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# Characteristics of peptide and major histocompatibility complex class I/$\beta_2$-microglobulin binding to the transporters associated with antigen processing (TAP1 and TAP2)

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**ABSTRACT** **The transporter proteins associated with antigen processing (TAP proteins) transport antigenic peptides across the endoplasmic reticulum membrane where they can assemble with newly synthesized major histocompatibility complex (MHC) class I/$\beta_2$-microglobulin ($\beta_2$m) dimers. We have shown previously that TAP possesses a peptide-recognition site with broad specificity and that MHC class I/$\beta_2$m dimers physically associate with TAP. Here, we further characterize the nature of the peptide-binding site on TAP, and the site of interaction of TAP with MHC class I/$\beta_2$m dimers. TAP photoaffinity labeling experiments revealed that both TAP1 and TAP2 are photolabeled by two distinct photopeptide analogues, suggesting that elements of both TAP1 and TAP2 compose the peptide-recognition site. TAP photolabeling analysis on transfectant cell lines that express TAP1 and TAP2 both individually and together revealed that efficient formation of the peptide-binding site occurs only when TAP1 and TAP2 are coexpressed, which correlates with the finding that peptide translocation via TAP occurs only in the presence of both TAP1 and TAP2. These data strongly support the notion that TAP functions as a heterodimer. MHC class I/$\beta_2$m dimers were shown to associate with individual TAP1 chains but were not detectable with individual TAP2 chains. This result suggests that the site of interaction for MHC class I/$\beta_2$m dimers with TAP is on TAP1.**

Traffic ATPases or ABC transporters (for ATP-binding cassette) constitute a large family of related molecules that are responsible for translocating a variety of compounds across membranes (for review, see ref. 1). Examples include the oligopeptide (OppABCDF) and hemolysin (HlyB) transporters in bacteria (2, 3), the STE6 (sterile 6) a-factor transporter in yeast (4), the eukaryotic P-glycoprotein associated with multidrug resistance (5, 6), and the cystic fibrosis transmembrane conductance regulator (7). Although many such transporters have been identified in prokaryotes and eukaryotes, limited information is available on the actual mechanism of translocation. Members of the ABC transporter family possess a characteristic structure consisting of two hydrophobic domains (each with six putative membrane-spanning segments) and two hydrophilic ATP-binding domains. The transporter proteins associated with antigen processing (TAP proteins) are recently identified members of the traffic ATPases (8–10). TAP molecules transport antigenic peptides across the endoplasmic reticulum (ER) membrane (11–13), an essential step in the major histocompatibility complex (MHC) class I antigen-presentation pathway. Through the action of TAP, peptides generated in the cytosol gain entry into the ER, where they can associate with MHC class I/$\beta_2$-microglobulin ($\beta_2$m) heterodimers. TAP molecules are made up of two distinct polypeptides, termed TAP1 and TAP2, each containing single hydrophobic and ATP-binding domains. Normal peptide loading and class I transport can be reconstituted in mutant cells that lack both TAP1- and TAP2-encoding genes only when both genes are reintroduced (14), suggesting that TAP1/TAP2 heterodimers constitute a functional transporter.

For some time we have been interested in the mechanisms involved in peptide translocation and assembly with MHC class I heavy chain and $\beta_2$m. Recently, we have gained insight into this process by showing that TAP translocates peptides in an ATP-dependent fashion (13) and possesses a peptide-binding site that exhibits a very broad peptide specificity (15). Although peptide translocation across the ER membrane requires ATP, peptide binding to TAP occurs in the absence of ATP, suggesting that peptide recognition and translocation are separate events. Furthermore, we have also shown that MHC class I/$\beta_2$m heterodimers physically associate with TAP before peptide loading (16). This interaction may facilitate peptide loading either by increasing the proximity of TAP and MHC class I/$\beta_2$m heterodimers, or by an unknown, more tightly coupled mechanism.

