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The Ins and Outs of Intracellular Peptides and Antigen Presentation by MHC class I Molecules

Adapted from Current Topics in Microbiology and Immunology

The Ins and Outs of Intracellular Peptides and Antigen Presentation by MHC Class I Molecules

Tom Groothuis and Jacques Neefjes

MHC class I molecules present small intracellular generated fragments to the outside surveying immune system. This is the result of a series of biochemical processes involving biosynthesis, degradation, translocation, intracellular transport, diffusion, and many more. Critical intermediates and end products of this cascade of events are peptides. The peptides are generated by the proteasome, degraded by peptidases unless transported into the ER where another peptidase and MHC class I molecules are waiting. Unless peptides bind to MHC class I molecules, they are released from the ER and enter the cytosol by a system resembling the ERAD pathway in many aspects. The cycle of peptides over the ER membrane with the proteasome at the input site and peptidases or MHC class I molecules on the output site are central in the MHC class I antigen presentation pathway and this review.

1 The Classical Model of Antigen Presentation by MHC Class I Molecules

The immune system uses MHC class I molecules to recognize intracellular residing pathogens. These MHC class I molecules present fragments of cytosolic or nuclear proteins of such a pathogen at the plasma membrane. This can only occur after at least three consecutive cell biological processes (Fig. 1). Since fragments of proteins are presented, intracellular degradation of proteins to peptide fragments should first occur. The vast majority of intracellular proteins are degraded by the proteasome into small fragments. These proteins may be degraded at the end of their normal life (old proteins), but a large fraction (up to even more than 70%) is degraded immediately after synthesis, probably due to a high failure rate in translation and folding (1-3). The

latter fraction is termed DriPs and couples viral infection and production of new viral proteins with immediate antigen presentation by MHC class I molecules (4). The endoplasmic reticulum (ER) of the cell operates a quality control system that identifies misfolded proteins, transports them into the cytosol and successively targets them for degradation by the proteasome. Aberrant protein degradation is the mechanism underlying many diseases, including cystic fibrosis and heritable forms of lung and liver disease. The pathways that orchestrate the destruction of aberrant proteins in the ER are collectively termed ER-associated degradation (ERAD) (5).

Still, both the old and the new proteins are degraded into smaller fragments by the proteasome, which is present in both the nucleus and the cytosol. Most of these fragments are larger than 15 amino acids and are further degraded by the cytosolic peptidase tripeptidylpeptidase II (TPPII, (6)) and subsequently other peptidases (7), until they are reduced to single amino acids.

To become immunologically relevant, the peptides have to bind to MHC class I molecules before they are reduced to single amino acids. The cytosolic protein degradation products are not spontaneously passing membranes, although this is required for association with MHC class I molecules, which are present in the lumen of the ER. Peptide translocation is driven by an ATP-dependent pump located in the ER membrane called TAP, for transporter associated with antigen processing. The pump is a member of the ATP-dependent transporter family that includes drug pumps such as multidrug resistance (MDR)

Abbreviations: ER, Endoplasmic reticulum; MHC, Major histocompatibility complex; TPPII, Tripeptidyl peptidase II; PDI, Protein disulfide isomerase; TOP, Thimet oligopeptidase; LAP, Leucine aminopeptidase; BH, Bleomycin hydrolase; MLC, MHC loading complex; PSA, Puromycin sensitive aminopeptidase; ERAD, ER-associated degradation; CFTR, Cystic fibrosis conductance regulator; MDR, Multidrug resistance; H-chain, Heavy-chain; ERAP, Endoplasmic reticulum aminopeptidase (also ERAAP); ERAAP, Endoplasmic reticulum aminopeptidase associated with antigen processing (also ERAP); BiP, Luminal binding protein; TAP, Transporter associated with antigen processing; ABC, ATP binding cassette; GFP, Green fluorescent protein; Hsp, Heat shock protein

and cystic fibrosis conductance regulator (CFTR) (8). TAP translocates many different peptides but excludes those with a modified N-terminus and those containing a proline residue at position 2 or 3 (9-11). TAP prefers peptides of 8–12 amino acids, but also handles longer peptides albeit less efficiently (12). Peptides shorter than 8 amino acids are not able to bind to TAP, which makes sense since these are of no immunological relevance because MHC class I molecules require a peptide of minimally 8 amino acids for a stable interaction.

