

# The *TL* Region Gene 37 Encodes a Qa-1 Antigen

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## Summary

Of all the biochemically defined mouse MHC class I molecules, the Qa-1 antigens are the only ones for which a gene has not been identified. Recent evidence has suggested that Qa-1 antigens are functional class I molecules and can function as restriction elements for  $\gamma/\delta$  T cells. We have examined the relationship between Qa-1 and the product of gene 37, a presumed novel class I antigen encoded within the *TL* region. Immunoprecipitation and polyacrylamide gel electrophoresis analysis of the molecules reactive with anti-Qa-1 and anti-37 sera show that the Qa-1 molecule of *Qa-1<sup>b</sup>* (*Qa-1.2*) mouse strains is identical to the product of gene 37 on the basis of molecular weight, pI, and strain distribution. Immunodepletion, biosynthetic labeling, and tunicamycin treatment confirm that the protein encoded by gene 37 in *Qa-1<sup>b</sup>* mice is *Qa-1.2*. In contrast, the anti-37 serum was unable to recognize the Qa-1 molecule in *Qa-1<sup>a</sup>* strains. Given the fact that the only allele to gene 37 thus far identified in a *Qa-1<sup>a</sup>* strain (*A/J*) has a termination codon in the  $\alpha 3$  domain, our data lead us to conclude that the Qa-1 molecule expressed in *Qa-1<sup>a</sup>* mice is not a true allelic product of the gene 37 encoded antigen of *Qa-1<sup>b</sup>* mouse strains.

The Qa-1 alloantigens are class I glycoproteins encoded by loci telomeric to *H-2* in the *TL* region of the MHC (reviewed in references 1-3). The Qa-1 antigenic system was first identified by Stanton and Boyse (4), who observed a novel reactivity in anti-*TL* serum for peripheral lymphocytes that was distinct from *TL* on the basis of tissue distribution. This newly identified antigen was designated Qa-1 and was assigned to the *TL* region (1, 4). Later, several laboratories independently demonstrated that Qa-1 could be detected by alloreactive CTL in an *H-2* nonrestricted manner suggesting that Qa-1 had functions similar to classical *H-2K,D* antigens (5-8). Subsequent biochemical studies revealed Qa-1 antigens to have structural properties characteristic of class I antigens, i.e., cell surface glycoproteins with molecular weight of  $\sim 48,000$  and associated with  $\beta 2$ -microglobulin (9-11). Four alleles (*a, b, c, d*) have been defined for the *Qa-1* locus by CTL reactions (12-15). The protein product in the *Qa-1<sup>a</sup>* strains is denoted as *Qa-1.1*, and that in *Qa-1<sup>b</sup>* strains as *Qa-1.2*. The *Qa-1* molecules from *Qa-1<sup>c</sup>* and *Qa-1<sup>d</sup>* strains are very similar to *Qa-1.2* as shown by serological, biochemical, and CTL analyses (12-16).

Original reports using serological techniques characterized the expression Qa-1 as limited to cells of hematopoietic origin, similar to other antigens encoded by the *Q-TL* region genes (1-3). Recent investigations using more sensitive monoclonal anti-Qa-1 CTL have revealed Qa-1 to be widely expressed, resembling the ubiquitously expressed *H-2K,D,L* antigens (17). Although the Qa-1 alloantigens can serve as targets of *H-2* nonrestricted cell-mediated killing analogous to *H-2K*

and *D* molecules, it was not until recently that "classical" class I molecule functions were reported for Qa-1. Vidovic et al. (18) demonstrated using antiserum blocking that Qa-1 can function in presentation of conventional antigen [copolymer (Glu-Tyr)] to a  $\gamma/\delta$  T cell hybridoma. In addition, there are at least two other described  $\gamma/\delta$  T cell hybridomas that recognize or are restricted to class I molecules encoded in the *TL* region (19, 20). Also, Aldrich et al. (17) have described a locus, *Qdm*, linked to the *H-2D* region that is required for the expression of certain CTL-defined Qa-1 determinants. A possible interpretation from these data is that Qa-1 may restrict the recognition of a self protein or peptide, the product of *Qdm*. Thus, Qa-1 and other *TL* region products may have a physiological role in presenting antigen to  $\gamma/\delta$  or  $\alpha/\beta$  T cells; the low level of polymorphism would be consistent with these molecules having a more limited and specialized role than classical class I antigens. Further support for possible class I-like functions of these molecules stems from computer aided models comparing the structure predicted from *Q* and *TL* gene sequences with the published structure of the HLA-A2 molecule (21-23). These molecules have sufficient homology to conventional class I antigens overall and in key regions of the molecule to predict that they have class I-like structure with a potential peptide binding site.

Of the 23-28 class I genes identified within the *Q/TL* region of the mouse MHC, none have been shown to encode Qa-1 to date. This has hindered further investigations into the possible role of Qa-1 as a restriction element, as well as any other functional properties it might possess. Previously,

Lalanne et al. (24) isolated a novel class I gene, *pH-2<sup>d</sup>-37*, while investigating the diversity of *H-2*-related transcripts in DBA/2 (*H-2D<sup>d</sup>*) liver. Gene 37 was subsequently shown to be located in the *TL* region and closely related to the *T10<sup>c</sup>* gene (25).<sup>1</sup> Analyses of gene 37 by RFLP and Northern blots have shown it to have a low degree of polymorphism and to be ubiquitously expressed (24, 25). Cochet et al. (27) have demonstrated in L cells transfected with gene 37 and in spleen cells that antisera against novel gene 37 peptide sequences specifically precipitate the 37 product expressed on the cell surface. The striking similarities between gene 37 and Qa-1, namely their shared ubiquitous tissue distribution and low polymorphism, have led us to investigate the possibility that Qa-1 is encoded by gene 37. To do so, we have used antisera specific for Qa-1.1, Qa-1.2, and a peptide predicted from the gene 37 sequence to immunoprecipitate proteins recognized by each from Qa-1<sup>a</sup> and Qa-1<sup>b</sup> mouse strains. Our results indicate that the Qa-1.2 antigen from Qa-1<sup>b</sup> strains is encoded by gene 37.

## Materials and Methods

**Mice.** B6.T1a<sup>a</sup> and A.T1a<sup>b</sup> mouse strains were bred in our mouse colony at Baylor College of Medicine. C57BL/6J (B6)<sup>2</sup> and all other mice were purchased from the Jackson Laboratory, Bar Harbor, ME.

