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Sequence of the mouse *Q4* class I gene and characterization of the gene product

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Abstract. The *Q4* class I gene has been shown to participate in gene conversion events within the mouse major histocompatibility complex. Its complete genomic nucleotide sequence has been determined. The 5′ half of *Q4* resembles *H-2* genes more strongly than other *Q* genes. Its 3′ end, in contrast, is *Q*-like and contains a translational stop signal in exon 5 which predicts a polypeptide with an incomplete membrane spanning segment. The presence of two inverted B1 repeats suggests that part of the *Q4* gene may be mobile within the genome. Gene transfer experiments have shown that the *Q4* gene encodes a β^2-microglobulin associated polypeptide of M_r 41 000. A similar protein was found in activated mouse spleen cells. The Q4 polypeptide was found to be secreted both by spleen cells and by transfected fibroblasts and was not detectable on the cell surface. Antibody binding and two-dimensional gel electrophoresis indicate that the Q4 molecule is identical to a mouse class I polypeptide, Qb-1, which has been previously described.

Introduction

The class I major histocompatibility complex (MHC) antigens are glycoproteins of relative mass 35 000–45 000 which are associated with β_2-microglobulin. In mice, the H-2K, D and L antigens are transmembrane proteins expressed on the surface of nearly all cells and are thought to direct T lymphocyte immune responses to viral and other cell surface antigens (Ploegh et al. 1981). 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). For this reason, it has been suggested that Qa and TL antigens may be markers of cell differentiation (Harris et al. 1984). In C57BL/10 mice, the *Qa* region contains 10 class I genes, *Q1–Q10* (Weiss et al. 1984). Three biochemically and immunologically distinct proteins associated with this gene cluster have been described; these are Qa-2, Q10 and Qb-1. Qa-2 is found on the surface of several types of hematopoietic cells and also occurs as a secreted molecule (Michaelson et al. 1981, Robinson 1987, Soloski et al. 1986). Q10 is produced only in the liver and is secreted into the blood (Lew et al. 1986). Qb-1 is synthesized by cells from several lymphoid tissues (Robinson 1985).

It was shown previously that the Qa-2 and Q10 molecules are encoded by *Q6–Q10* (Mellor et al. 1985, Devlin et al. 1985), but no polypeptide products of *Q1–Q5* have so far been described. Recently, *Q4* was shown to be the donor gene for the bm6 gene conversion event in C57BL/6 mice, since it has the bm6-specific nucleotide sequence in exon 3, which encodes the second protein domain (Geliebter et al. 1986). It has been shown previously that the flanking regions of *Q4–Q10* are highly homologous to those of the *H-2K* region genes, supporting the hypothesis that the *H-2K* gene pair may have arisen by duplication which involved *Qa-2* region genes (Weiss et al. 1984). It was therefore necessary to obtain the nucleotide sequence of *Q4* in order to understand how such gene conversion events could occur. The fact that the *Q4* gene is transcriptionally active (Geliebter et al. 1986) prompted us to identify the corresponding polypeptide. Our results show that *Q4* encodes a functional class I polypeptide in contrast to *Q5*, which carries a translational stop signal in exon 3.

Materials and methods

Nucleotide sequence determination. The *Q4* and *Q5* genes were subcloned from cosmid Bm1–2 (Weiss et al. 1984) and their nucleotide sequences determined according to the scheme shown in Figure 1 using procedures already published (Maxam and Gilbert 1977, Sanger et al. 1980). Enzymes were purchased from Boehringer Mannheim or New England Biolabs.

Gene transfer and antigen detection. Cosmid or plasmid DNA (10 μg) containing 1 μg pAG60neo was transfected into BALB/c3T3 cells using