Once in the ER, peptides can have different fates. The immunologically most relevant one is binding to MHC class I H-chain/ β_2m heterodimers. These heterodimers are mostly residing in the MHC class I loading complex (MLC), consisting of the peptide transporter, a dedicated chaperone tapasin, and at least two other, more common, chaperones Erp57 and calnexin (13). Peptide binding to the MHC class I heterodimer releases it from the MLC, allows passage along the ER quality system and transport to the

plasma membrane where it represents its cargo (the peptide) to the surveying immune system (14).

However, peptides can also undergo different fates. They can be trimmed by an ER-resident peptidase called ERAP (endoplasmic reticulum aminopeptidase), also named ERAAP (endoplasmic reticulum aminopeptidase associated with antigen processing) (15-17). This can trim ER resident peptides to the correct size for MHC class I binding and beyond (then destroying MHC class I binding peptides), but probably stops digesting peptides smaller than 8 amino acids.

Peptides can also bind to other ER proteins, mainly the ER chaperones PDI (protein disulfide isomerase), BiP (luminal binding protein), gp96 and gp170 (18-20). Whether peptides mimic unfolded protein segments and thus bind to chaperones or whether this interaction has another physiological meaning is unclear.

Finally, unbound peptides have to be removed from the ER, which otherwise would obtain

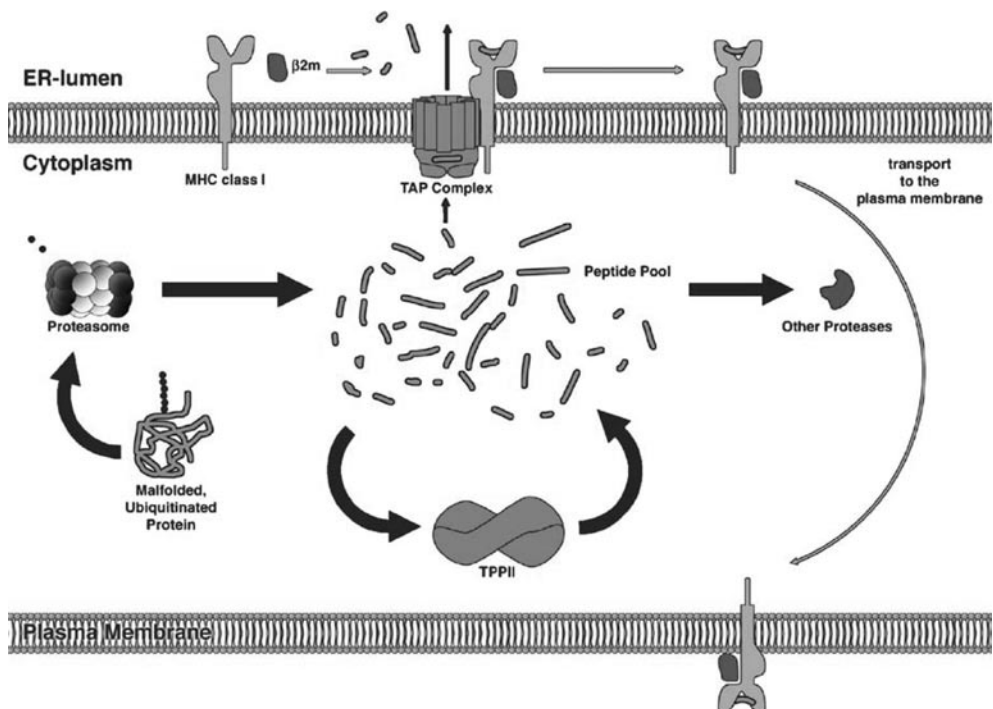


Figure 1. Antigen presentation by MHC class I molecules. Proteins that are destined for degradation by the proteasome are ubiquitinated. Polyubiquitinated proteins are recognized by proteasomes and cleaved into peptides. The majority of the peptides present in the peptide pool are successively trimmed by amino proteases, of which TPPII is the most dominant one. Peptides are trimmed until they are totally reduced to single amino acids. A small part of the peptides may escape the cytosolic proteases by translocation into the ER by the TAP transporter. Once in the ER lumen, the peptides may bind to MHC class I H-chain/ β_2m heterodimers. Peptides bound to these heterodimers form a stable complex that will be transported to the plasma membrane for antigen presentation to surveying cytotoxic T cells (CTLs)

high concentrations of peptides that subsequently may affect many other cellular processes. These ER peptides are not released by secretion through the normal secretory route (via ER to Golgi transport) but merely transported back into the cytosol by the same machinery as used for ER degradation of proteins employing Sec61/translocon mediated retrotranslocation back to the ER (21). These different processes ensure successful peptide loading of MHC class I molecules with peptides by employing various old, but highly conserved systems such as protein and peptide degradation, transporters, chaperones, the translocon, and many peptidases again. The end result, peptide presentation by MHC class I molecules, occurs in a very inefficient manner since less than 1% of the proteins degraded deliver a peptide for presentation by MHC class I molecules. The rest is completely turned over and of no immunological significance (2,22,23).

