Assembly and export of MHC class I peptide ligands

Antony N Antoniou[*†], Simon J Powis[*‡] and Tim Elliott[§]

During the assembly of MHC class I molecules with peptide, a series of transient interactions are made with endoplasmic reticulum-resident chaperones and MHC class I-specific accessory molecules. These interactions culminate in the trafficking of MHC class I molecules to the cell surface and presentation of peptide to $CD8^+$ T lymphocytes. Recent studies have revealed just how important these early interactions are, and how they influence the quality control of assembly and the optimisation of peptide–ligand binding.

Addresses
[*]Division of Cell Biology and Immunology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK
[†]e-mail: a.n.antoniou@dundee.ac.uk
[‡]e-mail: s.j.powis@dundee.ac.uk
[§]Cancer Sciences Division, School of Medicine, University of Southhampton, Southhampton General Hospital, Southhampton SO16 6YD, UK
e-mail: tje@soton.ac.uk

Current Opinion in Immunology 2003, **15**:75–81

This review comes from a themed issue on
Antigen processing and recognition
Edited by Andrea Sant and Jonathan Yewdell

0952-7915/03/$ – see front matter

DOI 10.1016/S0952-7915(02)00010-9

Abbreviations

β2m	β2 microglobulin
BiP	immunoglobulin binding protein
ER	endoplasmic reticulum
HLA	human leukocyte antigen
MHC	major histocompatibility complex
NBD	nucleotide binding domain
P domain	proline-rich domain
PLC	peptide-loading complex
TAP	transporter associated with antigen processing
TR	thioredoxin

Introduction

MHC class I molecules present peptides, derived mostly from intracellular polypeptides, to $CD8^+$ T lymphocytes. The assembly of MHC class I molecules with peptide is the culmination of a coordinated and regulated series of interactions with endoplasmic reticulum (ER)-resident chaperones and accessory molecules. Some interactions, for example those with calnexin, calreticulin and the oxidoreductase ERp57, are part of the normal pathway for assembly in the ER of asparagine-glycosylated proteins. During the later stages of assembly, associations are also formed with 'class I specific' accessory molecules, such as tapasin and the transporter associated with antigen processing (TAP). This stage of assembly is characterised by the formation of the peptide-loading complex (PLC). In this review, we highlight some of the recent progress made towards understanding the roles of chaperones and accessory molecules in MHC class I assembly. In addition, some emerging observations of the control of MHC class I trafficking from the ER will be discussed.

Early events in MHC class I assembly

Early events in MHC class I assembly are regulated by interactions with calnexin. This is initially a lectin interaction [1–4], occurring through the monoglucosylated $Glc_1Man_9GlcNAc_2$ (Man, mannose; Glc, glucose; GlcNAc2, N-acetylglucosamine) carbohydrate group found attached to the conserved Asn86 in the MHC class I heavy chain; the removal of Asn86 by mutagenesis, however, still permits some calnexin association, suggesting that protein–protein interactions also occur [5,6]. The recently resolved crystal structure of the ER-lumenal domain of calnexin has revealed a binding site for the glycan unit [7•], which could place a folding MHC class I peptide-binding domain within a microenvironment shielded by the protruding arm of the proline-rich domain (P domain).

Upon association of the MHC class I heavy chain with β2 microglobulin (β2m), calnexin is replaced by the homologous ER-soluble chaperone calreticulin [8], although prolonged calnexin associations can also be detected [9,10]. During these early events calnexin appears to have distinct requirements when binding to heavy chains in comparison to calreticulin. In the absence of β2m, MHC class I molecules bind calnexin, with little or no calreticulin association [11,12]. In calnexin-deficient cells, however, MHC class I molecules assemble and traffic to the cell surface [13,14], suggesting calnexin can be functionally replaced. A candidate chaperone for this role is immunoglobulin binding protein (BiP). Calnexin and BiP can cooperate in preventing the aggregation of unfolded proteins [15]. This may be the case with class I heavy chains, as the BiP–MHC heavy chain interaction is readily detected, both in β2m-deficient cells [16,17] and during assembly of the misfolding-prone HLA-B27 class I molecule (A Antoniou, unpublished data).

MHC class I heavy chains contain two conserved disulfide bonds: one within the α3 domain between residues Cys203 and Cys259; and a second in the peptide-binding domain between Cys101 and Cys164, tethering the α2 helix to the floor of the groove. Both disulfide bonds are

