

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

Peter J. Robinson¹, Dorian Bevec², Andrew L. Mellor³, and Elisabeth H. Weiss²

¹ Department of Immunology, German Cancer Research Center, Postfach 101949, D-6900 Heidelberg, Federal Republic of Germany

² Institute of Immunology, University of Munich, Goethestr. 31, D-8000 München, Federal Republic of Germany

³ Transplantation Biology Section, Clinical Research Centre, Harrow, HA1 3UJ, United Kingdom

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 41000. 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 twodimensional gel electrophoresis indicate that the O4 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 μ g) containing 1 μ g 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, \uparrow ; Hpa I, \P ; Kpn I, \P ; Sal I, \uparrow ; and the two Bam HI sites ($\stackrel{\bullet}{\square}$) used for subcloning. Restriction site symbols of the Q4 subclone are: Bam HI, $\stackrel{\blacksquare}{\square}$; Bgl II, $\stackrel{\square}{\square}$; Hind III, \P ; Kpn I, \P ; Pst I, \P . Only those Sau 3A (\downarrow), Hinf I (\downarrow), Pvu II (\downarrow), and Sac II ($\stackrel{\bullet}{\blacksquare}$) 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 μ g G418 were pooled, expanded, and labeled with ³⁵S-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 Q^4 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

