

WIDESPREAD TRANSCRIPTION OF A Qa REGION GENE IN ADULT MICE

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Mouse class I MHC antigens are encoded on chromosome 17 by a family of related genes grouped within four closely linked regions: H-2K, H-2D, Qa, and Tla. Most sequences map to the Qa and Tla regions. Class I proteins share extensive amino acid homology and the genes are similar in sequence, structure and organization of exons, introns and protein domains (reviewed in reference 1). All class I gene products identified thus far are 40–45 kD glycoproteins and are noncovalently associated with β_2 -microglobulin. In spite of these similarities, several features differentiate the H-2K/D genes from the Qa/Tla region genes. Foremost is the disparity in the degree of sequence diversity among alleles between K/D and Qa/Tla. >50 alleles of *K*, *D*, and *L* genes have been identified by serological analysis (2). The high degree of polymorphism is primarily due to sequence divergence in exons 2 and 3, which code for the $\alpha 1$ and $\alpha 2$ domains, respectively (1). In contrast, genes mapping to the Qa/Tla region seem to be simple allelic systems (reviewed in reference 3). For example, the allelic sequences of the *Q10* gene from SWR and B10 mice are >99% homologous (4). If the conservation of sequences holds true for other Qa genes, it implies that K/D and Qa/Tla region sequences are evolving in response to different selective pressures.

K/D class I genes and Qa/Tla class I genes also appear to differ in expression and presumably, function. K/D-encoded molecules are expressed in most somatic tissues while Qa/Tla gene expression has been detected primarily in hematopoietic cells (3). Functional studies of K/D-encoded class I molecules indicate that they provide the self component in interactions between foreign antigens and allospecific or virus-restricted cytotoxic T lymphocytes (5). The function of Qa/Tla-encoded class I molecules is unknown (3).

~10 class I sequences have been identified in the Qa subregions of B10 and BALB/c mice (1, 6, 7). While Qa-2-reactive products have been immunoprecipitated from the cytoplasm of L cells transfected with Qa genes (*Q6^b*, *Q7^b*, *Q8^b*, and *Q9^b*) (8), additional attempts to identify Qa region products by screening transfected cells for surface Qa antigens have met with limited success (9, 10). Whether the failure to detect Qa products on the surface of L cells is due to the peculiar features of Qa genes or to differences in tissue-specific regulation is not known.

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An alternative approach to detecting novel class I products is to make recombinant cDNA libraries from mRNA of different tissues to isolate and characterize transcription of class I genes. This approach was used to isolate a liver-specific secreted class I polypeptide (*Q10^a*) (11–13). More recently, LaLanne et al. (14) have used the same approach to study a collection of class I cDNA clones made from liver RNA of DBA/2 (*H-2^d*) mice. They found expression of eight class I mRNAs transcribed from six class I genes. In addition, they identified mRNAs from a gene equivalent to *Q7^d* and a *Tla* gene. Northern blot analyses of liver, spleen, and kidney mRNA from DBA/2 mice using low copy number probes derived from these two sequences suggest that these or closely related genes were transcribed in at least these tissues.

Our laboratory previously reported (15) that several non-K/D genes isolated from a B10.P sperm library can provoke weak antibody responses and be recognized by T cells when transfected into L cells. One gene, designated λ 3a, was chosen for further analysis. In this report, we have extended the analysis of λ 3a to the molecular level by using probes derived from the 3' noncoding region of λ 3a. Southern blot analysis of genomic DNA and dot blots of class I cosmid DNAs using the 3' noncoding probes confirms that λ 3a maps to the Q_a region of B10.P. RFLP analysis of genomic DNAs shows the λ 3a sequence is associated with a RFLP specific for *Q4* and that sequences corresponding to *Q6* and *Q8* are absent in B10.P mice. Comparisons of the DNA sequence of λ 3a and *Q4^d* are identical. Based on these data, we conclude that the λ 3a gene is *Q4^p*. We have examined the transcription of the λ 3a gene and found transcripts in a variety of B10.P tissues including brain, heart, kidney, liver, lung, lymph node, muscle, spleen, testes, and thymus. These data represent the first demonstration of widespread transcription of a Q_a region gene.

Materials and Methods

Preparation of DNA and RNA. Spleen DNAs from B10.P and B10 were prepared according to the described procedure (16). Liver DNA from B10, B10.P, B10.F(13R), B10.F(14R), and C3H/HeJ were prepared according to Blin and Stafford (17). Spleen DNA from AKR/J, BALB/cj, DBA/2J, SWR/J, and 129/J were purchased from The Jackson Laboratory (Bar Harbor, ME). The following BALB/c cosmid DNAs were generously provided by Dr. Michael Steinmetz (Basel Institute, Basel, Switzerland): 64.1, 2.1, 65.1, 52.3, 46.1, 8.2, 46.2, 16.1, 59.2, 1.1, 48.1, 12.2, 31.1, 59.1, 6.3, 47.1, 4.1, 50.2, 52.2, 20.1, 49.1, 8.3, 15.1, 15.3, 17.1, 22.1, 18.1. The following B10 cosmids or plasmids were generously provided by Dr. Andrew Mellor (MRC Clinical Research Center, Harrow, United Kingdom): B1.1, B2.17, B1.24, K^b, B1.4, B3.3, B2.7, B2.5, B4.8, H6, H11, H18, H26, K4, K5.1.