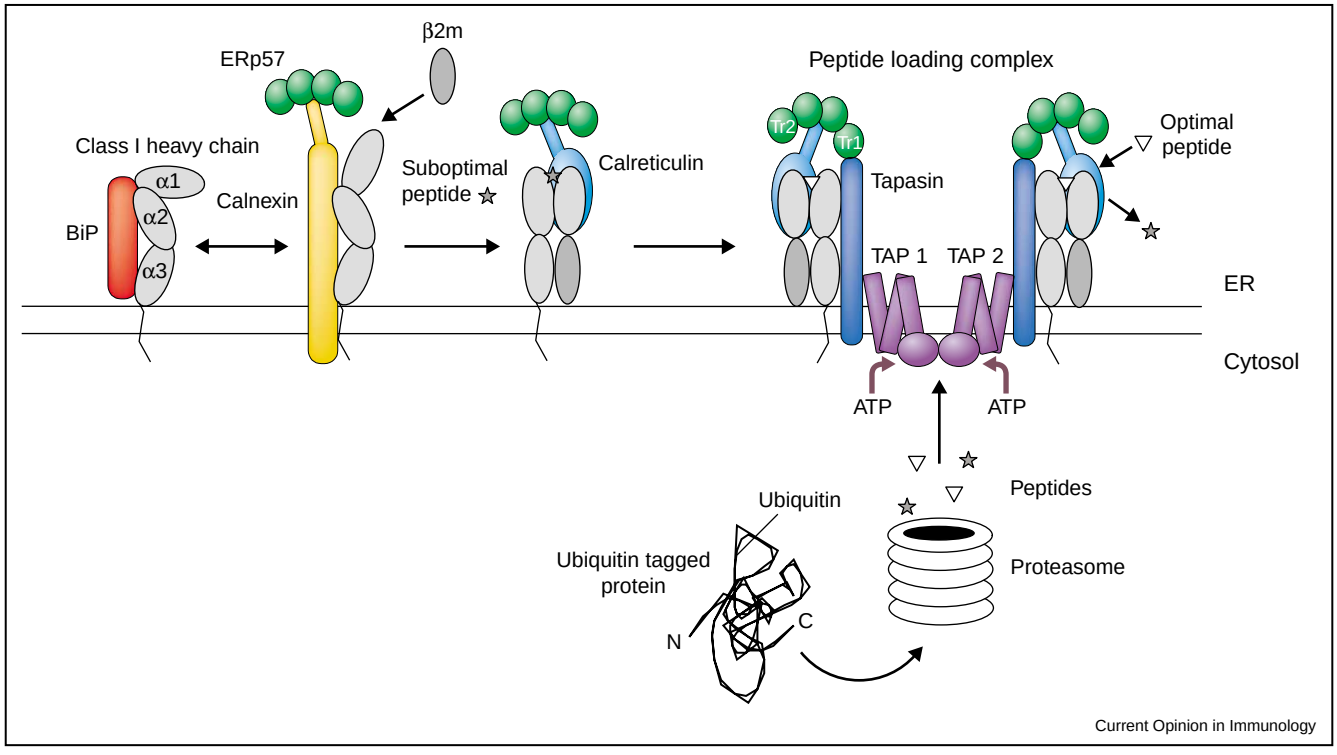
essential for the correct assembly of MHC class I molecules. Interaction of the MHC heavy chain with calnexin promotes the formation of intrachain disulfide bonds [18]; a likely candidate for this process is the oxidoreductase ERp57, which binds to both calnexin and calreticulin [3,19–21]. Interaction of the tip of the calreticulin P domain [22•] with ERp57 allows close proximity to the folding polypeptide. The affinity of this interaction is low, allowing for the rapid dissociation of ERp57 when it has participated in formation of the disulfide bonds. Calnexin and calreticulin again demonstrate differential associations with MHC class I heavy chains, as calnexin retains an interaction when Cys101 is changed to Ser101, whereas calreticulin binding is lost [6]. This is probably caused by the inability of disulfide mutants to bind β2m, thus they remain in a partially folded, calnexin-binding conformation.

The peptide-loading complex

With β2m associated and calnexin replaced largely by calreticulin, the stage is set for the assembly of the PLC, a multimolecular complex comprising MHC class I, β2m, calreticulin, ERp57, TAP and the class I-specific accessory molecule tapasin (Figure 1). The emerging function of the PLC is to retain MHC class I molecules within the ER until they become loaded with high-affinity peptides. In this respect the PLC, and in particular the role of tapasin, has been compared to the roles of HLA-DM and HLA-DO in the editing of peptides within the groove of MHC class II molecules [23]. Tapasin tethers the PLC to TAP through an interaction that relies on its carboxy-terminal region [12]. The reason for this is unknown; however, clustering the PLC next to the portal of peptide entry into the ER would presumably increase the efficiency of peptide binding. Expression of a soluble amino-terminal region of tapasin prevents TAP binding, but does permit the formation of the PLC. Tapasin also stabilises the TAP heterodimer, although how this occurs is not yet understood [24].

