TISSUE ANTIGENS
IMMUNE RESPONSE GENETICS

Tissue Antigens ISSN 0001-2815

REVIEW ARTICLE

The cell biology of major histocompatibility complex class I assembly: towards a molecular understanding

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Key words
Major histocompatibility complex, Antigen presentation, Peptide selection, Peptide loading complex, Tapasin, ERAAP, Calreticulin, Immunodominance

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doi: 10.1111/j.1399-0039.2010.01550.x

Abstract

Major histocompatibility complex class I (MHC I) proteins protect the host from intracellular pathogens and cellular abnormalities through the binding of peptide fragments derived primarily from intracellular proteins. These peptide-MHC complexes are displayed at the cell surface for inspection by cytotoxic T lymphocytes. Here we reveal how MHC I molecules achieve this feat in the face of numerous levels of quality control. Among these is the chaperone tapasin, which governs peptide selection in the endoplasmic reticulum as part of the peptide-loading complex, and we propose key amino acid interactions central to the peptide selection mechanism. We discuss how the aminopeptidase ERAAP fine-tunes the peptide repertoire available to assembling MHC I molecules, before focusing on the journey of MHC I molecules through the secretory pathway, where calreticulin provides additional regulation of MHC I expression. Lastly we discuss how these processes culminate to influence immune responses.

Introduction

Since we last discussed major histocompatibility complex class I (MHC I) antigen presentation in this journal (1) progress in the field has been extensive. In 2002, we knew that MHC I molecules are expressed stably at the cell surface only after acquiring a stable peptide cargo. MHC I assembly occurs rapidly within the endoplasmic reticulum (ER), with nascent MHC I heavy chains (HCs) firstly folding and forming the disulphide bridges necessary to associate non-covalently with beta 2 microglobulin (β_2m). These early events are aided by, but not dependent upon, the lectin-like chaperone calnexin and the thiol-oxidoreductase ERp57. HC:β_2m heterodimers quickly acquire a peptide cargo, which involves an initial cargo being bound which prevents degradation of unassembled HC:β_2m heterodimers. The composition of the initial cargo may be influenced mostly by the prevalence of peptides within the ER, and consequently may not be well suited to the MHC I peptide preference. Subsequently this initial cargo is exchanged for a higher affinity cargo better suited to the MHC I allele in a process requiring integration into a macromolecular peptide-loading complex (PLC), constituting the transporter associated with antigen processing (TAP), the lectin-like chaperone calreticulin, ERp57, and the chaperone tapasin (2–4).

In 2002, only the contribution of TAP and tapasin to MHC I peptide loading had begun to be assessed, with the role of calreticulin just emerging, while the function of ERp57 in the PLC remained unrecognised. TAP is the main source of peptides for most MHC I alleles, with both TAP subunits binding and hydrolysing ATP, allowing the transport of peptides into the ER in a process producing large changes in TAP structure. In contrast to the dominant role of TAP, MHC I alleles are differentially affected by the absence of tapasin. While a subset of MHC I alleles are seemingly unaffected by tapasin deficiency, most MHC I alleles exhibit severe deficiencies: fewer MHC I molecules reach the cell surface, where they have decreased stability and an impaired ability to present certain antigens to cytotoxic T lymphocytes (CTL) (5–8). These deficiencies suggest that without tapasin most, but not all, MHC I alleles fail to exchange their initial sub-optimal cargo for higher affinity peptides.

Thus, attention focused upon tapasin and functional roles for this newly identified molecule were rapidly established: tapasin acts as a molecular bridge crucial to the formation

of the PLC (4, 9, 10). Mutagenesis established that the ER luminal domains of tapasin interact with two solvent-exposed regions in the ER luminal domains of MHC I molecules. One region comprises a beta strand in the alpha 3 domain (residues 222–227/229), which is also the binding site for the CD8 co-receptor (11–13). The second binding site is a loop in the α2 domain (residues 128–136) which lies adjacent to the carboxyl-terminal end of the peptide-binding groove and connects the short α2-1 helix to the β sheets underlying the peptide-binding groove (14–16). Meanwhile residues in the C-terminal portion of tapasin associate with the TAP heterodimer allowing tapasin to: locate peptide-receptive MHC I molecules next to the peptide portal; increase TAP expression levels and consequently increase the amount, but not the rate, at which peptides enter the ER (17, 18). However, association of tapasin and TAP without an association with MHC I molecules does not restore MHC I expression, suggesting the ER luminal domains of tapasin facilitate optimal peptide selection (17). Other roles have been suggested: in some studies tapasin retains peptide-empty or sub-optimally loaded MHC I molecules within the ER (19, 20). However, this retention function is not consistently supported, most notably in tapasin knock-out (KO) mice where MHC I molecules mature at the same rate as in wild-type mice (7).

So, by 2002, we had an appreciation of the benefits that tapasin brings to MHC I peptide loading and possessed rudimentary knowledge of the regions of tapasin responsible for these functions. Formalising the molecular interactions occurring between PLC members and understanding how these interactions promote editing of the peptide cargo and expression of stable MHC I molecules have been the Holy Grail sought by numerous laboratories in the years since.