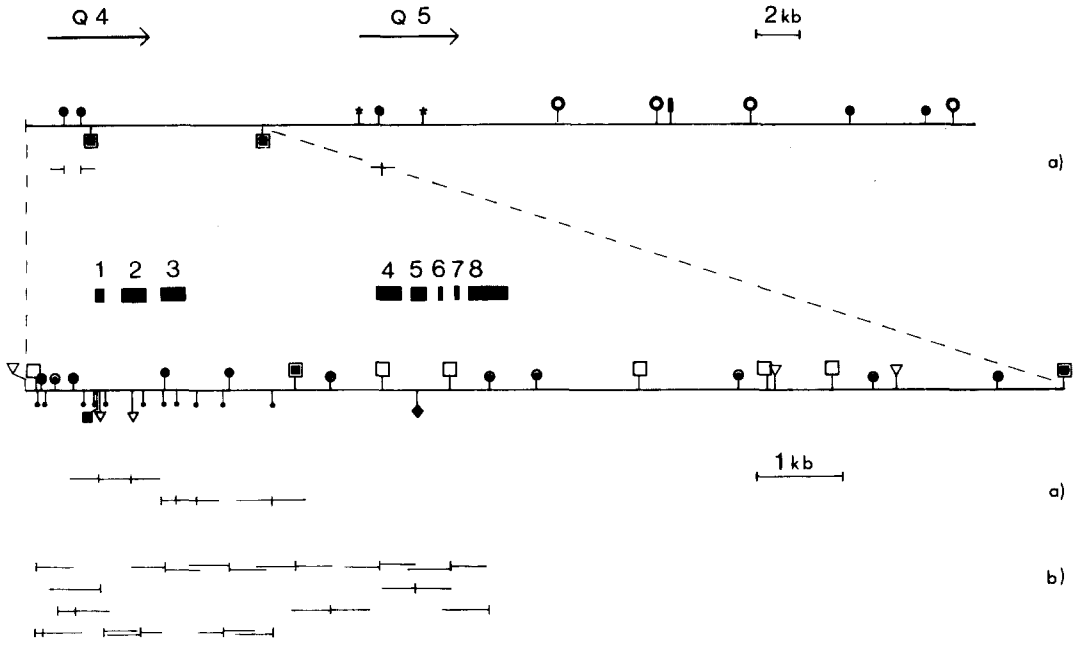

Fig. 1. Restriction maps of cosmid Bm1-2 and the subcloned *Q4* gene. Numbered solid boxes represent exons. Restriction site symbols in Bm1-2 are: Cla I, ; Hpa I, ; Kpn I, ; Sal I, ; and the two Bam HI sites () used for subcloning. Restriction site symbols of the *Q4* subclone are: Bam HI, ; Bgl II, ; Hind III, ; Kpn I, ; Pst I, . Only those Sau 3A (), Hinf I (), Pvu II (), and Sac II () sites used for sequencing are shown. (a) Sequences obtained by the chemical method of Maxam and Gilbert (1977). (b) Fragments sequenced by the dideoxy method of Sanger and co-workers (1980)

standard procedures (Wigler et al. 1977). Colonies resistant to 0.8 μg G418 were pooled, expanded, and labeled with ^{35}S-methionine as described (Mellor et al. 1985). Mouse spleen cells were cultivated with concanavalin A for 48 h prior to labeling (Robinson 1987). Class I molecules were detected by immunoprecipitation of cell lysates (Dobberstein et al. 1979) using protein A-sepharose immunosorbents (Robinson 1987). Pulse-chase analysis was performed as described by Dobberstein and co-workers (1979). Surface labeling of cells was performed according to Robinson and Schirrmacher (1979). Two dimensional gels were run as before (Robinson 1985).

Carbonate treatment of microsomes. Microsomal vesicles were isolated from transfectants as described by Dobberstein and co-workers (1979), and treated with sodium carbonate, pH 11.5, as described by Fujiki and co-workers (1982). Soluble and membrane fractions were treated with triton X-100 and immunoprecipitates prepared as before.

Molecular weight determinations. To estimate the molecular weights of class I molecules, immunoprecipitates were treated exhaustively with Endo F (Boehringer Mannheim) as described (Robinson 1987), reduced, and separated on 10–15% polyacrylamide gradient gel slabs using ovalbumin (M_r 43000), glyceraldehyde 3 phosphate dehydrogenase (M_r 36000) and carbonic anhydrase (M_r 29000) as markers. Estimates of molecular weight were obtained by plotting relative migration (R_f values) against log M_r. Actual M_r was calculated from the amino acid sequence.

Results

The entire *Q4* gene is present on two subcloned restriction fragments isolated from cosmid clone Bm1-2 (Weiss et al. 1984). As a result of the cloning procedure, the 5′ end of the gene is present on a 3.1 kb Hind III/Bam HI fragment and the 3′ end is on a genomic 9 kb Bam HI fragment. DNA sequence analysis was carried out according to the scheme outlined in Figure 1. The nucleotide sequence of *Q4* is shown in Figure 2, together with the partial sequence of *Q5*. In *Q4* no polyadenylation signal is present in the 210 bp of the 3′ untranslated region sequenced.