**Serological Reagents.** Antisera to products of the Qa-1<sup>a</sup> (anti-Qa-1.1) and Qa-1<sup>b</sup> (anti-Qa-1.2) alleles were prepared as previously described (16). The anti-37 peptide serum (207-1) directed against the cytoplasmic tail of the gene 37 product (amino acids 326–337) has been previously described (27), and was a gift from John E. Coligan (National Institutes of Health, Bethesda, MD). Anti-H-2K<sup>b</sup> [(A × B10.A)F<sub>1</sub> anti-B10.A(5R)] was obtained from the Research Resources Branch, NIH. Normal mouse serum (NMS) and normal rabbit serum (NRS) were purchased from Pel-Freez Biologicals, Rogers, AR. The anti-Qa-1.1, Qa-1.2, and NMS reagents used for immunoprecipitations were IgG fractions isolated on Affi-gel protein A (Bio-Rad Laboratories, Richmond, CA).

**Cell Culturing and Radiolabeling of Cells.** Con A-activated T cell blasts (CAB) were prepared as described (28). For internal labeling, 50 × 10<sup>6</sup> cells were incubated for 30 min in 1 ml HBSS plus 10% FCS containing 1.25 mCi of [<sup>35</sup>S]methionine (Amersham Corp., Arlington Heights, IL). At the end of the labeling period, cold methionine containing medium (supplemented Mishell-Dutton medium, sMDM) was added to stop the labeling reaction. Cells were then either washed three times with Dulbecco's PBS (D-PBS) and immediately lysed with 0.5% NP40 in D-PBS, or recultured at 37°C in sMDM at 2 × 10<sup>6</sup>/ml and chased for varying lengths of time. After each chase, cells were washed and lysed as above. For cell surface iodination, 50 × 10<sup>6</sup> cells were labeled in D-PBS with 2.5 mCi <sup>125</sup>I (Amersham Corp.) by the lactoperoxidase catalyzed procedure (10). At the end of the labeling period, the cells were washed and lysed as above. All cells were lysed in the presence of 1 mM PMSF and 33 μg/ml of aprotinin (Boehringer Mannheim

Biochemicals, Indianapolis, IN) to inhibit endogenous protease activity. To examine the contribution of N-linked carbohydrates to the cell surface expression of Qa-1 and 37, CAB were incubated in sMDM containing 0.5 μg/ml tunicamycin (Calbiochem-Behring Corp., La Jolla, CA) for 12 h at 37°C. The tunicamycin-treated blasts were iodinated and immunoprecipitated as above. For internal labeling, CAB were preincubated in sMDM-containing 10 μg/ml tunicamycin for 30 min at 37°C. At the end of the incubation period, cells were washed in methionine-free medium and labeled in the presence of tunicamycin (10 μg/ml) with [<sup>35</sup>S]methionine and immunoprecipitated as above.

**Immunoprecipitation, SDS-PAGE, and Two-Dimensional (2-D) Gel Analysis.** Cell lysates were depleted of Ig and "nonspecific" material by preclearing with BSA-sepharose, goat anti-mouse Ig-sepharose, and protein A-agarose (Pierce Chemical Co., Rockford, IL). Aliquots of the lysates (~12 × 10<sup>6</sup> cell equivalents) were incubated with specific or control antibodies at 4°C overnight. The immune complexes were recovered on protein A-agarose and washed four times with PBS containing 0.5% NP40, 0.25% sodium deoxycholate, 0.1% SDS, and twice with H<sub>2</sub>O. For 1-D gels, immunoprecipitates were eluted from protein A-agarose with solubilizing buffer and analyzed by SDS-PAGE on 12.5% gels under reducing conditions as described by Laemmli (29). For 2-D gels, immunoprecipitates were eluted from protein A-agarose in isoelectric focusing (IEF) solubilizing buffer and analyzed on a pH 5–7 IEF gel followed by SDS-PAGE on a 12.5% gel (16). BSA (68,000), ovalbumin (45,000), chymotrypsinogen (24,000), and cytochrome C (12,500) were used as molecular weight markers. Gels were dried and analyzed by fluorography or autoradiography.

**N-Glycanase Treatment.** Immunoprecipitates were eluted with 1 ml of SDS solubilizing buffer, dialyzed overnight against H<sub>2</sub>O, lyophilized, and resolubilized in 0.2 M sodium phosphate pH 8.6 and 1% NP40. Each sample was digested with a total of 2 U of N-glycanase (Genzyme Corp., Boston, MA) (1 U additions at 0 and 2 h), and the reaction was stopped at 4 h. Samples were then dialyzed against H<sub>2</sub>O overnight, lyophilized, and analyzed by 1-D and 2-D PAGE.

## Results

**The Molecules Reactive with the Qa-1-specific and 37 Peptide Antisera Are Biochemically Similar.** To address the relationship between the Qa-1.1 and Qa-1.2 antigens and the gene 37 product, we first examined the reactivity of the anti-37 and anti-Qa-1 sera with the *TL* region congenic mouse strains, B6 (Qa-1<sup>b</sup>) and B6.T1a<sup>a</sup> (Qa-1<sup>a</sup>). The anti-37 serum was raised in rabbits against a peptide corresponding to residues 326–337 in the cytoplasmic region deduced from the 37 gene sequence (27). Cochet et al. (27) reported that this antiserum precipitated a ~45.5-kD polypeptide associated with β2-microglobulin from the cell surface of splenocytes of *H-2<sup>b,d,k</sup>* (B6, BALB/c, and CBA) mice, and that this product of gene 37 was not found on the cell surface of splenocytes from *H-2<sup>a,s</sup>* (A/J and SJL/J) mice. It intrigued us that the three strains that were reactive with the anti-37 serum were Qa-1<sup>b</sup>, while the two negative strains were Qa-1<sup>a</sup>.

To investigate possible biochemical similarities between the Qa-1 molecules and the 37 product, lysates of <sup>125</sup>I surface-labeled T cell blasts from B6 and B6.T1a<sup>a</sup> were immunoprecipitated with the described antisera and analyzed via 1-D SDS-PAGE. Con A-activated T cells were used instead of

<sup>1</sup> Although located 3' and linked to BALB/c gene *T10<sup>c</sup>*, gene 37 has not as yet been given a *T* gene number; *T18<sup>c</sup>* has been assigned to a different gene that has not been linked to other *TL* genes (26).