Structure and composition of the peptide-loading complex

What is the function of ERp57 within the PLC?

ERp57 is a soluble protein disulphide isomerase (PDI) family member possessing the ability to make, break and rearrange disulphide bridges in substrate proteins. ERp57 has four thioredoxin-like domains (named a, b, b′, a′), with the a and a′ domains having canonical thioredoxin-like motifs (CxxC C = cysteine, x = any amino acid) conferring catalytic activity, separated by catalytically inactive b and b′ domains. The crystal structure of its orthologue PDI showed a twisted U-shaped conformation with the catalytic sites at the ends of the U and a hydrophobic patch with specificity for peptides and small misfolded proteins in the interior of the U bend (21). Typically PDI proteins form transient disulphide bridges with their substrate through the N-terminal cysteine of the thioredoxin motif, which is rapidly resolved in the ‘escape pathway’ by attack from the C-terminal cysteine, thus forming an intramolecular disulphide bridge and releasing the substrate. ERp57 interacts directly with some non-glycosylated proteins to assist their oxidative folding or via the lectin-like chaperones calnexin and calreticulin to assist oxidative folding of glycoproteins. In conjunction with calnexin ERp57 catalyses MHC I HC oxidative folding during early assembly events (22), and further confirming the importance of ERp57 in MHC I biogenesis, few MHC I molecules reach the surface of ERp57-deficient cells (23). In 2002, a significant step was taken towards revealing the PLC architecture when it became apparent that tapasin exists almost exclusively in the PLC as a semi-stable disulphide-linked heterodimer with ERp57, involving cysteine 95 (Cys95) in tapasin and Cys57 situated in the N-terminal thioredoxin motif of ERp57 (24). Thus the interaction formed with tapasin is unusual, with ERp57 effectively trapped within the PLC.

The presence of ERp57 in the PLC intuitively suggests that the thiol-oxidoreductase enzymatic activity was the function selected by evolution. Supporting this possibility are studies where ERp57 is excluded from the PLC by mutation of tapasin Cys95. In such cells, although PLCs assemble as normal, there are some deficiencies in MHC I surface expression, and significantly there is incomplete oxidation of some, but not all, MHC I alleles within the PLC (24). Further the detection of MHC I–ERp57 complexes in such cells suggested that by conjugating with tapasin, the disulphide bond within the MHC I peptide-binding groove was protected from being reduced by PLC-independent ERp57 (24, 25). However, subsequent findings suggested ERp57 does not perform any enzymatic function within the PLC:

(1) In ERp57-deficient mouse B cells MHC I molecules are fully oxidised, suggesting either redundancy exists among thiol oxidoreductases or non-enzymatic oxidative folding proceeds sufficiently (23). However, PLC complexes have deficient recruitment of MHC I and calreticulin, which may account for the decreased surface MHC I expression and impaired CTL responses (23, 26).
(2) Partial oxidation of some MHC I alleles in tapasin Cys95 mutant cells might instead represent failure to acquire a suitable peptide cargo. This may correlate with the increased propensity of these MHC I alleles to exist as β_2m-free HC (25), leading to the reduction of intramolecular disulphide bridges which precedes degradation of unassembled HC.
(3) The a′ domain thioredoxin motif of ERp57 forms an intramolecular disulphide bridge which is buried at the interface with tapasin (discussed further below) (27). Thus, this thioredoxin motif is seemingly unable to participate in oxidative folding. Indeed mutation of this motif, while preserving the tapasin–ERp57 conjugate (ERp57 Cys60/406/409 mutants) does not adversely affect functionality (22, 28, 29).

These studies suggest that ERp57 fulfils a structural role within the PLC; indeed in cellular lysates only the tapasin–ERp57 heterodimer, and not free tapasin, interacts with MHC I molecules and facilitates peptide loading (30). Interaction of ERp57 with both tapasin and calreticulin may strengthen the individually weak interactions that exist between these chaperones and MHC I molecules. However, all might not be quite so simple: Zhang et al. showed that ERp57 requires no interaction with calnexin or calreticulin to function, and is recruited to the PLC by tapasin alone (29). Additionally the authors showed that calreticulin does not require either the lectin or ERp57-binding sites to associate with the PLC, and is recruited by means of a poorly defined polypeptide binding site. These findings are hard to reconcile with the 'molecular glue' hypothesis. However, a recent report supports such a hypothesis: Del Cid et al. showed that calreticulin requires both its glycan and ERp57-binding specificity to be efficiently incorporated into the PLC (31). Furthermore, an *in vitro* study showed that the specificity of calreticulin for MHC I is strictly dependent upon the MHC I monoglucosylated N-linked glycan (32). The contradictory conclusions of these reports may result from different calreticulin mutants: while the lectin-deficient calreticulin mutant used by Zhang et al. had a greatly reduced ability to bind glycosylated substrates; the mutant used by Del Cid consistently had an absolute inability to bind glycans. Therefore, it is possible that ERp57 collectively strengthens the interactions within the PLC, a possibility reinforced by an *in vitro* study showing that tapasin was fully functional in the absence of ERp57 providing that tapasin and MHC I are artificially tethered (33).