In this report we take advantage of a series of TAP1/TAP2 transfectants derived from the processing mutant 721.174, which lacks both TAP1 and TAP2 (17). These variants contain TAP1 and TAP2 expressed individually and together. Our goals were to determine whether the individual TAP1 or TAP2 molecules could bind and translocate peptides or interact with MHC class I/$\beta_2$m dimers. We have found that peptide binding and translocation occur only when both TAP1 and TAP2 are present, supporting the idea that TAP functions as a heterodimer. Furthermore, based on photoaffinity labeling, it appears that the peptide-binding site is composed of elements of both TAP1 and TAP2. In contrast, MHC class I/$\beta_2$m dimers can associate with individual TAP1 molecules, as well as functional TAP1/TAP2 heterodimers. These data provide insights into the mechanism of action of the transporters associated with antigen processing.

## MATERIALS AND METHODS

**Cells and Cell Culture.** The B-lymphoblastoid cell line Swei (18) and the T × B lymphoblast hybrid T0 (19) were grown in Iscove's modified Dulbecco's medium (IMDM; GIBCO)/5% bovine calf serum (HyClone)/gentamicin at 20 $\mu$g/ml. The

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Abbreviations: TAP, transporter associated with antigen processing; MHC, major histocompatibility complex; ABC, ATP-binding cassette; ER, endoplasmic reticulum; HSAB, *N*-hydroxysuccinimidyl-4-azidobenzoate; $\beta_2$m, $\beta_2$-microglobulin.

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TAP1 and/or TAP2 transfectant lines derived from 721.174 (B6, J4, and C3) have been described (14). The transfectant lines were grown in IMDM/10% bovine calf serum/gentamicin at 20 μg/ml.

**Photopeptide Analogues.** The synthesis and use of the photopeptide nef7B–*N*-hydroxysuccinimidyl-4-azidobenzoate (HSAB) was described (15) (nef7B; QVPLRPMTYK). The same procedure was used for the generation of another photopeptide analogue EEF.1–HSAB (EEF.1; AKAYAAEEF). The lysine residue serves as the conjugation site for the photoreactive crosslinker for each peptide; this was confirmed by sequence analysis of the purified peptide conjugates. The EEF.1 peptide was synthesized and purified to 95% by Quality Controlled Biochemicals (Hopkinton, MA). The photoconjugates were iodinated to a specific activity of 20–40 cpm/fmol as described (13).

**Preparation of Crude Membranes and TAP Photoaffinity Labeling.** Cell pellets were either stored frozen at −70°C or harvested the day of the experiment and quick-frozen in liquid nitrogen. Pellets were thawed and resuspended in 2 ml of cold Tris-buffered saline (10 mM Tris·HCl, pH 7.4/150 mM NaCl)/0.02% $NaN_3$/0.5 mM phenylmethylsulfonyl fluoride/5 mM iodoacetamide per $10^8$ cells. The suspension was spun at low speed (2000 × *g*) for 5 min at 4°C. The supernatant was removed and saved, and the pellet was resuspended in 1 ml of 10 mM Tris·HCl, pH 7.4, to maximize membrane yields and spun again at 2000 × *g* for 5 min. The two supernatants were combined and spun at 100,000 × *g* for 45 min to pellet the membranes. The crude membranes were resuspended in 1 ml of warm intracellular transport buffer (ICT, see ref. 13), pH 7.0, containing bovine serum albumin at 4 mg/ml. The membrane suspensions were transferred to 3.5-cm dishes, and TAP photolabeling was initiated by the addition of iodinated photopeptide in the dark. Incubation in the presence of label was continued for 5 min at 37°C on a rocker platform. The dishes were then cooled on ice, and the membranes were exposed to UV light (254 nm, Ultraviolet Products) for 3 min. The photolyzed membranes were diluted with 2 ml of cold ICT buffer and spun at 100,000 × *g* for 45 min to wash and pellet the membranes. The labeled membranes were extracted in 1 ml of lysis buffer (Tris-buffered saline/1% Triton X-100/0.02% $NaN_3$/0.5 mM phenylmethylsulfonyl fluoride/5 mM iodoacetamide) per $10^8$ cell-membrane equivalents. Immunoprecipitation of photolabeled TAP molecules and analysis by SDS/PAGE was as described (15).