<sup>2</sup> Abbreviations used in this paper: B6, C57BL/6J; CAB, Con A-activated T cell blasts; NMS, normal mouse serum; NRS, normal rabbit serum; D-PBS, Dulbecco's phosphate-buffered saline; 1-D, one dimensional; 2-D, two dimensional.

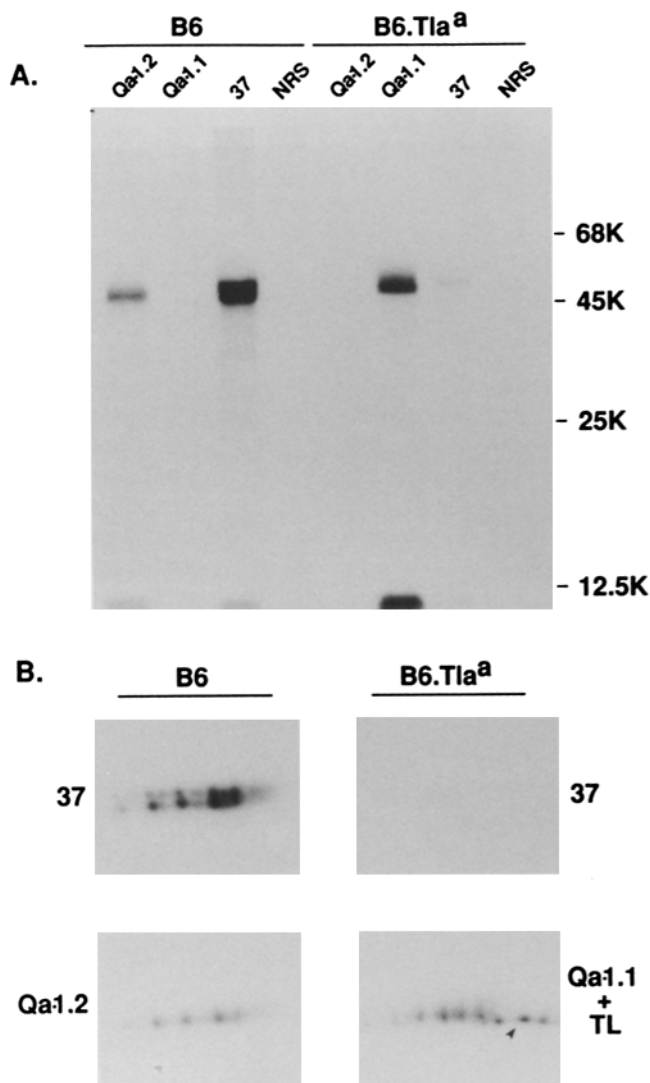
splenocytes due to increased Qa-1 expression on the cell surface (16). The anti-Qa-1.1 and anti-Qa-1.2 sera have specificity for Qa-1 molecules encoded by *Qa-1<sup>a</sup>* and *Qa-1<sup>b</sup>* strains, respectively (16). As depicted in Fig. 1 A, both the anti-Qa-1.1 and Qa-1.2 sera immunoprecipitated a ~48-kD protein associated with  $\beta$ 2-microglobulin (~12 kD) from the appropriate mouse strain. Anti-Qa-1.1 has been shown to react with the ~46-kD TL antigen present on activated T cells (30) and can not be distinguished from Qa-1.1 on the 1-D gel. Anti-37 also reacted with a protein in B6 of similar  $M_r$  (~48,000) as well as an additional slightly higher species of ~50,000. However, anti-37 only weakly precipitated

a protein product from B6.Tla<sup>a</sup> in the 48–50,000 mol wt range. Similarities between Qa-1 and 37 were further examined by 2-D gels. As shown in Fig. 1 B, the anti-Qa-1.2 and anti-37 reactive proteins have identical isoelectrophoretic patterns. A species of similar charge heterogeneity but slightly higher molecular weight was detected with the anti-37 serum, consistent with the above SDS-PAGE analysis. 2-D analysis of anti-Qa-1.1 precipitates from B6.Tla<sup>a</sup> revealed two proteins, Qa-1.1 and TL; the latter has a slightly lower molecular weight and is depicted with an arrow. As can be seen, neither Qa-1.1 nor TL were present in the anti-37 precipitates.

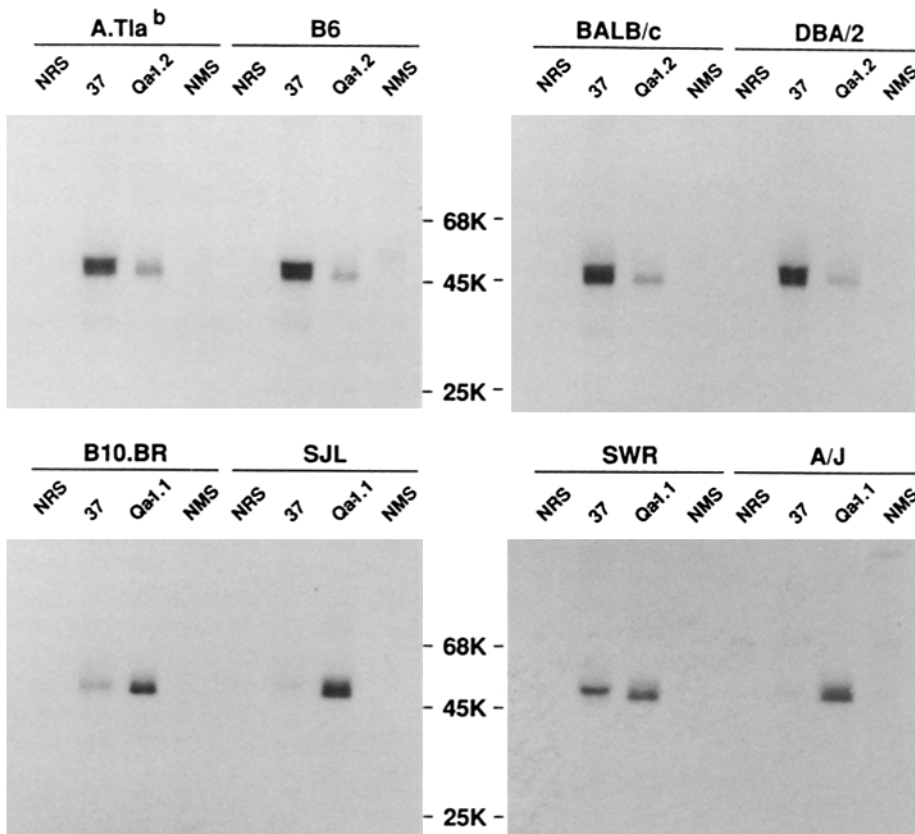
The identity of the molecule from B6.Tla<sup>a</sup> seen on the 1-D gels that is weakly precipitated by anti-37 remains unclear, but may represent weak cross-reactivity with H-2K<sup>b</sup>. The gene 37-derived peptide used to generate the antiserum shows little homology with other class I cytoplasmic regions. However, there is a four amino acid sequence (Gln-Thr-Ser-Asp) that is shared between 37 and H-2K<sup>d</sup>, -K<sup>b</sup>, and -K<sup>k</sup> cytoplasmic domains which could serve as a potential cross-reactive determinant (1). Thus, the anti-37 serum reacts strongly with a protein of the same molecular weight and pI as Qa-1.2 but fails to detect a molecule equivalent to Qa-1.1.