The tapasin–ERp57 conjugate may also subtly alter PLC structure or function, perhaps by maintaining a functional tapasin conformation. Interestingly, the cysteine residue in tapasin which conjugates with ERp57 is not conserved in birds or fish, implying that the ERp57–tapasin conjugate is a relatively recent adaptation. If the tapasin–ERp57 conjugate maintains a particular tapasin conformation, then in birds and fish tapasin may be less likely to adopt alternate conformations (perhaps on account of the extra N-terminal disulphide bridge), or may use other PLC chaperones for conformational support. Additionally the tapasin-ERp57 conjugate may preclude access to MHC I from PLC-independent ERp57 or other thiol oxidoreductases, thus preserving the fully oxidised MHC I status.

PDI: A second oxidoreductase in the PLC?

Contributing to the complexity of the PLC are the findings of Park et al., who showed a significant proportion of PDI associated with the PLC (34); however, this interaction may be extremely labile, as PLC-incorporated PDI is not consistently found (e.g. Ref (25)). Park et al. suggested PDI performs a crucial enzymatic role within the PLC, with PDI depletion resulting in: partial MHC I reduction within the PLC; decreased MHC I maturation and thermal stability; decreased surface expression of correctly folded MHC I and decreased ability to mount an antiviral response. Intriguingly while MHC I expression was severely compromised, the surface expression of several other disulphide bond containing glycoproteins were unaffected by PDI depletion, suggesting that MHC I is especially sensitive to the oxidative conditions within the ER.

The authors dissected the role of individual domains of PDI by expressing truncated PDI proteins in cells largely depleted of the endogenous protein. This showed that the a domain thioredoxin motif and b′ domain peptide-binding site fully complemented the deficiencies resulting from PDI depletion. While the peptide-binding capacity initially raised the possibility that PDI may protect peptides from degradation and deliver them to peptide-receptive MHC I molecules within the PLC, Park et al. subsequently established that peptide was a cofactor or was otherwise required for substrate interaction (35). Furthermore, PDI was found to interact non-covalently with ERp57 and tapasin as well as being disulphide bonded with tapasin. Through these interactions PDI was suggested to provide a quality-control checkpoint after optimal MHC I peptide loading whereby PDI controls the reduction of the tapasin–ERp57 conjugate, thus limiting release of peptide-loaded MHC I from the PLC. Surprisingly resolution of the conjugate and control of MHC I egress relied not upon the a domain thioredoxin motif, but instead upon the b′ domain peptide-binding capacity. However, this possibility is not consistent with studies where ERp57 is permanently conjugated to tapasin (Cys60Ala ERp57 mutants) and is fully functional (27, 30). Furthermore, a recent report suggests that PDI associates with the PLC in a distinct fashion, notably involving a direct interaction with TAP (36). Clearly there are significant contradictions in the literature; however, the possibility remains that PDI associates with PLC components, and by so doing PDI may fulfil a significant structural or functional role within the PLC.

The tapasin–ERp57 structure

Our understanding of antigen processing and presentation has been greatly enhanced by the crystal structures of some key proteins. Thus great anticipation greeted the crystal structure of tapasin, a feat requiring preservation of the semi-stable tapasin–ERp57 interaction (Cys60Ala ERp57 mutation) (27). Even though this long-awaited structure does not illustrate exactly how tapasin achieves editing of the MHC I peptide repertoire, the structure does however provide significant insight.

Despite being conjugated to tapasin, ERp57 adopts a similar conformation to the orthologous protein PDI, with the a and a′ domains using equivalent surfaces to bind to tapasin. There is the expected intermolecular disulphide bridge between ERp57 Cys57 and tapasin Cys95, while the a′ thioredoxin

motif forms an intramolecular disulphide bond buried at the interface with tapasin. The b and b′ domains arch away from tapasin to form a cavity 10–30 Å in diameter. Comparison of the a and a′ domains of ERp57 and PDI showed no significant alterations which might inactivate the a domain escape pathway. Instead, the stability of the tapasin–ERp57 conjugate may be a consequence of both the a and a′ domains interacting with tapasin, resulting in a large shared surface area with numerous non-covalent interactions. It is noteworthy that mutation of either thioredoxin motif ablates the association with tapasin (24). While the effect of mutating the a domain motif is obvious, the effect of mutating the a′ domain motif is more subtle, and suggests that this buried intramolecular disulphide bridge maintains ERp57 in a stable conformation permitting an interaction to occur.

Tapasin assumes an inverted L shape, with two intramolecular disulphide bridges (Cys7–Cys71 and Cys295–Cys362, Figure 1A) (27). As anticipated (37) there is a C-terminal immunoglobulin-like (Ig) domain which forms the (usually) membrane proximal base of the structure. Above this is a novel three tiered β-sandwich N-terminal domain, which is expected to be level with the MHC I peptide-binding groove and therefore potentially influencing peptide selection. Indeed a recombinant 87 amino acid N-terminal tapasin fragment possesses MHC I chaperone-like activity (38). Most of the loops linking β strands within this domain are well organised, but three loops are consistently insufficiently structured to permit crystallisation: these loops link the first three N-terminal strands and form a loop into the cavity betwixt tapasin and ERp57. It is possible that these loops usually interact with, and therefore influence, the MHC I peptide-binding platform.