The promoter region of the Q4 gene. The sequence of the *Q4* promoter reveals strong sequence homology to the 5′ flanking sequences of *H-2K^b* (Kimura et al. 1986) and the *Q* genes (Devlin et al. 1985). A more detailed inspection shows that, with one exception, the regulatory sequences (e. g., the interferon consensus sequence) are strongly homologous to those of *H-2K^b*. The *Q4* gene, however, contains only a mutilated copy of sequence IV, which is part of the *H-2K^b* enhancer. In *H-2K^b*, this en-

Fig. 2. Sequence of the *Q4* gene. Protein translations of the exons are given above the DNA sequence. The untranslated exons 6, 7, and 8 are ▶ shown in triplets. The partial sequence of the *Q5* gene (exons 2/3 and introns 2/3) is written below the Q4 sequence. – denotes nucleotide identity. The interferon consensus sequence, the CCAAT and the TATAAA boxes are indicated with broken lines. Sequences homologous to the B1 repeat, two copies in intron 3 and a portion in the 5′ flanking region, are underlined

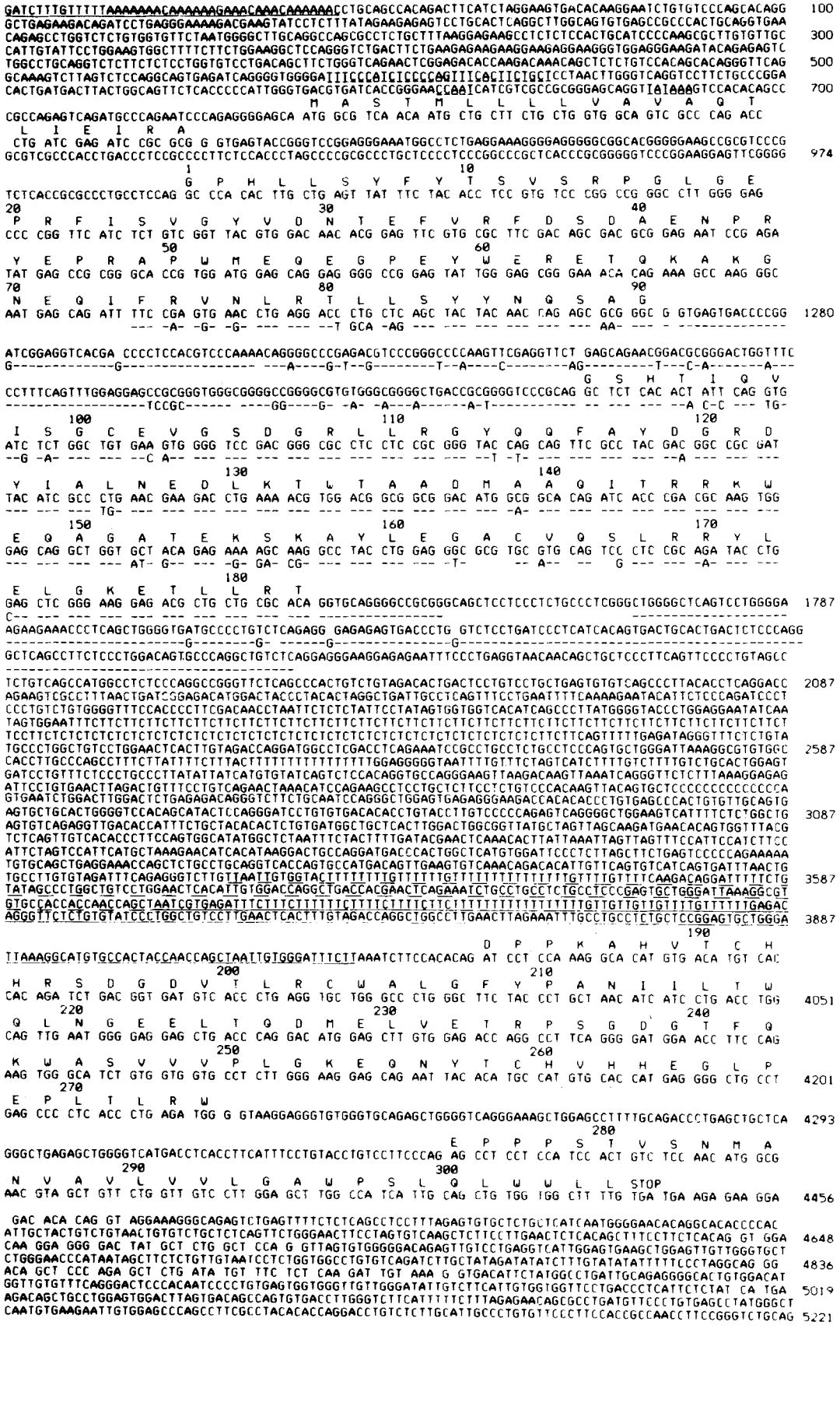

Table 1. DNA sequence homology

	Length	Changes					
	Q4	Q4/K^b	Q4/D^d	Q4/Q5	Q4/Q7	Q4/Q8	Q4/Q10
Exon 1	64	31.2	57.8		14.1	14.1	29.7
Intron 1	195	12.3	12.8		13.3	13.3	14.9
Exon 2	270	8.5	9.6		13.3	13.3	10.7
Intron 2	186	11.3	12.9	16.7	16.1	16.1	17.2
Exon 3	276	8.7	10.9	11.2	8.3	9.1	11.2
Exon 4	276	7.2	6.1		5.8	5.4	5.8
Intron 4	126	3.1	6.3		8.7	2.4	6.3
Exon 5	116	10.3	19.0		10.3	3.4	15.5
Intron 5	178	2.8	9.5		12.4	3.4	9.5
Exon 6	33	0	3.0		9.1	0	12.1
Intron 6	163	19.6	24.0		22.1	1.2	22.1
Exon 7	40	12.5	5.0		17.5	0	15.0
Intron 7	135	14.1	15.6		16.3	0.7	21.5
3′ UT	207	15.0	20.8		16.4	2.4	22.2

Nucleotide sequence data were obtained from the following sources: *H-2K^b^*, Weiss et al. 1983; *H-2D^d^*, Taylor Sher et al. 1985; *Q7, Q8,* Devlin et al. 1985; *Q10,* Mellor et al. 1984

hancer element is composed of two overlapping 18 bp repeats, only one copy of which is found in *Q4.*

Repetitive sequences in the Q4 gene. It is interesting that at the 3′ end of intron 3, the *Q4* gene contains two direct, but not exact, 280 bp copies of the B1 repeat (Kalb et al. 1983), as indicated in Figure 2. This element is not found in this position in any other *H-2* class I genes. The limited sequence data available shows that this repeat is present in the reverse orientation at the very 5′ ends of *Q7* and *H-2K^b^*, as well as in *Q4* (Steinmetz et al. 1981, Devlin et al. 1985, Kimura et al. 1986). Thus, exons 1, 2, and 3 of *Q4* are flanked by inverted DNA sequence repeats which may render the polymorphic 5′ half of the gene mobile within the genome.