Several other *Qa-1<sup>a</sup>* (A/J, B10.BR, SWR, SJL) and *Qa-1<sup>b</sup>* (B6, A-Tla<sup>b</sup>, BALB/c, DBA/2) strains were next examined by immunoprecipitation with the Qa-1 and 37 antisera and subjected to 1-D and 2-D analysis (Fig. 2, and data not shown). The results obtained with these additional strains are consistent with the data already shown for B6 and B6.Tla<sup>a</sup>. From each of the *Qa-1<sup>b</sup>* strains, anti-37 immunoprecipitated a doublet in the ~48,000 mol wt range, the lower of which had the same molecular weight as Qa-1.2. Analysis on 2-D gels demonstrated that for each of these strains the anti-37 reactive lower band was identical to Qa-1.2; as seen for B6, the higher molecular weight species had a pI profile and charge heterogeneity similar to the lower molecular weight species (data not shown). Examination of the *Qa-1<sup>a</sup>* strains revealed that anti-37 did not react with the Qa-1.1 molecule. The ~48–50-kD species visible in the anti-37 lanes from B10.BR and SWR were not Qa-1.1 as determined by 2-D gels (not shown) and again likely represent weak cross-reactivity with other class I molecules. Thus, the 37 gene product is indistinguishable in size, isoelectric point, and strain distribution from Qa-1 from *Qa-1<sup>b</sup>* strains, but is not equivalent to or detected in the different *Qa-1<sup>a</sup>* strains studied.

*Anti-Qa-1.2 and Anti-37 Sera Recognize the Same Class I Molecule by Immunodepletion Analysis.* To determine if the molecule isolated by immunoprecipitation with anti-Qa-1.2 and anti-37 were indeed the same, sequential immunoprecipitation experiments were performed. Lysates from iodinated B6 CAB were reacted with anti-37, NRS, anti-Qa-1.2, or NRS in two preclearing cycles and aliquots of these precleared lysates were then immunoprecipitated. As shown in Fig. 3, depletion of the cell lysates with anti-37 efficiently removed the 37 protein and all Qa-1.2 molecules as compared with the NRS controls. H-2K<sup>b</sup> molecules were unaffected by either treatment. Lysate depletion with anti-Qa-1.2 removed all Qa-1 molecules and effectively depleted the lower, but not the upper mol wt species reactive with anti-37 serum. Thus,



**Figure 1.** Comparative 1-D and 2-D gel analysis of Qa-1.2, Qa-1.1, and the product of gene 37 from activated B6 (*Qa-1<sup>b</sup>*) and B6.Tla<sup>a</sup> (*Qa-1<sup>a</sup>*) T cells. Iodinated B6 and B6.Tla<sup>a</sup> T cell blasts were immunoprecipitated with anti-Qa-1.1, anti-Qa-1.2, anti-37, and NRS and then analyzed by 1-D (A) and 2-D (B) gel electrophoresis. The band at ~12,000 mol wt represents  $\beta$ 2-microglobulin. Equal areas from each autoradiogram are shown in B; the basic end of the IEF first dimension gel is to the left.

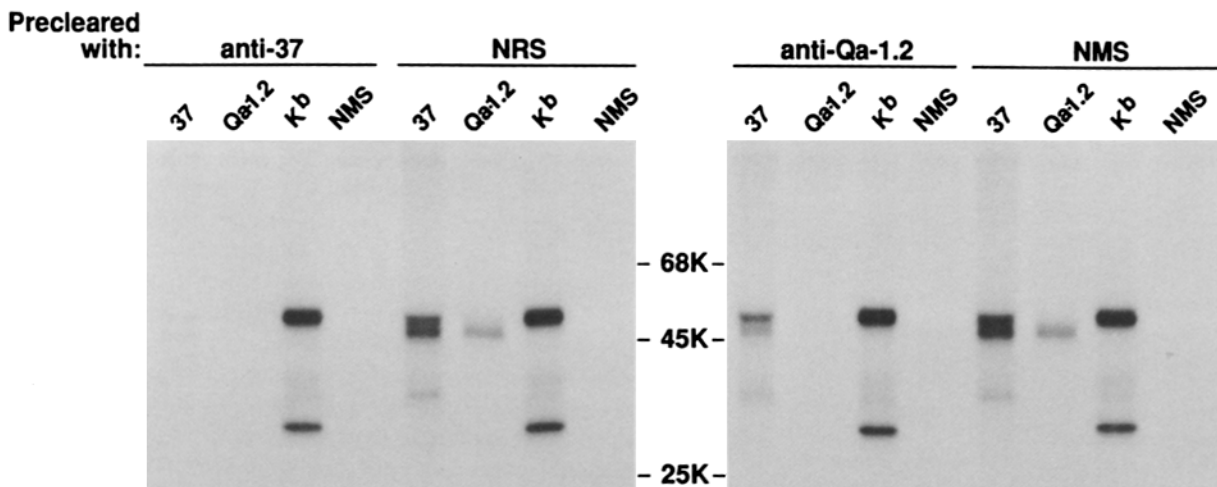


**Figure 2.** SDS-PAGE analysis of Qa-1 and the 37 gene product from different *Qa-1<sup>a</sup>* and *Qa-1<sup>b</sup>* mouse strains. Activated T cell blasts from *Qa-1<sup>b</sup>* (A.T1a<sup>b</sup>, B6, BALB/c, DBA/2) mice and *Qa-1<sup>a</sup>* (B10.BR, SJL, SWR, A/J) mice were iodinated and immunoprecipitated with NRS, anti-37, anti-Qa-1.2 or -1.1, and NMS and analyzed by 1-D SDS-PAGE.

determinants recognized by anti-Qa-1.2 and anti-37 must reside on the same molecule, confirming the 1-D and 2-D data indicating Qa-1.2 and 37 to be the same.