Furthermore in conjunction with the structure Dong et al. identified a conserved surface that they speculated may represent a functionally relevant MHC I interaction site. Importantly the authors showed that only mutations located in and around this conserved surface severely impaired association with MHC I and diminished MHC I peptide loading. Therefore, this conserved surface is likely to play a significant role in the peptide-editing mechanism.

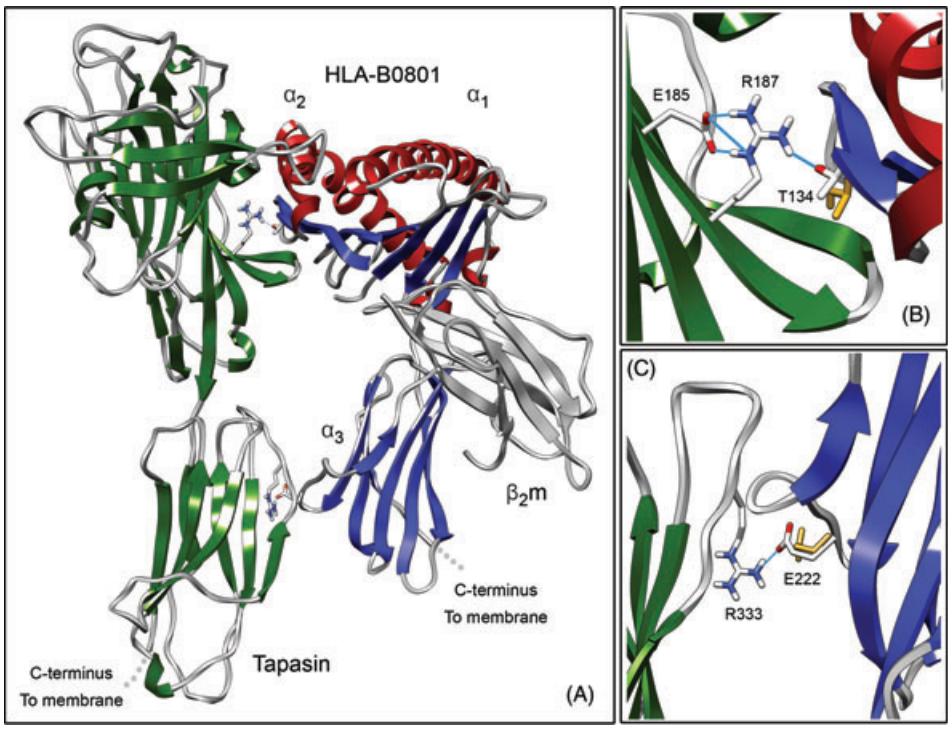

Figure 1 Modelling a functional tapasin and MHC I interaction. (A) Tapasin (green) was docked with an open MHC I conformation (HC in blue and red, β_2m in grey) using known sites of interaction: the TN6 region of tapasin identified in Ref (27) is docked with MHC I residue T134 [identified in Ref (14)] using HADDOCK (39). The open structure of human leukocyte antigen (HLA)-B*0801 (pdb ID: 1agb) (40) was generated as an average structure from an energy minimisation of the crystal structure with peptide absent (41). The human tapasin structure is completed from the tapasin–ERp57 crystal structure (pdb ID: 38fu) (27) using homology modelling (42). Images generated using Chimera (43). (B) Interactions involving the side chain of MHC I residue T134 of the peptide-bound structure of HLA-B*0801 (gold) are compared with an open structure (red and white). In the absence of peptide, the α_{2-1} helix (red) widens the peptide-binding groove by ≈3 Å (measured between residues 76 and 150) as previously observed (44). This movement corresponds with movement of T134 by ≈ 2 Å to find a more favourable interaction with tapasin residue R187. Tapasin residue E185 appears to form a structural interaction with R187. (C) The simulation independently identifies a favourable C-terminal interaction between tapasin residue R333 and MHC I residue E222 (coloured as above) which is more favoured with an open MHC I structure.

Conformational flexibility of empty MHC peptide-binding grooves

Numerous studies have shown that tapasin does not interact with peptide–MHC I complexes; in the absence of peptides HC:β_2m heterodimers are thought to transiently adopt alternate less structured conformations before becoming refractory to peptide binding (45). Thus, correlating known sites of interaction with the static structures of tapasin and peptide–MHC I complexes is challenging. It is likely that tapasin interacts with and stabilises alternate MHC I conformations to preserve the MHC I peptide-binding ability and allow preferential selection of high affinity peptides.

