Peptide Length and Sequence Specificity of the Mouse TAP1/TAP2 Translocator

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Summary

The transporter associated with antigen processing (TAP) delivers peptides to the lumen of the endoplasmic reticulum in an adenosine triphosphate (ATP) dependent fashion for presentation by major histocompatibility complex class I molecules. We show that the mouse TAP translocator (H-2^b haplotype) selects peptides based on a minimal size of nine residues, and on the presence of a hydrophobic COOH-terminal amino acid. The preponderance of COOH-terminal hydrophobic amino acids in peptides capable of binding to mouse class I molecules thus fits remarkably well with the specificity of the TAP translocator. In addition to transport in the lumenal direction, efflux of peptide in the cytosolic direction is observed in an ATP- and temperature-dependent manner. By maintaining a low peptide concentration at the site of class I assembly, this efflux mechanism may ensure that class I molecules are loaded preferentially with high affinity peptides.

The involvement of the MHC-encoded peptide transporter subunits associated with antigen processing (TAP1 and TAP2)[1] in class I MHC–restricted antigen presentation is well established. Mutant cell lines defective in the synthesis of TAP subunits fail to engage in self-restricted antigen presentation (1–4), and this defect is restored upon transfection with the corresponding wild-type cDNAs (5–10). However, these studies using *TAP* mutant and wild-type cell lines could not make a distinction between a peptide translocating and a class I loading function for the TAP gene products. Recently, mice with a targeted deletion in the *TAP1* gene were produced and shown to have drastically reduced expression of class I molecules (11) and consequently they fail to develop a normal $CD8^+$ T cell repertoire (12). Microsomes prepared from the liver of these *TAP1* mutant mice and a comparison with similar preparations from wild-type mice allowed us to demonstrate that peptide translocation is ATP dependent, and requires the presence of a functional TAP1 gene product (13). A similar ATP dependency and specificity of peptide uptake was observed in experiments using TAP^- cell lines and *TAP1/TAP2* transfectants thereof (14). These experiments have indicated that there is specificity in the TAP-mediated translocation mechanism because peptides of different length and sequence do not compete (13) and are not taken up (14) equally well.

The efficacy of antigen presentation might be enhanced if the specificity of the peptide translocator and class I MHC molecules were matched (15). Most mouse class I bound peptides contain a COOH-terminal hydrophobic amino acid (15, 16) (H-$2K^d$: L/I; H-$2D^b$: M/I; H-$2K^b$: L/M; H-$2K^k$: I; H-$2K^{bm1}$: I). Not all human class I alleles fit this pattern, as both Aw68 and B27 have a preference for COOH terminal R or K (17, 18). A similar preference for a particular type of amino acid sidechain is not seen for the NH_2-terminal residue or any other sequence position in class I–bound peptides. If peptides that bind to class I molecules would be immediate substrates for the TAP translocator (rather than larger precursors with COOH-terminal extensions), then mouse TAP and class I molecules might share a preference for a hydrophobic COOH terminus. We show here that the mouse TAP translocator preferentially accommodates peptides having a large hydrophobic COOH-terminal amino acid. We also show that the TAP translocator transports peptides with a minimum length of nine amino acids, consistent with the length preference of class I molecules.

[1] *Abbreviations used in this paper:* β_2m, β_2-microglobulin; ER, endoplasmic reticulum; TAP, transporter associated with antigen processing.

Along with determining the peptide specificity of the TAP translocator, we also establish the presence of an ATP-dependent efflux mechanism that allows exit of peptides from the endoplasmic reticulum (ER) to the cytosol. In this manner, a low steady-state peptide concentration in the ER may be maintained such that class I molecules are preferentially loaded with high affinity peptides.

Materials and Methods

Antibodies, Peptides, and Peptide Labeling. All peptides were synthesized on a multiple peptide synthesizer (model 350; Advanced Chemtech, Louisville, KY), using conventional Fmoc chemistry. Purity of all peptides was assessed by TLC and subsequent ninhydrin staining (*N*-butanol/H_2O: pyridine/HOAc, 2:1:0.75:0.25, on Silicagel 60 plates; EM Science, Gibbstown, NJ). Where required (for the FAPGNYPAX series X = C, X = N, and X = P), peptides were purified by HPLC. Amino acid composition of the peptides X = L and X = G was confirmed by amino acid analysis. The identity of the peptides, X = D, X = F, X = L, and X = R was confirmed by sequencing. The identity of peptides TYQRTRALV, acetyl-FAPGNYPAL, FAPGNYPAL-amide, APGNYPAL, PGNYPAL, and GNYPAL was confirmed by amino acid analysis. Peptides were dissolved in 0.1× PBS and filtered through 0.22-μm filters. All peptides were readily soluble in aqueous solution up to 2 mM. Peptide concentrations were determined by absorbance at 275.5 nm (19).

