q4 gene from an AKR cosmid library, we identified a class I sequence 3′ of the Q4 gene which has no direct counterpart in the C57BL or Balb/c genome (Fig. 1). The AKR Q4 gene expressed in 3T3 cells, was found to encode the Qb-1^a allele, whereas the Q4 gene of the H-2^b haplotype codes for Qb-1^b (Robinson *et al.*, 1988). Sequence analysis of the 5′ half of the gene (5′ flanking region to intron 3) did not show any differences in the exons compared to $Q4^b$. Only few substitutions are present in the introns, one leading to the polymorphic XhoI restriction site in intron 3 which is not present in other Q4 alleles, and

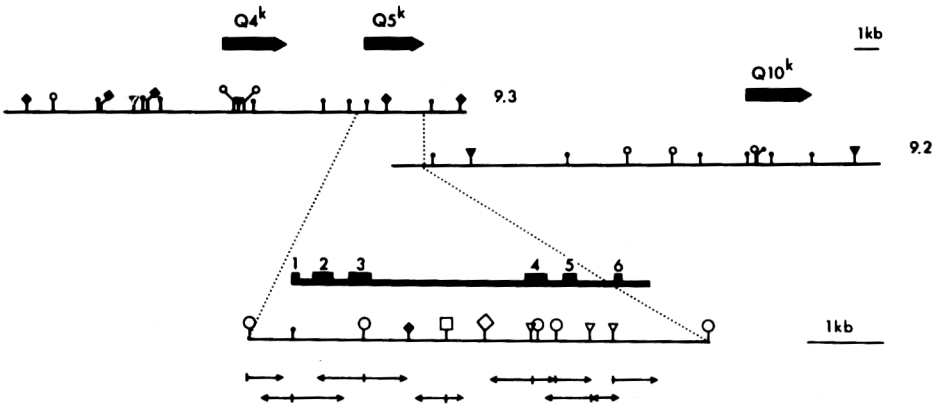

FIG. 1. Two overlapping cosmids spanning 90 kb, link the Q5 and the Q10 genes of the AKR mouse. The sequence of the AKR Q5 gene was established according to the strategy shown on the bottom. The sequences of exon 4 and exon 5 of the Q5 gene have been determined both from cosmids 9.3 and 9.2 to confirm linkage. Partial sequence of the Q10 gene (intron 4/5, exon 5) has also been obtained to unequivocally identify the telomeric class I gene as Q10 of the AKR strain. The 5′ half of the Q4 gene of cosmid 9.3 has been determined using a similar strategy as for the Q4 gene of the C57BL/10 mouse (Robinson *et al.*, 1988). The following restriction sites are shown: BamHI ♦; HpaI ♦; KpnI ♀; XhoI ▼; (no SalI or ClaI restriction sites are present). The sites of the restriction enzymes used in the sequencing protocol are only marked for the 5·6 kb of the gene sequence: BglII ▽; EcoRI ◇; HindIII ⍈; PstI ♀.

is also absent in the C3H sequence (Watts *et al.*, 1989). These authors could explain the more basic phenotype of the Qb-1 allele of the H-2^k haplotype by three amino acid substitutions in the third protein domain.

The AKR Q5 gene

The AKR Q5 gene was sequenced and as only cosmid 9.3 had been characterized initially, an oligonucleotide was chosen from the otherwise conserved α3 domain coding region to confirm the linkage to Q4. This oligonucleotide is specific for the Q5 gene and unique for H-2^k haplotypes (Fig. 3B). The Southern blot results also localize this gene to the Qa region (see B6K1 versus B6K2). The linkage was established by the characterization of cosmid 9.2 hybridizing to the oligonucleotide (Fig. 1). Previously an intriguing relationship between genes from the H-2K and Qa regions has been observed (Weiss *et al.*, 1984). Related genes could be identified by the hybridization pattern to 5′ flanking K-region probes (Fig. 3A). In the AKR genome four hybridization signals in addition to H-$2K^k$ and $K1^k$ are present. The nonpolymorphic HindIII fragment of 4·4 kb is localized between H-2D and Q1, and the nonpolymorphic 3·0 kb band is characteristic for the Q10 gene. The two polymorphic fragments (3·6 and 5 kb) are found in the 5′ flanking regions of Q4 and Q5, respectively. Interestingly, this sequence which is otherwise located more than 3 kb upstream of the coding regions, is more closely linked to the AKR Q5 gene (contained within the 1·5 kb BamHI fragment carrying the promoter).