While there is no direct structural knowledge of alternate MHC I conformations, it is possible to conduct molecular dynamic simulations (MDS) of peptide-free MHC I molecules (46). MDS suggest that while the membrane proximal Ig-like domains (α3 and β_2m) and the β sheet floor of the peptide-binding groove are not significantly altered by the absence of peptide, the α helices flanking the empty peptide-binding groove exhibit greater conformational flexibility. Interestingly, the two ends of the α helices are differentially affected by the absence of peptide; while the end that binds the N-terminus of peptide ligands remains in a conformation similar to that of peptide-occupied structures, the end that binds the C-terminus (including the F-pocket) exhibits significant conformational variation, especially the short α2-1 helix, which rolls outwards to distort the C-terminal portion of the binding groove. Supporting these simulations is that the α2-1 helix adopts slightly different conformations in various peptide–MHC I crystal structures, as well as in a ligand-free non-classical MHC structure (47, 48). Interestingly, the α2-1 helix lies above the loop that is a predicted binding site for tapasin (residues 128–136), and the likelihood that the newly identified conserved surface of tapasin interacts with this loop to stabilise a MHC I conformation with a more open C-terminal end of the groove is compelling. By stabilising the MHC I molecule in this way, the widened binding groove might disrupt interactions between peptide and MHC I, disfavouring the binding of peptides which do not form the full complement of interactions throughout the peptide-binding groove.

Peptide-free MDS analysis of two MHC I alleles differing only at position 116 showed allelic variation in their propensity to adopt alternate conformations (44). Although part of the F-pocket, the polymorphism does not affect peptide-binding preference (49) but does significantly alter the dependence upon tapasin to optimise the peptide repertoire (50). The tapasin-dependent B*4402 allele was predicted by MDS to favour a more open conformation than the tapasin-independent B*4405 allele, which predominantly remained in a conformation similar to the peptide-occupied structure, which may be amenable to peptide exchange. Analysis of the changes predicted to occur as peptide is drawn away from the F-pocket suggests that for both MHC I alleles the peptide-binding groove widens considerably during peptide dissociation. Crucially, however, B*4405 recovers from such a widened conformation, while B*4402 fails to recover, resulting in a gaping binding groove that is unlikely to bind peptides (44). This suggests that variation in MHC I dependency upon tapasin to optimise peptide repertoire correlates with the ability of MHC I alleles to maintain a 'receptive' conformation similar to that seen in the peptide-occupied crystal structures. As partially widened, peptide-free grooves may be more solvent-accessible than when a peptide is bound, the balance of hydrophobic and hydrophilic residues within the groove may govern the self-editing potential. In this respect it is interesting to note that the side chains of residue 116 for these alleles have different solubility: Y116 in B*4405 may preferentially remain 'buried', being much less prone to hydration than D116 in B*4402. Thus, B*4405 may preserve the integrity of the F-pocket in the absence of bound peptide.

The dynamic nature of empty MHC I peptide-binding grooves may also be shared with MHC II molecules (51–53). As for MHC I, the MHC II-α helices display most conformational variation in peptide-free MDS, notably that of the MHC II-β chain (which, like the MHC I α2 helix, can be split into three short α helices) which rolls outwards to distort the N-terminal end of the MHC II peptide-binding groove. Thus, there are interesting similarities shared by MHC I and II molecules: while the MHC I α2-1 helix might be maintained in a receptive conformation by tapasin; the N-terminal end of the MHC II groove may be maintained in a receptive partially open conformation by the chaperone DM, which interacts in this region. Furthermore, there is the possibility that the function of both tapasin and DM in MHC peptide loading is regulated: while the MHC-like protein DO attenuates DM function; tapasin may be regulated by the tapasin-related protein (known as TAPBP-R) (54).

Another similarity suggested by MDS are predicted conformations that may be averse to peptide binding, although no simulations show gross-structural alterations or large-scale-domain unfolding. For MHC I, the fully open C-terminal groove of B*4402 is unlikely to be receptive to peptide binding. While one MHC II simulation predicted a partial collapse at the N-terminus of the MHC II groove, with part of the α-chain folding inwards to occupy two MHC II anchor positions (P1 and P4), and form hydrogen bonds that usually exist between MHC and peptide (53). This MHC II conformation is unlikely to permit peptide binding. It is noteworthy that these simulations are on the nanosecond scale, longer simulations may predict large-scale structural changes including partial unfolding as suggested previously (45).

A model of the peptide-loading sub-complex

Dong et al. also generated a model of the MHC I, tapasin, ERp57, calreticulin PLC sub-complex by juxtaposing known or likely structures of PLC constituents with known sites of

intermolecular interaction (27). In this model, the MHC I α2 loop was proximal to the newly identified conserved surface of tapasin. Calreticulin was placed to form interactions with the MHC I N-linked glycan and with ERp57. A recent study suggested that the glycan-binding site of calreticulin, which resides in the globular domain of the protein, is essential for PLC incorporation (31). However, while this may be the case, earlier studies indicated that it is possible to reduce the affinity of the glycan-binding domain by 80%–95% without losing the ability to either recruit calreticulin to the PLC or to restore antigen-presenting function (29, 55). In the former study, a central role was given to the glycan attached to tapasin asparagine 233 for calreticulin recruitment. If this interaction was involved in stabilising the PLC, then it is likely that the PLC components would adopt different relative positions than proposed by Dong et al. The interaction between calreticulin and ERp57 is likely to occur via charged residues within the b and b′ domains of ERp57 associating with the flexible 'arm-like' proline rich P domain of calreticulin. This interaction involves the opposite face to the substrate-binding patch of the orthologous protein PDI, suggesting ERp57 has evolved to interact with substrates in a different fashion. The importance of the calreticulin–ERp57 interaction for PLC assembly is not fully resolved with one mutagenesis study indicating dependence (31) and another indicating independence (29). Thus, while the model proposed by Dong et al. is persuasive and consistent with the functions of PLC components, the sub-PLC may involve alternate interactions and possibly alternate conformations than are evident in protein crystal structures. Indeed this is highly probable for the elongated P domain of calreticulin, and the N-terminal domain of tapasin may also flex relative to the C-terminal Ig domain, although this may be restrained by conjugation with ERp57. Perhaps most significant though is that within the PLC the MHC I peptide-binding groove is unlikely to be in the conformation seen by protein crystallography so far (as discussed above).