The presence of ERp57 within the PLC suggests that it has a role in controlling disulfide bonds within MHC molecules. Somewhat unexpectedly, however, ERp57 and tapasin disulfide bond within the PLC [25••]. Covalent interactions of ERp57 with the MHC class I heavy

Figure 1



Formation of the peptide loading complex. Newly synthesised class I heavy chains can associate with calnexin and/or BiP. The sequence of these interactions is largely undetermined. The association of β2m and binding of suboptimal peptides can lead to conformational changes within the heavy chain and displacement of calnexin by calreticulin, which leads to the formation of the PLC. Proteins within the cytosol destined for proteasome degradation are targeted by ubiquitin. The resulting peptides are transported in an ATP-dependent manner via TAP into the lumen of the ER. Within the PLC optimal peptides are exchanged for suboptimal peptides by a tapasin-mediated event. In this figure, binding of suboptimal peptide is shown to precede loading with high affinity peptides. Although the loading of suboptimal and optimal peptides has not formally been distinguished temporally in normal APC, it is known that mutant class I molecules that do not interact with any components of the PLC (and are denied an opportunity to optimise their peptide cargo) are transported out of the ER bound to unstable peptides [61].

chain within the PLC have not so far been detected and, although ERp57–MHC heavy chain interactions can be detected, they probably represent an earlier pre-PLC folding event [26,27•]. ERp57 contains two thioredoxin-like (TR-like) motifs with the sequence CGHC (in amino acid one-letter code), which are the active sites of disulfide bond reduction/oxidation. Normally these TR motifs are involved in the formation of transient disulfide intermediates with the amino-terminal cysteine residue, which would then be disrupted by nucleophilic attack from the carboxy-terminal cysteine. In the case of the ERp57–tapasin conjugate, however, we may be witnessing a novel interaction for an oxidoreductase. Cys95 of tapasin, which forms a linkage with Cys57 in the TR1 motif of ERp57, is not thought to be involved in a structural intrachain disulfide. It is therefore worth considering whether tapasin has evolved the ability to 'trap' ERp57 to overcome the intrinsically low affinity of calreticulin to ERp57. The combination of interactions may permit an enhanced association of ERp57 with the PLC. In the absence of tapasin Cys95, ERp57 does not remain part of the PLC [25••]. In calreticulin-deficient cells, however, ERp57 associated with TAP can be detected in the PLC [28•]. This suggests that it is tapasin that recruits and maintains ERp57 in the PLC. The presence of calnexin, which also has a P domain arm, in the PLC–TAP complexes of calreticulin-deficient cells may, however, contribute to the presence of ERp57.

If the TR1 motif of ERp57 is occupied by a covalent interaction with tapasin, what is the function of the TR2 motif? We have recently examined this question and have determined that the redox status of the TR2 motif in ERp57–tapasin conjugates can be modified by oxidising reagents, suggesting that TR2 is available to undergo redox reactions with substrates (A Antoniou and S Powis, unpublished data). TR2 may therefore be able to act on MHC class I molecules. The recent study by Dick *et al.* [25••] provides a fascinating observation: the α1/α2 domain of MHC class I molecules folds in a peptide-dependent manner, indicating that the Cys101–Cys164 bond may be exposed before folding is completed. Mutagenesis of an intrachain disulfide bond in the amino-terminal region of tapasin, between Cys7 and Cys71, however, results in a partial reduction of the class I heavy chain. This result does not necessarily indicate that the Cys7–Cys71 disulfide bond in tapasin is directly involved in defining or maintaining the redox status of the MHC class I heavy chain. It may be that there is localised unfolding of an amino-terminal domain in tapasin, which prevents normal access to the groove by other polypeptides, including ERp57. Nevertheless, it does indicate that the redox status of MHC class I molecules can be influenced in the PLC.

Peptide optimisation by tapasin

MHC class I molecules that fail to interact correctly with the PLC often exit the ER prematurely in a peptide-receptive state [28•,29]. Thus, a two-step model for MHC class I peptide binding has been proposed [29]. Initially, MHC class I molecules may bind low-affinity, suboptimal peptides. Within the PLC, the suboptimal peptides are replaced with high-affinity optimal peptides, which stabilise the MHC class I–β2m heterodimer. Peptide binding and exchange within the PLC is probably mediated by the lumenal domain of tapasin. Utilising the relationship between thermostability of MHC class I–peptide complexes and peptide binding affinities, the MHC class I–peptide cargo has been shown to be optimised both qualitatively and quantitatively over time in the presence of tapasin [30••,31–33]. Some alleles, such as HLA-B*2705 and HLA-B*4405, demonstrate tapasin-independent optimisation, albeit inefficiently [31]. The precise reason for this is not known, but may reflect subtle differences in the molecular architecture of the peptide binding grooves of different alleles. Polymorphisms in residue 116, located in the F pocket of the peptide groove, may determine the mechanism of a tapasin-independent assembly pathway [34].

Organisation of the peptide-loading complex

Site-directed mutagenesis of MHC class I molecules is providing us with clues about the organisation of the PLC. Three main regions of the MHC class I heavy chain, outside the peptide binding groove, have been identified as influencing formation of the PLC.

The mutation of residues in the region 86–88 alters the Asn-linked glycosylation site and leads to PLC disruption through poor calreticulin association [5,6]. Mutations in an α2 domain loop between residues 122 and 136 have been studied extensively, and recent experiments performed with H-2D^d and H-L^d molecules indicate that this exposed loop is probably a tapasin interaction site, because residual interactions with calreticulin can still be detected [35–38]. In the α3 domain, residues 227–229 may also contribute a secondary tapasin interaction site. Thus, the α2 face of the MHC class I molecule may form interactions with tapasin, whereas the end of the α1 helix surrounding the Asn-linked glycosylation site may be the main point of contact for calreticulin. The recognition of partially unfolded MHC class I molecules in the PLC by a monoclonal antibody that recognises an epitope in the region of residue 48, underneath the opposite end of the α1 helix from the glycan site, suggests that this region of the MHC class I molecule may be partially exposed in the PLC [5,39].