Comparison of Q4 with other mouse class I genes. Since no *Q4* cDNA clones have been isolated so far, the boundaries of the protein-coding exons of *Q4* were assigned using consensus splice-donor and acceptor sequences, as well as by comparison with the *H-2K^b^* gene (Weiss et al. 1983). Thus the regions marked in triplets near the bottom of Figure 2 correspond to exons 6, 7, and 8 and the 3′ untranslated region of *H-2K^b^*. Table 1 shows percentage homologies between *Q4* and other class I genes. All comparisons are between genes of the *H-2^b^* haplotype, with the exception of *H-2D^d^*, which was used because only a cDNA sequence is available for *H-2D^b^* (Reyes et al. 1982). The percentage homology was not calculated for intron 3 because multiple insertions and deletions make alignment of the different sequences impossible. For simplicity, only comparisons with *Q4* are given in Table 1. Comparisons among other genes in Table 1 have been published elsewhere (Weiss et al. 1983, Mellor et al. 1984, Devlin et al. 1985, Arnold et al. 1984).

The 5′ half of the *Q4* gene (e. g., introns 1 through 3 and intron 4) shows particularly strong sequence homology to *H-2* genes. This level of homology is significantly greater than that normally found for nonallelic class I genes. Particularly strong homology to *H-2* genes is found in short stretches, for example in the last 80 bp of intron 2, the first 147 bp of exon 3 and the first 63 bp of exon 5. In exon 4, which encodes the conserved third protein domain, *Q4* is no more homologous to other class I genes than they are to each other.

Comparison of Q4 with other Q genes. Published comparisons of *Q* gene sequences suggest that *Q7* and *Q9* may be pseudo-alleles. In addition, the 5′ half of *Q8* is highly homologous to that of its odd-numbered partner, *Q7* or *Q9* (Devlin et al. 1985). In contrast, the 5′ half of *Q4* differs significantly from the corresponding regions of other *Q* genes. As was mentioned previously, this segment is more closely related to *H-2* genes (e. g., *H-2K^b^*, *H-2D^b^*, and *H-2D^d^*) than it is to other *Q* region genes. In its 3′ half, starting in intron 3, *Q4* is similar to its even-numbered counterpart *Q8,* and these two genes are virtually identical from intron 5 onwards.