Greater complexity is suggested by studies showing that four sub-complexes cluster around each TAP heterodimer (37), although other studies suggest higher (56) or lower (9) PLC stoichiometry. Tapasin interacts with the first three of four N-terminal transmembrane (TM) domains of both TAP proteins via residues in the TM and membrane proximal domains of tapasin (57, 58). TAP mutants lacking these 'N-domains' form functional peptide-binding and peptide-transporting heterodimers in the ER membrane (forming a core TM segment that is predicted for most ABC transporters), but are unable to bind tapasin (57, 59). For both TAP proteins, these tapasin-binding domains are poorly conserved when multiple species are compared, and in some species only one N-domain is present, suggesting lower PLC stoichiometry (60). Depending upon the stoichiometry, MHC I peptide loading must occur in a crowded molecular environment.

Peptide trimming in the ER

In the mid-1990s, it was shown that proteolysis is not restricted to the cytoplasm, and that optimal MHC I ligands can be generated in the ER (61, 62). The protease which trimmed sub-optimal N-terminally extended precursor peptides in the ER was identified in 2002 and named ERAAP (ER aminopeptidase associated with antigen processing) in mice, or ERAP1 in humans (ER aminopeptidase 1, ERAAP hereafter) (63, 64). Murine and human ERAAP share: homology (86% identity); Interferon-gamma (IFN-γ) induction; and substrate specificities, with most amino acids being hydrolysed except X–P peptides (X = any amino acid and P = proline), although trimming of aliphatic amino acids is most efficient (63–65). The inability of ERAAP to trim X–P peptides may resolve the unexpected prevalence of such peptides among human MHC I molecules given the inability of TAP to transport these peptides: it is likely that X–P peptides result from ERAAP-mediated trimming of TAP-transported N-terminally extended precursors (66–68).

In mice, ERAAP is solely responsible for ER aminopeptidase activity; however, a second ER aminopeptidase has been identified in humans; ERAP2 which has ~50% identity to ERAAP (69). ERAP2 has distinct substrate specificity and preferentially hydrolyses basic residues (70, 71). Interestingly ERAAP and ERAP2 can heterodimerise, thus combining specificities to generate peptides that the individual aminopeptidases can not. However, only a small proportion of ERAAP molecules exist as heterodimers and evidence for the role of ERAP2, or heterodimers, in the generation of antigenic peptides *in vivo* is not yet available. Additionally, the ERAP2 tissue expression pattern, unlike ERAAP, does not correlate with MHC I expression suggesting MHC I peptide ligands are primarily generated by ERAAP (69, 72).

The importance of ERAAP to MHC I restricted peptide presentation was further reinforced when ERAAP-deficient mice were generated. In such mice, classical and non-classical MHC molecule surface expression levels are reduced (73–76) although less so than with tapasin- or TAP-deficiencies. In particular, the murine MHC I molecule L^d is severely affected; an observation likely attractable to the peptide-binding preference of L^d (which favours X–P peptides).

Comparison of a range of endogenous peptides that are presented by MHC I in wild-type or ERAAP-deficient mice showed that peptides fall into three categories: (i) those that are dependent on ERAAP for their generation and are not seen in ERAAP-KO mice; (ii) peptides that are preferentially presented in ERAAP-KO mice, suggesting ERAAP limits their generation through over-processing; and (iii) peptides that are unaffected and presumably require no further proteolysis. Similarly ERAAP-KO mice exhibit variation in CD8 T-cell responses to a range of pathogens (mostly murine viruses); with some responses being increased, decreased or remaining unaffected. Interestingly ERAAP-KO mice resolve pathogen infections comparably to wild-type animals with the exception

of *Toxoplasma gondii*, where ERAAP-deficient mice are susceptible (77). This susceptibility results from the inability of ERAAP-KO mice to generate the immunodominant epitope, thus limiting T-cell response induction. Intriguingly when wild-type mice are immunised with ERAAP-deficient cells a robust CD8 T cell and antibody response develops (78). The precise antigens stimulating these responses are not known, but are only observed in the absence of ERAAP.

With its aminopeptidase activity it might be anticipated that MHC I peptide loading would be most optimal if ERAAP associated with the PLC, where the proximity of ERAAP, MHC I and TAP would allow immediate capture of optimal peptides generated by ERAAP following transport of N-terminally extended precursors. However, despite exhaustive investigations ERAAP has not been found associated with the PLC or other ER resident proteins, suggesting if such an association exists it is extremely transient and labile.