Degradation of MHC class I molecules

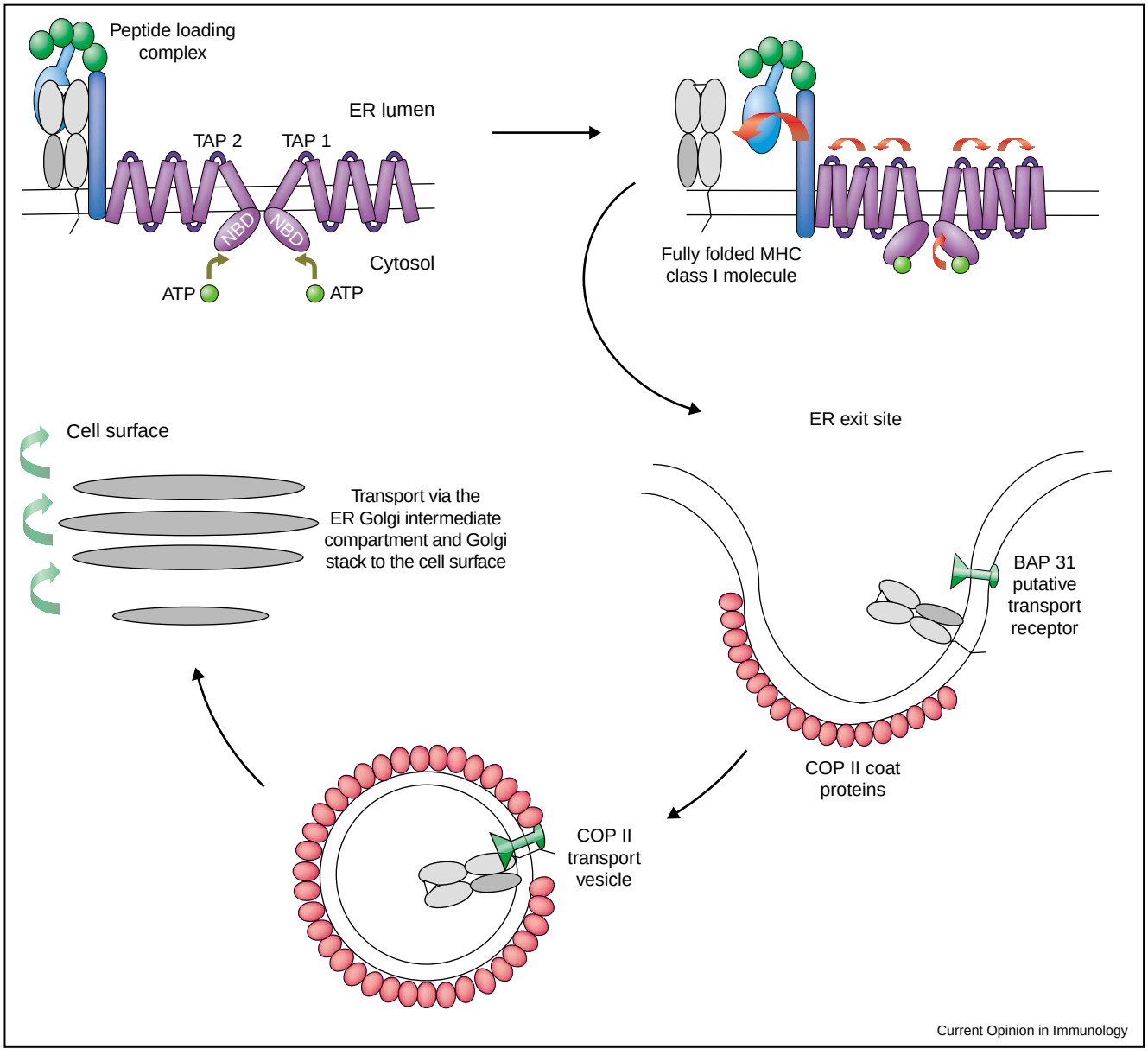
Misfolded MHC class I molecules are segregated from the folding environment of the ER, retrotranslocated and degraded in the cytosol, a process referred to as ER-associated degradation (ERAD). Ubiquitination then targets misfolded proteins for proteasome-mediated degradation [40]. Mannose trimming, unfolding and disulfide

bond reduction of heavy chains appears to occur before retrotranslocation [41,42].

In an *in vitro* assay system, recombinant ERp57 reduces disulfide bonded and partially folded MHC class I heavy chains [27•], suggesting that ERp57 could be active throughout the assembly pathway, not only in productive folding but also in the priming of MHC class I molecules for degradation. However, this prerequisite for unfolding requires further study. Recent data has demonstrated cytosolic fluorescence after retrotranslocation of GFP-tagged MHC class I molecules, suggesting that retrotranslocation of folded MHC class I molecules takes place [43].

