

Trimming of TAP-translocated Peptides in the Endoplasmic Reticulum and in the Cytosol during Recycling

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Summary

Cytosolic peptides are translocated to the endoplasmic reticulum (ER) lumen by the transporters associated with antigen processing (TAP), where major histocompatibility complex (MHC) class I molecules associate with peptides of about 8–10 amino acids. TAP translocates peptides of 9–13 amino acids with the highest relative affinity but also longer and shorter peptides. The fate of the peptides that fail to associate with class I molecules because of incorrect sequence or length, is unknown. Here we show that the bulk of the translocated peptides are rapidly released from the ER by a mechanism that requires adenosine triphosphate (ATP) and that could not be inhibited by GTP γ S. TAP does not appear to be involved in this process. Whereas free peptides are slowly trimmed in the ER lumen, they are rapidly degraded in the cytosol. A fraction of the peptides released from the ER escapes complete degradation in the cytosol and recycles back to the ER in a TAP-dependent fashion. These results suggest that peptides that are too long for binding to class I molecules in the ER can be trimmed further in the ER lumen or, alternatively, can be transported back to the cytosol where a fraction of the peptides is trimmed to a size suitable for association to MHC class I molecules and recycles back to the ER.

MHC class I molecules present fragments of proteins to CD8⁺ T cells. These fragments are generally derived from nuclear and cytosolic proteins (for review see 1, 2). Since the class I H chain/ β_2 -microglobulin (β_2m)¹ heterodimer associates with these peptide fragments in the endoplasmic reticulum (ER) lumen (3, 4), the peptides have to be translocated from the cytosol to the ER. Genetic studies have resulted in the identification of two multimembrane spanning proteins (TAP1 and TAP2) that are essential for peptide loading of class I molecules (5–11). TAP1 and TAP2 form a heterodimer (12, 13) and the hydrolysis of ATP is required for peptide translocation by TAP (14–17).

MHC class I molecules bind peptides that are usually 8–10 amino acids in length (1, 18), although longer sizes have been reported (19). Class I molecules bind peptides of a restricted size because the peptide NH₂ and COOH termini have to associate with the extremities of the class I peptide binding groove to facilitate a stable interaction (20–24). Furthermore, a defined class I allele does not associate with all possible peptides but selects for certain peptides because these peptides

contain amino acid side chains at defined positions that dock into pockets in the class I peptide binding groove (20–24). The amino acid side chains (anchor residues) have been identified by pool sequencing the set of peptides eluted from defined class I alleles (25).

Whether peptide translocation by TAP follows similar restrictions on peptide size and sequence, has recently been elucidated. Murine TAP and the rat TAP2^u allele selects for hydrophobic and aromatic COOH-terminal residues, whereas human TAP and the rat TAP2^a allele do not show such a selectivity (26–28). Also selectivity for amino acids at other positions in the peptide substrate has been observed (our unpublished observations). TAP shows a size selectivity that is broader than that of class I molecules. TAP efficiently translocates peptides of 9–13 amino acids (17, 27, 29), but shorter and longer peptides are also translocated albeit with decreasing affinity (29). Thus, although TAP prefers peptides with a length similar to the peptides binding to class I molecules, it does not restrict translocation of longer peptides.

The fate of the peptides once translocated to the ER, is unclear. Falk et al. (30) suggested that peptides that are too long to perfectly fit the class I peptide binding groove still associate and are trimmed accordingly to the correct size. It has recently been reported that the half-life of peptides in the ER lumen is relatively short (27, 29), but the fate of the

¹ Abbreviations used in this paper: β_2m , β_2 -microglobulin; ER, endoplasmic reticulum.

peptides (or fragments thereof) is unclear. Here, we followed the fate of two peptides that do not associate with resident class I molecules in our model system because of incorrect sequence or size. We show that peptides are more rapidly translocated from the ER to the cytosol than degraded in the ER lumen, although trimming in the ER can be visualized. The major site of peptidase activity is the cytosol. However, a fraction of the peptides released from the ER escapes complete degradation in the cytosol and is transported back to the ER lumen in a TAP-dependent fashion. Our data suggest that two mechanisms exist for the trimming of peptides to a size suitable for association with class I molecules: by trimming in the ER and by cytosolic peptidases during peptide recycling over the ER membrane.