The peptide FAPGN (^{3}H-)YPAL was prepared from a FAPGN-(di-iodo-)-YPAL precursor by reduction under ^{3}H-gas (13, 20) and purified as described (13); this peptide binds to H-2^b molecules (21). The peptide TYNRTRALI was labeled by chloramine T-catalyzed iodination. Anti-TAP1 antibodies were obtained by immunization of rabbits with purified mouse TAP1 ATP binding domain (the COOH-terminal 280 amino acids) obtained by expression in *Escherichia coli*. This serum has been described (22). The anti-TAP2 serum was raised against the ATP binding domain of mouse TAP2 and will be described elsewhere.

Preparation of Microsomes and Transport Assays. Microsomes were prepared from livers of 6–8-wk-old female C57Bl/6 mice, or C57BL/6, 129/SV, and β_2-microglobulin (β_2m)–negative mice (129/SV × C57BL/6 F_1), that had been primed with polyinosinic-polycytidylic acid (Sigma Chemical Co., St. Louis, MO) for 24–36 h as described (13, 23). Transport assay mixtures were assembled on ice as described (13). Each mixture contained 5 μl microsomes (corresponding to 80–120 μg protein), 50 mM Hepes, pH 7.2, 150 mM KOAc, pH 7.5, 5 mM MgOAc, 250 mM sucrose, 0.2 μM FAPGN[^{3}H]YPAL or 60 nM ^{125}I-TYNRTRALI, 1 mM dithiothreitol, and where applicable, ATP generating system (50 μM ATP, 250 μM UTP, 2.5 mM creatine phosphate, 8 U rabbit muscle creatine phosphokinase), and competitor peptides. Reactions were incubated at 23°C for 10 min, and subsequently transferred to ice. Samples were loaded on ice-cold 1-ml sucrose cushions (1 M KOAc, pH 7.5, 500 mM sucrose, 50 mM Hepes, pH 7.2, and 25 μM unlabeled FAPGNYPAL). Samples were spun for 15 min at 67,000 rpm in a rotor (TLA 100.2; Beckman Instruments, Inc., Fullerton, CA). Supernatants were aspirated and pellets were resuspended in 150 μl lysis mix (0.5% NP-40, 150 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$) by repeated pipetting. Debris was removed by centrifugation at 14,000 *g* for 4 min and soluble radioactivity was determined by scintillation counting. For all experiments shown, the ratio of peptide uptake in the presence and absence of ATP was at least a factor 3–3.5, and the total number of counts recovered varied between 1,500 and 6,000 cpm, varying both between different microsome preparations and different labeled substrates.

FACS® Analysis of Upregulation of MHC Class I Expression. Stabilization of class I molecules by peptide as measured by FACS® analysis (Becton Dickinson & Co., Mountain View, CA) was performed as described (1). Briefly, RMA-S cells were incubated overnight in the presence of 100 μM peptide for all peptides of the FAPGNYPAX series. Cells were subsequently stained with either B8-24-3 (anti-K^b; (American Type Culture Collection, Rockville, MD [ATCC]) or B22.249 (anti-D^b, ATCC) antibodies, and a second goat anti-mouse IgG FITC antibody. Mean fluorescence of labeled cells was determined by cytofluorimetric analysis. "–" indicates 0–33% of maximal class I upregulation, "+" indicates 33–67% of maximal upregulation, and "++" indicates 67–100% of maximal class I upregulation. Class I upregulation was calculated as (mean fluorescence in the presence of peptide) − (mean fluorescence in the absence of peptide). For both D^b and K^b, maximal upregulation (an approximate threefold increase in class I expression) was observed with the peptide FAPGNYPAV (Table 1).

