TISSUE-SPECIFIC EXPRESSION OF CELL-SURFACE Qa-2 ANTIGEN FROM A TRANSFECTED Q7b GENE OF C57BL/10 MICE

GERALD L. WANECK,* DAVID H. SHERMAN,* SUSAN CALVIN,* HAMISH ALLEN,* AND RICHARD A. FLAVELL*

From *Biogen Research Corporation, Cambridge, Massachusetts 02142; and the Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

While the murine K, D, and L class I transplantation antigens are expressed on virtually all adult mouse tissues, the structurally related Qa-2 molecule is expressed only on the surface of functional subpopulations of peripheral T cells, LPS-induced B cell blasts, and hematopoietic cells (1). Although this tissue distribution suggests an important role for Qa-2, the function of this molecule is unknown. Only two alleles of Qa-2 (positive and null) have been defined by serological analysis of inbred and wild mouse strains (reviewed in reference 2). Molecular cloning of the MHC from C57BL/10 (B10) and BALB/c, two independent Qa-2+ mouse strains, surprisingly has revealed that the B10 mouse (H-2b haplotype) has 10 Qa region genes (Q1 through Q10), and the BALB/c mouse (H-2d haplotype) has 8 (reviewed in reference 3).

The identity of the gene(s) encoding the Qa-2 cell-surface antigen has been obscure. Goodenow et al. (4) reported that Qa-2 antigen was expressed on the surface of L cells transfected with the BALB/c cosmid clone 50.2. However, it was found recently that the two genes on clone 50.2 are located between H-2D\(^d\) and H-2L\(^d\) (5), rather than in the genetically defined Qa region. Melloret al. (6) have implicated the Q6\(^b\), Q7\(^b\), Q8\(^b\), and Q9\(^b\) genes of B10 in Qa-2 expression; however, in this study, Qa-2 molecules were not expressed on the surface of L cells transfected with these genes, but were detected only in whole cell lysates. Exon-shuffling experiments by Stroynowski et al. (7) and Straus et al. (8) showed that a hybrid BALB/c Q7\(^d\)/L\(^d\) molecule, composed of \(\alpha1\) and \(\alpha2\) domains from Q7\(^d\), and \(\alpha3\), transmembrane and cytoplasmic domains from L\(^d\), was expressed on the surface of transfected L cells; but a reciprocal L\(^d\)/Q7\(^d\) molecule was not.\(^1\)

This suggested that Q7\(^d\) might encode the Qa-2 antigen in vivo, but that an element in the 3' part of this gene prevented its expression on the surface of L cells. DNA sequence analysis of several Qa region genes, including Q7\(^d\) and Q7\(^b\), has revealed that all contain premature stop codons in their transmembrane

\(^1\) The Q7\(^d\) gene of BALB/c was originally called 27.1 (9). Subsequently, it was called Q6 since BALB/c mice are missing a gene corresponding to Q3 of B10 (10). However, the BALB/c 27.1/Q6 gene is allelic to Q7\(^b\), and recently the nomenclature for Qa region genes of BALB/c has been changed to be consistent with that of B10 (5).

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regions, suggesting that they may be pseudogenes or encode secreted products rather than membrane-bound proteins (9, 11, 12). Recent evidence (13) that activated T cells synthesize a secreted form of Qa-2, in addition to the membrane-bound form, has added even more confusion to the issue.

Studies by Sherman et al. (14) of Qa-2⁺ cloned allorreactive BALB.B10 CTL lines, have distinguished two membrane-bound forms of 36,500 mol wt and 38,500 mol wt. Some CTL clones express only the smaller or larger form, while others express both forms of Qa-2 protein. We wanted to identify which Qa region gene is expressed by one of these CTL clones (2.1.1) that synthesizes the smaller form. We screened a 2.1.1 cDNA library with a class I gene probe, but isolated Qa region cDNA clones representative of the Q7b gene only. In this paper, we show that the Q7b cDNA can express Qa-2 on the surface of both R1.1 thymoma and L cells. In addition, we show that the Q7b chromosomal gene and a Q7b gene construct driven by the Moloney murine leukemia virus (M-MuLV)² long terminal repeat (LTR) can express Qa-2 in a tissue-specific manner on the surface of R1.1 cells, but not on L cells. We have mapped the tissue-specific element downstream of a site near the middle of Q7b exon 4 by exchanging segments of Q7b and H-2Db.