**Antibodies.** Affinity-purified anti-TAP1 and anti-TAP2 antisera (R.RING4C and R.RING11C, respectively) were raised in rabbits against C-terminal peptides derived from TAP1 or the shorter version of TAP2 (TAP2A) (20). The purification of R.RING4C using a peptide-affinity column has been described (21), and R.RING11C was purified similarly. The anti-TAP2 monoclonal antibody 435.3 (see ref. 22) was derived by immunizing mice with a 280-amino acid C-terminal fragment of TAP2.

**Immunoblots.** Blots were done by using enhanced chemiluminescence as described (23). Briefly, 2 × $10^6$ cells were lysed in Tris-buffered saline/1% digitonin/0.1 mM 7-amino-1-chloro-3-tosylamido-2-heptone (TLCK)/0.5 mM phenylmethylsulfonyl fluoride and precleared overnight with normal rabbit serum and rabbit anti human IgG (heavy plus light chain). TAP molecules were precipitated with either R.RING4C or the 435.3 (anti-TAP2) monoclonal antibody as described above. The precipitated proteins were separated by nonreducing SDS/12.5% PAGE and transferred onto Immobilon membrane (Millipore). Class I heavy chains were probed with the rat monoclonal antibody 3B10.7 (24). Bands were visualized with horseradish peroxidase-conjugated secondary antibody and epichemiluminescence substrate (Amersham).

## RESULTS

**Comparison of nef7B and EEF.1 Photoconjugates.** Our previous photolabeling analysis revealed that the labeled bands derived from TAP occurred as a doublet at $M_r$ ≈71,000 (15). This result suggested that both TAP1 and TAP2 were photolabeled by using the nef7B photoconjugate. To address the nature of the bands photolabeled by nef7B–HSAB and to attain a higher yield of photolabeled TAP, we devised a method of photolabeling TAP from a crude membrane preparation derived from Swei cells (see *Materials and Methods*). This allowed us to attain a much greater level of TAP labeling than was achieved when using streptolysin O-permeabilized cells. Fig. 1 shows the results of labeling membranes directly with two separate $^{125}$I-labeled photoconjugates and immunoprecipitating with antibodies to both TAP1 and TAP2. As shown, precipitation with either R.RING4C or 435.3 (anti-TAP2) resulted in labeled TAP species for each photoconjugate (lanes 1 and 2, nef7B; lanes 3 and 4, EEF.1). A clear doublet can be seen when labeling with the nef7B photopeptide with the lower band being the major labeled species. In contrast, for the EEF.1 peptide a doublet band was not as apparent, and the major labeled species corresponded to the upper band seen with the nef7B photolabeling. The EEF.1 peptide was engineered specifically for photolabeling TAP. The lysine provides the primary amino group for conjugation to the photoreactive crosslinker, and the tyrosine allows for labeling the peptide with $^{125}$I. The C-terminal EEF sequence is the linear epitope recognized by monoclonal antibody YL1/2 (25) and thus can be used as an epitope tag. Ultimately, we want to isolate a peptide fragment from the peptide-binding region of TAP using the EEF.1 photoconjugate. The EEF.1 photopeptide labels TAP to a much higher degree than the nef7B photopeptide (≈20-fold difference when the amount of labeled peptide added for each was similar).

**Both TAP1 and TAP2 Are Photolabeled.** To verify that the doublet seen in Fig. 1 (lanes 1 and 2) was indeed composed of TAP1 and TAP2, we denatured the labeled TAP species in SDS after initial immunoprecipitation with R.RING4C and reprecipitated the denatured species separately with R.RING4C and R.RING11C antibodies. The results shown in Fig. 2 (lanes 2 and 3) revealed that the doublet derived from $^{125}$I-labeled nef7B–HSAB photolabeling was composed of TAP1 and TAP2 chains (TAP1 being the lower band and