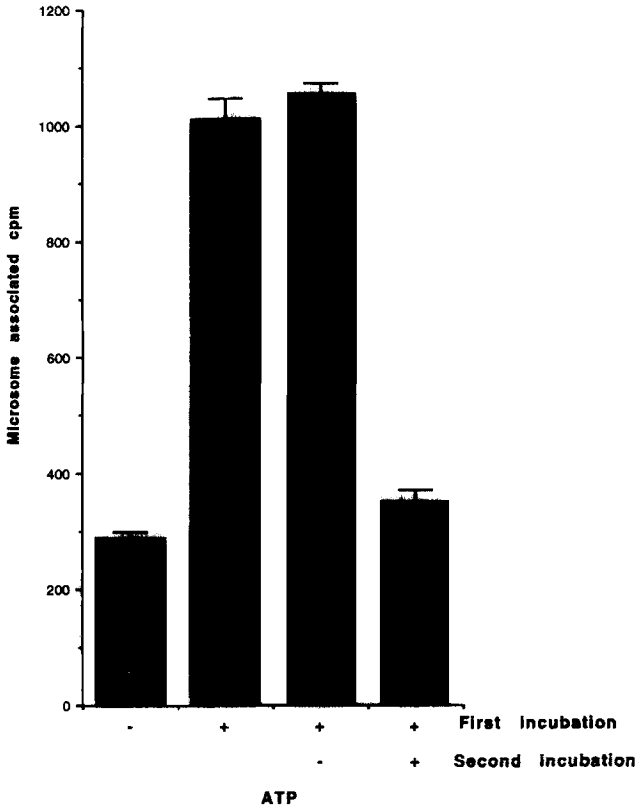

Figure 1. Peptide uptake by mouse microsomes is reversible in an ATP-dependent manner. Microsomes were loaded with [^{3}H]FAPGNYPAL at 23°C in the absence (sample *A*) or presence of ATP (samples *B*, *C*, and *D*), as described in Materials and Methods. After the first incubation, microsomes were spun down and resuspended in fresh, [^{3}H]FAPGNYPAL free incubation buffer containing 200 μM unlabeled FAPGNYPAL. Samples *C* and *D* underwent a second incubation at 23°C in the presence or absence of ATP. Microsomes were pelleted by centrifugation. Microsome-associated radioactivity was determined as described in Materials and Methods. Data show that efflux of labeled peptide occurs in an ATP-dependent manner. Values given are means of duplicates ± SD.

Results

Peptide Uptake by Mouse Microsomes Is Reversible in an ATP- and Temperature-dependent Manner. Peptide uptake in microsomal vesicles is a rapid process that reaches equilibrium within 10 min (13). This is not due to inactivation of TAP function in the course of incubation, because a 10-min preincubation in the absence of peptide does not affect subsequent peptide accumulation (data not shown). Rather, peptide efflux appears to be responsible. When microsomes are loaded with labeled peptide and subsequently transferred to fresh medium, efflux of labeled material is observed in a temperature (data not shown)- and ATP-dependent fashion (Fig. 1). The material accumulated by and released from microsomes in the presence of ATP is largely intact FAPGNYPAL, as shown by HPLC (data not shown).

Efflux of FAPGNYPAL may occur because the number of peptide molecules transported into the microsomes is in excess of the number of available class I binding sites, or because other proteins in the ER (such as chaperones) might keep class I molecules in a state that would prevent high affinity capture of substrate.

Peptide Accumulation in Mouse Microsomes Is Influenced by the Presence of MHC Class I Products. To test if manipulation of the number of available class I binding sites would affect peptide accumulation inside the microsomes, we made use of mice with a targeted disruption of the β_2m gene (24). As a consequence of the lack of β_2m, neither K^b nor D^b is expected to fold correctly. The resulting free H chains, while capable of binding peptide, do so only at very high concentrations of peptide (25). As shown in Fig. 2 *A*, microsomal preparations from β_2m^- mice fail to significantly accumulate the [^{3}H]FAPGNYPAL substrate in an ATP-dependent fashion. However, introduction of a consensus site for N-linked glycosylation in a derivative of a K^d-restricted epitope (TYQRTRALV→TYNRTRALI) results in a peptide that