Materials and Methods

Probes. A broadly reactive class I gene probe was prepared by nick translation of the 3' Bam HI fragment of the H-2Ka gene (15). Oligonucleotide probes were synthesized at Biogen Research Corp. on a 380A DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) and purified by PAGE. The oligonucleotides were end-labeled with γ-[³²P]ATP (7,000 Ci/mmol; New England Nuclear, Boston, MA) and polynucleotide kinase (New England Biolabs, Beverly, MA), and were purified by chromatography on Sephadex G50.

Southern Analysis. Southern blots and hybridizations were performed as described by Cate et al. (16), using Gene Screen nylon 66 membranes (New England Nuclear). Oligonucleotide probes were washed at low stringency in plaque screen buffer (PSB) at 45°C, and at high stringency in 3.2 M tetramethylammonium chloride (TMACL) containing 1% SDS, according to Cate et al. (16) and Wood et al. (17).

DNA Sequencing. The Q7b cDNA clone 69-2C2 was sequenced using the method of Church and Gilbert (18), as modified by R. Tizard and H. Nick (unpublished methods) and Cate et al. (16). Based on the Q7b genomic sequence (11), clone 69-2C2 DNA was cut to completion at various restriction enzyme sites, subjected to base-specific chemical cleavages (19-21), electrophoresed on DNA sequencing gels, transferred to Biotrans nylon 66 membranes (ICN Radiochemicals, Irvine, CA), UV-crosslinked, and hybridized to [³²P]-labeled oligonucleotides complementary to Q7b coding sequences. Membranes were reprobed to yield additional sequence information after stripping them with Church and Gilbert (18) wash buffer at 70°C.

Expression Vectors. Q7b pTCF was constructed by subcloning a 10-kb Hind III fragment from cosmid B2.17 (10) into the vector pTCF (22), which contains the amino glycosyl 3' phosphotransferase gene encoding G418 resistance. This Hind III fragment contains the entire Q7b gene with about 3 kb of flanking sequences at each end. The construct DⁿMo, containing the H-2Dⁿ gene in the Bam HI site of pTCF driven by the M-MuLV LTR, has been described previously (23). The construct Q⁷ⁿMo was made by inserting a 4.6-kb Not I–Bam HI (partial) Q7b fragment in place of a similar fragment from DⁿMo. The Not I site is located 32 bp on exon 1 of both Q7b (11) and H-2Dⁿ (Waneck, G., unpublished observations). This cloning strategy was used because there are no convenient restriction

² Abbreviations used in this paper: β2m, β-2-microglobulin; LTR, long terminal repeat; M-MuLV, Moloney murine leukemia virus; PSB, plaque screen buffer; TMACL, tetramethylammonium chloride.
enzyme sites in the 5' untranslated region of the Q7b gene. The hybrid exon 1 remains in frame and generates a normal leader sequence. The Q7b/D3Mo hybrid gene construct was made by inserting a 3.1-kb Not I–Stu I (partial) 5' gene fragment of Q7b in place of a similar 2.75-kb fragment of D3Mo, by using the Stu I site present in exon 4 of both genes. The D3/Q7bMo hybrid gene construct was made by inserting a 1.6-kb Stu I (partial)-Bam HI 3' gene fragment of Q7b in place of a similar 1.75-kb fragment of D3Mo. To express the Q7b cDNA, the 1.4-kb Eco RI insert of clone 69-2C2 was subcloned into the vector pBG312 (16). A 2.4-kb Bam HI fragment, composed of the Q7b cDNA linked to a 900 bp 3' cassette containing the SV40 splice and polyadenylation signals, was removed from the pBG312 construct and subcloned into pTCFMo, a vector generated from D3Mo by removing the H-2Dd gene. Constructions were made using standard techniques (24), and are illustrated in Fig. 1.