Comparison of the *Q4* sequence with other published mouse class I sequences reveals a possible analog in the BALB/c mouse. The partial DNA sequence of the genomic clone H-2D28.5 (Jaulin et al. 1985), covering exons

1, 2, and 3, shows only three nucleotide differences in the polymorphic exons 2 and 3. Several differences are found in exon 1, which encodes the signal sequence, as is commonly observed between allelic class I genes. An additional nucleotide in exon 1 of H-2D28.5 may cause a frameshift preventing expression of the polypeptide. Since no restriction map of H-2D28.5 has been published it is difficult to say conclusively whether this gene is allelic with *Q4*.

The Q5 gene is a pseudogene. The partial nucleotide sequence of the *Q5* gene from the cosmid clone Bm1–2 (Fig. 2) covering exon 2, intron 2 and exon 3 reveals a series of three small deletions in exon 3 resulting in an early translational stop signal at amino acid position 164. A mRNA derived from this gene would direct the synthesis of a short polypeptide lacking the third and subsequent protein domains.

Structure of the Q4 gene product. The nucleotide sequence predicts that *Q4* directs the synthesis of a polypeptide which differs significantly from other class I molecules in its third protein domain. In this conserved protein domain, several residues are present which are unique to *Q4*. In addition, at amino acid 296 in the membrane-spanning domain, a single nucleotide deletion causes a premature translational stop signal after amino acid 305. The same deletion is also found in the *Q8* gene (Devlin et al. 1985). Thus, the hydrophobic transmembrane domain of *Q4* is incomplete and may be incapable of providing an adequate membrane anchor for the polypeptide chain. To identify the Q4 polypeptide, the cosmid clone B2.5 (Weiss et al. 1984), containing both the *Q4* and *Q5* genes, was transfected into BALB/3T3 fibroblasts using the Ca_2PO_4 method (Wigler et al. 1977). In the same experiment, 3T3 cells were transfected with two other cosmid clones, B1.24 and H26, containing *Q1–Q3*. In addition, a plasmid subclone of the *Q4* gene on a 10 kb Eco RI fragment was also tested. Transfectants were labeled biosynthetically with a short pulse of ^{35}S-methionine and class I molecules were detected by immunoprecipitation of cell lysates with the rat monoclonal antibody, R1.9.6, which recognizes determinants common to several mouse class I molecules. Immunoprecipitates were analyzed by one- or two-dimensional gel electrophoresis. As shown in Figure 3a, 3T3 cells transfected either with cosmid B2.5 or with the Q4 subclone synthesized a polypeptide of M_r 41 000 which was not present in cells transfected with B1.24 or H26. A polypeptide of similar size was detected in lysates of ^{35}S-methionine labeled C57BL/6 spleen cells. The polypeptides of M_r 43 000 and 45 000 present in all 3T3 lysates are H-2D^d and H-2L^d respectively, both of which react with R1.9.6. In C57BL/6 spleen cell lysates, however, R1.9.6 detects only the Q4 polypeptide. Due to the co-precipitation of H-2 molecules by R1.9.6, it was not possible to obtain all the necessary biochemical data on Q4 using the transfectants alone and therefore spleen cells were used for some experiments. Two-dimensional gel analysis of class I material isolated from transfectants shows that the Q4 polypeptide is a homogeneous species which is smaller and more basic than the H-2D^d and H-2L^d antigens from the host cell line (Fig. 3b). No polypeptides attributable to the *Q1, Q2, Q3,* or *Q5* genes have so far been detected.

Biosynthesis of the Q4 polypeptide. Pulse-chase analysis was used to follow the biosynthesis of the Q4 polypeptide in transfected 3T3 fibroblasts and in C57BL/6 spleen