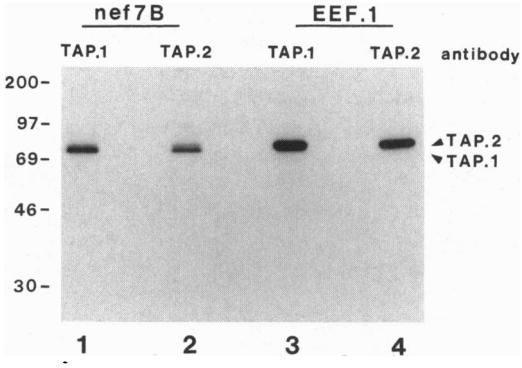


FIG. 1. TAP photolabeling using the nef7B and EEF.1 photoconjugates and crude membranes from Swei cells as a source of TAP. Crude membranes derived from Swei cells ($10^8$) were incubated with $^{125}$I-labeled nef7B–HSAB (0.5 μM) or $^{125}$I-labeled EEF.1–HSAB (0.2 μM) for 5 min at 37°C in the dark, as described. The membranes were exposed to UV light, washed, and extracted in detergent. The labeled TAP molecules were precipitated with R.RING4C (lanes 1 and 3) or the TAP2 monoclonal antibody 435.3 (lanes 2 and 4). The precipitated complexes were analyzed by SDS/10% PAGE and autoradiography. Arrowheads indicate locations of TAP1 and TAP2 subunits.

TAP2 being the upper band). Furthermore, for nef7B the major labeled band was TAP1, whereas TAP2 was a minor labeled species. When the same experiment was done on TAP photolabeled with the EEF.1 peptide, again we could detect both TAP1 and TAP2 bands; however, in this case TAP2 was the major labeled species (lane 3), whereas TAP1 was the minor labeled species (lane 2). In Fig. 1 TAP labeled with the EEF1 peptide did not appear to produce a doublet (lanes 3 and 4). However, the strong labeling of TAP2 most likely masked the weak labeling of TAP1, and only when the chains were precipitated separately did TAP1 labeling become apparent. Nevertheless, the data support the notion that the peptide-binding site on TAP is made up of elements of both TAP1 and TAP2.

**Peptide Binding and Translocation Occur Only in the Presence of TAP1 and TAP2.** To determine whether both TAP1 and TAP2 are required to form a functional peptide-binding site we used a series of transfectants derived from the antigen-processing mutant cell line .174 (17), which lacks the genes coding for TAP1 and TAP2. The transfectants contain TAP1 and/or TAP2 expressed separately or together. It was previously shown using these cell lines that reconstitution of peptide loading and MHC class I expression occurred only in the presence of both TAP1 and TAP2 (14), suggesting that to reconstitute a functional transporter both TAP1 and TAP2 must be expressed. We decided to carry this analysis further by measuring the reconstitution of the peptide-binding site on TAP in the .174 transfectants by photolabeling. The results of this analysis (Fig. 3) clearly show that to photolabel TAP molecules, both TAP1 and TAP2 must be present. Photolabeling of TAP1 and TAP2 occurred only in the C3 transfectant, which contains both TAP1- and TAP2-encoding genes (lanes 5 and 10) and in the wild-type Swei cells (lanes 1 and 6). The double-negative parental cell line .174 (lanes 2 and 7), and the single-chain transfectants B6 (lanes 3 and 8) and J4 (lanes 4 and 9) showed virtually no photolabeled species. The degree of TAP photolabeling in the double-chain transfectant C3 is lower than in the wild-type Swei cells (≈5-fold), which is probably indicative of lower expression levels of TAP in the transfectants. These data strongly suggest that a functional peptide-binding site is attained only when TAP1 and