A putative subcompartment of the ER, termed the 'quality control' compartment, has been described as a site for retrotranslocation of MHC class I molecules. Within this site, calnexin and calreticulin are detected but BiP and

Figure 2



MHC class I exit from the ER. ATP binding to the TAP NBD cytosolic domains, predominantly via the NBD of TAP 1, instigates conformational changes within the TAP complex. These conformational alterations lead to nucleotide-binding dependent, peptide independent release of the MHC class I–peptide complex. These molecules cluster at ER exit sites, which are the possible precursors of COP II coated transport vesicles. Within these vesicles, MHC class I molecules associate with the putative transport receptor BAP31. These vesicles later fuse with the ER Golgi intermediate compartment and traverse the Golgi apparatus to the cell surface.

protein disulfide isomerase (PDI) are absent [44•,45]. If ERp57 was also present within this compartment, this would place it in an appropriate environment to unfold MHC class I molecules.

The export of assembled MHC class I–peptide complexes

Optimal peptide binding by MHC class I molecules was initially thought sufficient for release from the PLC. Several other factors are now known to affect transportation of the MHC–peptide complex to the cell surface. These include the regulation of MHC class I–peptide complex release from the peptide transporter TAP and from ER exit sites (Figure 2).

Crystallographic data on the multitransmembrane domain containing ATP-binding cassette transporters such as TAP is rare; however, a recent structure of the BtuCD vitamin B_{12} importer from *Escherichia coli* indicates close contact between the nucleotide binding domains (NBDs) and also between the transmembrane spanning domains, with a clearly visible central translocation pathway [46]. The overall shape bears similarities to the structure of TAP obtained by single particle image analysis and electron microscopy [47]. TAP NBD mutants, which prevent the hydrolysis of ATP, and nucleotide-depletion experiments suggest that peptide-mediated dissociation of MHC class I molecules depends on conformational signals arising from TAP [48,49]. Precisely how the cycle of nucleotide binding and hydrolysis in the cytoplasm controls both peptide translocation and also the release of peptide-loaded class I molecules in the ER remains to be determined; however, large conformational changes are seen in the ABC transporter P glycoprotein [50], and similar changes may occur in TAP [51–53]. The ability of tapasin to increase the stability and therefore peptide translocation efficiency of TAP also indicates a close cooperativity between the function of these two molecules [24,54]. Further crystallographic data in addition to the structure of the NBD of TAP1 is therefore eagerly awaited [55].

Receptor mediated ER to plasma membrane transport

Upon dissociation from TAP, MHC class I molecules exit the ER and enter the Golgi apparatus via the ER Golgi intermediate compartment. Cargo proteins are thought to either enter and traverse the Golgi by default through a nonselective bulk flow mechanism; alternatively, signal sequences within the cytoplasmic tails of cargo proteins could mediate their selective and efficient incorporation into COPII coated vesicles, which mediate ER to Golgi trafficking (Figure 2; [56]).

Using GFP-tagged class I molecules and measuring lateral diffusion within the ER by fluorescence microscopy as a surrogate marker for their association with TAP (lateral diffusion is reduced when class I molecules are bound to TAP), peptide loading was shown to promote dissociation from TAP [57]. However, the rate of transport of MHC class I–peptide loaded complexes from ER to Golgi remains unchanged. Furthermore, peptide loaded MHC class I molecules cluster at ER exit sites and associate with a putative transport receptor BAP31 [57–59], a 28 kDa transmembrane protein previously found associated with IgD [60]. These exit sites exclude TAP and tapasin, strongly suggesting that MHC class I ER export is highly regulated and receptor mediated. Interestingly, a mutant MHC class I molecule that does not interact with the PLC but instead is rapidly exported from the ER bound to suboptimal peptides, clusters at different ER exit sites [59]. It is not known whether MHC class I egress from these so-called rapid exit sites is also BAP31-dependent.

Conclusion

MHC class I assembly is a highly regulated process, with coordinated ER chaperone and accessory molecule interactions. Soon after early folding of the heavy chain and its binding to β2m, integration of MHC class I into the PLC occurs. It is within the PLC that most MHC class I molecules achieve optimal peptide binding and stability. Although the chief protagonist within this complex is probably tapasin, the PLC fails to operate at full efficiency without all of its component parts. When loaded with optimal peptides, and after regulated release from TAP, MHC class I molecules must undergo further selection for exit from the ER.

Acknowledgements

SJP is supported by an MRC Senior Fellowship.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zhang Q, Tector M, Salter RD: **Calnexin recognizes carbohydrate and protein determinants of class I major histocompatibility complex molecules**. *J Biol Chem* 1995, **270**:3944-3948.

2. Zhang Q, Salter RD: **Distinct patterns of folding and interactions with calnexin and calreticulin in human class I MHC proteins with altered N-glycosylation**. *J Immunol* 1998, **160**:831-837.