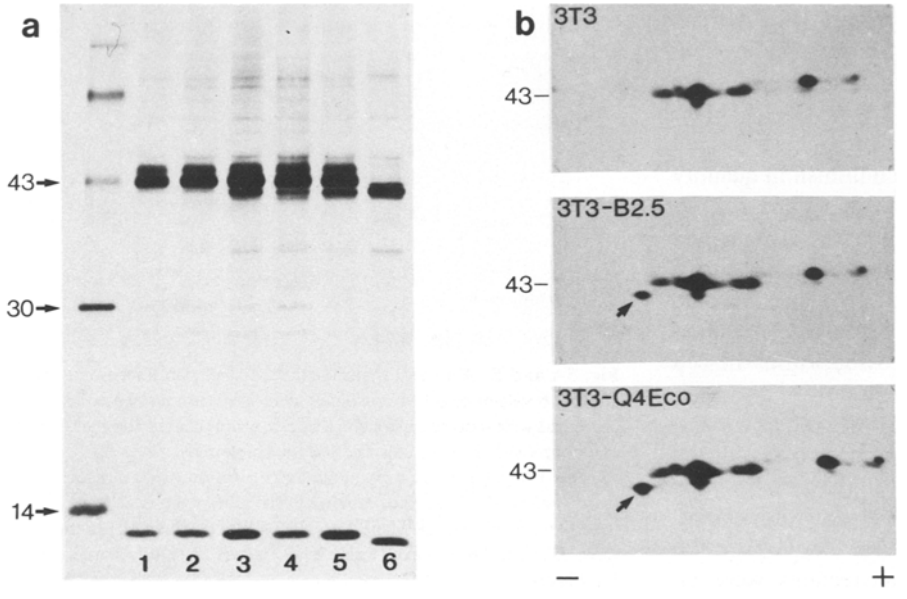

Fig. 3. Fluorograms of one- and two-dimensional polyacrylamide gels showing synthesis of ^{35}S-methionine labeled Q4 polypeptides. (a) 10–15% gradient SDS gel showing polypeptides precipitated from transfected 3T3 cells (lanes 1–5) and C57BL/6 spleen cells (lane 6) using antibody R1.9.6. DNAs transfected were cosmids B1.24 (*Q1*, lane 1), H26 (*Q2+Q3*, lane 2), B.2.5 (*Q4+Q5*, lanes 3 and 4), and a *Q4* Eco RI subclone (lane 5). (b) Two-dimensional gels of class I molecules synthesized by untransfected or transfected 3T3 cells and precipitated with R1.9.6. Arrows indicate Q4 polypeptides. Markers at border are kilodaltons and indicate running positions of ^{14}C-labeled marker proteins. Fluorograms were exposed for 3 days (a) or 7 days (b) using Kodak XAR-5 film

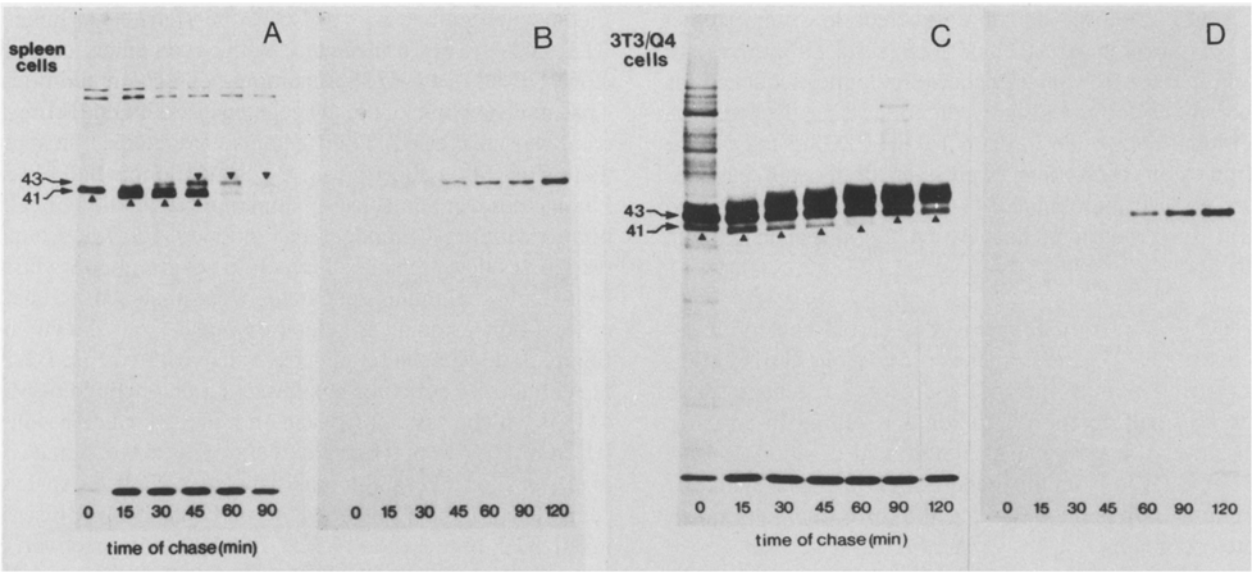

Fig. 4. Processing and secretion of Q4 polypeptides. C57BL/6 spleen cells (panels a and b) or 3T3 cells transfected with *Q4* Eco RI DNA (panels c and d) were labeled for 5 min with ^{35}S-methionine and chased for the times shown. Panels a and c show immunoprecipitates of cell lysates and panels b and d are from the corresponding supernates, using R1.9.6 antibody. Conditions as for Figure 3a. Pointers indicate running position of Q4 polypeptides