Materials and Methods

Peptides. The peptide SN14/13 (sequence IETVDNKASTRAY) has been synthesized with a free COOH terminus by t-boc chemistry on a peptide synthesizer (model SAM2; Biosearch, San Rafael, CA). Peptide no. 417 (sequence TVNKTERAY) has been synthesized with a free COOH terminus by f-moc peptide chemistry using a multiple synthesizer (ABIMED, Langenfeld, Germany). The peptides were analyzed by HPLC and were >90% pure.

10 μ g of peptide was radio iodinated using chloroamine T-catalyzed iodination (31) and free iodine was removed by anion exchange chromatography (Dowex[OH-]; Bio-Rad Laboratories, Richmond, CA). The specific activity of the iodinated peptides ranged from 20 to 50 μ Ci/ μ g. The iodinated peptides were stored at -20° C.

Preparation of Microsomes and Cytosol. Microsomes were prepared from 3×10^9 T2 or T2/TAP1 + 2 cells. Cells were pelleted and washed one time with 20 ml STKMM buffer (250 mM sucrose, 50 mM triethanolamine-HCl, pH 7.5, 50 mM KAc, 5 mM MgAc₂, and 0.1% β -mercaptoethanol). The cell pellet was resuspended in 10 ml H₂O and homogenized by douncing (15 \times). 30 ml STKMM buffer was added and the nuclei and cell fragments were removed by centrifugation for 10 min at 7,500 rpm (in a GA/18 rotor, Beckman Instruments, Palo Alto, CA). The supernatant was then pelleted by centrifugation for 40 min at 18,000 rpm and the resulting pellet was resuspended (by douncing) in 20 ml STKMM buffer. Microsomes were again pelleted by centrifugation for 40 min at 18,000 rpm. Pellets were aliquoted and stored at -70° C. The amount of microsomes was determined by measuring the OD at 280 nm.

Cytosol was generated by repeated freeze-thaw cycles followed by removal of the membranes by centrifugation. The cytosol was diluted approximately fourfold.

Peptide Translocation and Recycling. Peptide translocation was performed as described (15). Briefly, $2-5 \times 10^6$ T2 or T2/TAP1 + 2 cells/incubation were harvested and washed once with incubation buffer (130 mM KCl, 10 mM NaCl, 1 mM CaCl₂, 2 mM MgCl₂, 2 mM EGTA, and 5 mM HEPES, pH 7.3). Cells were permeabilized with 2 U/ml streptolysin O (Wellcome, Beckenham, UK) for 10 min at 37° C. Free streptolysin O and released cytosol was removed by washing the permeabilized cells twice with incubation buffer (2 min at 800 g) and 10 μ l radioiodinated peptide and 10 μ l 100 mM ATP were added to a final volume of 100 μ l. Translocation was performed at 37° C for the times indicated. Nonbound peptides were removed by pelleting the permeabilized cells (2 min at 800 g) followed by washing, and the cell pellet and supernatant fraction were extracted by phenol. The phenol phase (containing most peptides) was analyzed by TLC (Kieselgel 60; Merck, Darm-

stadt, Germany) run in *N*-butanol/pyridine/acetic acid/water (97:75:15:60) and exposed by autoradiography (XAR5 films; Eastman Kodak Co., Rochester, NY). To inhibit N-linked glycan addition, T2 or T2/TAP1 + 2 cells were cultured for 45 min in the presence of tunicamycin (Sigma Chemical Co., Mountain View, CA) at a final concentration of 10 μ g/ml and at a cell density of 10^6 per ml, before permeabilization with streptolysin O.