2 Behavior of Intracellular Peptides

Proteins are degraded by the proteasome in the cytosol and in the nucleus. The proteasome diffuses through but not between these compartments since it is excluded from transport through the nuclear pore. TAP is excluded from the nuclear site of the nuclear envelope, which implies that peptides generated in the nucleus have to access the cytosol before contacting the peptide transporter TAP (24). While diffusing through the various compartments in the cell, many peptides will be trimmed by aminopeptidase activities and only few (less than 2%) will contact TAP and enter the ER (2).

Peptides will be substrate to peptidases only in a free form. To gain more insight into the longevity of peptides, internally quenched peptides were injected into cells (6,24). Upon cleavage between the amino acids containing the two groups, the fluorophore is no longer quenched and fluorescence will appear. It turned out that peptides were rapidly and completely degraded within a few seconds. No additional pool of peptide degradation products was detected at later time points. Surprisingly, artificial N-terminal modifications of peptides sufficed to protect them from peptidase activity. This implies that cells contain only aminopeptidases and lack carboxy- and endopeptidase activity. In addition, the proteasome (which is an endopeptidase) does not digest peptides (24).

N-terminally protected (and thus stable) fluorescent peptides were introduced in living cells by microinjection. Their rate of diffusion was determined in FRAP experiments because mobility is in approximation proportional to $(\text{mass})^{-1/3}$ (25). The protected L-peptides (~1 kDa) moved faster than

GFP (green fluorescent proteins, 27 kDa) and GFP moved faster than proteasomes (an intact 20S proteasome is already 700 kDa) (24). This implies that the majority of peptide is moving in a free form rather than being associated to other proteins such as heat shock proteins (Hsps). Most likely, these peptides associate transiently to Hsps followed by rapid dissociation.

Do cells have a peptide sink? Closer examination revealed a significant pool of peptides dynamically associated to chromatin (in fact to histones). Whether this influences the MHC class I peptide-loading system in any way is unclear (24).

In conclusion, intracellular peptides are mostly free and rapidly moving by normal Brownian motion. In the cytosol, TAP and peptidases compete for these peptides. Peptidases are highly active and modify/destroy more than 99% of the TAP substrates, thus strongly reducing the number of peptides entering the ER.

3 Peptides and Peptidases

The proteasome degrades substrate proteins into fragments. In vitro studies suggest that these fragments are peptides of 4–20 amino acids (26). Peptides of 4–7 amino acids are excluded from TAP-driven ER import but longer ones (8–20 amino acids) can be transported into the ER (12,27). Peptides have a very short half-life in the cytosol/nucleus of intact living cells. Introduction of internally quenched peptides (as mentioned in Sect. 2) into cells to follow their turnover revealed that they are degraded within seconds and exclusively by aminopeptidases (6,24). The endopeptidase the proteasome is involved in the generation of peptides from proteins, but is irrelevant in peptide degradation. It can be calculated that under normal conditions, the collective cytosolic peptidase activities remove 1.5 amino acid/s (6). In other words, a 20-mer peptide will be fully degraded within 15 s but will be irrelevant for the immune system (that is shorter than 8 amino acids) within 8 s. Hence, for a peptide to become immunologically relevant, it should interact with TAP within 8 s after generation by the proteasome, or it will be degraded completely. Many peptides will fail to meet TAP before their destruction, which explains the inefficiency of the MHC class I antigen presentation pathway (7).

Various cytosolic peptidases have been identified (Fig. 2). These include leucine aminopeptidase (LAP) (28–30), bleomycin hydrolase (BH) (31–34), puromycin sensitive aminopeptidase (PSA) (35–38), thimet oligopeptidase (TOP) (7,39), neurolysin (40–42), and tripeptidylpeptidase II (TPPII) (43,44). LAP and TOP activity have been shown to affect the pep-

tide pool presented by MHC class I molecules (39). TOP as well as neurolysin is probably selective for peptides of 8 up to about 17 amino acids. Whether other peptidases show a defined substrate size selectivity is unclear, with the exception of TPPII (6).