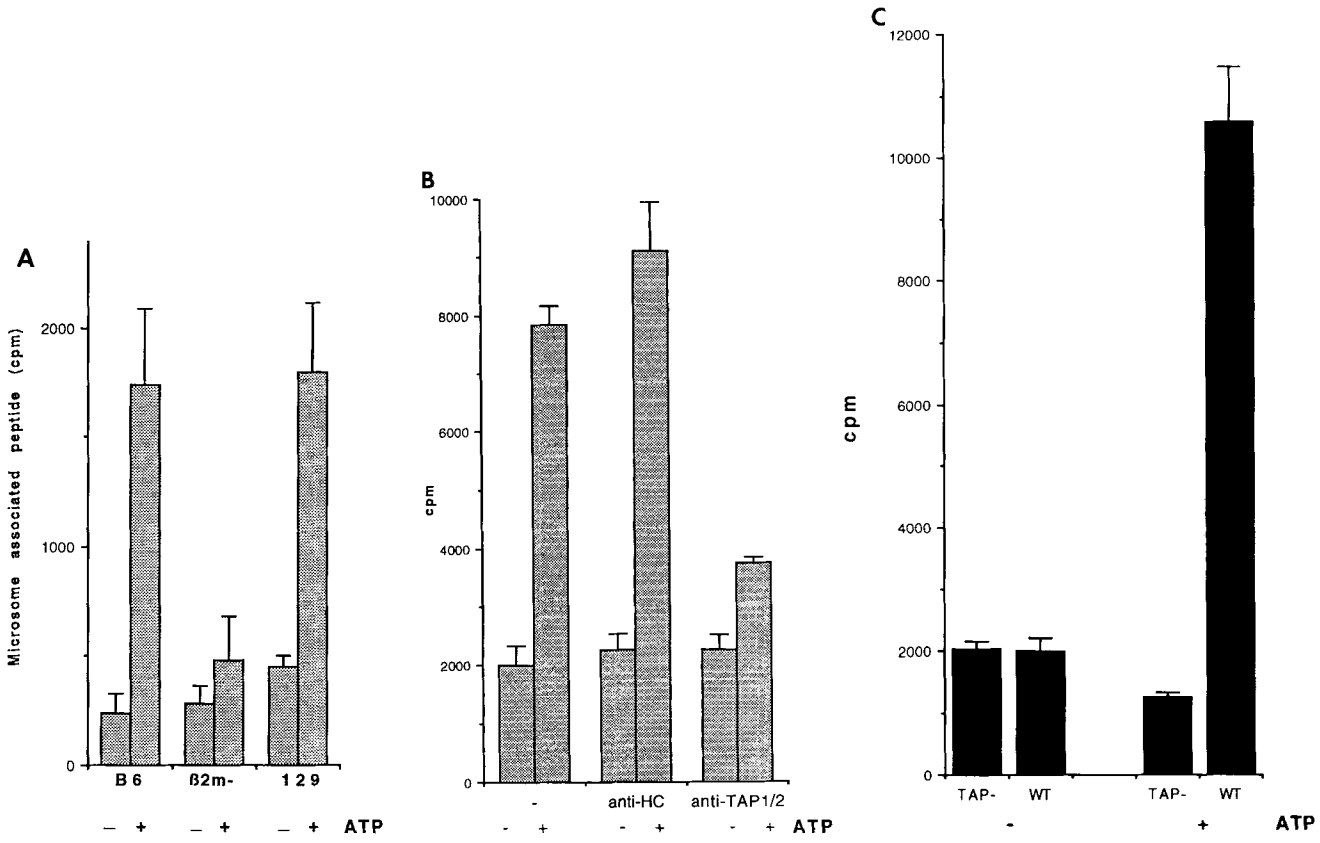

Figure 2. (*A*) [^{3}H]FAPGNYPAL accumulation in mouse microsomes is dependent on β_2m. C57Bl/6 (H-2^b) and 129/SV (H-2^b) derived microsomes but not C57Bl/6 × 129/SV (H-2^b) β_2m^- derived microsomes accumulate [^{3}H]FAPGNYPAL in an ATP-dependent manner. Microsomes were prepared as described in Materials and Methods, and tested for peptide accumulation, using the substrate ^{3}[H]FAPGNYPAL. Values are means of duplicates ± SD. (*B*) β_2m^- microsomes accumulate the glycosylation substrate ^{125}I-TYNRTRALI in an ATP- and TAP-dependent fashion. Microsomes were incubated as described in Materials and Methods in the presence of 60 nM ^{125}I-TYNRTRALI. Microsomes were preincubated on ice with 2 μl antiserum for 10 min in a total volume of 50 μl. Anti-HC is a rabbit antiserum raised against the human MHC class I H chain, rabbit anti-TAP1/2 sera are directed against the ATP binding domains of mouse TAP1 and TAP2. Values are means of duplicates ± SD. (*C*) TAP$^-$ microsomes do not accumulate the glycosylation substrate ^{125}I-TYNRTRALI. Microsomes were prepared from wild-type age-matched male C57Bl/6 and TAP$^-$ (C57Bl/6 × 129/SV) mice and tested for uptake of the peptide ^{125}I-TYNRTALI as described in Materials and Methods. Values are means of duplicates ± SD.

is accumulated by both wild-type and β_2m$^-$ microsomes (Fig. 2 *B*). This phenomenon is TAP dependent since it is not observed for microsomes derived from TAP1 mutant mice (Fig. 2 *C*), and peptide uptake can be inhibited by TAP1- and TAP2-specific antibodies (Fig. 2 *B*). The attachment of an N-linked glycan is observed for this peptide after uptake by rat microsomes, a property that could explain its retention (25a).

Thus, accumulation of FAPGNYPAL in microsomal vesicles requires an intact β_2m gene, and therefore binding to class I molecules affects the outcome of such translocation assays. Alternatively, the presence of a glycosylation site in the translocation substrate renders the assay independent of class I binding sites.