The AKR $Q5^k$ sequence is very similar to the recently published $Q5^k$ gene of the C3H mouse (Watts *et al.*, 1989). Only few nucleotide substitutions and insertions/deletions are found in the introns. One amino acid (aa) substitution is present in position 139 (CTG = L in AKR to GCT = A in C3H).

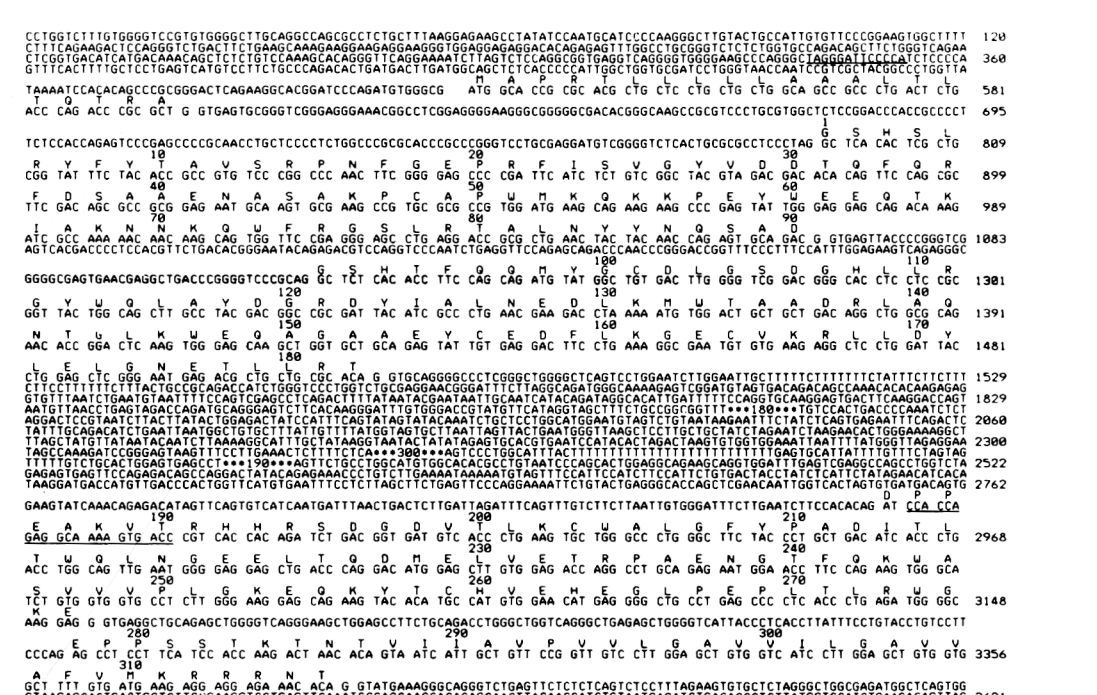

FIG. 2. Sequence of the Q5 gene of the AKR mouse. Protein translations of the exons are given in the one letter code above the DNA sequence. The sequences homologous to exon 7 and exon 8/3′ untranslated region are marked with one space at the beginning and the end. The sequence complementary to the $Q5^k$ oligonucleotide (aa 184–190) and nucleotides homologous to the H-2TF1 binding site are underlined.

The $Q5^k$ gene has some unique features as four potential N-linked glycosylation sites at aa 42, 86, 176 and 238. The most interesting finding in its sequence not noted before, is based on its classical transmembrane domain which is identical except aa 306 (V in $Q5^k$ and M in $H\text{-}2D^d$), to the hydrophobic membrane anchor of $H\text{-}2D^d$ (Sher *et al.*, 1985). Thus, in this respect, the $Q5^k$ gene differs from the genes Q4 to Q10 and should determine an integral cell surface glycoprotein of 328 amino acids. A translational termination codon is present at the end of exon 6. In addition to exon 5, the $Q5^k$ gene shows discrete homology to $H\text{-}2D^d$, particularly in exon 4 (aa 238–277) and in short stretches in exon 2 and 3.