The permeabilized and peptide-loaded cells were cultured for 10 min in the presence of 10 mM ATP and 5 μ l T2 or T2/TAP1 + 2 derived microsomes (of OD₂₈₀ = 105) in a final volume of 100 μ l. Glycosylated peptides were isolated from the microsomes with Con A-Sepharose after lysis in NP-40 lysis mix (15). The Con A-Sepharose beads were washed five times and quantitated by gamma counting. Additional cytosol was recovered from 10^7 T2 or T2/TAP1 + 2 cells after permeabilization with streptolysin O in a total volume of 100 μ l followed by removal of the cell pellet. 50 μ l of the supernatant fraction was added to each incubation.

Results

Peptide Release from the ER Lumen and Peptide Degradation. TAP preferably translocates peptides that are in part larger (8–13 amino acids; reference 17, 27, 29) than the peptides that are usually found associated with MHC class I molecules (8–11 amino acids, generally 9 amino acids) (1). We followed the 13-mer peptide SN14/13 after translocation in T2 cells, which lack the genes coding for the TAP subunits (32), and in T2 cells transfected with rat TAP1 and 2^a cDNA (T2/TAP1 + 2; reference 33). Any difference in peptide loading between T2 and T2/TAP1 + 2 cells should therefore be due to TAP-activity. In the assay used to follow TAP-dependent peptide translocation from the cytosol to the ER (15), the cells were first permeabilized with streptolysin O to gain access to the cytosol and then incubated with the 13-mer peptide in the presence of ATP. Peptides that had failed to become translocated into the ER were removed by washing. The cells were then cultured in the presence or absence of ATP for different times, followed by analysis of the cell-associated and the supernatant fraction by TLC (Fig. 1 A). The input 13-mer peptide was exclusively associated with the pellet fraction of T2/TAP1 + 2 cells, indicating that the cell-associated peptide was translocated into the ER by TAP. Note the presence of other peptide fragments associated with the pellet of T2/TAP1 + 2 cells, which are the result of trimming of the input peptide before translocation by TAP (29).

The peptide translocated in T2/TAP1 + 2 cells is efficiently released from the ER in the presence of exogenous ATP and arrives in the cytosolic fraction where the peptide was rapidly degraded by peptidases (Fig. 1 B; reference 29). In the absence of exogenous ATP, the translocated peptide slowly disappeared from the ER (the pellet fraction), suggesting that the retained peptides are only slowly degraded. However, since release of the peptide in the presence of exogenous ATP (which reflects the normal situation in cells) appears to be faster than possible degradation of the ER-retained peptides in the absence of ATP, a major portion of the peptides translocated to the ER will rapidly leave the ER under physiological conditions, and are consequently degraded by cytosolic peptidases (29).