The question still remained as to the identity of the higher molecular weight species recognized by anti-37 but not anti-Qa-1.2 in the *Qa-1<sup>b</sup>* strains. It is possible that the antiserum crossreacts with an additional protein product distinct from

Qa-1.2. A more reasonable explanation is that the higher molecular weight species represents a modified glycosylated form of Qa-1.2 which only the anti-37 peptide serum can detect. It is not known what determinants on the Qa-1 molecule the Qa-1.2 antiserum recognizes, but it has been documented that carbohydrates can influence Qa-1 allodeterminants as detected by CTL (31). It is therefore possible that a modified



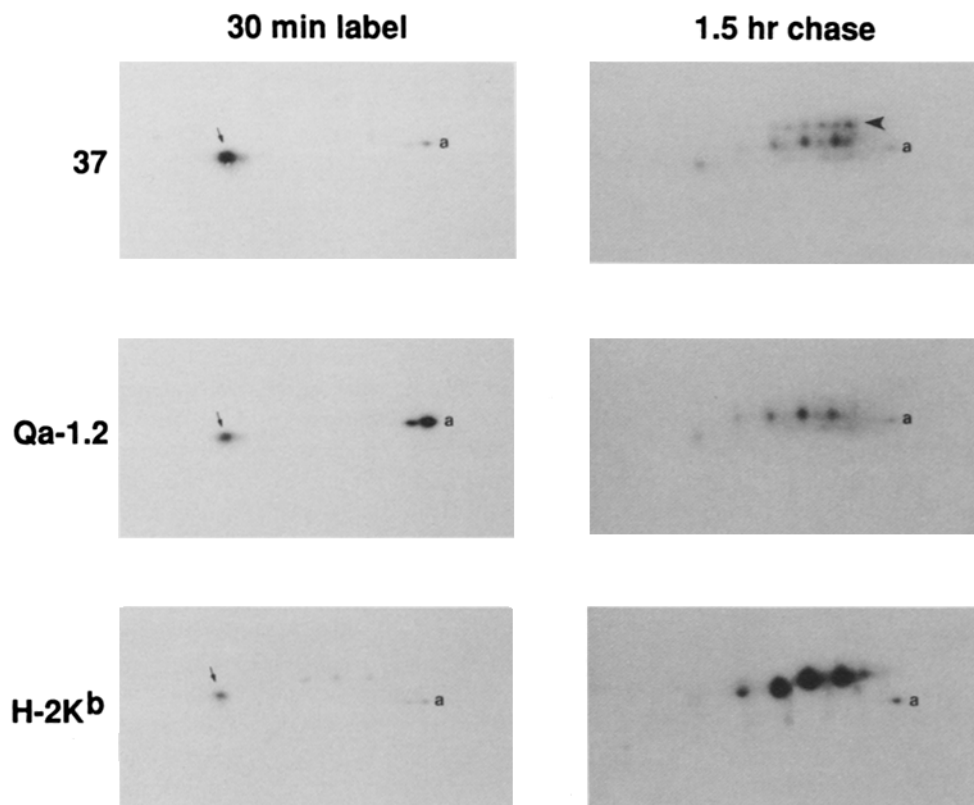
**Figure 3.** Immunodepletion analysis of Qa-1 and the 37 gene product. B6 lysates were precleared with anti-37, NRS, anti-Qa-1.2, or NMS. Samples of lysates were then immunoprecipitated with anti-37, anti-Qa-1.2, anti-H-2K<sup>b</sup>, or NMS and analyzed by 1-D SDS-PAGE.

N-linked oligosaccharide could sterically prevent the anti-Qa-1.2 serum from recognizing this form of Qa-1.2. The following experiments will address this question.

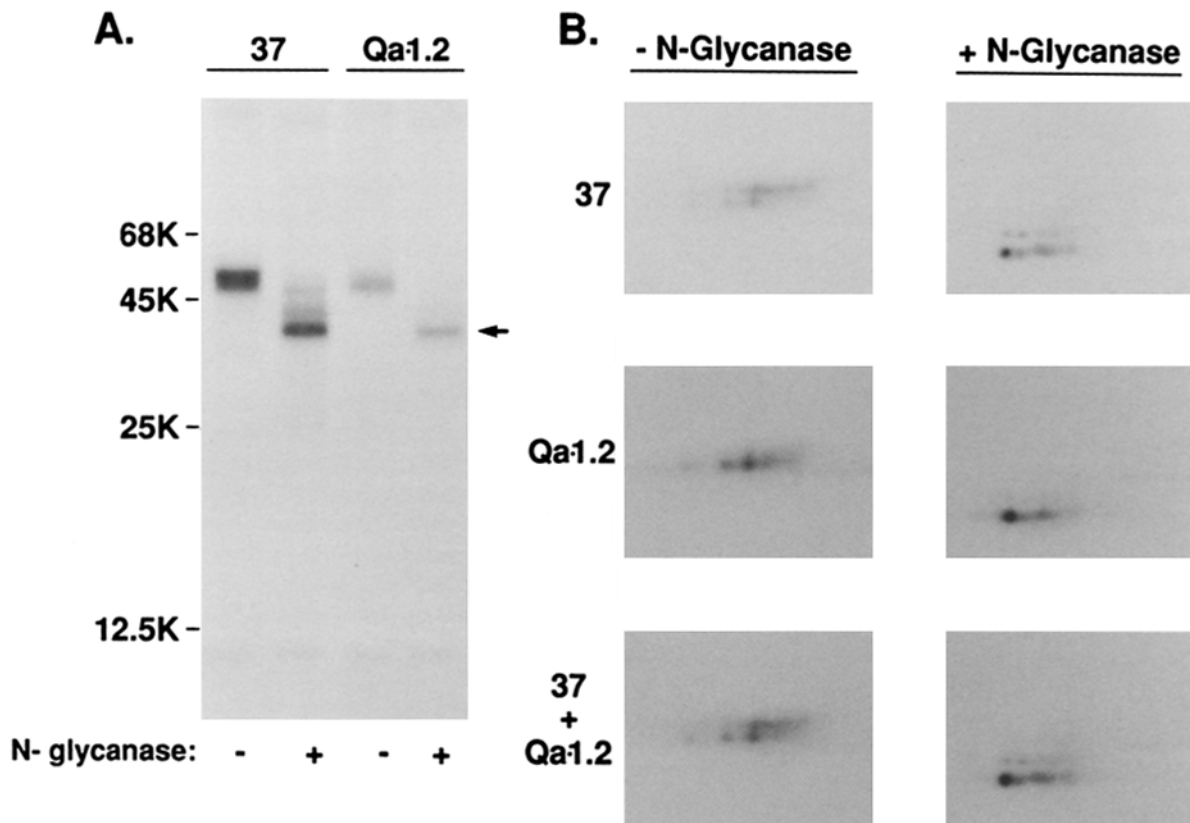
**The 37 Antiserum Recognizes a Single Protein Product Identical to Qa-1.** To investigate whether one or two protein products were recognized by the anti-37 serum, we used a short term biosynthetic label followed by immunoprecipitation to allow visualization of molecules that were minimally processed (Fig. 4). B6 CAB were internally labeled with [<sup>35</sup>S]methionine for 30 min or labeled for 30 min and then chased for 1.5 and 4 h. The resulting lysates were immunoprecipitated with anti-37, anti-Qa-1.2, anti-H-2K<sup>b</sup>, or NRS (not shown) and analyzed by 2-D gels. The H-2K<sup>b</sup> precipitated from the internally labeled cells was seen in predominantly unprocessed form at 30 min (denoted by arrow) and processed form after the chase. After the 30-min label, only a single co-migrating protein species was detected by both the Qa-1.2 and 37 peptide antisera (denoted by arrow), representing a glycosylated, probably high mannose, immature form of Qa-1.2. Processing of Qa-1.2 to its mature, complex sialylated form, could be detected after a 1.5-h chase by both antisera. As seen in the anti-37 serum panel, in addition to the species identical to Qa-1.2, a higher molecular weight species emerged, presumably the result of additional post-translational modifications (denoted by arrowhead). After a 4-h chase, only a small amount could be detected with either antisera, indicating Qa-1 has a more rapid turnover rate than H-2K<sup>b</sup> which could still be detected at 4 h (not shown; reference 11). Consistent with