Expression of $Q5^k$

We employed the $Q5^k$ oligonucleotide to search for transcription of the gene. No Q5 transcripts were detected in lung, spleen, liver, heart and kidney of adult AKR mice. We therefore introduced cosmid 9.3 into Baby Hamster Kidney cell line (BHK), and transfectant cell lines and clones were tested for transcription of $Q5^k$. A 1·6 kb $Q5^k$ specific mRNA is present in the AKR 9.3 transfectant cells (Fig. 4). We failed to detect cell surface expression of an H-2 class I heavy chain in three mRNA-positive BHK/9.3 cell lines or clones using the

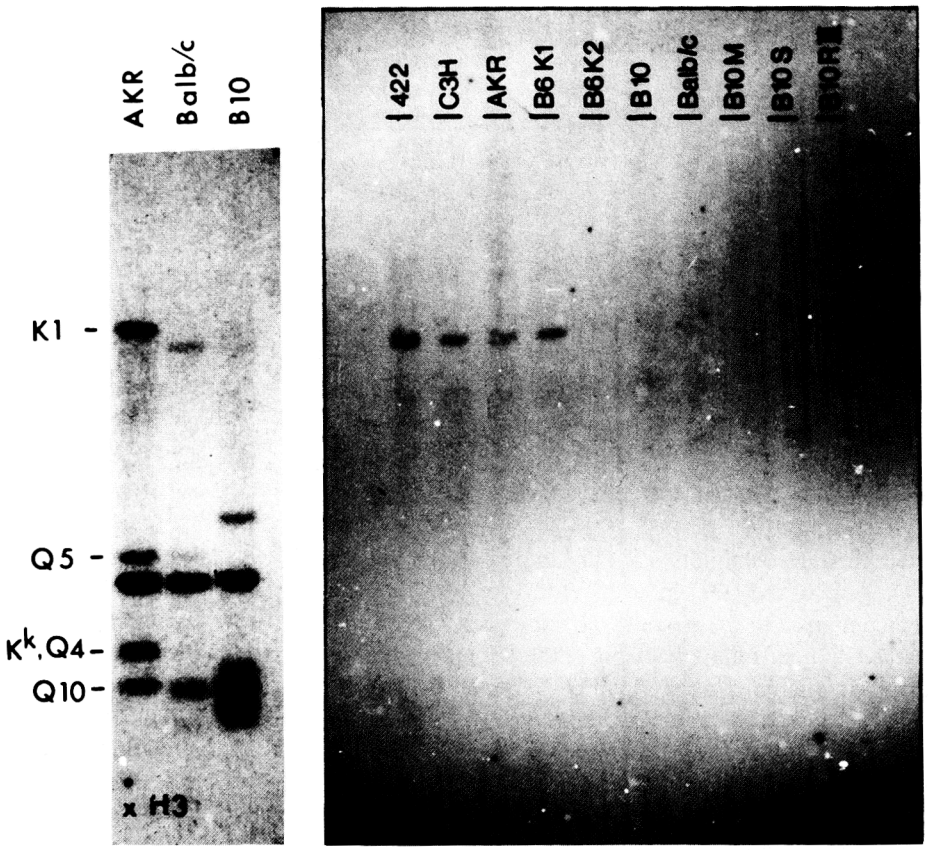

FIG. 3. Southern blot analysis of different digests of mouse DNA with the H-2K 5′ flanking probe in A and with the Q5 oligonucleotide in B. Liver or spleen DNA was isolated from various mouse strains: C3H (H-2^k); AKR (H-2^k); B6K1 (H-2^b/Qa^k); B6K2 (H-2^b/Qa^b); B10 (H-2^b); Balb/c (H-2^d); B10.M (H-2^f); B10.S (H-2^s) and B10.RIII (H-2^r). 422 is an AKR Gross virus leukemic cell line. The class I genes most closely linked to the five AKR bands are given on the left.

rat anti-H2 class I monoclonal antibody 42.3.9.8 (obtained from the American Type Culture Collection).

DISCUSSION

We have established a linkage map of the Q4, Q5 and Q10 genes in the AKR (H-2^k) mouse. Thus, this H-2^k haplotype has an identical organization in the Qa region as the C3H strain. The H-2D^k–Q1 linkage in the AKR MHC has been described by Stephan *et al.* (1986). No Q6–Q9 genes are present in the AKR Qa region, confirming previous genomic Southern blot results (Eastman O'Neill *et al.*, 1986; Weiss, 1987), and the Q5^k gene is directly linked to Q10^k.