Cectore from the formation of the forma	GATCTTTGTTTTTAAAAAAAAAAAAAAAAAAAAAAAAAA	100 300 500 700
terteactegestertegettegettegettegettegesteres ter and ter and tert being and ter	CGCCAGAGTCCAGAGTCCCAGAGCCCAGAGGGGAGCA ATG GCG TCA ACA ATG CTG CTT CTG CTG GTG GCA GTC GCC CAG ACC L 1 E 1 R A CTG ATC GAG ATC CGC GC G GTGAGTACCGGGTCCGGAGGGGAAATGGCCTCTGAGGAAAGGGGAGGGGGGGG	97 4
CEC CGE TIC ALC TET DEL GET TAK DE GAL AAL ALD GAL TIC DE LA TIC DA AL CLO DAR AT LEG BAR TAT GAR CEG CGE GE GAL CEG TEG ATG GAR CAG GAG GAG GEG GE GA TAT TIGE GAR CEG CAA CAG AAA GEA AAG GE TAT GAR CEG CGE GE GAL CEG TEG ATG GAR CAG GAG GAG GEG GE GA TAT TIGE GAR CEG GAA AC CEG AAA GEA GAG GAG GE TAT GAR CEG CGE GE GAL CEG TEG ATG GAR CAG GAG GAG GEG GE GA TAT TIGE GAR CEG GAG AAA GEA GAA GEG GAA GEG TAT GAR CEG CEG SCA CEG TEG ATG GAR CAG GAG GAG GEG GE GA TAT TIGE GAR CEG GEG GAA CAC CEG GAAA GEGAAGCGGGACTGGGAT TAT GAG CAG ATT TIT CEGA GTG AAC CTG AGG ATC LTG TICC AGC TAL TAA AAA CAG AG AG CAAA GEG GAAG GEGAAGCGGGACTGGAT TAT GAA CAG ATT TIT CEGA GTG AAC CTG AGG ATC LTG TICC AGC TAL TAAA CAG AG AG CAGAAACGGAACGGGGACTGGATGTGGACTGGGAG TAT GAA CAG ALCCLTCAACGTCCAAAGGGGGCCGGAGCGGGGGGGGGGG	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
74 E O I F R V N L R 99 AT SAG CAG AT T TE CAG AGA CIG AGA CIG TAG CE AGA CIG TAG CAG AGA CAG GE G GGAGTGACECCCGG 1220 AT GGGAGGTACCACAGA CECCGAGACGAGCCGGAGAGCCCGGAGAGCCCCCAGACGCGAGAGCGAGCAGGAGCGGGAGGA	$\begin{array}{c} \text{CCC} \ \text{CGG} \ \text{FIC} \ \text{AIC} \ \text{CG} \ C$	
ATCEGEGUTIAGEG CICCICLCANACAGEGUGGUCGUGGUGGUGGUCGUCGUGGUCTAGUCTUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGG	700 900 N E Q I F R V N L R T L L S Y Y N Q S A G AAT GAG CAG ATT TTC CGA GTG AAC CTG AGG ACC CTG CTC AGC TAC TAC AAC LAG AGC GCG GGC G GTGAGTGACCCCCGG AGG GCA -AG AA	1280
CCTTLCAGTTIGEAGGAECCECCEGE IGGECGGGGGGGGCGGGGGGGCGGGGGGCCCCCCCGGGGGG	ATCGGAGGTCACGA CCCCTCCACGTCCCAAAAACAGGGGGCCCGAGACGTCCGGGGCCCCCAAGTTCGAGGTTCT GAGCAGAACGGACGGGGGGGGGG	
$ \begin{bmatrix} 1 & S & G & C & E & V & G & S & D & G & R & L & L & R & G & Y & Q & F & A & Y & D & G & R & D \\ Tac art tor tor tar dar one constraints and the set of the set o$	CCTTTCAGTITGGAGGAGCCGCGGGTGGGCGGGGCGGGGC	
$ \begin{array}{cccc} \mathbf{y} & \mathbf{L} & \mathbf{A} & \mathbf{L} & \mathbf{N} & \mathbf{E} & \mathbf{D} & \mathbf{L} & \mathbf{T} & \mathbf{L} & \mathbf{T} & \mathbf{A} & \mathbf{A} & \mathbf{D} & \mathbf{T} & \mathbf{A} & \mathbf{A} & \mathbf{G} & \mathbf{I} & \mathbf{T} & \mathbf{R} & \mathbf{R} & \mathbf{H} & \mathbf{H} \\ \mathbf{J} & \mathbf{A} & \mathbf{A} & \mathbf{C} & \mathbf{E} & \mathbf{A} & \mathbf{A} & \mathbf{C} & \mathbf{E} & \mathbf{A} & \mathbf{A} & \mathbf{C} & \mathbf{E} & \mathbf{G} $	I S G C E V G S D G R L L R G Y Q Q F A Y D G R D ATC TCT GGC TGT GAA GTG GGG TCC GAC GGG CGC CTC CTC CGC GGG TAC CAG CAG TTC GCC TAC GAC GGC CGC GAT -G -A	