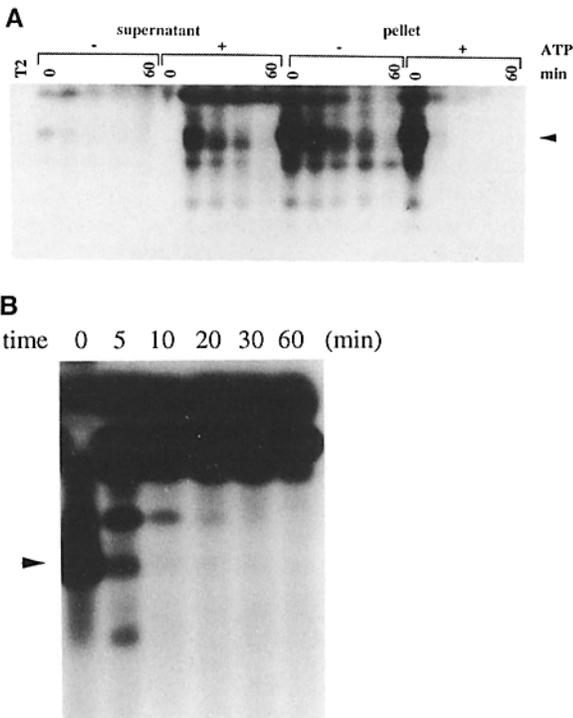


Figure 1. Fate of TAP-translocated peptides. (A) Release of peptides from the ER. T2 or T2/TAP1 + 2 cells were permeabilized with streptolysin O, the cytosol was removed followed by incubation for 10 min with the radioiodinated peptide SN14/13 in the presence of 10 mM ATP at 37°C. Free, nontranslocated peptides were removed by washing at 0°C and the cells were cultured at 37°C in the presence or absence of 10 mM ATP for 0, 10, 20, 30, or 60 min. At the respective timepoints, cells were pelleted and the supernatant and pellet fraction were analyzed by TLC. The arrow indicates the position of the input peptide. The iodotyrosine degradation product that associates nonspecifically with membranes (see also Fig. 4 A) has been removed from the TLC. After peptide incubation for 10 min, no peptide is associated with the pellet of T2 cells (lane T2), indicating that the peptides associated with T2/TAP1 + 2 cells are retained as the result of TAP activity. ATP induces rapid release of the peptide from the pellet fraction of T2/TAP1 + 2 cells and appearance of the peptide in the supernatant fraction. In the absence of ATP, the peptide is retained in the pellet fraction of T2/TAP1 + 2 cells and is slowly degraded. (B) Degradation of peptide SN14/13 by cytosolic peptidases. The radioiodinated peptide SN14/13 was incubated at 37°C with cytosol derived from T2 cells. At the timepoints indicated, a sample was extracted with phenol and analyzed by TLC. The arrow indicates the position of the input peptide. The peptide is quantitatively degraded by cytosolic peptidases within 10 min.

Glycosylated Peptides Are Not Released from the ER Lumen. To show that the peptides are released from the ER by a specific mechanism and not by membrane leakage or budding of vesicles from the ER, we followed ER-release of the glycosylated 9-mer peptide no. 417 and its nonglycosylated counterpart in T2/TAP1 + 2 cells cultured in the absence or presence of the drug tunicamycin that inhibits N-linked glycosylation (34). The translocated peptides were analyzed by TLC (Fig. 2 A). The cells were permeabilized with streptolysin O, peptide no. 417 was translocated by TAP (see also Fig. 4 A), and the nontranslocated peptides were removed. The nonglycosylated peptide is rapidly released from the cell pellet (the ER) in an ATP-dependent fashion. Note