TPPII is a huge homomultimeric protease (with a calculated size of 5–9 MDa, which is larger than the proteasome!) (45). Reits and colleagues have shown that TPPII represents the major proteolytic activity for peptides of 16 amino acids or larger (6). Since inhibition of TPPII by a specific compound called butabindide (46) inhibits peptide loading of MHC class I molecules and simultaneous inhibition of the proteasome does not further inhibit this, the current concept of peptide generation for MHC class I molecules is in consecutive order:

1. Generation of peptides from proteins by the proteasome. These peptides are mainly larger than 15 amino acids. The proteasome degrades protein substrates into peptide fragments mainly larger than 15 amino acids.
2. TPPII trims these into smaller fragments. This will be achieved by the removal of small (2–3 amino acids long) N-terminal sequences or longer (>8 amino acid) N-terminal fragments.

In the latter case, peptides with a new C-terminus are generated for MHC class I molecules.

3. The TPPII substrates will be further trimmed by other peptidases. Possibly TOP is specialized to trim TPPII substrates (it “likes” substrates smaller than ~17 amino acids), but the possible contribution of other peptidases is unclear.

These steps precede peptide import into the ER by a dedicated system, the peptide transporter TAP. A small fraction of peptides (probably less than 1% of peptides and less than 0.01% when starting from protein) survives the collective proteolytic activity by colliding into TAP and are transported to a less hostile environment, the ER lumen. And cytosolic peptidases become relevant again for those peptides failing to bind to MHC class I molecules in the ER lumen, since they will be retrotranslocated back in the cytosol for further trimming and destruction.

4 Peptide Import in the Endoplasmic Reticulum: The Transporter Associated with Antigen Processing

Peptides usually do not pass lipid bilayers. Consequently, a dedicated system has been developed to transport cytosolic peptides into the ER

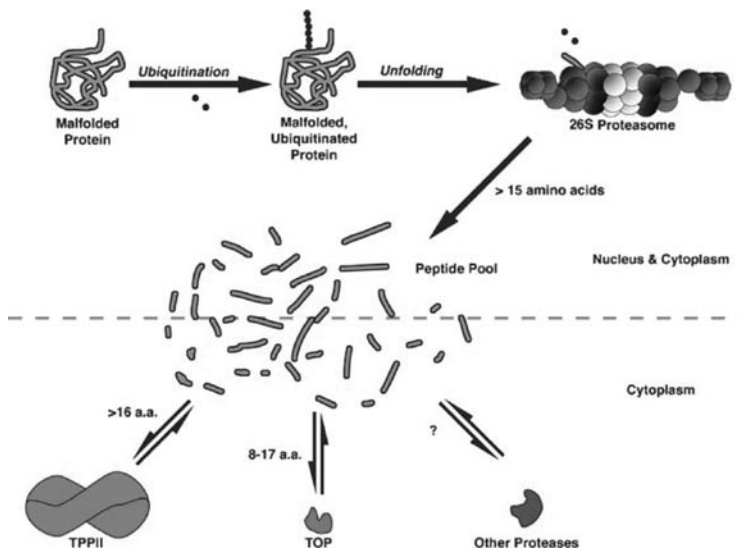


Figure 2. Antigen processing in the cytosol. Before proteins can be recognized by the proteasome, they have to be polyubiquitinated in a process called ubiquitination. After binding of more than four ubiquitins to a single ubiquitin chain the cap of the 26S proteasome (blue) is able to bind the ubiquitin chain and the attached substrate protein. The protein is unfolded by the 19S cap and successively degraded by the 20S core into peptides that contain more than 15 amino acids. The peptides form a heterogeneous pool and can diffuse freely through the nucleus and the cytosol. In the cytosol, the peptides are prone to aminopeptidases with different specificities: TPPII trims peptides that are longer than 16 amino acids; TOP is thought to cleave peptides containing 8–17 amino acids; other proteases, including neurolysin, LAP, and PSA, have a thus far unknown specificity.