$ \begin{bmatrix} 1 & 186 \\ 6A & CA & G & A & T & E & M & S & M & V & 180 \\ CA & CA & G & G & C & V & 0 & S & L & R & 170 \\ A & -A & -G & G & -C & & & & & $	Y I A L N E D L K T W T A A D M A A Q I T R R K W TAC ATC SCC CTG AAC GAA GAC CTG AAA ACG TGG ACG GCG GCG GCA ATG GCG GCA CAG ATC ACC CGA CGC AAG TGG	
E L G K E T L G K E T L L R T A GAB GAB CIC TO CIG CIG CIG CA CA GATA GATGAGGGGCAGGCGGGGCAGCTCCTCCCTCGGGCCGGGGCCAGTCCTGGGGGCCAGGCCCGGGGGCAGGCCGGGGCCAGGCCCGGGGGCCAGTCCTCGGGGGGCCAGGCCCGGGGGCCAGGCCCGGGGGCCAGGCCCGGGGGCCAGGCCCGGGGGCCAGGCCCCGGGGGCCCGGGGGCCCGGGGGCCCCGGGGGCCCC	E Q A G A T E K S K A Y L E G A C V Q S L R R Y L GAG CAG GCT GCT ACA GAG AAA AGC AAG GCC TAC CTG GAG GGC GCG TGC GTG CAG TCC CTC CGC AGA TAC CTG AT- G G- GA- CG G	
AGAAGAAACCETCAGETGGGGTGATGCCCCTGTTCTCAGGGGAGGGAAGGAAGGAA	E L G K E T L L R T GAG CTC GGG AAG GAG ACG CTG CTG CGC ACA GGTGCAGGGGCCGGCGGGCAGCTCCTCCTCTGCCCTCGGGCTGGGGGCTCAGTCCTGGGGA C	1787
LEGICAGCCATGGCCTTCCCAGGCCGGTTCCCAGCCCCACTGTCTGT	AGAAGAAACCCTCAGCTGGGGGGGATGCCCCCTGTCTCAGAGG GAGAGAGTGACCCTG GTCTCCTGATCCCTCATCACAGTGACTGCACTGC) -
CCCTGCTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	TCTGTCAGCCATGGCCTCTCCCAGGCCGGGTTCTCAGCCCACTGTCTGT	2087
TGLED LIGGELIGUELIGUELIGUELIGUELIGUELIGUELIGUE	CCCTGTCTGTGGGGGTTTCCACCCCTTCGACAACCTAATTCTCTCTATTCCTATAGTGGTGGTCACATCAGCCCTTATGGGGGTACCCTGGAGGAATATCAA TAGTGGAATTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT	
BIGAALLIGGACTLIGAGAGACAGGAGGAGGAGGGGGGGGGGGGGGGGGGGGG	CACCTIGECCAGECTITCTIATITCTIATITATCATGTGATAGECTICAGEGGGGGTAATITGICTICTGCTCTGCAGTGGGGTACTIAAGGCGGGGGGAGTTAGAGTGCACTGGGGTGCTCCTCTTAATGTGTGTG	2.387
ATTOTAGTECATTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTA	GIGAATEIGGACTEGGACTETGAGAGACAGGGTETTETGCAATECAGGGETGGAGTGAGAGGGAAGACCACACACECTGTGAGCECACTGGTTTGCAGTG AGTGCTGCACTGGGGTCCACAGCATACTECAGGGATECTGTGTGACACACEGTACTTGCCTCGAGGTCAGGGGCTGGAAGTCATTTTTETCTGGETG AGTGTCAGAGGTTGACACCATTTCTGCTALACACTECTGGTGGGCTGCTCACTTGGCTGGCTGGCTTATGCTAGGTAGG	3087
TATAGCCCIGGCIGTCCCCGGACCTGCCCACGACCCCGGACCCCGGACCCCGGACCCCCGGACGCCCCGGGACGCCCCGGACCCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCGACCCCCC	ATTCTAGTCCATTCATGCTAAAGAACATCACATAAGGACTGCCAGGATGACCCACTGGCTCATGTGGATTCCCTCTTAGCTCCCCAGGAAAAA TGTGCAGCTGAGGAAACCAGCTCTGCCTGCAGAGGTCACCAGTGCCATGACGGCTGACAGCAGCACATTGTICAGCTCCCCCGGAAAAAA TGTGCAGCTGAGGAAACCAGCTCTGCCTGCCGCAGGCCACCATGCCATGACGTGCAAACAGACAACAGTGCATCAGTGCACCAGTGCACCAGTGCTACAGTTTAACTG	3587