**Figure 1.** Exon/intron structure and restriction map of the Q7b, H-2Dd, and hybrid gene constructs. The Q7b exons and 3' untranslated region (3'UT) are indicated by open boxes; H-2Dd exons and 3'UT by closed boxes; introns, 5', and 3' flanking sequences by solid lines. The MuLV LTR is indicated by a hatched box. For the Q7cDNA/Mo construct, the SV40 3' cassette is indicated by an open box broken by a line representing the 64-bp intron.

Restriction sites: B, Bam HI; N, Not I; R, Eco RI; U, Stu I.

**Transfection of DNAs and Cell Culture.** L cells were transfected by calcium phosphate coprecipitation (25), as performed previously in our lab (26). R1.1 cells were transfected by electroporation (27) as described previously (28). Transfected cells were selected and grown in medium containing 10% FCS and 1 mg/ml G418 Sulfate (Gibco Laboratories, Grand Island, NY). Growth medium was DME for L cells, and RPMI 1640 for R1.1 cells.

**Radioimmunoassay of Transfectants.** G418-resistant clones were assayed for expression of surface protein by binding of mAbs D3.262 (28), 20-8-4 (29, 30), and B22.249 (31). 125I-labeled F(ab')2 fragments of sheep anti–mouse Ig (New England Nuclear) were used to detect bound antibodies.

**Extraction of RNA and Northern Analysis.** Total RNAs were extracted from cultured cells using the lithium chloride method of Auffray and Rougeon (32), as modified by J. Bertonis and W. Schleuning (unpublished results). RNAs were denatured and electrophoresed through formaldehyde/1% agarose gels (20 μg/lane), transferred to nylon membranes, and hybridized to 32P-labeled oligonucleotides, as described by Cate et al. (16).
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TABLE I

| Oligonucleotide Probes for C57BL/10 Class I Genes |
|--------------------------------------------------|
| Probe  | Stringency* |
|        | Low (45°C PSB) | High (TMACL) |
| D6     | K            | K           |
| D67    | K, D, Q2/5, Q10, T2, T7/8 | T7/8 |
| D68    | D, Q2/5, Q10, T2, T7/8 | Q4, Q6, Q8 |
| D69    | K, Q7, Q9    | D, T7/8     |
| D70    | K            | T7/8        |
| D73    | D            | D, T7/8     |
| D74    | K, Q2, Q5, Q7, Q8, Q9, T2, T3, T11, T12, T13 | T7/8, D, T7/8, T7/8, T7/8, T7/8, T7/8, T7/8, T7/8, T7/8, T7/8, T7/8, T7/8 |
| D75    | D, T7/8, T7/8, T7/8 | T7/8 |
| D76    | K            | Q5, Q7, Q9 |
| D78    | K           | K, Q1, Q2, Q3, Q4/5, Q9, T9, T4/5 |
| D79    | K           | Q9, Q10, T4/5 |
| D80    | D, T7/8     | D          |
| D124   | ND, K7, Q8  | ND         |

* Based on the published sequences of H-2Kb (15), H-2Dd (33), Q7b and Q8b (11).