for binding to MHC class I molecules (9,10,47). A heterodimeric ER-located transporter that performs peptide translocation consists of transporter-associated with antigen processing 1 (TAP1) and TAP2 and is a member of the ATP binding cassette (ABC) transporter superfamily. The TAP1 and TAP2 genes are located in the MHC locus on chromosome 6, very close to two proteasome subunits (48,49). The expression of these genes is upregulated by interferon- γ , like the expression levels of MHC H chains and β_2m . Like most ABC transporters, TAP is made of a multimembrane-spanning segment that forms the pore required for actual passage of the ER membrane. This segment is formed by the N-terminal parts of TAP1 and TAP2 and ensures ER retention of the complex. A second area where the peptide is binding then follows this. Both TAP1 and TAP2 contribute to this second segment with TAP2 determining the sequence of the C-terminus, as became apparent by comparing two different rat TAP alleles. Two ATP binding cassettes (ABC), one from TAP1 and the other from TAP2, conclude the transporter. These two ABC domains are essential for the alternating cycles of ATP hydrolysis that drive the different conformational changes in TAP. Probably ATP is first hydrolyzed by TAP1 to open the pore and deliver the peptide in the ER, and then a second ATP is hydrolyzed by TAP2 to close the pore again and return to the ground state (50-54). These alternating cycles of ATP hydrolysis then drive the conformational changes required for a continuous pumping of peptides. Studies following the lateral mobility of TAP under various conditions have indicated major conformational changes during this cycle. TAP moves “quickly” when inactive and “slowly” when in the process of pumping peptides (1). This may be surprising, but it has been shown that TAP can handle peptides with extended side chains of around 70Å (which is similar to the size of an elongated 8-mer peptide) (55). The mobility assay has been used to monitor the intracellular peptide pool in living cells. This revealed that, under normal conditions, TAP is getting limited amounts of peptides and can handle more substrate. Only upon conditions like stress or virus infection, saturating amounts of peptides are generated. Since most MHC class I molecules are not fully loaded with peptides (and ultimately degraded through the ERAD pathway) (56), more peptides will enhance the loading and consequently the expression of MHC class I molecules. Probably, a reservoir/excess of MHC class I H-chain/ β_2m heterodimers is produced to handle fragments produced under these conditions.

The peptide transporter TAP is unique among the ABC transporter family of pumps. First of all, it is exclusively located in the ER. Second, it acts as a docking protein for a unique chaperone tapasin and MHC class I H-chain/ β_2m heterodimers. The heterodimer is further stabilized by other chaperones (Erp57 and calnexin). In fact, probably four tapasin-MHC class I heterodimer-Erp57-calnexin complexes dock onto one TAP protein, thus forming a approximately 1 MDa MHC class I loading complex or MLC (57-59). In concept, this architecture may support efficient peptide loading of MHC class I molecules when peptides imported by TAP are immediately loaded onto the associated MHC class I heterodimers. However, this is likely not too important since:

1. Many peptides require trimming in the ER before being suitable for MHC class I binding (15,16).
2. Many MHC class I alleles are not associated with the TAP complex and are still efficiently loaded (60).
3. MHC class I molecules are still loaded with peptides in tapasin-deficient cells and mice although the quality of the MHC class I associated peptide pool is clearly affected (61,62). However, whether this reflects a difference in chaperoning activity of tapasin or the recruitment to TAP, is unclear.

MHC class I molecules are polymorphic proteins and every human expresses between three and six different alleles. The polymorphic residues cluster around the peptide-binding groove of MHC class I. Consequently, different MHC class I molecules bind different sets of peptides (63,64). TAP is not polymorphic and thus has to supply all the different MHC class I molecules with peptides. This is only possible when TAP is able to translocate peptides in a fairly sequence-independent manner. Various studies have tested this. TAP translocates peptides with a minimal length of 8 amino acids (MHC class I molecules usually bind peptides of 8 or 9 amino acids). However, peptides of over 40 amino acids are translocated by TAP as well, albeit less efficiently (12,65). Although some small differences in sequence selectivity have been observed, TAP translocates peptides with only minor distinction for sequence, with two exceptions. Peptides with the imino acid proline at position 2 or 3 are poorly handled, but still presented by particular MHC class I alleles. In these cases, probably longer peptides (that reposition the proline

in the sequence) are translocated by TAP followed by further trimming in the ER lumen (11). Furthermore, selectivity for the C-terminal amino acid residue is found in particular species. Murine TAP as well as a rat TAP allelic form selectively translocate peptides with a hydrophobic or aromatic C-terminal residue, whereas another rat allele and human TAP are very nonselective for amino acids at this position (9). If the amino acid side chains of a peptide are not recognized by TAP, what is? Further peptide modifications revealed that the N- and C-termini are critical for recognition. Incorporation of amino acid stereoisomers showed that the peptide bond contributed to the interactions with TAP (55). This resembles the situation for peptide binding to MHC class I molecules where most interactions between the MHC class I molecule and peptide are made through hydrogen bonds to the ends of the peptide as well as the peptide's peptide bond (66).