IIAAAGGCAIGTGCCACTACCAACCAGCIAALIGIGGGAIIICIIAAATCTTCCACACAG AT CUT CCA AAG GCA CAT GTG ACA TGT CAC IIAAAGGCAIGTGCCACTACCAACCAGCAGCAGAGTIALIGIGGGAIIICIIAAATCTTCCACACAG AT CUT CCA AAG GCA CAT GTG ACA TGT CAC 200 200 210 CAC AGA TCT GAC GGT GAT GTC ACC CTG AGG TGC TGG GCC CTG GGC TTC TAC CCT GCT AAC ATC ATC CTG ACC TGG 4051 4051 220 240 240 Q L N G E E L T Q D M E L V E T R P S G D G T F Q 240 CAG TTG AAT GGG GAG GAG CTG ACC CAG GAC ATG GAG CTT GTG GAG ACC AGG CCT TCA GGG GAT GGA ACC TTC CAG 250 K W A S V V V V P L G K E Q N Y T C C H V H H E G L P 220 270 220 220 CAG TTG GTG GTG GTG GTG GTG CCT CTT GGG AAG GAG CAG AAT TAC ACA TGC CAT GGC GAC CAT GAG GGG CTG CCT 4201 270 270 280 GAG CCC CTC ACC CTG AGA TGG G GTAAGGAGGGGTGTGGGGTGCAGAGGCTGGGGCTCAGGGGAAAGCTGGAGCCTGGAGCCTGAGGCGCTGAGGCGCCGAGCCCCGAGACTCCCACC TCA ACC ATG GG GG CTA CCT CTC ACC CTG AGA TGG C GT GTT CTC GTAACCTCCCACCTTCATTTCCTGTACCTGTCCTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG GGGCTGAGAGCTGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCGCCTCCCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG A V V V V L G A W P S L Q L W L L STOP AAC GTA GCT GTT GTG GTT GTC CTT GGA GCT TGG CCT TGG CCTCTTTGCAAGCTTTGCTAACGCCTTCTCTCTACAGGCACACGGCACACCCCAC A GAC GAG GGG GCT GTT GTC GTT GGA GCT TGG CCT TGG GCCTGTGTCCAGGTGGGGGACAACTGGCGGACAACCGCGCTGCTGAAGCCCCCACA	TATAGCCCIGGCIGTCCIGGAACTCACAITGIGGACCAGGCTGACCACGAACTCAGAAAICTGCCTCCGCCICCCGAGIGCTGCGGAITAAAGGCGT GIGCCACCAACCAGCTAAICGIGAGAIIICIIICIIIIIIIIII	3887
H R S D G D V T L R C W A L G F V P A N I I L T W 4051 CAC AGA TCT GAC GGT GAT GTC ACC CTG AGG TGC TGG GCC CTG GGC TTC TAC CCT GCT AAC ATC ATC CTG ACC TGG 4051 240 Q L N G E E L T Q D M E L V E T R P S G D G T F Q CAG TTG AAT GGG GAG GAG GAG CTG ACC CAG GAC ATG GAG GCT TG GGA ACC TTC AGG GGA ACC TTC CAG 250 K W A S V V V P L G K E Q N V T C H V H H E G L P AAG TGG GCA TCT GTG GTG GTG GTC CTT GGG GAG GAG GAG GAG AAT TAC ATG GCG AT GGA ACC TTC CAG 270 E P L T L R W GAG CTC CTC ACC CTG AGA TGG G GTAAGGAGGGGTGTGGGGTGCAGAGCTGGGGGTCAGGGGAAAGCTGGAGCCTGTGGACCCTGAGCTGCTCA 270 GGGCTGAGAGCTGGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCCTTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 270 GGGCTGAGAGCTGGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCCTTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 270 GGGCTGAGAGCTGGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCCTTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 270 GGGCTGAGAGCTGGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCCTTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 