cells. Cells were labeled with a short pulse of ^{35}S-methionine and chased for various times in complete medium. Both cells and supernates were treated with R1.9.6 to precipitate class I polypeptides. As shown in Figure 4, Q4 molecules, in contrast to H-2 antigens, undergo a small but characteristic reduction in M_r after about 15 min of chase. This shift was not observed if the samples were treated with endoglycosidase F (Elder and Alexander 1982) prior to electrophoresis, indicating that modification of N-linked carbohydrates is responsible (data not shown). A similar shift was observed in Q4 polypeptides derived from C57BL/6 spleen cells (Fig. 4). The processing of Q4 precursors to their corresponding mature forms could best be followed in spleen cells, since in transfectants the mature forms are masked by H-2 molecules. In spleen cells, Q4 precursor polypeptides are processed to M_r 43 000 forms which diminish in quantity during the chase period. After about 45 min of chase, soluble class I molecules of M_r 43 000 were detected in the supernates of spleen cells and fibroblasts expressing the *Q4* gene, but not in supernates of control transfectants. Thus, both transfected fibroblasts and spleen cells synthesize Q4 polypeptides which are processed within the cell and are secreted into the culture medium.

It was shown recently that Qa-2 exists both as a membrane-associated and as a secreted molecule (Robinson 1987, Soloski et al. 1986). The data suggest that soluble Qa-2 is derived from the membrane form by processing. To determine whether secreted Q4 was derived from a membrane-associated precursor, transfectants were la-

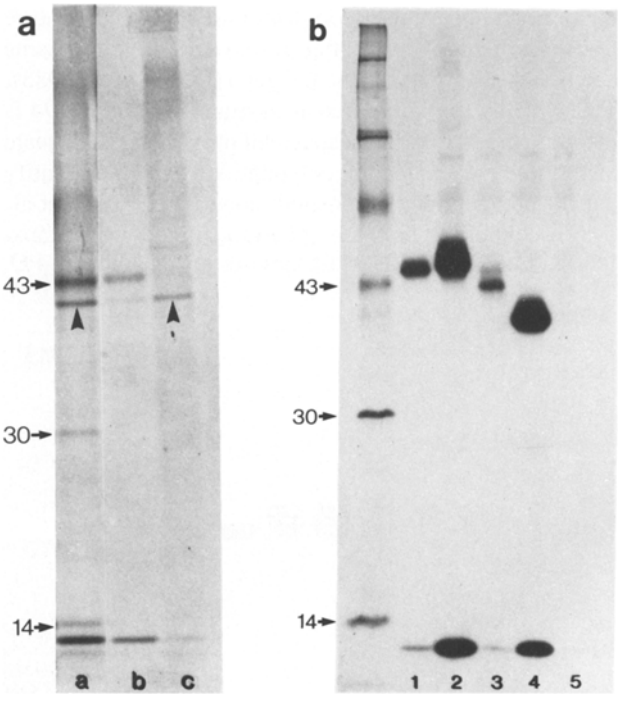

Fig. 5A and B. A Class I molecules associated with microsomes from C57BL/6 spleen cells. Microsomes were used unfractionated (lane a), or treated with sodium carbonate pH 11.5. Lanes b and c show molecules associated with the insoluble and soluble fractions respectively. Pointers indicate Q4 polypeptides. **B** Immunoprecipitation of class I molecules from lysates of ^{125}I-surface-labeled C57BL/6 spleen cells. Antibodies used were 9.178 (H-2K^b lane 1), B22.249 (H-2D^b lane 2), anti-Qa-1^b (lane 32), anti-Qa-2 (lane 4) and R1.9.6 (lane 5). Other conditions as for Figure 3

beled for 5 min with ^{35}S-methionine and the microsomal vesicles were then isolated and subjected to treatment with sodium carbonate at pH 11.5 (Fujiki et al. 1982). This procedure opens sealed microsomal vesicles, thereby releasing soluble proteins, but lipid-associated molecules generally remain membrane bound. In Figure 5a the carbonate treatment is shown to partially separate H-2 and Q4 molecules. The entire H-2 remains membrane-associated under these conditions, while a major part of the Q4 is released into the supernate. The complete release of Q4, however, was never observed. This experiment reveals that most of the newly-synthesized Q4 molecules are water soluble under these conditions, but does not rule out the possibility that at physiological pH, Q4 molecules may associate with the plasma membrane. To test this possibility, C57BL/6 spleen cells were surface-labeled with ^{125}I using the lactoperoxidase method (Robinson and Schirrmacher 1979). As shown in Figure 5b, lysates of surface-labeled cells contained H-2K^b, H-2D^b, Qa-2, and Qa-1 molecules, but no Q4 material could be detected with R1.9.6. These results strongly suggest that Q4 is a soluble class I molecule which is not present on the cell surface.