Total cellular RNA was prepared by a modification of the guanidinium thiocyanate procedure of Chirgwin et al. (18). After sedimentation through cesium chloride, RNA pellets were suspended in 8 M guanidine HCl and precipitated by addition of one-tenth volume of 2 M sodium acetate and one-half volume absolute ethanol. RNA pellets were resuspended in diethylpyrocarbonate-treated water, heated to 70° for 10 min and spun at 4° at 10,000 rpm for 10 min. The supernatant was removed and the procedure was repeated twice. $A_{260/280}$ was measured and supernatants containing RNA were pooled and precipitated with one-tenth volume 3 M sodium acetate and three volumes absolute ethanol. Samples were resuspended in diethylpyrocarbonate-treated water and stored in aliquots at -20°C.

Construction of *pMJP-1* and *pMJP-2* Plasmids. 640-bp Sac I and 190-bp Pvu II–Pst I fragments were gel purified from 6% polyacrylamide gels after fractionation. After gel

purification and ethanol precipitation, the 190 Pvu II–Pst I fragment was repaired with T4 polymerase (New England Biolabs, Beverly, MA) and Eco RI linkers attached (Bethesda Research Laboratories, Gaithersburg, MD). The 200-bp Eco RI fragment was ligated into the Eco RI site of linearized pSP65 (19) and the 640-bp Sac I fragment was ligated into the Sac I site of linearized pGEM-1 (19). Recombinant clones were obtained in both 5' to 3' and 3' to 5' orientations with respect to the SP6 promoter. Transcripts complementary to mRNA were obtained by transcribing linear templates of the 3' to 5' orientation.

DNA Blot Hybridization. 10 μ g of genomic DNA samples were digested for 3 h at 37°C with 30–60 U Xba I or Pst I (International Biotechnologies, Inc., New Haven, CT). Samples were electrophoresed in 0.8% agarose gels at 500 V·h. Electrophoresed DNA was transferred to either nitrocellulose or Z-probe nylon filters (Bio-Rad Laboratories, Richmond, CA) by the method of Southern (20). Before hybridization, nylon filters were prewashed in 30 mM NaCl, 4 mM sodium citrate, 0.5% SDS at 65°C for 30 min each. Both Nylon and nitrocellulose filters were hybridized to ³²P-labeled RNA transcribed from pMJP-2 (1–2 \times 10⁶ cpm/ml). Filters were exposed to Kodak XAR-5 film with Lightening Plus screens (DuPont Co., Wilmington, DE) for 5–10 d at –70°.

Dot blots of 10 ng of BALB/c and B10 cosmid DNAs were performed in duplicate as described (21).

RNA Blot Hybridization. Total cellular RNA (20 μ g) or poly(A)⁺ RNA (5 μ g) was subjected to electrophoresis through formaldehyde (1.6% agarose) gels at 400 V·h and transferred to nitrocellulose as described by Maniatis et al. (22). Hybridization to single-strand ³²P-labeled RNA transcribed from pMJP-2 (2 \times 10⁶ cpm/ml) was performed according to DeLeon (23). Filters were exposed to Kodak XAR-5 film with Lightening Plus screens (DuPont Co.) at –70. After exposure for 24–48 h, filters were treated with RNase A (1 μ g/ml) according to DeLeon (23) to reduce nonspecific binding of the probe.

RNAase mapping (RNAase protection) with pMJP-1 was performed according to Zinn, et al. (24). Fixed gels were exposed to Kodak XAR-5 film with DuPont lightening plus screens at room temperature for 16–20 hours.

Synthesis of Complementary-Strand RNA Probes. pMJP-1 and pMJP-2 plasmids were linearized with Eco RI and Bam HI, respectively, extracted successively with phenol/chloroform (1:1), chloroform, and ether, before ethanol precipitation. Linearized DNA was resuspended at a concentration of 1 μ g/ml in diethylpyrocarbonate-treated water. Transcription of linearized DNA with SP6 polymerase (Boehringer Mannheim Diagnostics, Inc., Houston, TX) was performed as described for the synthesis of high specific activity probes by Promega Biotec (Madison, WI) and modified as follows: 100 μ Ci of α [³²P]UTP (800 Ci/mM, New England Nuclear, Boston, MA) was added per 20 μ l reaction with 1 μ l of 240 μ M UTP. After transcription, the mix was treated with RNase-free DNase, extracted, and precipitated. Transcripts were gel purified by fractionation on 6% polyacrylamide/urea sequencing gels at 30 V/cm. Full-length transcripts were cut out of the gels and eluted for 2–4 h in 0.5 M sodium acetate (pH 5.2), 0.1% SDS, and 20 μ g/ml of tRNA. Because of the rapid degradation of the ³²P-labeled single-stranded probes (24), the probes were used immediately after elution.