Results

Isolation of a Full-Length Q7b cDNA from CTL Clone 2.1.1. As described previously (14), the cloned alloreactive BALB.B10 CTL line 2.1.1 (H-2b haplotype) expresses only a 36,500 mol wt form of Qa-2 protein on its cell surface. To determine which Qa region gene(s) is expressed by these cells, we screened a bacteriophage Agt10 library (a generous gift of E. Riley and H. N. Eisen, Massachusetts Institute of Technology, Cambridge, MA) prepared from CTL clone 2.1.1 cytoplasmic mRNA using a nick-translated probe derived from the 3' Bam HI fragment of the H-2Kb gene. This probe contains exon 4, which encodes the conserved a3 protein domain, and therefore hybridizes to all known class I genes. A total of 42 cDNA clones were isolated.

DNAs from these clones were spotted onto nylon membranes, UV-crosslinked, and analyzed using a panel of synthetic oligonucleotide probes (Table I) specific for individual or limited numbers of the 26 C57BL/10 class I genes isolated previously by Weiss et al. (10). The specificity of these probes was predicted by computer comparisons of the coding region sequences of H-2Kb, H-2Dd, Q7b, Q8b, and Q10b (11, 12, 15, 33), and was tested by hybridization at low and high stringencies to Southern blots of cosmid clones representing all 26 class I genes. Of the 42 cDNA clones analyzed by this method, six were identical as H-2Kb, five were H-2Dd, and four were Q7b. The identity of the remaining 27 clones has not yet been established; however, none of these hybridized to probes that would detect any other Qa region gene.

Of the four clones identified as Q7b, clone 69-2C2 contained the largest insert (1.4 kb), and was sequenced. Because the genomic sequence of Q7b had been determined previously (11), we prepared oligomers that hybridize adjacent to

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* Not tested against genomic cosmid clones since these probes recognize spliced exons.

† At low stringency, this probe reacts with nearly every class I sequence.
**Q7b ENCODES CELL-SURFACE Qa-2 ANTIGEN**

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**FIGURE 2.** Nucleotide sequence and translation of Q7b cDNA clone 69-2C. The first nucleotide of each exon is indicated by an asterisk followed by the number of the exon. UT refers to the 3' untranslated region. The amino acid translation is shown above the dashed number line. Positions of the two potential asparagine-linked glycosylation sites are overlined.

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various restriction enzyme sites present in Q7b exons, and used Church-Gilbert sequencing (18) to show that clone 69-2C is in fact Q7b cDNA. The sequence of 69-2C2 is shown in Fig. 2, with the exon boundaries indicated. This clone begins 13 nucleotides upstream of the initiation codon of exon 1, and the reading frame ends at a termination codon in exon 5 (the transmembrane region).
remainder of the 3′ untranslated region is composed of sequences derived from
the splicing of exon 6 to an exon 8 containing only five nucleotides. Hybridization
experiments using D79, an oligomer complementary to the exon 6/exon 8 splice
junction (see Table I), indicate that the three other Q7b cDNA clones isolated
from the CTL 2.1.1 library are spliced similarly. This pattern is expected since
the exon 7 splice acceptor signal of Q7b appears to be defective (11).

The polypeptide predicted from this Q7b cDNA is shown above the nucleotide
sequence in Fig. 2. The primary translation product is 334 amino acids, and the
molecular weight of the unglycosylated mature polypeptide is predicted to be
35,600. Based on the amino acid sequence of other class I cell-surface proteins
(reviewed in reference 34), the NH2-terminus is predicted to be glycine at position
22, and the mature Q7b polypeptide to be 313 amino acids in length. Potential
asparagine-linked glycosylation sites are present at amino acid positions 107 (α1)
and 277 (α3).

Expression of the Q7b cDNA. The Q7b cDNA insert was subcloned into the
expression vector pTCFMo, as described in Materials and Methods. This vector
has been used previously in our laboratory to obtain stable transfectants expressing
high levels of class I mRNA in L cells and R 1.1 cells (23). When R 1.1 cells
transfected with the Q7b cDNA construct (see Fig. 1) were screened with the
anti-Qa-2 mAb D3.262 and the Qa-2-crossreactive mAb 20-8-4, we detected
high levels of Qa-2 on the cell surface (see Table II). When L cells transfected
with this construct were screened using the same antibodies, surprisingly, we
found that they also expressed significant but lower levels of Qa-2 on their
surface (see Table II). The clones shown in Table II were selected for maximum
binding of the anti-Qa-2 mAbs; however, these clones are representative of the
uncloned G418-resistant populations (data not shown).