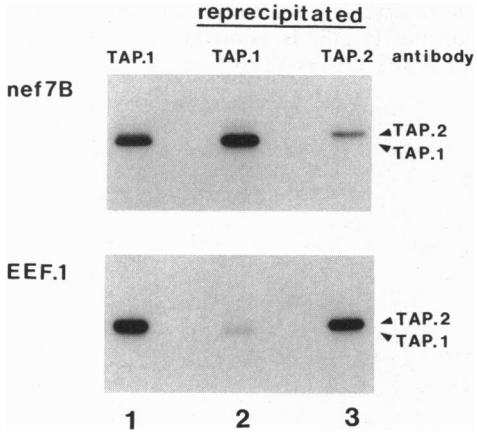


FIG. 2. The nef7B and EEF.1 photoconjugates label both TAP1 and TAP2. Crude Swei membranes were photolabeled with $^{125}$I-labeled nef7B–HSAB or $^{125}$I-labeled EEF.1–HSAB as described for Fig. 1. Labeled TAP was precipitated with R.RING4C (lane 1). Another aliquot of R.RING4C-precipitated material was denatured in 100 μl of Tris-buffered saline, pH 7.4/2% SDS/4 mM dithiothreitol by boiling for 5 min. The denatured TAP subunits were diluted into 1 ml of lysis buffer/1% Triton X-100/5 mM iodoacetate (see ref. 13). The samples were then divided in half, and the individual subunits were reprecipitated with R.RING4C (lane 2) or R.RING11C (lane 3). The samples were analyzed by SDS/8% PAGE and autoradiography. (*Upper*) nef7B. (*Lower*) EEF.1.

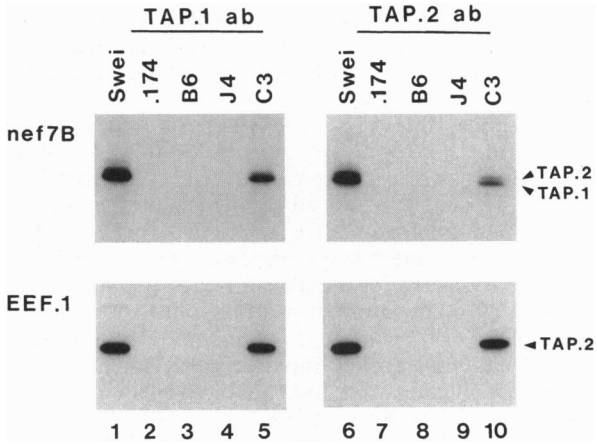


FIG. 3. A heterodimer of TAP1 and TAP2 is required to constitute a peptide-binding site. Crude membranes were prepared from $10^8$ Swei, .174, B6, J4, and C3 cells. The membranes were photolabeled with $^{125}$I-labeled nef7B–HSAB or $^{125}$I-labeled EEF.1–HSAB as described for Fig. 1. After detergent extraction of the membranes, the samples were divided in half, and labeled TAP from each half was precipitated with either R.RING4C (lanes 1–5) or 435.3 (lanes 6–10). 435.3 was used in these experiments because the TAP2 allele transfected into .174 is not reactive with R.RING11C. The precipitated species were analyzed by SDS/10% PAGE and autoradiography. Lanes: 1 and 6, Swei (wild type); 2 and 7, .174 (TAP1$^-$/TAP2$^-$); 3 and 8, B6 (TAP1$^+$/TAP2$^-$); 4 and 9, J4 (TAP1$^-$/TAP2$^+$); 5 and 10, C3 (TAP1$^+$/TAP2$^+$). (*Upper*) nef7B. (*Lower*) EEF.1. ab, Antibody.

TAP2 associate to form a heterodimer. TAP1 or TAP2 expressed alone possess no capability or very limited capability to bind peptide.