The fact is that TAP is the only peptide transporter in the ER, especially designed to support the MHC class I antigen presentation pathway and in some respects resembling MHC class I. Like other ABC transporters, TAP supports unidirectional transport of its substrate, a peptide.

5 Peptides and Peptidases in the Endoplasmic Reticulum

TAP translocates peptides preferentially of 8–12 amino acids but also longer peptides (27). Peptides containing a proline at position 2 or 3 are not handled by TAP but these peptides are still found associated to various MHC class I alleles. It is assumed that in these cases N-terminally extended peptides are transported into the ER followed by trimming in the ER by resident peptidases. Peptidase activity has been observed in the ER (67,68) and only recently the corresponding peptidases have been identified and characterized. The ER-aminopeptidase ERAAP (or ERAP1) has been found and is critical in the handling of many peptides in the ER before they can bind to MHC class I molecules (15–17). In fact, ERAP trims peptidases from the amino terminus until fragments of 8–9 amino acids are left. ERAP thus acts as a sort of molecular ruler, trimming peptides to sizes fitting MHC class I molecules. Interestingly, as mentioned in Sect. 4, peptides containing a proline at position 2 or 3 are not handled by TAP unless the proline residue is repositioned by N-terminal extension. ERAP1 is designed to handle these peptides because it stops further N-terminal trimming when encompassing a proline at position 2 (16). ERAP is a protease and does not “know” which peptides should bind to the resident MHC class I molecules. Consequently it simply trims these peptides and

thus creates but also destroys potential MHC class I binding peptides (16,17). Whereas ERAP1 is the first ER-located peptidase identified, it is probably not the only one. Other peptidases (called ERAP2, 3 etc.) may also contribute, but their relative contribution still has to be established.

Thus peptides can bind in the ER to MHC class I molecules (if containing the correct anchor residues and length) and ERAP (albeit transiently). Peptides associated with the ER-resident chaperone gp96 have been used for vaccination purposes, even over an MHC barrier (69,70). This suggested that gp96 was able to accumulate the blueprint of peptides before selection by the endogenously expressed MHC class I molecules. To visualize ER proteins able to bind peptides, radioactive labeled peptides with a photo affinity label were introduced in the ER by TAP-mediated import followed by UV-catalyzed cross-linking. Various proteins were found to associate with these peptides, which were identified as the chaperones PDI (protein disulfide isomerase), calnexin, Erp72, gp96, and gp170. Some of these proteins (calnexin, gp170) were subsequently shown to also induce peptide specific immune responses upon vaccination (18–20,71). That chaperones associate with peptides is not too surprising since peptides can be considered resembling stretches of unfolded protein, the normal substrates for chaperones. Still, PDI was by far most efficient in binding peptides in the ER. The reason for this is unclear. It may be, but this is not shown, that PDI delivers the peptides for consideration by MHC class I molecules. However, PDI may also deliver the peptides to the SEC61/translocon for export out of the ER (see below), especially because PDI has been proposed to be the lid of the SEC61/translocon complex that opens upon nascent protein import (72), but possibly returns peptides for export back into the cytosol.

6 Peptide Export from the Endoplasmic Reticulum

Peptides are apparently not degraded to single amino acids in the ER. Consequently, they have to be removed at one point for destruction. Initial experiments following TAP-dependent import showed transient accumulation of peptides unless these obtained N-linked glycans (21,73). Further experiments showed that peptides transiently entered the ER microsomal lumen and re-entered the cytosol after some 1–3 min, at least in *in vitro* experiments. Using glycosylation-deficient and normal microsomes, it was shown that peptides were able to enter the ER by TAP transport, were released from the ER through another activity and re-enter the ER again by TAP activity. The limiting factor in this peptide recycling over the ER membrane was the cytosolic

peptidase activity that trimmed the peptides to a size too small for TAP handling (less than 8 amino acids) (68). The activity that removed peptides from the ER required ATP, was (unlike TAP) not pH-sensitive and could not be competed with exogenously added peptides (68). Furthermore, a viral inhibitor (ICP47) that inhibited TAP was unable to simultaneously inhibit export as well (21). Collectively this indicated that an activity different from TAP released the peptides from the ER.