the cell surface iodination experiments, the 37 antiserum was unable to recognize a product from biosynthetically labeled Qa-1<sup>a</sup> strains (data not shown).

**Contribution of N-linked Carbohydrates to the Recognition of Qa-1<sup>b</sup> by Anti-Qa-1.2 and Anti-37.** Since the biosynthetic experiments indicated that two different protein products were probably not being recognized by anti-37, we next examined whether the higher molecular weight species was the result of a modified N-linked glycosylation, that only the peptide antiserum could detect. Anti-Qa-1.2 and anti-37 immunoprecipitates from B6 were digested with N-glycanase, an enzyme that hydrolyzes N-linked oligosaccharides from glycoproteins. Fig. 5 A demonstrates that most of the higher molecular weight species and the 48-kD molecules of both precipitates were reduced to a single peptide of ~38-kD by digestion with N-glycanase; this is the size predicted for the nonglycosylated gene 37-encoded molecule and consistent with the size determined previously for Qa-1.2 (28). The fact that both of the anti-37 reactive species were reduced to the same molecular weight by deglycosylation again indicates that the higher molecular weight species is not a protein different from Qa-1.2. Rather, it is the result of a post-translational modification that blocks reactivity of the Qa-1.2 antiserum. Fig. 5 B shows the 2-D profiles of N-glycanase-treated anti-37 and anti-Qa-1.2 precipitates. Both precipitates again showed identical isoelectrophoretic patterns. When the digested anti-37 and anti-Qa-1.2 reactive molecules were mixed and analyzed together, they completely comigrated, as seen on the bottom



**Figure 4.** 2-D gel analysis of metabolically labeled lysates from B6 Con A blasts. Cells were labeled with [<sup>35</sup>S]methionine for 30 min and or labeled for 30 min and then chased for 1.5 h. The lysates were immunoprecipitated with anti-37, anti-Qa-1.2, anti-H-2K<sup>b</sup>, or NRS (not shown). The high mannose form of the isolated molecules is denoted by a downward arrow. Actin is denoted (a). Equal areas for each fluorogram are shown; the basic end of the IEF first dimension gel is to the left.



**Figure 5.** Analysis of Qa-1.2 and the 37 gene product after removal of N-linked carbohydrates. Iodinated B6 CAB lysates were immunoprecipitated with anti-37, anti-Qa-1.2, or NRS (not shown), and then treated with 2U of N-glycanase for 4 h. (A) SDS-PAGE analysis of treated (+) and nontreated (-) immunoprecipitates. The nonglycosylated form of each molecule is denoted by an arrow. (B) 2-D analysis of the N-glycanase treated and nontreated immunoprecipitates. Equal areas of each autoradiogram are shown; the basic end of the IEF first dimension gel is to the left. The bottom panels show the isoelectrophoretic patterns obtained after mixing equivalent portions of 37 and Qa-1.2 immunoprecipitates.

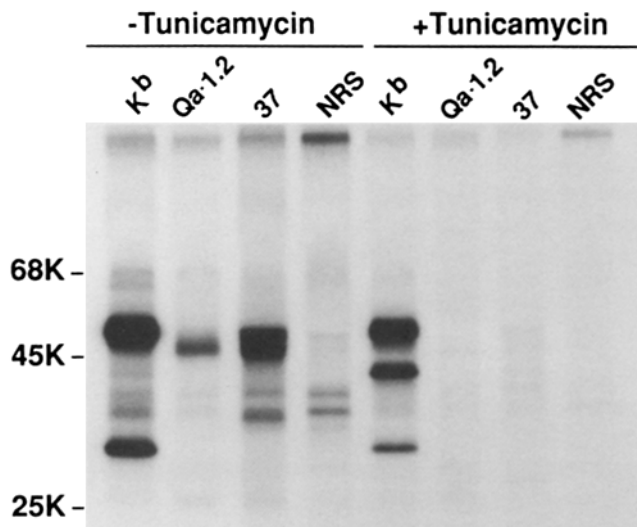
two panels of Fig. 5 B. Thus, the molecule reactive with anti-37 and the Qa-1.2 molecule from Qa-1<sup>b</sup> strains appear to be identical.