In previous experiments (13) we observed that the K^d-restricted peptide TYQRTRALV was a far worse competitor than the K^b-restricted peptide FAPGNYPAL when tested for competition of uptake of [^{3}H]FAPGNYPAL by H-2^b microsomes. These results are in contrast with the observation that the TYQRTRALV peptide is presented efficiently, and without a need for flanking sequences, when expressed in the cytosol of murine cells (26). When these two peptides are tested for competition of ^{125}I-TYNRTRALI uptake using β_2m$^-$ microsomes, no differences in efficiency of competition are observed (Fig. 3). Identical IC$_{50}$s (competitor concentration at which substrate uptake is half-maximal) for these two peptides (3 μM) are also found when tested using H-2^d–derived microsomes (Schumacher, T. N. M., unpublished observations).

Thus in assays where the labeled substrate can bind to the class I molecules present, peptide accumulation in microsomes may reflect the combined effects of two parameters: competition for TAP-mediated transport and competition for class I binding sites in the microsomes. To exclude a bias introduced by class I binding, all further experiments were performed using β_2m$^-$ microsomes and the glycosylation substrate.

The Mouse TAP Translocator Prefers Peptides with a Hydrophobic COOH Terminus. The peptides bound to all mouse class I products studied thus far are characterized by a hydrophobic COOH-terminal amino acid (15, 16). To explore the possibility that class I molecules and the TAP translocator might have a shared specificity for a hydrophobic COOH terminus, we synthesized FAPGNYPAL and the 19 other possible COOH-terminal substitutions and assayed them for their

Table 1. *IC$_{50}$ Values of FAPGNYPAL and FAPGNYPAL Derivatives in βm$^-$ Microsomes*

Competitor X =	Class I binding		IC$_{50}$ FAPGNYPAX
	K^b	D^b	
			μM
Leucine*	+ +	+	5.2
Methionine	+ +	+	6.4
Tyrosine	–	–	6.4
Tryptophan	–	–	7.1
Isoleucine	+ +	+ +	7.2
Phenylalanine	–	–	8.3
Valine	+ +	+ +	28.0
Glutamine	–	–	36.0
Cysteine	+ +	+	57.0
Threonine	–	+	>100.0
Alanine	+	+ +	>100.0
Histidine	–	–	>100.0
Lysine	–	–	>100.0
Asparagine	–	–	>100.0
Proline	–	+	>100.0
Serine	–	–	>100.0
Glutamic acid	–	–	>100.0
Aspartic acid	–	–	>100.0
Glycine	–	–	>100.0
Arginine	–	–	>100.0

* Measured using β_2m$^-$ microsomes, and ^{125}I-TYNRTRALI substrate.