Cell Lines. The C3H (H-2^k) mouse fibroblast cell line Ltk[–] is a thymidine kinase-deficient mutant of L929 cells. The BDP/J SV40 transformed splenic fibroblast line was supplied by Dr. Barbara Knowles, Wistar Institute, Philadelphia, PA.

Cells were maintained in RPMI 1640 medium supplemented with 5% FCS, 10 mM Hepes, penicillin, and streptomycin.

Results

Characterization of Class I Organization Using a Low Copy Number Probe from a B10.P Gene. To study the transcription of λ 3a, a Q region gene isolated from a B10.P λ library (25), we isolated probes from the 3' noncoding region of the λ 3a genomic clone. We elected to derive probes from this region because class I RNAs differ in their 3' noncoding regions (12, 26). We initially localized the 3' noncoding region of λ 3a to a 640-bp Sac I fragment that also included presump-

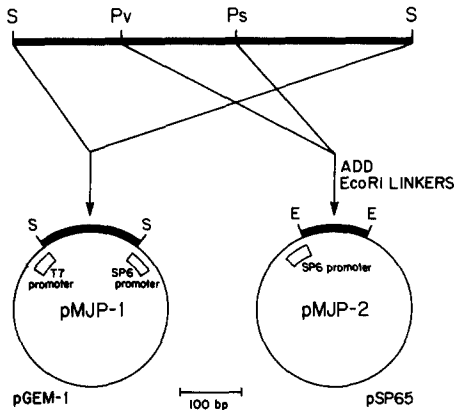


FIGURE 1. Construction of pMJJP-1 and pMJJP-2 plasmids. A 640-bp Sac I fragment from the 3' region of the λ 3a genomic clone (described in Schepart et al. [15]) was subcloned into the Sac I site of pGEM-1, producing the plasmid pMJJP-1. A 190-bp Pvu II-Pst I fragment (included within the 640-bp Sac I fragment) was subcloned into the EcoRI site of pSP65 after the addition of EcoRI linkers, producing the plasmid pMJJP-2. Colonies were screened with nick-translated 190-bp Pst I-Pvu II fragments and checked for orientation with respect to the SP6 promoters of pGEM-1 and pSP65.

tive exons 7 and 8. This fragment was subcloned into pGEM-1. The resulting plasmid is designated pMJJP-1 (Fig. 1). A 190-bp Pvu II-Pst I restriction fragment from pMJJP-1 was subcloned into pSP65 and designated pMJJP-2 (Fig. 1).

To map the position of the λ 3a sequence, liver DNAs from B10 ($H-2^b$), B10.P ($H-2^p$), and two reciprocal intra- $H-2$ recombinants, B10.F(13R) [$H-2^{bu}(K^pD^b)$] and B10.F(14R) [$H-2^{bu}(K^bD^p)$], were digested with Xba I, electrophoresed on agarose gels, transferred to nitrocellulose, and probed with pMJJP-2 in Southern blot experiments. An Xba I digest is shown in Fig. 2. Four of the six hybridizing fragments were invariant (arrows), one (~11 kb vs. 14 kb) differed in position, and one (~3.7 kb) was absent in B10.P and B10.F(14R). The absence of the 3.7-kb band has been noted previously (27, 28) in Xba I digests of DNA from $Qa-2^-$ strains. This corresponds to the absence of the $Q5-Q9$ sequences in $H-2^p$. From analysis of the λ 3a phage clone we know that the corresponding genomic fragment must be >10 kb since there are no Xba I sites located within the mouse sequences in the λ clone. Therefore, the 14-kb band in B10.P and B10.F(14R) DNAs must correspond to the λ 3a gene, and the 11-kb band in B10 and B10.F(13R) presumably represents a RFLP of the λ 3a sequence. The results confirm previous RFLP analysis localizing the λ 3a gene to the D end of B10.P (15). Further, the hybridization patterns show that λ 3a is homologous to at least one Qa -related sequence that is absent in B10.P mice.