We have shown that the Q7b cDNA derived from CTL clone 2.1.1 can encode
the Qa-2 antigen, and for the first time, have shown that a Qa-2 molecule,
encoded entirely by sequences from a Qa-region gene, can be expressed on the
surface of L cells. Since previous attempts by us (see below) and others (6–8) to
express Qa-2 antigens on the surface of L cells using Qa genomic clones have
been unsuccessful, it appears that the use of a cDNA spliced by T cells may
account for this difference. However, since the level of expression on L cells is
lower than that on R 1.1 cells, it is possible that other factors, such as posttransla-
tional modification, also may be responsible for the tissue-specific expression of
Qa-2.

Expression of the Q7b Genomic Gene. R 1.1 and L cells were transfected with
the Q7b pTCF construct containing the entire Q7b gene and endogenous regulatory
sequences, or the Q7bMo construct containing the Q7b gene with a hybrid
exon 1 and the M-MuLV LTR, a strong enhancer and promoter (see Fig. 1).
Both constructs expressed high levels of Qa-2 antigen on the surface of R 1.1
cells (see Table II), as determined by antibody-binding studies using mAb D3.262
and mAb 20-8-4. Neither of these constructs, however, expressed detectable Qa-
2 on the surface of L cells (Table II). Thus, the tissue-specific expression of cell-
surface Qa-2 is not overcome by use of the M-MuLV LTR.

Expression of the Hybrid Genes. Exon-shuffling experiments by Stroynowski
(7) and Straus (8) and their coworkers showed that hybrid BALB/c genes,
TABLE II

Binding of Specific Antibodies to R1.1 and L Cell Transfectants

| Transfectants*          | Antibody binding†                       |
|-------------------------|-----------------------------------------|
|                         | 20-8-4 (Crossreactive) | B22.249 (D^dα1) | D5.262 (Qa-2,3) | Normal ascites |
| R1.1 thymoma            |                          |                  |                  |                |
| Q7^b cDNA Mo (Q7c26R)   | 14,204                    | 357              | 2,179            | 86             |
| Q7^b Mo (A6R)           | 8,529                     | 317              | 1,057            | 81             |
| Q7^b pTCF (Q7g2R)       | 3,713                     | 229              | 606              | 96             |
| D^b Mo (DbMoK)          | 315                       | 16,142           | 163              | 114            |
| R1.1                    | 288                       | 230              | 177              | 87             |
| Q7^b/D^b Mo (B5R)       | 10,078                    | 282              | 2,222            | 376            |
| D^b/Q7^b Mo (C18R)      | 437                       | 8,035            | 474              | 480            |
| L cells                 |                          |                  |                  |                |
| Q7^b cDNA Mo (Q7c4L)    | 789                       | 173              | 478              | 359            |
| Q7^b Mo (A7L)           | 135                       | 83               | 184              | 304            |
| Q7^b pTCF (Q7g2L)       | 86                        | 131              | 307              | 269            |
| D^b Mo (D4L)            | 113                       | 9,759            | 291              | 282            |
| Ltk^-                   | 182                       | 56               | 227              | 293            |
| Q7^b/D^b Mo (B5L)       | 11,545                    | 144              | 2,859            | 356            |
| D^b/Q7^b Mo (C1L)       | 112                       | 272              | 313              | 340            |