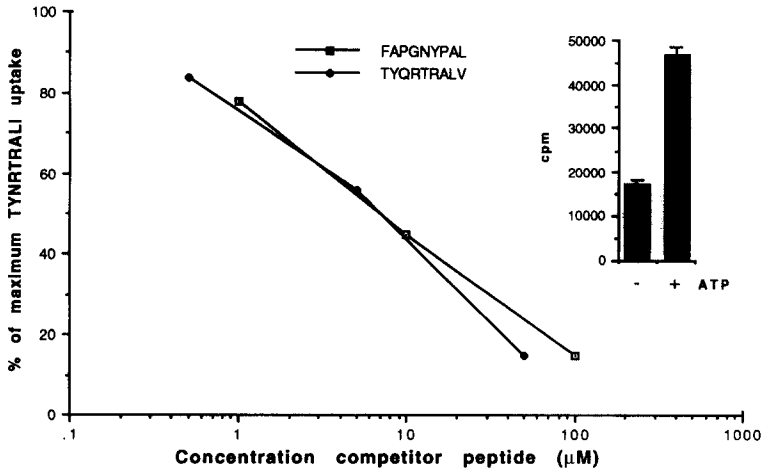

Figure 3. Two peptides that only share a hydrophobic COOH terminus compete with similar efficiency for TAP-mediated peptide uptake. The peptides FAPGNYPAL and TYQRTRALV were tested for competition in uptake of ^{125}I-TYRTRALI as described in Materials and Methods. Values are given as a percentage of maximal ^{125}I-TYNRTRALI uptake and were calculated as described in Table 2. Data show that unlike for wild-type microsomes (13), an H-2^d– and an H-2^b–restricted epitope compete with similar efficiency for uptake of an H-2K^d-restricted epitope when tested in β_2m$^-$ microsomes.

ability to compete for uptake of ^{125}I-TYNRTRALI by β_2m$^-$ liver microsomes (Table 1). A strong correlation between the ability of an analog to compete for uptake, and the presence of an aliphatic or aromatic residue at the COOH terminus is observed. Among those peptides that compete for uptake of ^{125}I-TYNRTRALI, only those ending in a nonaromatic, hydrophobic residue (L,I,V,M), and Cys are able to bind to and stabilize H-2 D^b and K^b molecules (Tables 1 and 2).

The effect of competition for class I binding sites in this type of translocation assay was examined using wild-type microsomes and the H-2^b binding peptide ^{3}H-FAPGNYPAL. Under these conditions, where the ability of the substrate to combine with ER-resident class I molecules is crucial to observe peptide accumulation (Fig. 2 *A*), those peptides that will bind to the class I molecules present compete far more efficiently than those that do not (Table 3).

The TAP Peptide Translocator Prefers Peptides with Free α-NH$_2$ and -COOH Groups of a Minimum Length of Nine Amino Acids. Replacement of a free COOH terminus with a COOH-terminal amide reduces efficacy of competition, as does acetylation of the NH$_2$ terminus (Table 2), suggesting that the presence or proper positioning of free α-NH$_2$ and -COOH groups may facilitate transport.

Truncation of FAPGNYPAL from the NH$_2$ terminus leads to a dramatic loss of inhibitory capacity such that the octamer APGNYPAL is no longer a good competitor for uptake of ^{125}I-TYNRTRALI (Fig. 4). However, peptides carrying NH$_2$-terminal extensions of up to four residues compete as efficiently as does the nine-residue peptide (Fig. 4).

COOH-terminal extensions of FAPGNYPAL consisting of Gly or Gly-Gly greatly impair the ability of these longer peptides to serve as substrates for the TAP translocator (Table 2), consistent with the preference for a large hydrophobic COOH-terminal residue. Thus, the presence within a longer peptide of a nonamer that is itself translocated efficiently, is not sufficient to drive translocation.