As an alternative method to examining the contribution of oligosaccharides to the higher molecular weight species detected with anti-37 serum, we used the inhibitor of N-linked glycosylation tunicamycin (32). Tunicamycin inhibits the first step in the processing of asparagine-linked oligosaccharides, which involves the synthesis of dolichol N-acetylglucosamine pyrophosphate (32, 33). The cell surface expression of Qa-1, unlike other class I molecules, can be dramatically inhibited by tunicamycin. Cells cultured in the presence of low concentrations of tunicamycin (0.5  $\mu\text{g}/\text{ml}$ ) for 8–12 h express undetectable levels of Qa-1 as assessed by serological and biochemical techniques (28). Fig. 6 demonstrates the effect of tunicamycin on the cell surface expression of Qa-1.2. Cells were incubated with or without tunicamycin for 12 h, radio-labeled with <sup>125</sup>I, and then immunoprecipitated. In agreement with previous data, cell surface Qa-1.2 was decreased to a level undetectable by immunoprecipitation with either 37 or Qa-1.2 antiserum. Also, the higher molecular weight species reactive with anti-37 serum was not detected. For

H-2K<sup>b</sup>, both normal cell surface forms (molecules that did not turnover or escaped the tunicamycin treatment) as well as nonglycosylated forms were detected. Thus, tunicamycin, which inhibits the addition of N-linked oligosaccharides, completely blocked the cell surface expression of Qa-1.2 and the 37 protein.

We next used biosynthetic labeling in the presence of tunicamycin to examine whether the same polypeptide in the absence of glycosylation was recognized by both anti-Qa-1.2 and anti-37 sera. Control and tunicamycin-treated cells were labeled for 30 min with [<sup>35</sup>S]methionine, lysed, immunoprecipitated, and analyzed on 2-D gels (Fig. 7). After a 30-min labeling in the presence of tunicamycin, much of Qa-1 present was in the nonglycosylated form (*up arrow*), as opposed to the “high mannose” form in nontreated cells (*down arrow*). As can be seen, the 2-D profiles of both the Qa-1.2 and 37 immunoprecipitates were identical for both the control and tunicamycin treated samples.

These data further argue that the Qa-1.2 antigen from Qa-1<sup>b</sup> strains is encoded by gene 37. Like Qa-1, both molecular weight species reactive with anti-37 serum were exquisitely sensitive to tunicamycin treatment and were not ex-



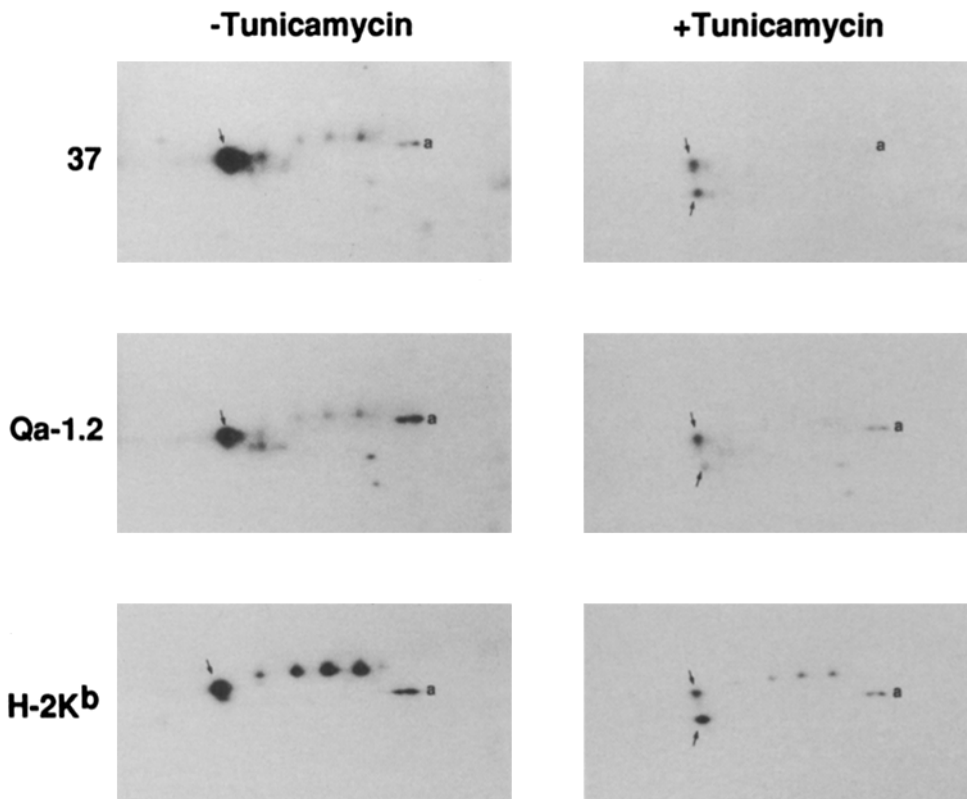
**Figure 6.** Effect of tunicamycin on the cell surface expression of anti-Qa-1.2 and anti-37 reactive molecules. B6 Con A blasts were cultured in the presence or absence of 0.5  $\mu\text{g/ml}$  tunicamycin for 12 h and then cell surface labeled with  $^{125}\text{I}$ . Lysates were immunoprecipitated with anti-H-2K<sup>b</sup>, anti-Qa-1.2, anti-37, and NRS and analyzed by 1-D SDS-PAGE.

pressed on the cell surface. Also, the polypeptides synthesized in the presence of tunicamycin were identical as shown by the 2-D gels.

## Discussion

We have demonstrated that the product of gene 37 is identical to the Qa-1 antigen of *H-2K<sup>b,d,q</sup>* and *k(Qa-1<sup>b</sup>)* mouse strains. Biochemical analysis of molecules reactive with Qa-1.2 antisera and antisera directed against a peptide deduced from the 37 gene sequence revealed that these molecules have identical molecular weight, charge heterogeneity, and comigrate with one another on 2-D gels. Sequential immunoprecipitation experiments confirmed that the determinants recognized by both antisera reside on the same class I molecule.