To determine directly the functional activity of the transfected TAP molecules, we measured the translocation of a labeled reporter peptide by using streptolysin O-permeabilized cells, as described (15). The reporter peptide possesses an N-linked glycosylation site that allows the peptide to become glycosylated upon peptide entry into the ER. The level of glycosylated peptide is measured by precipitation with Con A–Sepharose. It was previously shown that peptide glycosylation in permeabilized cells is TAP- and ATP-dependent (11). The results of the peptide-translocation assay in the transfectants is shown in Fig. 4. As revealed by the histograms, the level of peptide translocation directly correlates with the level of TAP photolabeling for each transfectant and the wild-type Swei cells. That is, the level of peptide translocation is essentially background in .174 cells and in the single-chain transfectants, whereas peptide translocation is reconstituted in the double-chain transfectant C3. The wild-type Swei cells possess a higher level of translocation than the C3 transfectant, which mirrors the results of the photolabeling analysis. Together, all of the above data suggest that a functional peptide transporter consists of a TAP1/TAP2 heterodimer. Implicit in this conclusion is that TAP1 or TAP2 homodimers do not form functional transporters.

**MHC Class I Heavy-Chain/$\beta_2$m Dimers Associate with TAP1, but Do Not Associate with TAP2, When the Chains Are Expressed Individually.** We showed earlier that peptide-free MHC class I heavy chain/$\beta_2$m dimers associate with the TAP1/TAP2 complex in human cells. Here we took advantage of the TAP1- or TAP2-transfected cell lines to study the contribution of the individual TAP subunits to the binding of class I/$\beta_2$m dimers. Cells were lysed in buffer containing digitonin and precipitated with R.RING4C, R.RING11C, or 435.3. After gel electrophoresis and blotting, the MHC class I heavy chain was detected with rat monoclonal antibody 3B10.7. The results (Fig. 5) reveal that MHC class I heavy chain coprecipitates with TAP from the transfectant that

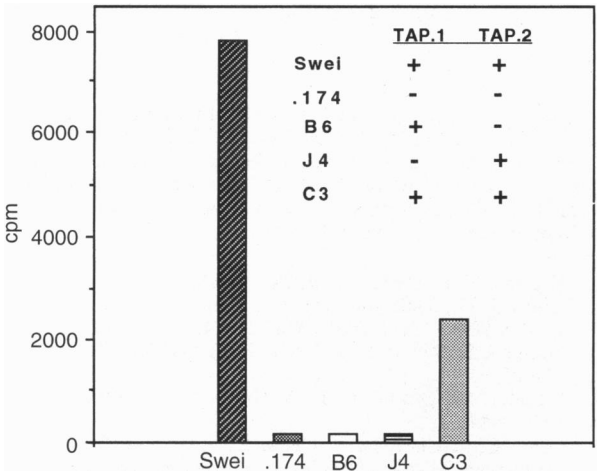


FIG. 4. A heterodimer of TAP1 and TAP2 is required for peptide translocation. Swei, .174, B6, J4, and C3 cells were permeabilized with streptolysin O, and the translocation of a reporter peptide ($^{125}$I-labeled B27#3*) into the ER was measured (see ref. 15). The histograms represent the mean of duplicate points with error ranges of 10% or less. The *y* axis (cpm) represents the amount of reporter peptide translocated as determined by its degree of glycosylation.

expresses TAP1 only (B6) (lane 3). The levels of coprecipitated heavy chain from the B6 transfectant are comparable to that coprecipitated from the wild-type Swei cell (lane 1), as well as from the TAP1/TAP2-expressing transfectant C3 (lane 5). However, class I heavy-chain coprecipitation does not follow the same pattern in cells that express TAP2 alone. As shown in lane 9, no TAP–class I association was detected in the J4 transfectant that expresses TAP2 only, whereas class I protein was coprecipitated from the wild-type cells (lane 6) and from the TAP1/TAP2 transfectant C3 (lane 10). The TAP expression levels in the transfectants were assayed by immunoblotting and revealed that each cell contained the expected TAP subunits, although the TAP levels were slightly lower in the cells that expressed single chains (data not shown). This result is most likely due to instability of the individually expressed chains. These data suggest that MHC class I/$\beta_2$m dimers do not associate with TAP2 when it is expressed individually.