The Q5 gene is polymorphic

The unique restriction map of the AKR Q5 gene already pointed to the absence of a direct counterpart in other haplotypes. Southern blot results also indicated (Fig. 3) that this gene is

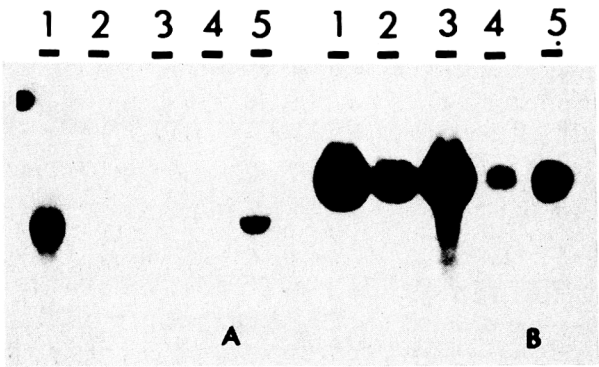

FIG. 4. Expression of the AKR Q5 gene. 10 μg of total RNA of BHK cells transfected with cosmid 9.3 were probed by Northern blot analysis with either the Q5 oligonucleotide (A) or with a human actin cDNA as control (B). Lane 1: BHK/AKR9.3 transfectant pool; lanes 2, 4 and 5: BHK/AKR9.3 transfectant clones; lane 3: untransfected BHK cells.

present in all H-2^k haplotypes. This finding was confirmed by the isolation and sequencing of the Q5 gene of the C3H mouse (Watts *et al.*, 1989). No strong sequence similarity is found when compared to the partial $Q5^b$ sequence (Robinson *et al.*, 1988). Some features indicate that the $Q5^k$ gene might be allelic to the Q5 gene of the Balb/c mouse (Steinmetz *et al.*, 1982). The restriction maps in the 3′ half of the genes share an unusual HpaI restriction site in intron 3 and the close XhoI and HpaI sites 3′ of the gene. The similarity in the 3′ gene region is stressed by the recently determined (S. W. Hunt III, personal communication) sequence of exon 5 from the $Q5^d$ gene which is identical to the $Q5^k$ sequence. Similar results have been obtained by Duran *et al.* (1989) who used an H-$2D^d$ specific oligonucleotide derived from exon 5, on genomic Southern blot analysis. This probe detected the H-$2D^d$ and $Q5^d$ genes in the Balb/c genome and the $Q5^k$ gene (5·1 kb BamHI fragment) in B10.BR DNA. Additional haplotypes but not H-2^b, code for a Qa gene with an H-$2D^d$-like exon 5. Thus to our knowledge, no strong evidence supports the notion that the $Q5^k$ gene arose by recombination of Q5 with Q9, as proposed by Watts *et al.* (1989). The restriction maps in the 5′ half differ significantly between $Q5^d$ and $Q5^k$, and the $Q5^k$ oligonucleotide did not hybridize to any Balb/c class I gene. Thus, the Q5 gene might constitute the first polymorphic class I gene localized outside of the H-2 complex proper.

The $Q5^k$ gene codes for a 1·6 kb class I mRNA

It has been suggested that the $Q5^k$ gene might not be expressed due to a potential premature polyadenylation signal present in intron 3 of $Q5^k$, both in the C3H and the AKR strains. We could show by transfection experiments that the AKR Q5 gene codes for a full length 1·6 kb transcript, detected both with the $Q5^k$ oligomer (exon 4) and the 1·5 kb PstI fragment (exon 1, 2, 3). No $Q5^k$ transcript was detected in tissues of adult AKR mice, but a $Q5^k$ transcript has been detected in AKR thymomas (Labeta *et al.*, 1989). Much is known about the complex regulatory sequences (CRE) of classical H-2 class I genes, and nuclear factors binding to discrete enhancer elements have been characterized (Kimura *et al.*, 1986). A comparison of the 5′ flanking region of the $Q5^k$ gene (500 bp) with other class I promoter regions shows that $Q5^k$ has a disrupted region of dyad symmetry—T*A*GGGATTCCCCA—in region I which binds H-2 transcription factor I (H-2TF1, Baldwin & Sharp, 1987/KBF1,

Yano *et al.*, 1987). A similar substitution in the CRE of Q10 (TG*A*GGA*C*TCCCCA) abolished the constitutive expression of the now liver specific class I gene (Handy *et al.*, 1989). In analogy, the G to A mutation in region I of $Q5^k$ might prevent its expression in normal tissues of the AKR mouse through the loss of binding of H-2 transcription factor I. In cell lines which do transcribe the $Q5^k$ gene, this factor which is responsible for basal class I expression, might be replaced by another regulatory DNA binding protein. An alternative explanation would be that under certain conditions the premature polyadenylation signal in intron 3 is not recognized by polymerase II resulting in a full length 1·6 kb $Q5^k$ mRNA. We are currently investigating the regulation of the $Q5^k$ gene expression in normal and tumor cells.

In conclusion the fact that the $Q5^k$ gene retained the potential to code for a class I antigen with a hydrophobic polypeptide membrane anchor and its polymorphism suggest that a gene product might have immune modulatory functions.

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