* Shown for each DNA construct. The transfectant cell clones selected for this assay are shown in parentheses. R1.1 and Ltk^- are the untransfected parent cell populations.
† 20-8-4 is the crossreactive mAb that recognizes Qa-2 and other H-2 antigens, but not D^b or the K^d and D^b antigens of R1.1 and Ltk^- (29, 30); B22.249 is specific for the α1 domain of D^b (26); D5.262 is specific for Qa-2,3 (28). 20-8-4 and B22.249 were used as cell supernatants. D5.262 was used as an ascites. Results are expressed as cpm of 125I bound. Each result is the average of triplicate samples, and the variation in all cases was not >10%.

composed of a 5' fragment from Q7^d and a 3' fragment from H-2K^d, H-2D^d, or H-2L^d, expressed Qa-2 determinants on the surface of transfected L cells; a reciprocal L'/Q7' construct did not. These experiments suggested that an element in the 3' part of Q7^d prevented its cell-surface expression.

These BALB/c hybrid genes were constructed by exchanging fragments 5' and 3' of a Bam HI site in the third intron. This intron contains B1 and B2 repeats that differ between H-2 genes and Qa genes (35). The B2 repeats, which may be developmentally regulated (36), are found 3' of the Bam HI site of Qa genes but not H-2 genes. Thus, to exclude the possible effects of these repeats on cell-surface expression, we constructed hybrid genes from Q7^b and H-2D^b using a conserved Stu I site in exon 4 (see Fig. 1). This enabled us to leave intron 3 intact, and to assay the expression of hybrid polypeptides that differ in their external domains from the parent molecules by only five amino acids. This difference is localized to the COOH-terminal half of the α3 domain.

The Q7^b/D^b Mo and D^b/Q7^b Mo hybrid constructs were transfected into R1.1 and L cells and assayed by antibody binding. As shown in Table II, the Q7^b/D^b hybrid is expressed on the surface of both cell types, while D^b/Q7^b is expressed only on the surface of R1.1 cells. Our ability to express cell-surface Qa-2 from an intact Q7^b gene in R1.1 thymoma cells, but not L cells, suggests that the 3'
element actually regulates the tissue-specific expression of $Q^7_b$. This element maps downstream of the Stu I site in exon 4.

**Northern Analysis of Transfected Cells.** It was possible that the failure of L cells transfected with the $Q^7_b$ genomic constructs to express $Qa-2$ on their surface might correlate with quantitative or qualitative differences in mRNA levels. We therefore extracted total RNA from R1.1 and L cell transfectants, and performed Northern analysis using specific oligonucleotide probes. Clones of all R1.1 transfectants, and of L cells transfected with $Q^7_b$ cDNA Mo, were selected on the basis of high levels of antibody binding. For L cells transfected with $Q^7_b$ genomic constructs that failed to express $Qa-2$ on their surface, RNAs from six independent G418-resistant clones were examined for each construct. These L cell clones all produced similar species of $Q^7_b$ mRNA, and those producing the highest levels are presented in Fig. 3.

Fig. 3 shows a Northern blot of RNAs from selected clones probed with the $^{32}$P-labeled oligomer D124 (see Table I). As expected, D124 hybridizes only to RNA from cells transfected with $Q^7_b$ constructs, but not to RNA from the $D^b$ Mo transfectants (panel 1) or the untransfected R1.1 and L cells (panel 5). From cells transfected with $Q^7_b$ Mo (panel 2), D124 detects an abundant 1.2-kb mRNA species (double arrow), in addition to a less abundant 1.5-kb species (single arrow) that is expected from the splicing pattern of $Q^7_b$ cDNA (Fig. 2). Another band of 1.7-kb is partially obscured by the saturation of RNA binding sites on the membrane by 18S ribosomal RNA. Although the sizes and quantity of $Q^7_b$-
specific RNA appear to be similar in both Q7\textsuperscript{b} Mo transfectants, only the R1.1 transfectant expresses detectable levels of cell-surface Qa-2 antigen (Table II). L cells transfected with Q7\textsuperscript{b} pTCF (lanes 4L and 7) predominantly transcribe a 1.2-kb mRNA, and possibly another species of ~1.0 kb. The 1.2-kb species is found at very low levels in the R1.1 transfectant (Fig. 3, lane 4R), and is barely visible on the autoradiogram. In spite of this quantitative difference, the R1.1 transfectant, but not the L cell transfectant, expresses cell-surface Qa-2 (Table II).