To assign the bands observed in the Xba I digests to the physical map derived from cosmid cloning, we used pMJJP-2 to screen a series of cosmid clones that contain the 26 class I genes isolated from B10 DNA (7). We also screened a series of cosmid clones that contain the 36 class I genes isolated from BALB/c (29). To simplify discussion, Qa genes will be referred to by the nomenclature established by Weiss et al. (7). The results of these studies are summarized in Table I. Only cosmids that hybridized are shown. The other cosmids tested are listed in Materials and Methods. Four overlapping cosmids from the Qa region of B10 containing $Q4^b-Q9^b$, and four overlapping cosmids from BALB/c containing $Q4^b-Q8/Q9^d$ hybridized to pMJJP-2. In addition, cosmid 50.2 from cluster 6 of BALB/c also hybridized to pMJJP-2. 50.2 contains two D region genes ($D2^d$ and $D3^d$) located between D^d and L^d (6), which were probably derived from the

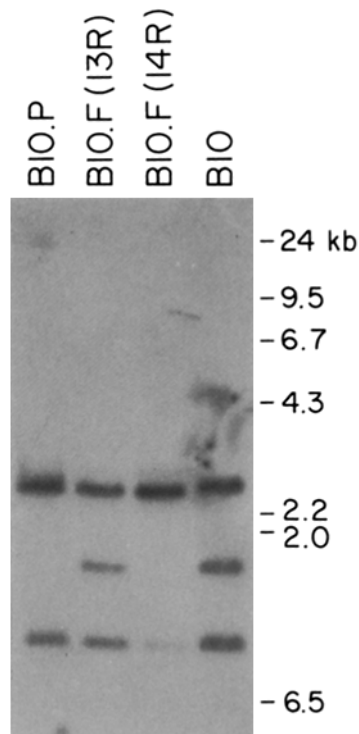


FIGURE 2. Southern blot analysis of genes hybridizing to pMJP-2. The mouse strain from which the DNA was extracted is indicated in each lane. Xba I digest of 10 μ g of genomic DNA. Sizes of λ Hind III markers are indicated in kilobases on the right.

TABLE I
BALB/c and B10 Cosmid DNAs Hybridizing to pMJP-2

BALB/c cosmid	Gene pair	B10 cosmid	Gene pair
65.1	Q4 ^d + Q5 ^d	B2.5	Q4 ^b + Q5 ^b
46.2	Q5 ^d + Q6 ^d	B2.17	Q6 ^b + Q7 ^b
8.2	Q6 ^d + Q8/9 ^d *	B3.3	Q7 ^b + Q8 ^b
46.1	Q8 ^d /Q9 ^d	B4.8	Q8 ^b + Q9 ^b
50.2	D2 ^d + D3 ^d †		

* Q8/Q9^d is a fusion gene.

† There is no equivalent to D2^d-D3^d in B10 DNA.

Qa region by an unequal crossover event. pMJP-2 does not hybridize to the cloned *DP* gene (data not shown). Further, analysis of B10.F(14R) DNA using *D* flanking region probes, confirms that the *D*^P region contains a single class I gene (6). These data eliminate the possibility that pMJP-2 hybridizes to the *D*^P gene. Based on the hybridization to Qa region sequences and the lack of hybridization to genes in other regions, we assigned λ 3a to the Qa region of B10.P.

The λ 3a Gene is Q4P. To precisely identify the λ 3a gene, we used the Pustell DNA sequence analysis Program (IBI, New Haven, CT) to compare the sequence of the 3' end of λ 3a genomic clone with the sequences of several class I genes including *K*^b (30), *K*^k (31), *D*^P (32), *D*^d (33), *L*^d (34), *Q7*^d (35), *Q8*^b (36), *Q10*^b (4), and the transmembrane sequence of *Q4*^d (provided by Dr. Steve Hunt, California

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1      10      20      30      40      50      60
AGCCTCCTCCATCCACTGTCTCCAACATGGCGAACGTAGCTGTTCTGGTTGTCCTTGGAG Q4d
|||||
AGCCTCCTCCATCCACTGTCTCCAACATGGCGAACGTAGCTGTTCTGGTTGTCCTTGGAG Q4p
1      10      20      30      40      50      60

70      80      90      100     110     117
CTTGCCATCATTCGAGCTGTGGTGGCTTTTGTGATGAAGAGAAGGAGACACACAGG Q4d
|||||
CTTGCCATCATTCGAGCTGTGGTGGCTTTTGTGATGAAGAGAAGGAGACACACAGG Q4p
70      80      90      100     110     117

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FIGURE 3. Comparison of the nucleotide sequence of the highly polymorphic exon 5 sequences of $Q4^d$ and $Q4^p$. Sequences were aligned to provide maximum match.¹