TAP requires triphosphonucleotides, but not necessarily ATP for driving peptide transfer into the ER (73). Momburg and colleges used this fact to show that peptides can be translocated by TAP in the presence of GTP, but GTP did not drive export (68). In fact, they show that ATP in the ER lumen was required to drive peptide export. Various bacterial toxins enter the ER by retrograde uptake. These toxins use the ERAD system and the SEC61/translocon to enter the cytosol where they are toxic. Momburg introduced exotoxin A in the ER lumen of microsomes and showed that this competed with peptide export (21). Since exotoxin bound the translocon, these data suggested that peptides also used this part of the ERAD system to leave the ER lumen, unless they are captured by the various chaperones.

The fate of peptides in the ER is:

1. Peptides enter the ER after translocation by TAP. Here peptides have different possibilities (Fig. 3).
2. They bind to MHC class I molecules.
3. They are trimmed by ER aminopeptidases and some of them bind to MHC class I molecules.
4. They have the incorrect sequence for binding to MHC class I molecules and bind ER chaperones.
5. They bind to PDI, which may target them to the SEC61/translocon followed by ATP hydrolysis-driven peptide export back into the cytosol.

Peptide cycling over the ER lumen thus resembles the ERAD pathway in many aspects: it requires chaperones, ATP hydrolysis, and retrotranslocation back into the cytosol by the SEC61/translocon followed by degradation. Peptides are targeted by cytosolic aminopeptidases; proteins are first degraded by the proteasome and then by cytosolic aminopeptidases.

7 The Equilibrium of Protein and Peptide Degradation, Peptide Cycling and Peptide Binding by MHC Class I Molecules

It seems obvious that the process of protein degradation, peptidase trimming, TAP-mediated peptide translocation, peptide trimming in the ER, peptide loading onto MHC class I molecules, and peptide export are constructed such that optimal peptide generation and loading of MHC class I molecules occurs. This is, however, not the case. The process of antigen presentation, i.e., the resultant of these different steps, is highly inefficient. Recently the kinetics and efficiency of various steps in this process were determined (2,23). Proteasomes do not know where to cleave in a protein substrate to generate the correct MHC class I peptides (there are only a standard, non-polymorphic proteasome and a specialized immunoproteasome, and many different MHC class I molecules). The proteasome probably generates peptides of various lengths but usually the correct C-terminus of a peptide (26). It has to, since cells lack carboxypeptidase activities (24). In addition, the proteasome will destroy many potential MHC class I binding peptides.

The resulting proteasomal-produced peptide fragments diffuse through the cell and are targeted first by TPPII and subsequently by other aminopeptidases (6). The half-life of many peptides is in the range of 5 s. This activity is such that more than 99% of the peptides generated are destroyed before they can be translocated into the ER (24). In the ER, many peptides are destroyed by ERAAP and/or removed by the translocon, while only a few peptides will interact with MHC class I molecules. In fact, cells contain 10^9 proteins of which about 2×10^6 are degraded per minute whereas only 10^4 MHC class I molecules per minute will be loaded with peptides (2,74). This means that only 0.5% or less of the peptides can maximally bind MHC class I molecules. The rest is destroyed unless many more peptides are made than can be loaded onto MHC class I molecules (1). It should be noted that many MHC class I molecules are made but not loaded with peptides. Consequently, under conditions of increased peptide generation (for example following a viral infection), the other MHC class I reservoir can be loaded with peptides as well, resulting in an increased expression of MHC class I molecules. The inefficiency of the system may be essential to generate this pool of peptide recipient MHC class I molecules, lost unless the intracellular peptide pool increases, for example as the result of a viral infection. In conclusion, only few substrates make it into MHC class I binding peptides. The rest are fully turned over into amino acids, not being of any immunological relevance.