Further properties of Q4 polypeptides. The nucleotide sequence of *Q4* predicts potential sites of N-glycosylation at amino acid positions 86 and 256. Partial digestion of Q4 molecules with endoglycosidase F gives rise to two additional smaller forms, indicating the presence of two N-linked glycans (data not shown). Estimation of the molecular weight of deglycosylated Q4 by SDS gel electrophoresis gives a value of M_r 37 000 ± 500. This is significantly larger than the value of 34 684 determined from the protein sequence. This discrepancy cannot be attributed to anomalous behavior of class I molecules on SDS polyacrylamide gels, since deglycosylated H-2D^b migrates according to its calculated molecular weight of 38 406. The apparent discrepancy for Q4 may be explained by unorthodox processing or modification of the polypeptide.

A limited enhancement of Q4 expression (approximately twofold) was achieved by treating the transfectants for 72 h with 20 units/ml recombinant gamma interferon. This is consistent with the presence of an intact interferon consensus sequence in the 5′ flanking region of the *Q4* gene.

Similarity between Q4 and Qb-1. The Qb-1 polypeptide was originally detected in lysates of ^{35}S-methionine labeled spleen cells using anti-β_2-microglobulin antibodies. Two allelic forms, Qb-1^a and Qb-1^b, are distinguishable by 2D gel electrophoresis. It was found that the Q4 molecule, isolated from C57BL/6 spleen cells using R1.9.6, co-migrates precisely with Qb-1^b (data not shown). A *Q4* allele was isolated from an AKR (Qb-1^a) cosmid library. When expressed in 3T3 cells, this gene was found to encode a Qb-1 molecule. This result supports the notion that Qb-1 is encoded by the *Q4* gene.

Discussion

We chose to determine the nucleotide sequence of *Q4* because of increasing evidence that this gene is a particularly active contributor to gene conversion events within the mouse major histocompatibility complex. Transfer of sequence information by *Q4* has been shown by sequencing *H-2K* alleles from the *H-2K^{bm}* mutant mice of the bg series (Geliebter et al. 1986, Nathenson et al. 1986). The high frequency with which *Q4* acts as a donor gene may be attributed to a high degree of sequence homology between *Q4* and *H-2K^b*, which could promote such exchange events. It has been suggested that the *H-2K* region may have been generated by duplication and translocation of a pair of *Q* genes (Weiss et al. 1984). Thus, in C57BL/10 mice, *H-2K^b* would be the homolog of an even-numbered *Q* gene, for example *Q4, Q6, Q8,* or *Q10.* The strong overall homology between *Q4* and *H-2K^b* suggests an ancient relationship between these genes. The fact that several stretches of strong sequence homology are located in regions of allelic variability may be explained by gene conversion. The fact that the 5′ region of *Q4* is far more similar to *H-2* genes than to *Q5–Q10* may explain the observation that the R1.9.6 antibody binds several H-2 molecules and *Q4* but is non-reactive with *Qa-2* or other *Q* gene products.

We have used a gene transfer approach to show that *Q4* encodes a secreted class I polypeptide. A similar polypeptide was found in C57BL/6 spleen cells. Secretion of the Q4 molecule can be explained by the presence of a translational stop signal in exon 5, which results in a protein with an incomplete membrane spanning segment. Similar defects are found in all the even-numbered *Q* genes so far sequenced, namely *Q4, Q8,* and *Q10.* The same single base deletion is found in *Q4* and *Q8,* but in *Q10,* 13 bases are deleted suggesting that this defect has an independent origin. The Q10 product is secreted by the liver (Lew et al. 1986).

The general properties of the Q4 polypeptide are as predicted by the Q4 nucleotide sequence, except that the apparent molecular weight of the deglycosylated chain is about 3000 daltons greater than expected. Inspection of the Q4 nucleotide sequence does not allow an alternative exon-intron organization which would explain such a large discrepancy in the molecular weight. One possible explanation is that the signal peptide is not removed after synthesis. This would result in a polypeptide with a different N-terminus and a calculated molecular weight of 36 942 which is close to the value of 37 000 estimated by gel electrophoresis.

Our data show that the Q4 molecule and Qb-1 are identical. This conclusion is supported by the fact that Q4

molecules precipitated by R1.9.6 are indistinguishable from Qb-1 by two-dimensional gel electrophoresis. Furthermore, Q4 molecules from spleen cells and from transfected fibroblasts are secreted together with β_2-microglobulin. It may be significant that the *Qa* region encodes at least three secreted class I molecules. It is therefore possible that the *Q* genes, in addition to being important for gene conversion, may also encode molecules of biological importance.

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