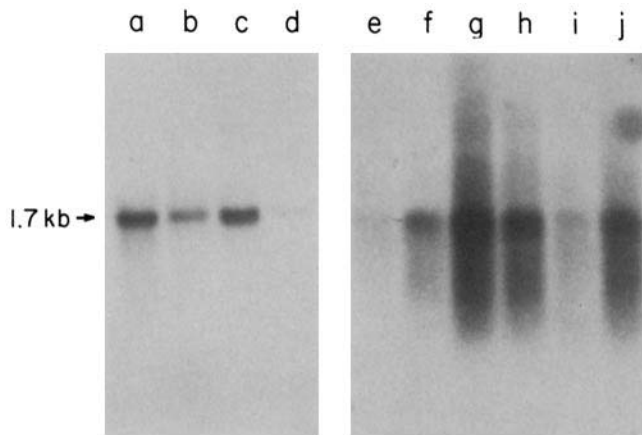


FIGURE 4. Expression of RNA related to pMJP-2 in various tissues of the B10.P mouse. Northern blots of 20 μ g total RNA hybridized with pMJP-2. (a) Ltk⁻, (b) spleen, (c) liver, (d) kidney, (e) brain, (f) heart, (g) lymph node, (h) lung, (i) muscle, (j) testes. Length of hybridizing RNA species is indicated in bases. a-d and e-j are separate experiments, so intensities can not be directly compared.

Institute of Technology, Pasadena, CA). The comparisons show that the $\lambda 3a$ sequence is most homologous to Qa region sequences, as expected. Most striking is the identity of the highly polymorphic transmembrane sequences of $\lambda 3a$ and $Q4^d$ (shown in Fig. 3). The $\lambda 3a$ and $Q4^d$ sequences are identical at 117/117 nucleotide positions. This demonstrates that the $\lambda 3a$ gene is $Q4^p$.

Class I Genes Homologous to pMJP-2 are Widely Transcribed in B10.P Tissues. To determine if $Q4^p$ and/or related B10.P class I genes are transcribed in vivo we used pMJP-2 to probe RNA blots from adult B10.P tissues. We performed Northern blot analysis of total RNA isolated from several B10.P tissues and C3H ($H-2^k$)-derived Ltk⁻ cells. Representative blots from two experiments are shown in Fig. 4. pMJP-2 detects a RNA species of ~1.7 kb in all tissues tested (brain, heart, kidney, liver, lung, lymph node, muscle, spleen, testes, and thymus). To confirm that the signals detected in total RNA were reflective of poly(A)⁺ mRNA levels, Northern blots of 20 μ g total RNA or 5 μ g of poly(A)⁺ RNA from brain, kidney, spleen, and Ltk⁻ were hybridized to pMJP-2 and the hybridization patterns were compared (data not shown). Since the patterns of hybridization were comparable, total RNAs were used in subsequent experiments. Collectively, these results demonstrate that $Q4^p$ or a closely related Qa gene(s) is transcribed in most B10.P tissues.

In attempts to generate antibodies against the $Q4^p$ gene product by immunizing

¹ These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00631.

mice with $Q4^P$ -transfected (L3a) cells (Schepart, B., and J. A. Frelinger, unpublished results), we repeatedly produced antibodies that were crossreactive with Ltk^- cells. To determine if the crossreactivity was due to the expression of a homologous gene in Ltk^- cells, we performed Northern blot analysis of Ltk^- RNA to test for the presence of related class I transcripts. We detected a strongly hybridizing band in RNA isolated from Ltk^- cells (see Fig. 4a). Thus, Ltk^- cells appear to express at least one $Q4$ -related transcript.

The $Q4^P$ Gene is Transcribed in Most B10.P Tissues. Since pMJP-2 hybridizes to cosmids containing several Qa region genes, it is possible that pMJP-2 detects multiple Qa transcripts in some tissues. To discriminate between transcription of the $Q4^P$ gene and transcription of related class I genes, we used ^{32}P -labeled RNA complementary to $Q4^P$ to assay protection of RNA isolated from adult B10.P tissues. In these experiments, the 640-bp Sac I fragment was inserted into pGEM-1 (pMJP-1) so that transcription of RNA complementary to $Q4^P$ mRNA was initiated at the SP6 promoter. The size of the protected fragment can be accurately predicted in a manner analogous to S1 nuclease mapping (37). The results of these experiments are shown in Fig. 5. Comparisons of the pMJP-1 sequence to the D^P (32), K^k (31), $Q7^d$ (35), and $Q10^b$ (4) sequences, showed multiple regions of nonidentity that should be cleaved by RNase. However, as depicted schematically in Fig. 5, a fragment of 500 nucleotides should be protected by $Q4^P$ mRNA. Transcripts from the alternate strand of pMJP-1 are not protected by B10.P RNA (data not shown). The results in Fig. 5 show one major protected fragment of 500 nucleotides was easily detected in most RNA from B10.P tissues (kidney, heart, liver, lung, lymph node, muscle, spleen, testes, and thymus). We do observe patterns of a variety of minor protected fragments that vary among the tissues tested. This is consistent with the protection of smaller regions of identity between pMJP-1 and other class I transcripts. In addition, the variation in minor protection patterns is also consistent with differences in the expression pattern among class I genes.