Table 2. *The TAP Transporter Prefers Peptides with Free Peptide Termini*

Competitor	IC_{50}
	μM
FAPGNYPAL*	5.2
FAPGNYPALG	>100.0
FAPGNYPALGG	>100.0
Acetyl-FAPGNYPAL	>100.0
FAPGNYPAL-amide	>100.0

The peptides FAPGNYPAX (in which *X* is any of the 20 naturally occurring amino acids) were tested for inhibition of uptake of ^{125}-I TYNRTRALI. Peptides are denoted by their COOH-terminal residues in single letter code. All values are means of duplicates and were expressed and plotted as a percentage of maximal FAPGN[^{3}H]YPAL uptake: 100% × [uptake (+ATP, +competitor)] − [uptake (−ATP)]/[uptake (+ATP)] − [uptake (−ATP)]. IC_{50} values were calculated based on curves as shown in Fig. 3 IC_{50} is defined as the concentration of competitor at which uptake of the ^{125}I-TYNRTRALI peptide is half maximal. Class I stabilization was assayed as described previously (1). The peptide TYNRTRALI was labeled by chloramine T–catalyzed iodination to a specific activity of ~30 Ci/mmol, and was used under conditions described in Materials and Methods, at a concentration of 60 nM. Note that the FAPGNYPAL peptide, but not the TYNRTRALI/TYQRTRALX peptides, contain the anchor residues found in both K^b and D^b bound peptides.

* Measured using β_2m$^-$ microsomes, and ^{125}I-TYNRTRALI substrate.

Discussion

The Peptide Preference of the TAP Translocator and Class I Molecules Is Similar but Not Identical. The TAP1–TAP2 complex translocates peptides with an aromatic or large aliphatic COOH terminus. Peptides having an aromatic COOH-terminal residue, although effective competitors for transport (Table 1), are seldom seen associated with mouse class I molecules (15). Conversely, some peptides that can bind to class

Table 3. *IC_{50} Values of FAPGNYPAL and FAPGNYPAL Derivatives in B6 Microsomes*

Competitor FAPGNYPAX, *X* =	IC_{50}
	μM
Isoleucine	0.1
Leucine	0.1
Methionine	0.1
Valine	0.4
Cysteine	0.6
Phenylalanine	0.8
Tryptophan	1
Tyrosine	3
Threonine	7
Alanine	14
Glutamine	36
Histidine	66
Lysine	66
Asparagine	82
Proline	>100.0
Serine	>100.0
Glutamic acid	>100.0
Aspartic acid	>100.0
Glycine	>100.0
Arginine	>100.0

IC_{50} is defined as the concentration of competitor at which uptake of the [^{3}H]FAPGNYPAL peptide is half maximal. Competition experiments were performed using B6 microsomes and [^{3}H]FAPGNYPAL substrate. Data show (together with the data in Table 1) the effect of competition for MHC class I binding sites on peptide accumulation in microsomes.

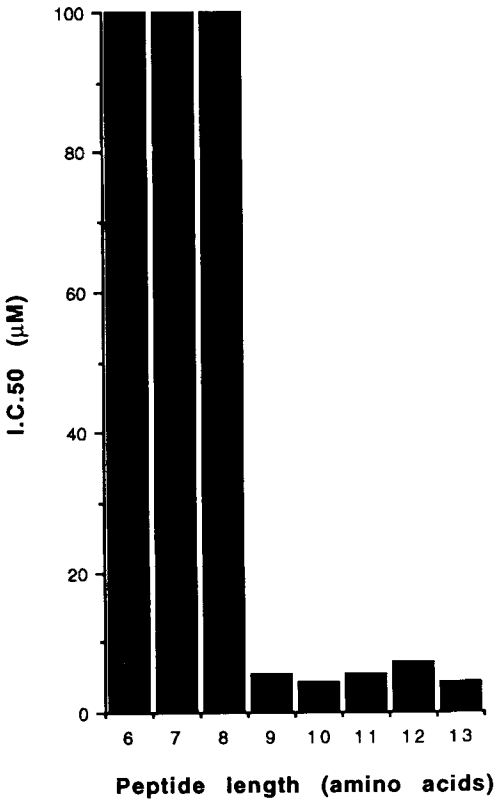

Figure 4. The TAP peptide translocator prefers peptides of a minimum length of 9 amino acids. Peptides tested were the following: GGTTFAPGNYPAL, GGTFAPGNYPAL, GGFAPGNYPAL, GFAPGNYPAL, FAPGNYPAL, APGNYPAL, PGNYPAL, and GNYPAL. IC_{50} values were determined from titration curves as shown in Fig. 3. Peptide uptake experiments were performed as described in Materials and Methods.

I molecules appear to be poor substrates for the peptide transporter (e.g., FAPGNYPAA, Table 1). TAP may therefore act as a restriction point in antigen presentation (27, 28).