3. Helenius A, Aebi M: **Intracellular functions of N-linked glycans**. *Science* 2001, **291**:2364-2369.

4. Ellgaard L, Molinari M, Helenius A: **Setting the standards: quality control in the secretory pathway**. *Science* 1999, **286**:1882-1888.

5. Harris MR, Yu YY, Kindle CS, Hansen TH, Solheim JC: **Calreticulin and calnexin interact with different protein and glycan determinants during the assembly of MHC class I**. *J Immunol* 1998, **160**:5404-5409.

6. Harris MR, Lybarger L, Yu YY, Myers NB, Hansen TH: **Association of ERp57 with mouse MHC class I molecules is tapasin dependent and mimics that of calreticulin and not calnexin**. *J Immunol* 2001, **166**:6686-6692.

7. • Schrag JD, Bergeron JJ, Li Y, Borisova S, Hahn M, Thomas DY, Cygler M: **The structure of calnexin, an ER chaperone involved in quality control of protein folding**. *Mol Cell* 2001, **8**:633-644.

The three-dimensional structure of the ER lumenal domain of calnexin reveals the lectin binding site and also the elongated P-domain arm composed of proline-rich motifs, interacting in a head-to-tail orientation.

8. Farmery MR, Allen S, Allen AJ, Bulleid NJ: **The role of ERp57 in disulfide bond formation during the assembly of major histocompatibility complex class I in a synchronized semipermeabilized cell translation system**. *J Biol Chem* 2000, **275**:14933-14938.

9. Diedrich G, Bangia N, Pan M, Cresswell P: **A role for calnexin in the assembly of the MHC class I loading complex in the endoplasmic reticulum**. *J Immunol* 2001, **166**:1703-1709.

10. Cresswell P, Bangia N, Dick T, Diedrich G: **The nature of the MHC class I peptide loading complex**. *Immunol Rev* 1999, **172**:21-28.

11. Solheim JC, Harris MR, Kindle CS, Hansen TH: **Prominence of beta 2-microglobulin, class I heavy chain conformation, and tapasin in the interactions of class I heavy chain with calreticulin and the transporter associated with antigen processing**. *J Immunol* 1997, **158**:2236-2241.

12. Sadasivan B, Lehner PJ, Ortmann B, Spies T, Cresswell P: **Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP**. *Immunity* 1996, **5**:103-114.

13. Scott JE, Dawson JR: **MHC class I expression and transport in a calnexin-deficient cell line**. *J Immunol* 1995, **155**:143-148.

14. Sadasivan BK, Cariappa A, Waneck GL, Cresswell P: **Assembly, peptide loading, and transport of MHC class I molecules in a calnexin-negative cell line**. *Cold Spring Harb Symp Quant Biol* 1995, **60**:267-275.

15. Stronge VS, Saito Y, Ihara Y, Williams DB: **Relationship between calnexin and BiP in suppressing aggregation and promoting refolding of protein and glycoprotein substrates**. *J Biol Chem* 2001, **276**:39779-39787.

16. Paulsson KM, Wang P, Anderson PO, Chen S, Pettersson RF, Li S: **Distinct differences in association of MHC class I with endoplasmic reticulum proteins in wild-type, and beta 2-microglobulin- and TAP- deficient cell lines**. *Int Immunol* 2001, **13**:1063-1073.

17. Nossner E, Parham P: **Species-specific differences in chaperone interaction of human and mouse major histocompatibility complex class I molecules**. *J Exp Med* 1995, **181**:327-337.

18. Tector M, Salter RD: **Calnexin influences folding of human class I histocompatibility proteins but not their assembly with beta 2-microglobulin**. *J Biol Chem* 1995, **270**:19638-19642.

19. Zapun A, Darby NJ, Tessier DC, Michalak M, Bergeron JJ, Thomas DY: **Enhanced catalysis of ribonuclease B folding by the interaction of calnexin or calreticulin with ERp57**. *J Biol Chem* 1998, **273**:6009-6012.

20. Oliver JD, Roderick HL, Llewellyn DH, High S: **ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin**. *Mol Biol Cell* 1999, **10**:2573-2582.

21. Elliott JG, Oliver JD, High S: **The thiol-dependent reductase ERp57 interacts specifically with N-glycosylated integral membrane proteins**. *J Biol Chem* 1997, **272**:13849-13855.

22. • Frickel EM, Riek R, Jelesarov I, Helenius A, Wuthrich K, Ellgaard L: **TROSY-NMR reveals interaction between ERp57 and the tip of the calreticulin P-domain**. *Proc Natl Acad Sci USA* 2002, **99**:1954-1959.

The use of a chemical crosslinker together with NMR indicates the interaction between the tip of recombinant calreticulin P-domain and recombinant ERp57 protein disulfide isomerase (PDI) appears not to form a similar interaction in this system.

23. Brocke P, Garbi N, Momburg F, Hammerling GJ: **HLA-DM, HLA-DO and tapasin: functional similarities and differences**. *Curr Opin Immunol* 2002, **14**:22-29.