270 AAC GTA GCT GTT CTG GTT GTC CTT GGA ACT TGG CCA TCA TTG CAG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG AC CA CAG GT AGGAAAGGGCAGAGTCTGAGTTTTCCTCAGCCTCCTTTAGCAGGTGGGGCACACCGGGAAAGCTCGGGAAAACTCGCCACAGGCACACCGCCA ATTGCTACTGTTGTAACTGTGTCTGCTCTCAGTTGGGGAACTTCCTGTGTCAAGCGTTGTCTGCAACTGGCGGAAAGCTGGGGACAACGGGCACACCGCCAC ATTGCTACTGTTGTAACTGTGTCTGCTCTCAGTTGGGGAACTTCCTAGGCTGTGCTCTGCTCTGCTAACTAGTGGAACTGGGGAACACCGGCACACCGCCA ATTGCTACTGTTGTAACTGTGTCTGCTGCTGCAGTTGGGGCTGTGTGCTGTGCTGCTGCTCTGCTAACTGGCTGAACTGGGGAACACCGGCACACCGCCAC ATTGCTACTGTGTACTGTGTCTCTCAGTTGGGGACTTGGGCCTGTGTCGGGACAAGCTTGCTGCTGCTGCTGCTGCTGCTGACTTGGGAACTTGCTGGGGGGCAACTTGCTGGGAACTTGCTGGGGGCTGAACTTGGGGGACAACGCGGCTGATGGACTTGGGGGGGG	D P K A H V T C H IIAAAGGCAIGIGCCACIACCAACCAGCIAALIGIGGGAIIICIIAAATCTTCCACACAG AT CCT CCA AAG GCA CAI GIG ACA IGI CAC	
Q L N G E E L T Q D H E L V E T R P S G D G T F Q CAG TIG AAT GGG GAG GAG GAG CTG ACC CAG GAC ATG GAG CTT GTG GAG ACC AGG CCT TCA GGG GAT GGA ACC TTC CAG 250 K M A S V V V P L G K E Q N Y T C H V H H E G L P AAG TGG GCA TCT GTG GTG GTG CCT CTT GGG AAG GAG GAG AAT TAC ACA TGC CAT GTG CAC CAT GAG GGG CTG CCT 4201 270 T L R W GAG CCC CTC ACC CTG AGA TGG G GTAAGGAGGGGTGTGGGGTGCAGGAGGCTGGGGAAGGCTGGGAAGCCTGGAGCCCTGAGCTGCTCA 4293 280 GGGCTGAGGCTGGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCCTCCCAG AG CCT CT CC ACT GTC TCC ACC ATG GCG 270 S N H A GGGCTGAGGGGGTCATGACCTCACCTTCATTTCCTGTACCTGTCCTTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 270 300 N V A V L V U L G A W P S L Q L W L L STOP AAC GTA GCT GTT CTG GTT GTC CTT GGG AGCTTGGG CCT CTTCTGTAGCTGTCTGCTCTGC	H R S D G D V T L R C W A L G F Y P A N I I L T W CAC AGA TCT GAC GGT GAT GTC ACC CTG AGG 1GC TGG GCC CTG GGC TTC TAC CCT GCT AAC ATC ATC CTG ACC TGG 230	4051
K W A S V V P L G K E O N Y T C H V H H E G P AGG TGG GGG CAT CAT GGG CAT CAT GGG GGG CAT CAT GGG GGG CAT CAT GGG CAT CAT GGG CAT CAT GGG GGG CAT CAT CAT CAT GAG CAT	Q L N G E E L T Q D M E L V E T R P S G D G T F Q CAG TTG AAT GGG GAG GAG CTG ACC CAG GAC ATG GAG CTT GTG GAG ACC AGG CCT TCA GGG GAT GGA ACC TTC CAG 250	
E P L T L R W GAG CCC CTC ACC CTG AGA TGG G GTAAGGAGGGTGTGGGTGGGGTG	K W A S V V V P L G K E Q N Y T C H V H H E G L P AAG TGG GCA TCT GTG GTG GTG CCT CTT GGG AAG GAG CAG AAT TAC ACA TGC CAT GTG CAC CAT GAG GGG CTG CCT 270	4201
GGGCTGAGAGCTGGGGGTCATGACCTCACTTCATTTCCTGTACCTGTCCTCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 290 300 N V A V L V V L G A W P S L G L W L L STOP AAC GTA GCT GTT CTG GTT GTC CTT GGA GCT TGG CCA TCA TTG CAG CTG TGG IGG CTT TTG TGA TGA AGA AGGA 4456 GAC ACA CAG GT AGGAAAGGGCCAGAGTCTGAGTTTTCTCTCAGCTCCTTCTAGAGTGGTGCTCTGCTCACAGGGGAACACAGGCACACCCCAC ATTGCTACTGTTCTGTALACTGTCTCTGGTCACTCGGGGACATGGGGGGGGGG	E P L T L R W GAG CCC CTC ACC CTG AGA TGG G GTAAGGAGGGTGTGGGTGCAGAGCTGGGGGCAGGGGAAAGCTGGAGCCTTTTGCAGACCCTGAGCTGCTCA 280	4293