The observation that the preference of the TAP translocator for a hydrophobic COOH terminus matches the preference of mouse class I products suggests that the COOH terminus of peptides that are translocated might be direct substrates for the class I molecules present. However, the human HLA-B27 class I product, which has a strong preference for peptides terminating in Lys or Arg (17), can present peptides efficiently when transfected into mouse cells (Rowland Jones, A., personal communication). As peptides terminating in Lys and Arg are not transported well by the mouse TAP peptide translocator (Table 1), (a) precursor(s) with COOH-terminal extensions could be transported, and trimmed to the proper length after arrival in the ER. It remains to be determined whether in human cells, those alleles that prefer basic residues at the COOH terminus (thus far shown for Aw68 and B27) rely on COOH-terminal trimming of peptides in the ER, or that the specificity of the human transporter includes those basic COOH-terminal residues.

The Transport of Peptides Longer than Nine Amino Acids Suggests the Possibility of ER Processing. Presentation of a 12-residue peptide encoded by a minigene has been shown to require both a functional TAP complex and ER-directed expression of a carboxypeptidase (26). Furthermore, TAP-dependent association of a subpopulation of HLA-B27 molecules with peptides up to 30 residues long in human cells has been observed (28a). Both these observations suggest that TAP may translocate peptides longer than those usually found associated with class I molecules. The experiments described here show directly that TAP transports peptides with a minimum length of nine amino acids. Consequently, many of the peptides that arrive in the lumen of the ER will require additional trimming by amino-peptidases and carboxypeptidases in order to convert them to high affinity, class I binding peptides. Since longer peptides can combine with class I molecules, albeit it with lower affinity (29), such trimming may well occur after binding.

The ability of TAP to transport peptides longer than those usually found associated with class I molecules may serve to increase the efficiency of antigen presentation. If only peptides of 8–10 amino acids ending in a hydrophobic residue could be transported, most of these sequences would contain only part of a presentable peptide. For instance, the Sendai nucleoprotein sequence KDPVHGE**FAPGNYPAL**WSYA could produce many peptides of 8–10 amino acids ending in a hydrophobic amino acid compatible with translocation (underlined) of which only three (**EFAPGNYPAL**, **FAPGNYPAL**, and **FAPGNYPALW**) would contain a sequence that can bind to H-2^b molecules, the others containing only part of the H-$2K^b$–restricted epitope. Thus, the transport of peptides longer than those usually found associated with class I molecules increases the chance of transporting fragments containing a peptide that can bind to the class I molecules present.

Peptide Efflux Provides a Mechanism for the Accumulation of High-affinity Peptides by Class I Molecules. The accumulation of peptide in mouse microsomes is dependent on retention of the substrate. In the case of the FAPGNYPAL peptide this retention is likely to be caused by combination with MHC class I molecules, as it is dependent on the presence of β_2m. However, unlike for cell surface–disposed class I molecules, the binding of this peptide to the class I molecules that reside in the microsomal ER equivalents is rapidly reversible: a temperature- and ATP-dependent efflux of peptide is observed. Association of ER-resident class I molecules with a heat shock protein type-chaperonin might result in a population of class I molecules with a more relaxed binding specificity, capable of exchanging peptides rapidly, until dissociation of the chaperone would lead to a quasi "irreversible" capture of substrate. In this model, the observed ATP dependency for peptide egress either could be imposed by the putative class I bound chaperonin, or by the proteins involved in delivery of peptide back to the cytosol. This/these protein(s) may not be the TAP1-TAP2 complex, as antibodies directed against TAP1 and TAP2 fail to inhibit the efflux of peptide (Schumacher, T. N. M., unpublished observations).

If TAP were to transport a very similar set of peptides regardless of MHC haplotype (the rat is an exception [8, 25a), as suggested by the very limited polymorphism in the TAP genes

(30), then most of the peptides in the ER would be of low affinity or would not fit the class I molecules present. By continuously perfusing the ER with peptides at low steady state concentrations—a possibility suggested by our data—ER-resident empty class I molecules can sample the peptides that pass and retain those with the best fit. This mode of accumulation of high-affinity peptides bound to class I molecules could explain the occurrence of class I–restricted epitopes only in the presence of the corresponding restriction element (31).

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