The mechanism by which ERAAP trims precursors to an optimal length for MHC I remains unresolved. Two mechanisms have been proposed: firstly that MHC I binds N-terminally extended peptides which are subsequently trimmed by ERAAP. Thus MHC I acts as a template, allowing ERAAP to generate optimal peptide ligands. Evidence supporting this mechanism is provided by the observations that optimal peptides are only generated in the presence of an appropriate MHC I molecule (79); and that when ERAAP is inhibited, N-terminally extended intermediate peptides can be eluted from MHC I molecules. In the second mechanism ERAAP trims the precursor peptide to an optimal length for subsequent binding to MHC I, and thus ERAAP acts as the molecular ruler (70). This was proposed from observations that ERAAP is unable to digest peptides <8 or 9 residues and trimming by ERAAP is affected by the N- and C-terminal residues, suggesting ERAAP may bind peptide in a similar fashion to MHC I. These mechanisms are not mutually exclusive, although the apparently contradictory findings suggest that the results obtained reflect the different systems used and currently preclude comprehension of a definitive mechanism.

Recently it has become apparent that ERAAP is polymorphic, and that a correlation exists between particular ERAAP alleles and the autoimmune condition ankylosing spondylitis (AS) (80). While AS is strongly associated with human leukocyte antigen (HLA)-B27, a molecular explanation for this association has remained elusive (81). One intriguing finding is the propensity of B27 molecules to form HC homodimers at the cell surface, where they may contribute to the initiation of AS (82). B27 homodimers are generated in endosomes, presumably following the loss of peptide and the dissociation of β_2m. Interestingly, addition of high affinity B27 epitopes into cells substantially reduces the formation of homodimers (83), which may identify the ability to generate B27 binding peptides as the link between B27 and AS. Thus, the differential activity of particular ERAAP alleles to generate B27 epitopes may underpin the association of particular ERAAP alleles with AS.

The molecular mechanism of MHC I peptide loading

Tapasin quantitatively and qualitatively improves MHC I antigen presentation

Since 2002 knowledge of how tapasin enhances MHC I peptide loading has been significantly advanced. Williams et al. laid the foundations for this progress by using an experimental system where MHC I loading and peptide editing was visualised in wild-type or tapasin-deficient cells to establish key concepts (50):

(1) Editing of the MHC-bound peptide repertoire involves replacement of sub-optimal fast off-rate peptides with higher affinity, slower off-rate peptides. This peptide optimisation mechanism may involve iterative peptide-binding/release cycles culminating in the selection of peptides that bind most stably. However, peptide trimming may also contribute, as discussed above.
(2) Peptide editing is an intrinsic property of MHC I molecules which occurs in the absence of tapasin to varying extents for different MHC I alleles.
(3) Tapasin augments this intrinsic peptide-editing ability, leading to:
    a. An increased rate of optimal peptide loading, which was apparent even for those alleles capable of self-editing.
    b. An increased amount of loaded MHC I molecules, although the increase that tapasin afforded varied. While tapasin significantly boosted the expression level of tapasin-dependent MHC I alleles, tapasin effected a much smaller increase in the expression of MHC I alleles which can self-edit. Thus self-editing may be beneficial if tapasin expression is suppressed, as may occur in viral infections or in tumours [e.g. Refs (84–87)] but may carry the cost of receiving a smaller benefit from tapasin under physiological conditions.
(4) Peptide editing occurs most efficiently as part of a fully formed PLC rather than in TAP-free sub-PLC complexes.

Tapasin selects peptides according to the stability they confer to MHC I molecules

Howarth and colleagues established that tapasin plays the most significant role in dictating which peptides are presented by MHC I (88). In this study, a series of peptides conferring different half lives when presented by MHC I molecules at the cell surface were expressed in wild-type or tapasin-deficient

cells. In wild-type cells a hierarchy existed in which peptides conferring greatest MHC I stability were preferentially presented at the expense of peptides conferring decreased stability. While in the absence of tapasin the most significant alteration concerned the relative presentation levels; with suboptimal peptides benefiting relative to the optimal peptide, a finding that has been observed *in vivo* (89, 90).

Editing of the MHC I peptide repertoire in favour of higher affinity peptides was also shown to be a function of tapasin in a study where tapasin-deficient lysates were supplemented with recombinant tapasin–ERp57 molecules (30). Furthermore, the ability of tapasin to stabilise peptide-receptive MHC I molecules prior to peptide binding was also shown, as suggested previously (19).

Although only a small sample of peptides has been investigated the results suggest tapasin exponentially favours the presentation of high affinity peptides. Thus, a peptide–MHC I complex with a slightly slower off-rate may have a dramatically increased surface expression level relative to a faster off-rate peptide–MHC I complex, which may be relevant to CTL response induction. Indeed, the significant contribution of tapasin to the immunodominance hierarchy of competing viral epitopes was recently shown and is discussed later (90).