N V A V L V V L G A W P S L Q L W L L STOP AAC GTA GCT GTT CTG GTT GTC CTT GGA GCT TGG CCA TCA TTG CAG CTG TGG IGG CTT TTG TGA TGA AGA GGA 4456 GAC ACA CAG GT AGGAAAGGGCCAGAGTCTGAGTTTTCTCTCAGCCTCCTTCAGAGTGGTCCTCTCTCACACAGCTTTCCTCTCACAG GT GGA ATTGCTACTGTTCTGTAALTGTGTCTCGCTCCAGTTCTGGGGACTTCCTGTGAGGCTCTTGCTCTGACACGCTTTTCCTTCTCACAG GT GGA CAA GGA GGG GAC TAT GCT CTG GCT CCA G GTTAGTGTGGGGGCAGAGAGTTGTTCGGTGGTTGTTGGTAGCTCTGCTGAGTCGTTGTCTGTAGCCTCTGTGTGAACTCCTGGGGGCACAGAGTTGTTGGGGGCACTGGGGGGCCG CTGGGGACCCATAATAGCTTCTCTGTTGTAATCCTCTGGTGGCCTGTGTCGAGACTGTGGGGGTAATTGTCTTGTAATATTTTTTCCCTAGGCAG CCA GG GG GAC TAT GCT CTG GCT CCA G GTTAGTGTGGGGGACAGAGTTGTTGGCAGTTGTTAGGCTGGGAGGGGGCACGGGG ACA GCT CCC AGA GCT CTG TAT TGT TTC TTC AG AGT TGT AAA G GTGCACATTCTATGGCCTGATGGAGGGGGCACTGGGGACAT GGTTGTGTTTCCAGGGACTCCCACAATCCCCTGTGAGTGGGGGGGTGTTGGGGACATTGTCTTCATTGGGCTGGAGGGGGCCCTGATGGGGCACTGGGGACAT GGTGGGTTGCCTGGGAGTGGACCTTAGTGACGCCAGTGTGGACGCTCTCATTTTTTTT	GGGCTGAGAGCTGGGGTCATGACCTCCACTTCATTTCCTGTACCTGTCCTTCCCAG AG CCT CCT CCA TCC ACT GTC TCC AAC ATG GCG 290 300	
GAC ACA CAG GT AGGAAAGGGCGGAGAGICIGAGITITICTICAGCCICCITIAGAGIGIGCTCIGUICATCAATGGGGAACACAGGCACACCCCAC ATIGCTACIGTCIGTAALIGIGICIGCICTCAGITIGGGGACATCICCIAGAGICICICTIGAACICICACAGCITICCITCICACAG GI GGA CAA GGA GGG GAC TAI GCT CTG GCT CCA G GITAGIGIGGGGGCAGAGGTGICCIGAGGACGIGGAACGIGGGGGGGGGG	N V A V L V V L G A W P S L G L W W L L STOP AAC GTA GCT GTT CTG GTT GTC CTT GGA GCT TGG CCA TCA TTG CAG CTG TGG TGG CTT TTG TGA TGA AGA GAA GGA	4456
ACA GCT CCL AGA GCT CTG ATA TGT TIC TCT CAA GAT TGT AAA G GTGACATICATIGGCCTGATGCAGAGGGGCACTGTGGACAT GGTTGTGTTTCAGGGACTCCCACAATCCCCTGTGAGTGGGTGG	GAC ACA CAG GT AGGAAAGGGCAGAGTCTGAGTTTTCTCTCAGCCTCCTTTAGAGTGTGCTCTGUTCATCAATGGGGAACACAGGCACACCCCAC ATTGCTACTGTCTGTAALTGTGTCTGCTCTCAGTTCTGGGAACTTCCTAGTGTCAAGCTCTTCCTTGAACTCTCACAGCTTTCCTTCACAG GT GGA CAA GGA GGG GAC TAT GCT CTG GCT CCA G GTTAGTGTGGGGGACAGAGTTGTCCTGAGGTCATTGGAGTGAAGCTGGGAGGTGTGTGGGGGGC CTGGGGAACTCATAATGGTTCTCTGTGTGTCTGCTGCTGCTGCTGCTGCTGCTGC	4648
THE	ACA GCT CCL AGA GCT CTG ATA TGT TTC TCT CAA GAT TGT AAA G GTGACATICTATGGCTGATATATTTTTLCCTAGGCAG GG GGTTGTGTTTCAGGGACTCCCACAATCCCCTGTGAGTGGTGGGGGGTTGTTGGGATATTGTCTTCATTGGGTGGTTGCTGAGGGGGCACTTGCGAG AGACAGCTGCCTGGGGGCCCACAATCCCCCGTGAGTGGTGGCTGGGGTTCTTCATTGTGGTGGTTCCCTGACCCTCATTCTCTAT CA TGA AGACAGCTGCCTGGGGCCCGAGTGGCCTTGGCCTGGGCTGGCCTCCTTTTTTTT	4836 5019