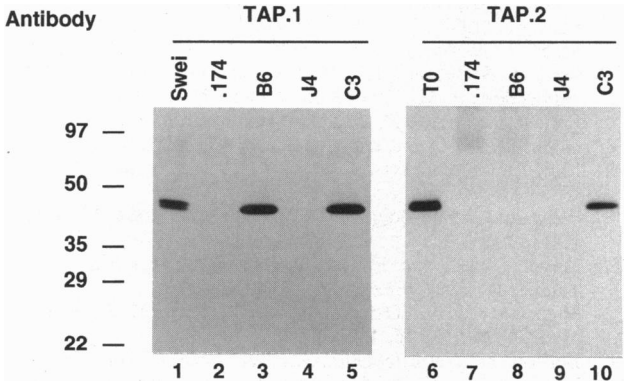


FIG. 5. MHC class I/$\beta_2$m binds to TAP1 but does not bind detectably to TAP2. Cells were lysed in digitonin, and TAP-associated class I heavy chain/$\beta_2$m was precipitated with either R.RING4C (*Left*), R.RING11C (*Right*, T0), or 435.3 (*Right*, mutant and transfectants) as described. Heavy chain was detected by immunoblotting with the specific antibody 3B10.7. Lanes: 1 and 6, Swei or T0 (wild type), respectively; 2 and 7, .174 (TAP1$^-$/TAP2$^-$); 3 and 8, B6 (TAP1$^+$/TAP2$^-$); 4 and 9, J4 (TAP1$^-$/TAP2$^+$); lanes 5 and 10, C3 (TAP1$^+$/TAP2$^+$).

## DISCUSSION

We have used transfectants that contain individual TAP1 and TAP2 molecules to address questions pertaining to the nature of the peptide-binding site on TAP and the association of MHC class I/$\beta_2$m heterodimers with TAP. Our studies reveal that the peptide-binding site on TAP is a combinatorial one, requiring elements from TAP1 and TAP2. This result is supported by the observation that both TAP1 and TAP2 are photolabeled when the chains are expressed together, and they are not photolabeled when expressed individually. In addition, peptide translocation into the ER via TAP occurs only when TAP1 and TAP2 are present. In support of these findings, individual TAP1 and TAP2 chains expressed in insect cells have also been shown not to bind or translocate radiolabeled peptides; however, when both chains are expressed together, then efficient binding and translocation occur (22, 26). These data argue that a functional TAP is composed of a TAP1 and TAP2 heterodimer and that homodimers of either TAP1 or TAP2, if they form, are nonfunctional. Furthermore, we find that MHC class I heavy chain/$\beta_2$m heterodimers can associate with individually expressed TAP1 molecules but apparently cannot associate with individual TAP2 molecules. This result suggests that the site of interaction between TAP and MHC class I/$\beta_2$m dimers resides on TAP1.

The observation that functional TAP transporters exist only as heterodimers provides some insight into the structure and function of TAP. Interestingly, TAP is one of the few defined eukaryotic ABC transporters encoded by two separate genes. The sterile 6, multidrug resistance, and cystic fibrosis transmembrane conductance regulator transporters are all encoded by a single gene. Nevertheless, the domain organization of TAP and the other transporters is very similar; each is basically composed of two homologous halves. Therefore, one might expect that the mechanism of action of these transporters would be conserved. There are examples of ABC transporters that can function as homodimers, such as the HlyB transporter in bacteria (3) and a truncated version of the cystic fibrosis transmembrane conductance regulator, which possesses the nucleotide-binding fold 1 (NBF1), the transmembrane domain 1 (TM1), and the regulatory domain (27). However, "half molecules" of the sterile 6 transporter cannot function individually, but when expressed together, they can reconstitute sterile 6-mediated a-factor transport (28). Whether the majority of ABC transporters can function as homodimers of "half molecules" is not yet clear. Our data indicates that for TAP the heterodimeric form must be maintained. We suspect that when a transporter is encoded by a single gene that possesses homologous halves or is encoded by two separate but related genes, then the heterodimeric form will be required to achieve a functional transporter. Cystic fibrosis transmembrane conductance regulator is unique in the sense that it possesses a large regulatory domain, and it is not clear how this affects substrate recognition and translocation. At the very least, the TAP heterodimer is required to achieve an active peptide-recognition site, but it could also be critical in facilitating other aspects of TAP function, such as peptide translocation.