24. Lehner PJ, Surman MJ, Cresswell P: **Soluble tapasin restores MHC class I expression and function in the tapasin-negative cell line .220**. *Immunity* 1998, **8**:221-231.

25. •• Dick TP, Bangia N, Peaper DR, Cresswell P: **Disulfide bond isomerization and the assembly of MHC class I-peptide complexes**. *Immunity* 2002, **16**:87-98.

ERp57 is shown to be disulfide bonded to tapasin, a result that significantly alters our view of the PLC. The disulfide association occurs between the TR1 motif of ERp57 and the unpaired Cys95 of tapasin. Mutation of an internal tapasin disulfide pairing between Cys7 and Cys71 results in incomplete disulfide bonding of MHC class I molecules in the PLC.

26. Lindquist JA, Hammerling GJ, Trowsdale J: **ER60/ERp57 forms disulfide-bonded intermediates with MHC class I heavy chain**. *FASEB J* 2001, **15**:1448-1450.

27. • Antoniou AN, Ford S, Alphey M, Osborne A, Elliott T, Powis SJ: **The oxidoreductase ERp57 efficiently reduces partially folded in preference to fully folded MHC class I molecules**. *EMBO J* 2002, **21**:2655-2663.

Recombinant ERp57, when added to radiolabelled immunoprecipitates of MHC class I molecules, is able to act as a reductase only on partially folded or unfolded heavy chains, and not on folded MHC class I molecules.

28. • Gao B, Adhikari R, Howarth M, Nakamura K, Gold MC, Hill AB, Knee R, Michalak M, Elliott T: **Assembly and antigen-presenting function of MHC class I molecules in cells lacking the ER chaperone calreticulin**. *Immunity* 2002, **16**:99-109.

Calreticulin-deficient cells assemble MHC class I molecules with inefficient peptide optimisation. The presence of tapasin permits MHC association with TAP, but cannot compensate fully for the lack of calreticulin.

29. Lewis JW, Elliott T: **Evidence for successive peptide binding and quality control stages during MHC class I assembly**. *Curr Biol* 1998, **8**:717-720.

30. •• Williams AP, Peh CA, Purcell AW, McCluskey J, Elliott T: **Optimization of the MHC class I peptide cargo is dependent on tapasin**. *Immunity* 2002, **16**:509-520.

Immunoprecipitation of folded MHC class I molecules from heat-treated lysates of tapasin-deficient and tapasin-expressing cell lines indicates a time-dependent acquisition of a stable MHC class I phenotype (equating to the presence of high affinity peptides) in the presence of tapasin.

31. Purcell AW, Gorman JJ, Garcia-Peydro M, Paradela A, Burrows SR, Talbo GH, Laham N, Peh CA, Reynolds EC, Lopez De Castro JA *et al.*: **Quantitative and qualitative influences of tapasin on the class I peptide repertoire**. *J Immunol* 2001, **166**:1016-1027.

32. Lybarger L, Yu YY, Chun T, Wang CR, Grandea AG III, Van Kaer L, Hansen TH: **Tapasin enhances peptide-induced expression of H2-M3 molecules, but is not required for the retention of open conformers**. *J Immunol* 2001, **167**:2097-2105.

33. Chun T, Grandea AG III, Lybarger L, Forman J, Van Kaer L, Wang CR: **Functional roles of TAP and tapasin in the assembly of M3-N-formylated peptide complexes**. *J Immunol* 2001, **167**:1507-1514.

34. Neisig A, Wubbolts R, Zang X, Melief C, Neefjes J: **Allele-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing**. *J Immunol* 1996, **156**:3196-3206.

35. Yu YY, Turnquist HR, Myers NB, Balendiran GK, Hansen TH, Solheim JC: **An extensive region of an MHC class I alpha 2 domain loop influences interaction with the assembly complex**. *J Immunol* 1999, **163**:4427-4433.

36. Suh WK, Derby MA, Cohen-Doyle MF, Schoenhals GJ, Fruh K, Berzofsky JA, Williams DB: **Interaction of murine MHC class I molecules with tapasin and TAP enhances peptide loading and involves the heavy chain alpha3 domain**. *J Immunol* 1999, **162**:1530-1540.

37. Turnquist HR, Vargas SE, Reber AJ, McIlhaney MM, Li S, Wang P, Sanderson SD, Gubler B, van Endert P, Solheim JC: **A region of tapasin that affects L(d) binding and assembly**. *J Immunol* 2001, **167**:4443-4449.

38. Paquet ME, Williams DB: **Mutant MHC class I molecules define interactions between components of the peptide-loading complex**. *Int Immunol* 2002, **14**:347-358.

39. Solheim JC: **Class I MHC molecules: assembly and antigen presentation**. *Immunol Rev* 1999, **172**:11-19.

40. Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL: **Viral subversion of the immune system**. *Annu Rev Immunol* 2000, **8**:861-926.