	Length Q4	Changes						
		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	

Table 1. DNA sequence homology

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 evennumbered 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 Ca₂PO₄

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 ³⁵S-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 oneor 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 41000 which was not present in cells transfected with B1.24 or H26. A polypeptide of similar size was detected in lysates of ³⁵S-methionine labeled C57BL/6 spleen cells. The polypeptides of Mr 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 twodimensional polyacrylamide gels showing synthesis of 35S-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 (OI, lane 1), H26 (Q2+Q3, lane 2), B.2.5 (Q4+Q5, lane 2)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 ¹⁴C-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 ³⁵S-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 ³⁵Smethionine 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 Mr 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 ¹²⁵I-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 ³⁵S-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 membraneassociated 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 ¹²⁵I 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 O4 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 $37\,000\pm500$. 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 38406. 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 ³⁵S-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 O4 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-Q10may 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.

Acknowledgments. The authors thank Margot Lang for help with sequencing and Karin Reimbold for performing the transfections. We are indebted to Peter Altevogt and Ivan Lefkovits for 2D gel analyses, and to Jan Geliebter and Sarah Spencer for helpful comments. This work was supported by grants We1069 and Ro692 from the Deutsche Forschungsgemeinschaft.

References

- Arnold, B., Burgert, H. G., Archibald, A. L., and Kvist, S.: Complete nucleotide sequence of the murine H-2K^k gene. Comparison of three H-2K locus alleles. Nucleic Acids Res. 12: 9472-9487, 1984
- Devlin, J. J., Weiss, E. H., Paulson, M., and Flavell, R. A.: Duplicated gene pairs and alleles of class I genes in the *Qa-2* region of the murine Mhc: a comparison. *EMBO J. 4*: 3203–3207, 1985
- Dobberstein, B., Garoff, H., Warren, G., and Robinson, P. J.: Cell free translation and membrane insertion of the H-2D^d histocompatibility antigen and β_2 microglobulin. *Cell* 17: 759–769, 1979
- Elder, J. H. and Alexander, S.: Endo-β-N-acetylglucosaminidase F: Endoglycosidase from *flavobacterium meningosepticum* that cleaves both high mannose and complex glycoproteins. *Proc. Natl. Acad. Sci. U.S.A.* 79: 4540–4554, 1982
- Flaherty, L.: Tla region antigens. In M. E. Dorf (ed.): The Role of the Major Histocompatibility Complex in Immunology, pp. 33-57, Garland STPM Press, New York, 1981
- Fujiki, Y., Hubbard, A. L., Fowler, S., and Lazarow, P. B.: Isolation of intracellular membranes by means of sodium carbonate treatment. Application to endoplasmic reticulum. J. Cell Biol. 93: 97-102, 1982
- Geliebter, J., Zeff, R. A., Schulze, D. H., Pease, L. R., Weiss, E. H., Mellor, A. L., and Nathenson, S. G.: Interaction between K^b and Q4 gene sequences generates the K^{bm1} mutation. Mol. Cell. Biol. 6: 645–652, 1986
- Harris, R.A., Hogarth, P.M., Penington, D.G., and McKenzie, I. F. C.: Qa antigens and their differential distribution on lymphoid, myeloid and stem cells. J. Immunogenet. 11: 265–281, 1984
- Jaulin, C., Perrin, A., Abastado, J. P., Dumas, B., Papamatheakis, J., and Kourilsky, P.: Polymorphism in mouse and human class I H-2 and HLA genes is not the result of random independent point mutations. Immunogenetics 22: 453–470, 1985
- Kalb, V. F., Glasser, S., King, D., and Lingrel, J. B.: A cluster of repetitive elements within a 700 base pair region of the mouse genome. *Nucleic Acid Res.* 11: 2177–2184, 1983
- Kimura, A., Israel, A., Le Bail, O., and Kourilsky, P.: Detailed analysis of the mouse $H-2K^b$ promoter: enhancer-like sequences and their role in the regulation of class I gene expression. *Cell* 44: 261–272, 1986