Although there is some variation in the intensity of the signal in the Northern blots from various tissues, there is a 1.7-kb RNA species detected in all tissues. The signal in brain RNA however, is consistently weaker than other tissues. Similarly, in the RNase protection experiments the $Q4^P$ transcript is easily detected in RNA from all tissues, except brain. The data from several Northern blot and RNA protection experiments are summarized in Table II. The values in Table II are based on the comparison of hybridization intensities to Ltk^- or liver RNAs that were run as internal standards. Therefore, these values are corrected for variability among experiments and more accurately reflect RNA expression in different tissues. These data demonstrate that the $Q4^P$ gene, unlike other characterized Qa genes, is transcribed in most, if not all, B10.P tissues.

Because we detected homologous transcripts in Ltk^- cells by Northern blotting, we also performed RNase analysis of Ltk^- RNA. After hybridization of pMJP-1 RNA to RNA from Ltk^- cells, we observed a major protected fragment of 290 nucleotides. Among other faint bands is a fragment of 500 bases, the size expected for protection of an mRNA to the $Q4^P$ gene. Because all the sequence of $Q4^k$ is not available, we could not be certain that the 290 nucleotide fragment represents the product of the $Q4^k$ allele. It is possible that the faint band at 500

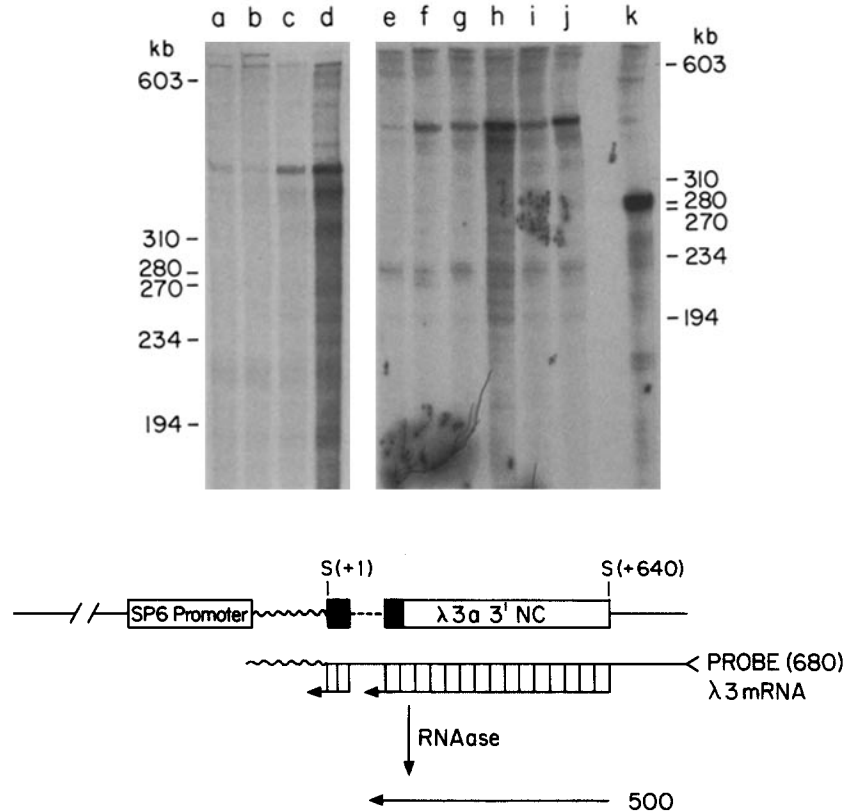


FIGURE 5. Detection of $Q4$ -specific RNAs by RNase analysis. Total cellular RNA ($20 \mu\text{g}$) from B10.P tissues and Ltk^- cells was hybridized to a >20 fold excess ($\sim 20 \text{ fmol}$; 10^6 cpm) of a 680 nucleotide complementary SP6 RNA probe ($\sim 5 \times 10^8 \text{ cpm}/\mu\text{g}$). (a) Liver, (b) lymph node, (c) spleen, (d) thymus, (e) brain, (f) heart, (g) kidney, (h) lung, (i) muscle, (j) testes, (k) Ltk^- .

The structure of the probe and the predicted protected fragments after hybridization and RNase treatment are shown in the diagram below the figure. Indicated lengths are in nucleotides. (S) Sac I sites of insert from which the probe was made; (+1) the start of pMJP-2 complementary RNA; (+640) the termination of pMJP-2 complementary RNA. (Boxes) Exons 7 and 8. The dotted line indicates intervening sequence. (Wavy lines) 5' polylinker sequence. a-d and e-k are from different experiments, so that the signal intensities can not be directly compared.

nucleotides in Ltk^- RNA represents a low abundance transcript from the $Q4^k$ gene. However, we believe that the 290 nucleotide fragment is $Q4^k$ since a single major RNA species of 500 nucleotides is protected in SV40-transformed BDP/J ($H-2^b$) fibroblasts (Fig. 6), a similar cell type. These results strongly suggest that $Q4$ is transcribed in transformed fibroblast cell lines.