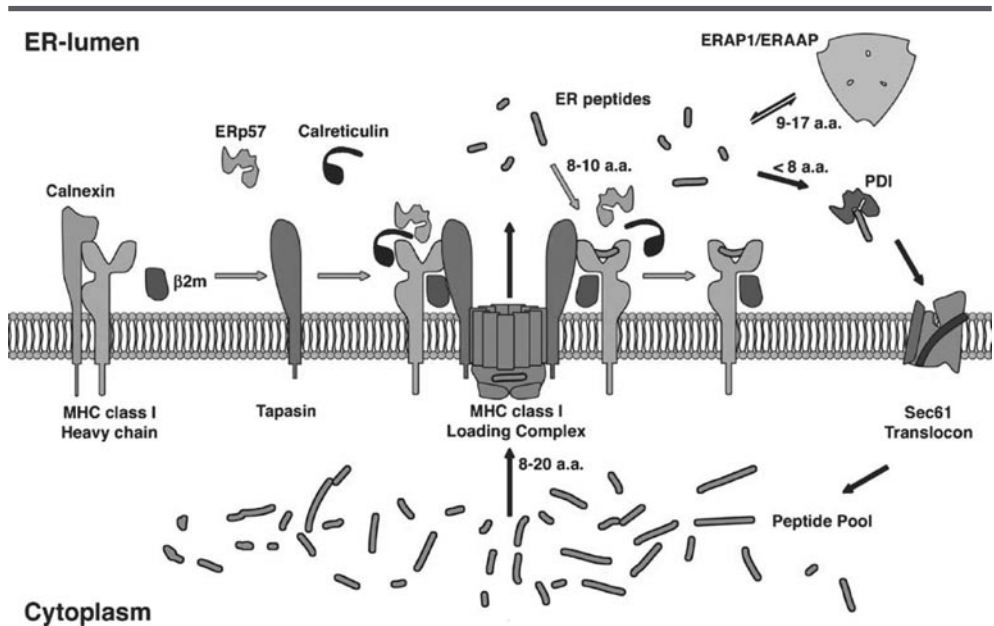


Figure 3. Peptide cycling over the ER membrane. Cytosolic peptides may escape cytosolic degradation by binding to the peptide transporter TAP. TAP can only bind peptides that are 8–20 amino acids and will transport them in an ATP-dependent process over the ER membrane. Once in the ER, the endoplasmic reticulum aminopeptidase (ERAP), which is specific for peptides of 9–17 amino acids, trims most peptides further. When peptides are not bound to MHC class I molecules, PDI and the Sec61/translocon guide them back to the cytosol. Only peptides of 8–10 amino acids with correct anchor residues are able to bind to MHC class I H-chain/ β_2m dimers. The heavy chain / β_2m dimer is initially formed with the help of the ER chaperone calnexin, after initial formation the unstable heterodimer is transferred to the MHC class I loading complex (MLC) where it is bound to other ER specific chaperones such as Erp57, calreticulin, and tapasin. Binding of a peptide to the heterodimer stabilizes it and will be granted transport to the plasma membrane by the ER quality control system

8 How the MHC Class I Route Is Manipulated

Obviously, it is fairly beneficial for pathogens to interfere with the system of antigen presentation by MHC class I molecules. This indeed often happens, as discussed in detail elsewhere in this volume. The major viral targets are proteins that are dispensable for household processes. More specifically, viral TAP inhibitors are frequently found, inhibitors affecting the dedicated chaperone tapasin and viral inhibitors targeting MHC class I H-chains or H-chain/ β_2m heterodimers for degradation, usually through the ERAD system (75-78). Viral inhibitors affecting the proteasome, TPPII, TOP or other peptidases, ERAP, ER chaperones, or the SEC61/translocon have not been defined, not unexpectedly, because inhibition of these would affect cell viability, thus being a disadvantage for the pathogens rather than an advantage.

Obviously, chemical inhibitors may be used to affect antigen presentation by MHC class I molecules. Inhibitors for TAP have been designed (being peptides with long side chains), but these cannot be used in living cells or organisms because they do not

pass membranes (55). This is also true for the active 35-amino acid fragment of the viral TAP inhibitor ICP47 (79). Inhibitors for the proteasome have been designed and clinically used in anti-cancer treatments (80-82). These inhibitors prevent proteasome-mediated protein degradation (possibly generating aggregates as a consequence) and peptide loading of MHC class I molecules. Whether these compounds can be used to inhibit/treat MHC class I-related autoimmune diseases such as Bechterew and Reiter's syndrome, is unclear.

9 The Eternal Cycle of Events, or Not ...

In conclusion, proteins are degraded by the proteasome and further degraded by TPPII and other peptidases into single amino acids. These single amino acids can then be used to build new proteins that—immediately in the case of DriPs or slowly for successful proteins—are degraded again into single amino acids. The MHC class I antigen presentation system samples a small amount out of this cycle for presentation purposes. Degradation intermediates

that are simultaneously substrate to peptidases in the cytosol and ER lumen and to MHC class I molecules are presented. Critical steps in acquiring peptides by MHC class I molecules are the peptide cycle over the ER membrane with TAP pumping peptides into the ER and the SEC61/translocon transporting them out. Although the dynamics of this cycle are not fully understood, the end result is again that a small fraction of imported peptides make it into something immunologically useful: an MHC class I peptide complex. The remainder is ultimately degraded into single amino acids, as are MHC class I molecules failing to obtain peptides during their biosynthesis.

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