The anti-37 serum also detected a slightly higher  $M_r$  product which did not react with anti-Qa-1.2 serum. Several lines of evidence indicate that this species results from a modification of N-linked oligosaccharides on Qa-1. Deglycosylation with N-glycanase reduced the molecular weight to that seen for Qa-1.2. Also, the biosynthesis experiments in the presence and absence of tunicamycin revealed that the nonglycosylated and the subsequent glycosylated forms of the polypeptide detected by anti-Qa-1.2 and anti-37 sera were the same; the higher molecular weight species precipitated by anti-37 serum was visualized only after a 1–1.5-h chase. The inability of the anti-Qa-1.2 sera to react with the higher molecular weight form is most likely due to a modification of the N-linked oligosaccharides that somehow alters folding of the molecule or sterically blocks recognition of allodeterminants. The modified glycosylation would not be expected to hinder binding by the anti-peptide serum because it is directed against the cytoplasmic domain. The specific oligosac-



**Figure 7.** Effect of tunicamycin on the biosynthesis of Qa-1.2 and the 37 gene product. B6 Con A blasts were labeled with [ $^{35}\text{S}$ ]methionine in the presence or absence of tunicamycin (10  $\mu\text{g/ml}$ ). The lysates were immunoprecipitated with Qa-1.2 and 37 antisera and analyzed by 2-D gel electrophoresis. The high mannose form of the isolated molecules is denoted by a downward arrow. The nonglycosylated form of the molecules is denoted by an upward arrow. The *a* denotes actin. Equal areas of each fluorogram are shown; the basic end of the IEF first dimension gel is to the left.



charide modification responsible for this higher  $M_r$  species is not clear. The addition of sialic acids alone are not the cause as demonstrated by neuraminidase digestion experiments (data not shown).

The Qa-1 product encoded by gene 37 is unusual among class I antigens in its extreme dependence on N-linked glycosylation for cell surface expression. Also novel is the apparent blocking of Qa-1 allodeterminants by oligosaccharides described above. Data in this report using the anti-37 peptide serum reiterate our previous findings using tunicamycin (28) and imply that Qa-1 stability, folding, peptide binding, association with  $\beta 2$ -microglobulin, and/or transport are dramatically influenced by N-linked glycosylation. However, some Qa-1 molecules are exported to the cell surface during 12–18-h treatment with tunicamycin as demonstrated by anti-Qa-1 CTL analyses (31); some CTL-defined determinants were found to be tunicamycin sensitive whereas others were not (31).

Of particular interest is the lack of reactivity of the anti-37 serum for the Qa-1.1 antigen from the  $Qa-1^a$  strains. There are two possible explanations for this finding. First, the Qa-1 molecule from these strains may be encoded by a gene that is allelic to gene 37 but whose cytoplasmic domain has diverged such that it is not detected by the anti-37 peptide serum. Second, the Qa-1 antigens from  $Qa-1^a$  and  $Qa-1^b$  strains may not be the products of allelic genes. There are several lines of evidence that support the latter possibility. A candidate gene allelic to gene 37 has been recently partially characterized using PCR in the  $Qa-1^a$  strain A/J (34). Based on the 541 bp analyzed, this gene appears to be a hybrid or chimeric gene with its 5' portion (exon II) closely related to gene 37 and 3' regions (exon IV, exon V, intron 4) identical to the  $T10^c$  gene in BALB/c. Gene 37 and  $T10^c$  demonstrate 95% homology by nucleotide sequence, have identical cytoplasmic domains, and appear to be the result of gene duplication. The  $T10^c/37$  hybrid gene is transcribed but like  $T10^c$  is presumably incapable of translation to a normal class I antigen due to a termination codon in exon IV (34). By Southern blots and RNase protection experiments the  $T10^c/37$  chimeric gene is the only 37-related gene found in A strain mice (34). It is possible that the  $T10^c/37$  hybrid gene encodes Qa-1 only if the termination codon in exon IV is read through, and the exon encoding the cytoplasmic domain has mutated such that the protein can no longer be recognized by the anti-37 serum. Therefore, it seems unlikely that the Qa-1.1 antigen in  $Qa-1^a$  mice is encoded by a gene allelic to gene 37. Instead, it seems to be encoded by an undefined gene that is pseudoallelic.

The possibility that Qa-1.1 and Qa-1.2 molecules are products of different genes is consistent with a previous report from this laboratory regarding Qa-1 expression in  $Qa-1^d$  strains. Distinct Qa-1 antigens reactive with Qa-1.1 and Qa-1.2 sera could be immunoprecipitated from these strains (35);  $Qa-1^d$  cells also expressed both  $Qa-1^a$  and  $Qa-1^b$  CTL-defined determinants (15). Even though we do not yet know which gene encodes Qa-1.1 in  $Qa-1^a$  strains, the data convincingly argue that it is not a true allele of gene 37 which encodes Qa-1.2.

At present, it is not known which gene from the  $H-2^b$  strains B6 or B10 is equivalent to gene 37 from BALB/c (25). Based on Southern blots there are two genes in B10 that hybridize to a 37-specific probe (25). One is the 37 gene equivalent and the other is  $T11^b$ , which is allelic to  $T10^c$ ; the  $T11^b$  is present in the described B10 cosmid library (36), while the gene 37 equivalent is not (25). A recent report has demonstrated that the  $T11^b$  is transcribed in lymphoid tissues and in L cells transfected with the gene (37). A protein product of  $\sim 41$  kD could be immunoprecipitated from the transfectants with several mAbs that react with various H-2K/D as well as Qa-2 molecules. However, a protein product could not be detected on lymphoid cells by immunoprecipitation or immunofluorescence, demonstrating that if a  $T11^b$ -encoded protein product is expressed in vivo it is at undetectable levels (37). Efforts are underway to screen a new  $H-2^b$  cosmid library to identify the gene allelic to BALB/c and DBA/2 gene 37 that encodes Qa-1.2.

With the elucidation of the gene that encodes a Qa-1 antigen it should be more feasible (e.g., transgene and mutagenesis studies) to explore the biological functions of the Qa-1 alloantigens, including their roles as classical peptide binding/presentation molecules, their restriction of  $\gamma/\delta$  and/or  $\alpha/\beta$  T cells, or possibly the description of novel functions. Based on the HLA-A2 crystal structure there are no features that would be predicted to preclude the gene 37-encoded Qa-1 from assuming a normal class I conformation complete with a peptide binding cleft (21–23). The fact that the  $\alpha 1$  and  $\alpha 2$  domains of gene 37 are quite divergent from most other class I genes coupled with the low polymorphism imply that the peptides bound may be distinct, or limited as compared with those bound by conventional class I antigens. Such functions, namely, the binding of mitochondrial N-formylated peptides, have been ascribed to the maternally transmitted antigen encoded telomeric to  $TL$  (38, 39). Thus, the non-classical class I antigens may have unusual peptide binding properties and subserve specialized functions.

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Note added in proof: Ito et al. (40) recently identified a new TL region gene designated 27<sup>b</sup> that is located on a previously undefined TL gene cluster. The H-2<sup>b</sup> equivalent of gene 37 (37<sup>b</sup>) is in this cluster and lies 7 kb downstream of 27<sup>b</sup>.

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