41. Tortorella D, Story CM, Huppa JB, Wiertz EJ, Jones TR, Bacik I, Bennink JR, Yewdell JW, Ploegh HL: **Dislocation of type I membrane proteins from the ER to the cytosol is sensitive to changes in redox potential**. *J Cell Biol* 1998, **142**:365-376. [Published erratum appears in *J Cell Biol* 1999, **145**:642].

42. Wilson CM, Farmery MR, Bulleid NJ: **Pivotal role of calnexin and mannose trimming in regulating the endoplasmic reticulum-associated degradation of major histocompatibility complex class I heavy chain**. *J Biol Chem* 2000, **275**:21224-21232.

43. Fiebiger E, Story C, Ploegh HL, Tortorella D: **Visualization of the ER-to-cytosol dislocation reaction of a type I membrane protein**. *EMBO J* 2002, **21**:1041-1053.

44. • Spiliotis ET, Pentcheva T, Edidin M: **Probing for membrane domains in the endoplasmic reticulum: retention and degradation of unassembled MHC class I molecules**. *Mol Biol Cell* 2002, **13**:1566-1581.
In $\beta 2m^{-/-}$ cells, unassembled MHC class I heavy chains fail to accumulate at ER exit sites. After ATP depletion, segregation occurs into a calnexin- and COPII-containing compartment, which may be a distinct site of quality control and/or degradation.

45. Kamhi-Nesher S, Shenkman M, Tolchinsky S, Fromm SV, Ehrlich R, Lederkremer GZ: **A novel quality control compartment derived from the endoplasmic reticulum**. *Mol Biol Cell* 2001, **12**:1711-1723.

46. Locher KP, Lee AT, Rees DC: **The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism**. *Science* 2002, **296**:1091-1098.

47. Velarde G, Ford RC, Rosenberg MF, Powis SJ: **Three-dimensional structure of transporter associated with antigen processing (TAP) obtained by single particle image analysis**. *J Biol Chem* 2001, **276**:46054-46063.

48. Knittler MR, Alberts P, Deverson EV, Howard JC: **Nucleotide binding by TAP mediates association with peptide and release of assembled MHC class I molecules**. *Curr Biol* 1999, **9**:999-1008.

49. Alberts P, Daumke O, Deverson EV, Howard JC, Knittler MR: **Distinct functional properties of the TAP subunits coordinate the nucleotide-dependent transport cycle**. *Curr Biol* 2001, **11**:242-251.

50. Rosenberg MF, Velarde G, Ford RC, Martin C, Berridge G, Kerr ID, Callaghan R, Schmidlin A, Wooding C, Linton KJ *et al.*: **Repacking of the transmembrane domains of P-glycoprotein during the transport ATPase cycle**. *EMBO J* 2001, **20**:5615-5625.

51. Lacaille VG, Androlewicz MJ: **Herpes simplex virus inhibitor ICP47 destabilizes the transporter associated with antigen processing (TAP) heterodimer**. *J Biol Chem* 1998, **273**:17386-17390.

52. Hewitt EW, Gupta SS, Lehner PJ: **The human cytomegalovirus gene product US6 inhibits ATP binding by TAP**. *EMBO J* 2001, **20**:387-396.

53. Antoniou AN, Ford S, Pilley ES, Blake N, Powis SJ: **Interactions formed by individually expressed TAP1 and TAP2 polypeptide subunits**. *Immunology* 2002, **106**:182-189.

54. Li S, Paulsson KM, Chen S, Sjogren HO, Wang P: **Tapasin is required for efficient peptide binding to transporter associated with antigen processing**. *J Biol Chem* 2000, **275**:1581-1586.

55. Gaudet R, Wiley DC: **Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing**. *EMBO J* 2001, **20**:4964-4972.

56. Gruhler A, Fruh K: **Control of MHC class I traffic from the endoplasmic reticulum by cellular chaperones and viral anti-chaperones**. *Traffic* 2000, **1**:306-311.

57. Spiliotis ET, Manley H, Osorio M, Zuniga MC, Edidin M: **Selective export of MHC class I molecules from the ER after their dissociation from TAP**. *Immunity* 2000, **13**:841-851.

58. Pentcheva T, Spiliotis ET, Edidin M: **Cutting edge: tapasin is retained in the endoplasmic reticulum by dynamic clustering and exclusion from endoplasmic reticulum exit sites**. *J Immunol* 2002, **168**:1538-1541.

59. Pentcheva T, Edidin M: **Clustering of peptide-loaded MHC class I molecules for endoplasmic reticulum export imaged by fluorescence resonance energy transfer**. *J Immunol* 2001, **166**:6625-6632.

60. Adachi T, Schamel WW, Kim KM, Watanabe T, Becker B, Nielsen PJ, Reth M: **The specificity of association of the IgD molecule with the accessory proteins BAP31/BAP29 lies in the IgD transmembrane sequence**. *EMBO J* 1996, **15**:1534-1541.

61. Lewis JW, Neisig A, Neefjes J, Elliott T: **Point mutations in the alpha 2 domain of HLA-A2.1 define a functionally relevant interaction with TAP**. *Curr Biol* 1996, **6**:873-883.