Analysis of the 1.2-kb species by SP6 RNAse protection experiments, and by hybridization to oligonucleotide probes spanning exon junctions, has revealed that this mRNA is unusually spliced for a class I gene transcript (37). This novel mRNA is formed from the joining of exon 3 to exon 6, so that exons 4 and 5 are spliced out. The missing exons respectively encode the a3 domain, which binds \(\beta_2\)m, and the transmembrane region, which anchors the protein in the membrane. Using the D80 oligonucleotide that recognizes the exon 3/exon 6 junction, further analysis of the three other Q7\textsuperscript{b} cDNA clones isolated from the 2.1.1 library revealed that they are shorter than clone 69-2C2 because they are spliced this way, and not because they are truncated (data not shown).

Fig. 3, lanes 3R and 3L, contain RNA from R1.1 and L cells, respectively, transfected with the Q7\textsuperscript{b} cDNA Mo construct. Due to the addition of the 3' cassette containing the SV40 64-bp intron and polyadenylation signal, a transcript of 2.4 kb is expected. In lane 3R, the upper band migrates at 2.6 kb, while the upper band in lane 3L migrates at 2.3 kb. The 2.3-kb transcript is not unique to the clone analyzed in lane 3L, but is observed in other L cell clones transfected with this construct that express cell-surface Qa-2 antigen (data not shown). The reason for the difference in size between the 2.6- and 2.3-kb transcripts is not known; however, examination of the SV40-derived sequence reveals the presence of two potential splice acceptors 370 bp and 420 bp downstream of the splice donor site. Thus, it is possible that differential RNA processing by R1.1 and L cells may account for the tissue-specific expression of Qa-2 antigen.

**Discussion**

We have isolated four Q7\textsuperscript{b} cDNA clones from a library prepared from CTL clone 2.1.1, which expresses cell-surface Qa-2 antigen (14). The four cDNA clones were identified unambiguously as Q7\textsuperscript{b}, using a panel of gene-specific oligonucleotide probes. It appears that the Qa-2 protein expressed by CTL clone 2.1.1 is the product of the Q7\textsuperscript{a} gene based on the following lines of evidence. First, no other Qa region clones were found, even though >100,000 plaques were screened with a class I gene probe, and the 42 clones isolated with this probe were rescreened with oligonucleotides capable of detecting all other Qa region genes known for the b haplotype. Second, transfection of Q7\textsuperscript{b} genomic constructs resulted in the tissue-specific expression of high levels of Qa-2 antigen on the surface of R1.1 thymoma cells, but not on L cells. Third, transfection of one of the four Q7\textsuperscript{b} cDNAs resulted in the expression of cell-surface Qa-2 on both R1.1 and L cells.

The most abundant species of Q7\textsuperscript{b} mRNA present in cells transfected with Q7\textsuperscript{b} genomic constructs is the alternately spliced form identified by Fahrner et al. (37). The alternate form has spliced out exon 4, which encodes the a3 domain.
that binds $\beta 2m$, and exon 5, which encodes the transmembrane region that anchors the protein in the membrane. The three other $Q^7b$ cDNA clones isolated from the CTL 2.1.1 library are spliced this way, while only $Q^7b$ cDNA clone 69-2C2 is spliced normally. Since the Qa-2 protein of CTL 2.1.1 can be immunoprecipitated by antibodies to $\beta 2m$ (14), and because it is believed (but not proven) that $\alpha 3$ is required for binding $\beta 2m$, it seems likely that the normal mRNA gives rise to cell-surface Qa-2. However, the inability to obtain higher levels of Qa-2 expression from the normal cDNA construct in L cells, compared to R1.1 cells, suggests that a number of posttranscriptional factors may be involved in the tissue-specific expression of $Q^7b$.