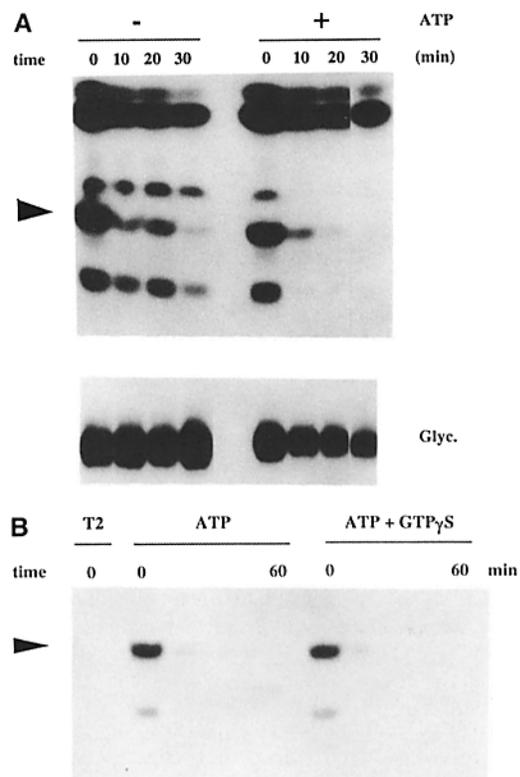


Figure 2. Specificity of peptide release from the ER. (A) Release of glycosylated peptides. T2/TAP1 + 2 cells were cultured in the absence or presence of tunicamycin and subsequently permeabilized with streptolysin O. Radioiodinated peptide no. 417 and 10 mM ATP were added for 10 min followed by removal of the free, nontranslocated peptide. The cells were then cultured in the presence or absence of ATP. After 0, 10, 20, and 30 min (as indicated) a sample was taken and the pellet fraction was extracted with phenol and analyzed by TLC. The arrow shows the position of the input peptide no. 417. The nonglycosylated translocated peptides are rapidly released from the ER of tunicamycin-treated cells (top) in an ATP-dependent fashion, and is only slowly trimmed in the absence of ATP. However, when peptide no. 417 was translocated by control T2/TAP1 + 2 cells, the resulting glycosylated peptide was retained in the pellet fraction both in the presence and in the absence of ATP (panel glyce). (B) Effect of GTP γ S on peptide-export. Tunicamycin-treated T2 and T2/TAP1 + 2 cells were permeabilized with streptolysin O and loaded with peptide no. 417 for 10 min in the presence of 10 mM ATP. Free peptides were removed and the T2/TAP1 + 2 cells were cultured for 0, 5, 10, 30, and 60 min with ATP (10 mM) in the presence or absence of GTP γ S (1 mM), as indicated. Samples were taken at the timepoints indicated and the pellet was analyzed by TLC. GTP γ S does not affect the release of peptide no. 417 from the ER lumen of T2/TAP1 + 2 cells.

that, in the absence of ATP, the 9-mer peptide is relatively stable in the ER. When cells were not pretreated with tunicamycin, the glycosylated peptide was retained in the cell-bound fraction during culture, indicating that only the nonglycosylated peptides are selectively released from the ER (Fig. 2 A, bottom). To further exclude release of peptides from cells by transport through the exocytic pathway, we again loaded streptolysin O-permeabilized T2/TAP1 + 2 cells with peptide and inhibited vesicular transport from the ER with 1 mM GTP γ S (35). ATP-dependent peptide release from the pelletable fraction is not influenced by GTP γ S (Fig. 2 B).

Is TAP Involved in Peptide Export? Since peptides are imported in the ER by TAP (14–16), the possibility that TAP is involved in peptide export is analyzed. TAP-dependent peptide import can be inhibited by exogenously added competing peptides (Fig. 3 A). Release of peptides from the ER, however, is not influenced by exogenously added excess peptides, which might have been envisaged if TAP was able to translocate peptide ligands from either side of the ER membrane (Fig. 3 B). Furthermore, TAP-dependent peptide translocation is inhibited at low pH (Fig. 3 A), whereas peptide export is not (Fig. 3 B), suggesting that peptides are selectively released from the ER by a mechanism not involving TAP activity.

Peptide Recycling over the Membrane of the ER. Peptides of up to 13 amino acids are translocated to the ER lumen and a fraction of these peptides are exported back to the cytosol (Fig. 1 A). These peptides may be further trimmed by cytosolic peptidases (Fig. 1 B) and a fraction of the peptides, which may now have the correct size for binding to MHC class I molecules, may again enter the ER. To show recycling of peptides over the membrane of the ER, T2 and T2/TAP1 + 2 cells were cultured in the presence of tunicamycin to inhibit N-linked glycosylation. The cells were permeabilized and subsequently incubated with peptide no. 417 that contains an N-linked glycosylation consensus sequence. The input peptide is rapidly degraded by cytosolic and membrane peptidases (Fig. 4 A). However, a peptide migrating at the position of the input peptide is selectively retained in permeabilized T2/TAP1 + 2 cells after extensive washing. This must be due to TAP-dependent peptide translocation into the ER, because the peptide is not retained in T2 cells (Fig. 4 A). Note the breakdown product (iodotyrosine) that associates with the membrane of permeabilized T2 as well as T2/TAP1 + 2 cells. The washed and permeabilized T2 or T2/TAP1 + 2 cells were subsequently cultured in the presence of equal amounts of microsomes derived from T2 or T2/TAP1 + 2 cells. Nonglycosylated peptides released from the ER of tunicamycin-pretreated permeabilized cells will obtain an N-linked glycan only after entering the microsomes. The glycosylated peptides were then isolated by Con A-Sepharose and quantitated by gamma counting (15) (Fig. 4 B). No glycosylated peptides are recovered from T2 cells incubated with microsomes from either T2 or T2/TAP1 + 2 cells. Glycosylated peptides are isolated by Con A-Sepharose only when peptide-loaded T2/TAP1 + 2 cells are cultured in the presence of T2/TAP1 + 2 microsomes but not T2 microsomes. Thus, peptides are released from the ER lumen of streptolysin O-permeabilized T2/TAP1 + 2 cells and a fraction of the peptide is able to enter microsomes in a TAP-dependent fashion, implying peptide recycling over the ER membrane. We then repeated the experiment of Fig. 4, A and B and followed transport of peptides from tunicamycin-treated cells to microsomes in the absence or presence of additional cytosol and thereby added peptidase activity (Fig. 1 B; reference 29). Additional cytosol decreases the recovery of glycosylated peptides (Fig. 4 C). Since TAP-activity as well as the mechanism that releases peptides from the ER is inde-