P.J. Robinson et al.: Structure and expression of the Q4 gene

- Lew, A. M., Maloy, W. L., and Coligan, J. E.: Characteristics of the expression of the murine soluble class I molecule, Q10. J. Immunol. 136: 254–258, 1986
- Maxam, A. M. and Gilbert, W.: Sequencing end-labeled DNA with base-specific chemical cleavages. *Proc. Natl. Acad. Sci. U.S.A.* 74: 560–564, 1977
- Mellor, A. L., Weiss, E. H., Kress, M., Jay, G., and Flavell, R. A.: A non-polymorphic class I gene in the murine MHC. *Cell 36*: 139-144, 1984
- Mellor, A. L., Antoniou, J., and Robinson, P. J.: Structure and expression of genes encoding murine Qa-2 class I antigens. *Proc. Natl. Acad. Sci. U.S.A.* 82: 5920–5924, 1985
- Michaelson, J., Flaherty, L., Bushkin, Y., and Yudkowitz, H.: Further biochemical data on Qa-2. *Immunogenetics* 14: 129–140, 1981
- Nathenson, S. G., Geliebter, J., Pfaffenbach, G. M., and Zeff, R. A.: Murine major histocompatibility complex class I mutants: molecular analysis and structure-function implications. *Annu. Rev. Immunol.* 4: 471–502, 1986
- Ploegh, H. L., Orr, H. T., and Strominger, J.: Major histocompatibility antigens: the human (HLA A, B, C) and murine (H-2K, H-2D) class I molecules. *Cell* 24: 287–299, 1981
- Reyes, A. A., Schöld, M., and Wallace, B.: The complete amino acid sequence of the murine transplantation antigen H-2D^b as deduced by molecular cloning. *Immunogenetics 6:* 1–9, 1982
- Robinson, P. J.: Qb-1, a new class I polypeptide encoded by the Qa region of the mouse H-2 complex. Immunogenetics 22: 285–289, 1985
- Robinson, P. J.: Two different pathways for the secretion of *Qa* region associated class I antigens by mouse lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 84: 527–531, 1987
- Robinson, P. J. and Schirrmacher, V.: Differences in the expression of histocompatibility antigens on mouse lymphocytes and tumour cells: immunochemical studies. *Eur. J. Immunol.* 9: 61–66, 1979
- Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. G. H., and Roe, B. A.: Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. 143: 161–178, 1980
- Soloski, M. J., Vernachio, J., Einhorn, G., and Lattimore, A.: Qa gene expression: biosynthesis and secretion of Qa-2 molecules in activated T cells. Proc. Natl. Acad. Sci. U.S.A. 83: 2949–2953, 1986
- Steinmetz, M., Moore, K. W., Frelinger, J. G., Sher, B., Shen, F. W., Boyse, E. A., and Hood, L.: A pseudogene homologous to mouse transplantation antigens: transplantation antigens are encoded by eight exons that correlate with protein domains. *Cell* 25: 683–692, 1981
- Taylor Sher, B., Nairn, R., Coligan, J. E., and Hood, L. E.: DNA sequence of the mouse H-2D^d transplantation antigen gene. Proc. Natl. Acad. Sci. U.S.A. 82: 1175–1179, 1985
- Weiss, E. H., Golden, L., Zakut, R., Mellor, A. L., Fahrner, K., Kvist, S., and Flavell, R. A.: The DNA sequence of the *H-2K^b* gene: evidence for gene conversion as a mechanism for the generation of polymorphism in histocompatibility antigens. *EMBO J. 2:* 453–462, 1983
- Weiss, E. H., Golden, L., Fahrner, K., Mellor, A. L., Devlin, J. J., Bullman, H., Tiddens, H., Bud, H., and Flavell, R. A.: Organisation and evolution of the class I gene family in the major histocompatibility complex of the C57BL/6 mouse. *Nature 310:* 650–655, 1984
- Wigler, M. S., Silverstein, L. S., Lee, A., Pellicer, Y., Cheng, T., and Axel, R.: Transfer of purified herpes virus thymidine kinase gene into cultured mouse cells. *Cell 11*: 223–232, 1977

Received June 15, 1987