Discussion

The analysis of individual class I genes has been difficult because of the large number of homologous class I sequences and the scarcity of allele specific probes. Molecular probes isolated from the 3' noncoding regions of class I genes have made it possible to identify sub-families of class I genes and in some instances,

TABLE II
*Signal Intensities Seen in Northern Blot and RNAase Analysis of
 B10.P RNAs Using pMJP-2 and pMJP-1*

Tissue	Northern blot analysis	RNAase analysis
Brain	+*	+/-
Heart	++	++
Kidney	++	++
Liver	++++	+++
Lung	+++	+++
Lymph node	+++	+++
Muscle	+	+
Spleen	+++	+++
Testes	+++	+++
Thymus	+++	+++
Ltk ⁻	++++	++++

* Indicates relative intensity of signal from +/- (weak) to ++++ (strong).

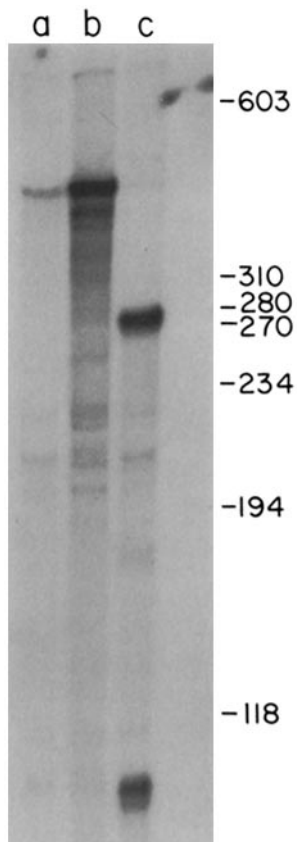


FIGURE 6. Detection of *Q4*-specific RNA in transformed fibroblasts. Total cellular RNA (20 μ g) from B10.P liver (a), SV40 BDP/J fibroblasts (b), and Ltk⁻ (c). Markers indicate size in bases.

individual genes. These probes can also be used to study the transcription of new class I genes as well as tracing the lineages of members of the class I gene family.

To explore the transcription of one B10.P class I gene, $Q4^P$ we isolated two low copy number probes from the 3' noncoding region of the $\lambda 3a$ genomic clone. Hybridization of pMJP-2 to cosmid DNAs from BALB/c and B10 partial DNA sequencing and restriction fragment length polymorphism mapping of genomic DNA indicate that the $\lambda 3a$ gene is $Q4^P$. Northern blot and precise RNase mapping experiments indicate that the $Q4^P$ gene, is transcribed in a variety of B10.P tissues. Thus, our experiments suggest that at least one Qa region gene may be transcribed ubiquitously in the tissues of B10.P mice.

$\lambda 3a$ is $Q4^P$. We have compared the sequence of pMJP-1 and the transmembrane sequence of $\lambda 3a$ (our unpublished observations) to the sequences of several class I genes including K^b (30), K^k (32), D^p (32), D^d (33), L^d (34), $Q7^d$ (35), $Q8^b$ (36), $Q10^b$ (4) and the transmembrane sequence of $Q4^d$ (Hunt, S., III, personal communication). These comparisons show that the sequence of pMJP-1 is most homologous to Qa region sequences, as expected. Most striking is the identity (117/117) of the highly polymorphic transmembrane sequence of $\lambda 3a$ and $Q4^d$, which, along with RFLP data, demonstrates that $\lambda 3a$ is $Q4^P$. Also interesting is the homology between pMJP-1 and the 3' noncoding region of $Q8^b$. With the exception of several nucleotide substitutions and two deletions, the corresponding sequences are identical for 357 nucleotides (not shown). This high degree of homology between nonallelic $Q4$ and $Q8$ sequences is probably the result of duplications and deletions in the Qa region that arise by homologous but unequal recombination (6, 8) or perhaps by gene conversion events among Qa sequences. It is particularly interesting since B10.P lacks the $Q8$ sequence.

Transcription of the $Q4$ Gene in B10.P Mice. The results reported here demonstrate that the $Q4^P$ gene is transcribed in all B10.P tissues tested (brain, heart, kidney, liver, lung, lymph node, muscle, spleen, testes, and thymus). There have been few systematic surveys of the transcription of individual class I genes due to the paucity of molecular probes. In fact, the only reported survey of transcription of a Qa gene is the $Q10$ gene, which is transcribed exclusively in liver (11). Our results differ from a preliminary report of the transcription of the $Q4^b$ gene in which $Q4^b$ was found to be transcribed in spleen but not liver (38). One explanation for this discrepancy is the difference in length and specific activities of the probes as well as the stringencies used in our assays. We have found that single-stranded RNA probes used in our experiment increase the sensitivity of detection several-fold over end-labeled and nick-translated probes. Alternately, we may be detecting the transcriptions of a B10.P gene that is not truly allelic to $Q4^b$. Such a difference is possible since DNA sequences in the region surrounding $Q4$ in B10 differ from those in B10.P DNA (Fig. 2). Duplication, deletion, and gene conversion events involving Qa genes have all been reported. Thus we could be detecting mRNA from a homologous but nonallelic sequence that is regulated differently from $Q4^b$. It is also possible that the transcription patterns of $Q4$ differ in B10 and B10.P mice.