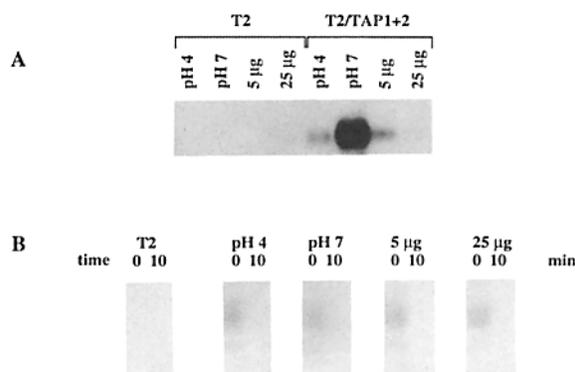


Figure 3. Is peptide export from the ER TAP-dependent? (A) Inhibition of TAP-dependent peptide translocation. T2 or T2/TAP1 + 2 cells were permeabilized with streptolysin O and incubated with the radioiodinated peptide no. 417 in the presence of ATP at either pH 4 or 7 or in the presence of 5 or 25 µg of the unlabeled peptide no. 417, as indicated. The cells were washed, the peptides extracted with phenol and analyzed by TLC. TAP-dependent peptide translocation can be inhibited at pH 4 and with exogenous competing peptides. (B) Competition for peptide-export from the ER lumen. Tunicamycin-treated T2 and T2/TAP1 + 2 cells were permeabilized with streptolysin O and incubated with peptide no. 417 for 10 min in the presence of 10 mM ATP. Free, untranslocated peptides were removed by washing and the peptide-loaded cells were cultured in the presence of ATP at either pH 4, pH 7, or in the presence of O (lane pH 7), 5, or 25 µg of the competing peptide no. 417, as indicated. Samples were analyzed at the respective timepoints by TLC. The translocated peptides were released within 10 min under all tested conditions. As expected, peptides were not associated with the cell pellet of streptolysin O-permeabilized T2 cells (lane "T2").

pendent of cytosol (not shown), this suggests that the efficiency of peptide recycling is inversely correlated with cytosolic peptidase activity.

Discussion

An essential step in the process of antigen presentation by MHC class I molecules is the translocation of peptides from the cytosol to the ER lumen. This is mediated by a heterodimer of two multimembrane spanning proteins, termed TAP, and requires the hydrolysis of ATP (14–16). A subset of the translocated peptides is subsequently selected by the MHC class I H chain/ β_2m heterodimer and presented at the cell surface to CD8⁺ T cells. MHC class I molecules select peptides on sequence and on size (for review see 1). However, this set of peptides may already be biased by the peptide generation system (that is still ill defined) and by the substrate selectivity of TAP. TAP has a broad, but certainly not indiscriminate, sequence specificity (26–28). Furthermore, TAP has a broader size selection than MHC class I molecules (27–29). It has long been a question what the fate of the peptides is that, because of inappropriate size or sequence, fail to associate with class I molecules in the ER lumen. One possibility would have been removal of the peptides from the ER by bulk flow (36). Alternatively, peptides are removed from the ER lumen by transport to the cytosol. This has been shown for