The ability to express significant levels of Qa-2 on L cells transfected with a $Q^7b$ cDNA derived from T cells, favors the notion that the tissue-specific expression of cell-surface Qa-2 may be regulated at the level of RNA processing. Also, Northern analysis of R1.1 and L cells containing the $Q^7b$ cDNA construct reveals differential RNA processing. In contrast, analysis of R1.1 and L cells transfected with the $Q^7b$ genomic construct reveals no obvious differences. We cannot exclude the presence of minor RNA species, however, or differences in polyadenylation or RNA secondary structure that might affect the efficiency of translation. Clearly, the total amount of $Q^7b$-specific mRNA in each transfectant does not correlate with the level of Qa-2 antigen expressed on the cell surface.

By using hybrids between $Q^7b$ and $H-2D^b$ genes, we have extended observations made by others (7, 8) that the element responsible for tissue-specific expression of cell-surface Qa-2 maps to the 3' part of $Q^7b$. Since the constructs used in our study differ in well-defined regions of these genes, some conclusions can be drawn about possible mechanisms, other than RNA processing, that might regulate their expression. At the protein level, the $Q^7b/D^b$ hybrid expressed on L cells differs in the $\alpha 3$ domain from the nonexpressed $D^b/Q^7b$ by only five amino acids. A comparison with amino acid sequences of $\alpha 3$ domains of other class I proteins (34) reveals no obvious reason why these five residues should affect cell-surface expression. Although the cytoplasmic tail of the $D^b/Q^7b$ polypeptide is truncated due to the premature termination codon in $Q^7b$ exon 5, the cell-surface expression of $Q^7b$ on R1.1 cells is not blocked. Also, experiments by Zuniga and Hood (38) have shown that mutant L$^d$ molecules having truncated COOH-termini, even those lacking a cytoplasmic tail, can be expressed on the surface of L cells, although at a lower level than wild type. A major difference between $Q^7b$ and other class I proteins is the presence of a negatively charged aspartate residue in the middle of the $Q^7b$ transmembrane region, which may require a positively charged sidegroup of some accessory protein to be stabilized in the membrane. R1.1 cells may have such a protein, while L cells may not. For example, a salt bridge between the transmembrane aspartate of the T3 protein and lysines of the T cell receptor $\alpha$ and $\beta$ chains is believed to play a role in the required coexpression of these molecules (discussed in references 39–41).

In conclusion, our results suggest that the tissue-specific expression of Qa-2 may involve a combination of posttranscriptional factors at the level of both RNA and protein. Current experiments in our laboratory are directed at understanding the basis for our observations.
Summary

We screened a cDNA library prepared from a BALB.B10 CTL clone that expresses Qa-2 antigen, and isolated four clones derived from Q7b, a Qa region gene of C57BL/10. One of these Q7b cDNAs and the Q7b chromosomal gene were subcloned into expression vectors and transfected into L cells and R1.1 thymoma cells. We found that the chromosomal Q7b gene expresses Qa-2 on the surface of R1.1 cells, but not on L cells while the Q7b cDNA expresses protein on the surface of both cell types. The levels of Qa-2 expression do not correlate with the total levels of Q7b mRNA in these transfectants. Our results suggest that the tissue-specific expression of Qa-2 may be controlled, in part, by mechanisms of alternate RNA splicing. By using hybrid gene constructs, we have mapped the tissue-specific element to the 3' part of the gene, downstream of a site near the middle of exon 4. The hybrid polypeptides differ significantly in their transmembrane and cytoplasmic regions. These portions of the protein also may play a role in the tissue-specific expression of Qa-2.

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