One result worth noting is the presence of a 500 nucleotide protected fragment in brain RNA. Brain is considered an immunologically privileged tissue and expresses only low levels of K and D molecules (2). The correlation of K/D and $Q4$ expression is interesting. The identity of the cells producing the $Q4^P$ transcript is unknown. Perhaps the signal is due to a small number of brain cells, such as

astroglia, that also synthesize conventional class I products (39). Alternatively, it is possible that this signal could be due to vascular endothelium and/or hematopoietic cells in preparations of brain RNA.

A surprising result was the presence of $Q4^P$ transcripts in testes. Although germ cells represent only a fraction of the testes, $Q4^P$ expression in testes is consistent with the expression of $Q4^P$ in germ cells. The $Q4^b$ gene has been suggested (38) to provide donor sequence for the generation of a mutant K^b gene, K^{bm6} . In addition to genetic interactions between K^b and $Q4^b$ at the DNA level, it has been suggested that the K^b mutants could have been generated through a donor gene transcript or cDNA intermediate (30, 40). As suggested by Geliebter et al. (38), this would require that the donor gene be transcriptionally active in the germline. Our results demonstrate that at least $Q4^P$ is transcriptionally active in the testes.

Our results also indicate that similar Qa genes are transcribed in Ltk^- cells. RNase protection experiments of RNA isolated from a transformed BDP/J($H-2^P$) fibroblast line reveal a major protected fragment identical to that seen in other B10.P tissues (Fig. 6; and Badley, J., and J. A. Frelinger, unpublished observations). Thus, it appears that at least the $Q4^P$ gene is transcribed in transformed fibroblasts. This result has important implications for the use of transformed fibroblast lines in studying the products of exogenously introduced Qa region genes. Reagents must be specific for the allele as well as the locus for detection of transfected gene products.

Systematic analyses of Qa -encoded products have found that the expression of serologically defined Qa region products is limited to cells of hematopoietic lineage (3). Thus far, at least five genes from the Qa region of B10 mice have been shown to code for polypeptides ($Q6^b$ - $Q10^b$, inclusively). Of these $Q6^b$ - $Q9^b$ code for polypeptides that are immunoprecipitated with Qa -2,3 antisera (8). The $Q10$ gene encodes a liver-specific, secreted class I molecule that is found not only in B10 but also in most other species of *Mus* (13, 41, 42). More recently, eight class I cDNAs from DBA/2 liver mRNA have been cloned. Using a low copy number probe derived from $Q7^d$, LaLanne et al. (14) showed that $Q7$ (27.1) and/or homologous genes are transcribed in at least liver, spleen, and kidney. Thus at least $Q7$, whose product can be identified with a conventional Qa -2 antibody, is transcribed outside of hematopoietic cells. Thus, there is now evidence that many Qa sequences are transcriptionally active.

The results reported here, along with those of Cosman et al. (11) and LaLanne et al. (14), imply that many of the Qa class I genes are transcribed, and in some cases, correspond to unusual class I polypeptides (12, 41). A recent study of Qa -2 expression in activated T cells (43) suggests that while Qa -2 molecules are expressed on the surface in resting T cell populations, activation results in the secretion of newly synthesized molecules. Cosman et al. (11) have speculated that secreted Qa molecules may perform soluble effector functions. Alternately, Qa genes may encode molecules whose functions are not immune related.

We have found that unlike previous studies of Q region genes and products, that the $Q4^P$ gene is widely transcribed in adult tissues.

Summary

The mouse MHC class I family includes genes encoded in four regions: H-2K, H-2D, Qa and Tla. While *K/D* genes are well characterized, relatively little is known about *Qa* or *Tla* genes. We have studied the transcription of a B10.P Qa region gene. DNA sequence comparisons of the transmembrane region, supported by Southern blot analysis of cosmid and genomic DNAs from BALB/c and C57BL/10, demonstrate the $\lambda 3a$ gene corresponds to $Q4^P$. In both Northern blots and RNA protection experiments using probes derived from the 3' non-coding region, we found that *Q4*, like the *H-2K* and *H-2D* genes, is widely transcribed in B10.P tissues. These data demonstrate for the first time widespread transcription of a Qa gene.

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