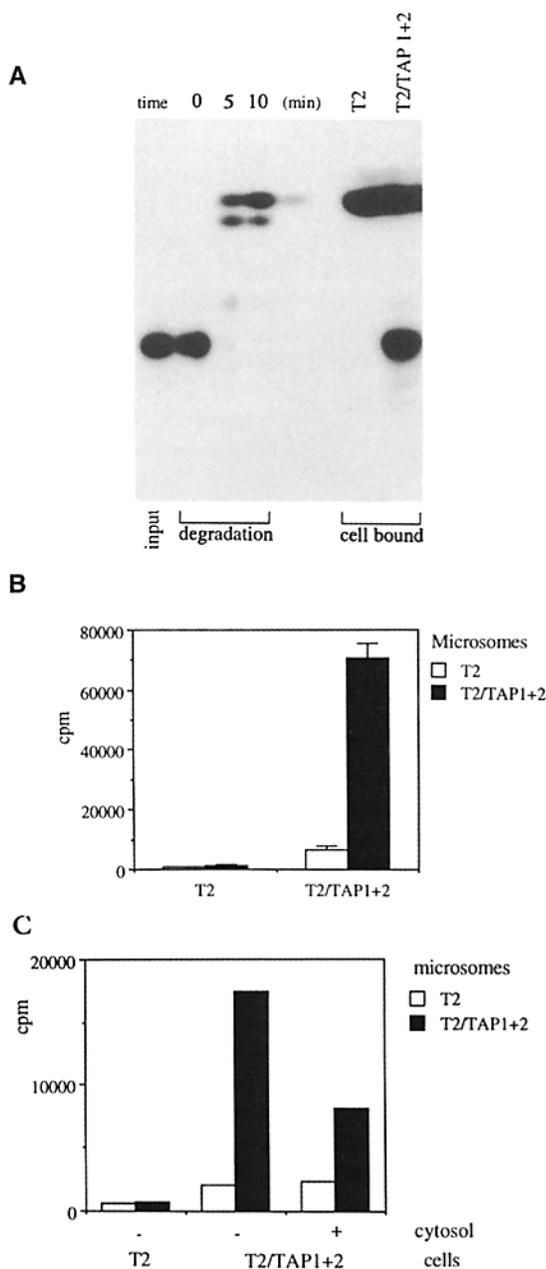


Figure 4. Peptide recycling over the membrane of the ER. (A) Peptide uptake by streptolysin O-permeabilized T2/TAP1 + 2 cells. Tunicamycin-pretreated T2 or T2/TAP1 + 2 cells were permeabilized by streptolysin O. The permeabilized cells were incubated for 10 min with the radioiodinated peptide no. 417 in the presence of 10 mM ATP. After 0, 5, and 10 min incubation, a sample of the incubation mixture was analyzed by TLC after phenol extraction. The input peptide (lane “input”) is rapidly degraded (panel *degradation*). After 10 min of incubation, the permeabilized T2 and T2/TAP1 + 2 cells were washed to remove the peptides in the supernatant and a fraction of the cell pellet was extracted with phenol and analyzed by TLC. The input peptide is selectively retained in the cell-bound fraction of T2/TAP1 + 2 cells. (B) Recycling of peptides. The tunicamycin-treated and permeabilized T2 or T2/TAP1 + 2 cells (as indicated on the *horizontal axis*) loaded with radioiodinated peptide no. 417 (see Fig. 4 A) were cultured for 10 min in the presence of 10 mM ATP and microsomes from either T2 cells (*open bars*) or T2/TAP1 + 2 cells (*solid bars*), followed by lysis. The glycosylated peptides were isolated by