It is clear, based on the photoaffinity labeling data, that elements of both TAP1 and TAP2 are localized in the peptide-binding region. These results are consistent with photolabeling experiments done on the multidrug resistance transporter, where regions of both the amino and carboxyl halves of the transporter become labeled by photoreactive drug substrates (29). In addition, our data indicate that TAP1 and TAP2 are differentially labeled, depending upon the particular photoconjugate used. The nef7B photoconjugate predominantly labels TAP1, whereas the EEF.1 conjugate predominantly labels TAP2 (see Fig. 2). It may not be

coincidental that the photoreactive group for nef7B–HSAB is located at the C terminus, whereas that for EEF.1–HSAB is located near the N terminus. The N-terminal portion of the peptide may be closely associated with TAP2, and the C-terminal end may be closely associated with TAP1, with TAP1 and TAP2 each constituting one-half of the peptide-binding site. However, another possibility is that elements of TAP1 and TAP2 may line the recognition site in a lengthwise fashion, reminiscent of the contributions of the $\alpha$ and $\beta$ chains to the MHC class II peptide-binding groove. In this model, the photoreactive group may be oriented primarily toward one TAP chain or the other, depending upon the particular peptide conformation. Further analysis of a number of different photoreactive peptides with the photoreactive group located at different positions within the peptide may provide some insight into the structure of the peptide-binding site.

Our finding that MHC class I heavy chain/$\beta_2$m heterodimers associate with TAP1 is consistent with what has previously been shown for TAP/MHC class I association in the mutant murine cell line RMA-S (30). This antigen-processing-defective cell line expresses a normal TAP1 molecule but expresses a truncated form of TAP2, which renders the TAP complex nonfunctional. Association of MHC class I/$\beta_2$m dimers with TAP can be detected in RMA-S cells, suggesting that the interaction with the TAP complex is through TAP1. It is not yet clear in the murine system whether or not TAP2 can bind MHC class I/$\beta_2$m dimers. We might also infer from our results that only one MHC class I/$\beta_2$m dimer is likely to be bound by a single TAP1/TAP2 complex because the sequence of TAP1 shows no obvious repetitive sequence motifs that could serve as multiple binding sites for more than one class I molecule. However, even though TAP2 expressed individually does not appear to bind class I, it may still bind class I/$\beta_2$m dimers when part of a functional TAP1/TAP2 complex. Further studies on the structure of native TAP/class I complexes will be required to answer this question.

There remain many unanswered questions with regard to the TAP/MHC class I association. For example, does the TAP/class I association play a physiological role in class I antigen presentation, and in particular, does the association facilitate peptide loading *in vivo*? Clearly, the binding of class I/$\beta_2$m dimers to TAP is not required for TAP activity, as peptides can be translocated normally by TAP in MHC class I-negative cells (M.J.A., unpublished observations). Conversely, the TAP/class I association is not absolutely required for the loading of class I/$\beta_2$m dimers, as shown by isolation of signal-sequence-derived peptides from class I molecules taken from TAP-negative cells (19). Therefore, there is no direct evidence to suggest that MHC class I association with TAP is required for successful class I antigen presentation. Class I association with TAP1 expressed in the absence of TAP2 suggests that TAP1 may simply provide a link for MHC class I/$\beta_2$m dimers to the peptide-translocation machinery. The finding that TAP/class I association is not dependent upon the existence of functional transporters suggests that association is not a tightly regulated step. However, the dissociation of class I/$\beta_2$m dimers from TAP may be tightly coupled to peptide loading, as indicated by previous experiments (16, 30). We suggest that, at the very least, MHC class I association with TAP provides a "microdomain" within the ER, where the major players involved in peptide loading can interact. In this scenario, newly translocated peptides could be sampled almost immediately by MHC class I/$\beta_2$m dimers for peptides that contain the correct binding motif. Peptide loading would therefore be "optimized" by the close association of TAP and MHC class I/$\beta_2$m dimers.

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