glycosylated trimer peptides in yeast-derived microsomes (37). Finally, it has been proposed that peptides that are too long to allow efficient binding to class I molecules, are still binding and are subsequently trimmed to a proper size while still associated with class I molecules (30). To follow the fate of free peptides after arrival in the ER lumen and eliminate any effect of binding to class I molecules on the kinetics of ER release and recycling of peptides, we have followed the fate of peptides that do not associate with the resident class I molecules in T2 cells (not shown). Since stable association to class I molecules of peptides with incorrect size does not occur (38), our model peptides plausibly also describe the fate of peptides that require trimming before they can bind to class I molecules. We directly show that peptide trimming in the ER occurs albeit at a considerably lower rate than in the cytosol. In addition, we provide evidence for an alternative route of peptide trimming and show that a considerable fraction of ER-located peptides are efficiently secreted into the cytosol by a mechanism that probably does not involve TAP activity. The secreted peptides are efficiently trimmed after arrival in the cytosol but, as we show, a fraction escapes cytosolic degradation and recycles back to the ER lumen in a TAP-dependent fashion. The efficiency of peptide recycling is unclear and depends on the rate of cytosolic peptidase activity. Possibly, TAP competes with cytosolic peptidases for peptides and it is therefore likely that the local concentration of TAP (which is probably high at the location of peptide release, the ER) also determines the efficiency of recapture of released peptides.

Under our experimental conditions, intracellular transport from the ER is inhibited (data not shown and indicated by the observation the peptides glycosylated after translocation contain exclusively high mannose carbohydrates [29]). Consequently, peptides are released from the ER. It is at present unclear which molecules are involved in the process of peptide release. Because we follow peptide release from the ER after the peptides have entered the ER due to TAP activity, we have not been able to identify the substrate specificity of the ‘back-transport system’. At least, peptide lengths that are efficiently imported in the ER by TAP (9–13 amino acids, references 27, 29) are efficiently released as well.

Con A-Sepharose from the lysis mixture and quantitated by gamma counting. When peptide no. 417 is transported from the tunicamycin-treated cells to the microsomes, it will obtain an N-linked glycan. No glycosylated peptides can be recovered when permeabilized T2 cells are incubated in the presence of microsomes from T2 or T2/TAP1 + 2 cells. Glycosylated peptides can only be recovered when peptide-loaded T2/TAP1 + 2 cells are cultured in the presence of microsomes from T2/TAP1 + 2 and not T2 cells, indicating that peptides are transported from the ER lumen of permeabilized tunicamycin-treated T2/TAP1 + 2 cells into the microsomes of T2/TAP1 + 2 cells, where peptide no. 417 is glycosylated. (C) Effect of additional cytosol on the efficiency of peptide recycling. The experiment shown in 4, A and B was repeated but transport of peptide no. 417 from the ER of tunicamycin-treated T2 or T2/TAP1 + 2 cells to the respective microsomes was assayed in the presence or absence of additional cytosol. Additional cytosol decreased the recovery of glycosylated peptides.

What is the relevance of peptide release from the ER? We have shown that peptides are efficiently transported from the ER into the cytosol. Besides peptides are degraded in the ER. Thus, peptides (including signal sequences) that fail to bind to MHC class I molecules because of an incorrect size or sequence, are not secreted into the surrounding extracellular medium but efficiently translocated to the cytosol. Consequently, peptide loading of MHC class I or II molecules at the cell surface with endogenously derived (cytosolic) peptides is prevented. The peptides that are secreted from the ER lumen into the cytosol are rapidly degraded by cytosolic peptidases. A fraction of the peptides that were initially too long for efficient binding to class I molecules may be trimmed by cytosolic peptidases, escape complete degradation, and recycle back to the ER lumen for association with MHC class

I molecules. Peptide release from the ER lumen requires ATP, but it is unclear whether this is essential for the activity of a putative peptide back-transporter and/or the release of peptides from accessory molecules in the ER lumen. We have, however, been unable to show peptide binding to ER-resident proteins after NP-40 detergent lysis of streptolysin O-permeabilized cells loaded with peptide (data not shown).

Cytosolic peptide trimming during recycling appears to be an attractive option for preventing high protease activity in a compartment (the ER) that contains many proteins in the process of folding that would have been good substrates for proteases. The relative contribution in the generation of properly sized class I-binding peptides of peptide trimming in the ER vs. in the cytosol during recycling over the membrane of the ER, still has to be determined.

We thank Dr. C. Boog for discussions and E. Wojcik-Jacobs for the microsome preparation.

This research was supported by grant 900-509-155 from the Netherlands Organization for Scientific Research.

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Received for publication 14 June 1994.

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