Tapasin stabilises an open MHC I conformation allowing rapid peptide exchange

Until recently the ability to analyse tapasin function *in vitro* was precluded by the low affinity with which tapasin and MHC I molecules interact. However, a breakthrough came when leucine zippers were used to tether recombinant soluble tapasin and MHC I proteins together allowing detailed investigation of the mechanism by which tapasin augments peptide loading/release (33). In this study several observations were made:

(1) Peptide association: as previously shown, tapasin increases both the loading rate and the amount of assembled MHC I molecules.
(2) Peptide dissociation: tapasin accelerates peptide dissociation. However, there was no correlation between peptide half life and the extent to which tapasin accelerated dissociation. Furthermore not all peptides were susceptible to the action of tapasin.
(3) Additionally tapasin was shown to enable MHC I to preferentially select a high affinity peptide over a lower affinity peptide.

Analysis of peptide–MHC I dissociation rates collected in the absence of tapasin provided additional indirect evidence of the heterogeneous conformational nature of MHC I molecules: while a small proportion of MHC-bound peptide dissociated rapidly, most of the MHC-bound peptide dissociated significantly more slowly. This is consistent with two conformations of peptide–MHC I complexes; with peptides dissociating rapidly from a small proportion of open peptide–MHC I complexes, with the majority being properly folded, stable peptide–MHC I complexes which slowly convert into an open conformation.

In contrast to the biphasic dissociation rates discussed above, when tapasin was mixed with peptide–MHC I complexes there was a single, fast rate of peptide dissociation. This suggests that by associating with tapasin peptide–MHC I complexes uniformly adopted an open conformation; consistent with the increased rate and amount of peptide–MHC I complexes produced when tapasin-assisted peptide-association assays. The ability of MHC I to transit between conformations, and hence tapasin function, was shown to depend principally upon the conserved hydrogen bond network at the C-terminus of the peptide-binding groove as suggested by MDS, although interactions throughout the peptide sequence also contributed.

The observation that tapasin function involves disruption of conserved hydrogen bonds between the peptide and MHC I F-pocket (33) suggests that tapasin may catalyse peptide binding in a similar way to how DM enhances MHC II peptide loading [reviewed in Ref (91)]. DM is thought to bind to an 'open' MHC II conformation and disrupt a conserved hydrogen bond between the peptide backbone and histidine 81 of the MHC II β chain, thereby accelerating peptide association and dissociation rates, bringing peptide binding under thermodynamic control more rapidly.

Precisely how tapasin achieves its function remains to be seen but the tapasin–ERp57 structure provides a platform for investigating how the dynamics of MHC I protein structure relates to peptide editing; and how tapasin assists this function. For example, we have docked a refined model of the tapasin structure to an energy-minimised peptide-free MHC I structure (HLA-B*0801). This open MHC structure was generated from the average structure of an 8-ns molecular dynamics simulation (Figure 1A). Docking was driven by the functional hotspot in tapasin (TN6) identified by Dong et al. (27) and the functional hotspot in MHC I identified by the T134K mutation (14). This showed the potential for a broad binding 'pocket' on tapasin for the α2-1 helix of MHC I (comprising the TN6 region of tapasin, the eight residue loop between residues 77 and 84 and the 10-21 loop which was not resolved in the crystal structure). The docking identifies a potential side-chain interaction between tapasin R187 and MHC I T134 which is more favourable with an open, peptide-free MHC I conformation (Figure 1B). Additionally the model finds an interaction between the membrane proximal domain of tapasin and the α3 domain of MHC I focussed on R333 of tapasin and E222 of MHC I. This is significant because mutations at position 222 prevent MHC I from interacting with the PLC. Interestingly in this model the putative R333–E222 interaction is also more favourable when MHC I is peptide-free (Figure 1C).

Overall, this supports a model in which physical approximation of MHC I and tapasin is achieved via co-operative interactions within the PLC, allowing weak functionally important interactions to occur. These may involve discrete side-chain interactions that are sensitive to relatively small allosteric shifts as suggested above, but they might also include interactions covering a much larger conformational space explored by the MHC I molecule. Such a quasi-stable intermediate of the MHC I HC has been proposed (45). Particularly interesting in this respect is that the N-terminal 87 tapasin fragment (which although not including the whole of the TN6 region identified by Dong et al., does contain both of the loops mentioned above) increases the yield of peptide–MHC I complexes from an *in vitro* refolding assay without affecting the rate at which peptide–MHC I complexes are formed (38). It is tempting to speculate that this distinguishes a chaperone-like function of tapasin from a catalytic function. If this were the case, it is possible that chaperone-like interactions between tapasin and a partially unfolded peptide-binding groove alter the energy landscape of the empty MHC I molecule in such a way as to 'funnel' it towards a conformation amenable to specific side-chain interactions that promote peptide exchange.

Thus, considerable effort has enhanced knowledge of how tapasin manipulates MHC I peptide selection. However, complete comprehension of the MHC I peptide selection mechanism requires: structural definition of the peptide-receptive MHC I conformation; knowledge of the mechanism by which MHC I molecules transit between conformations; and definitive identification of points of contact functionally relevant to the tapasin–MHC I interaction.

Post-ER quality control

Peptide–MHC I complexes are exported from the ER irrespective of peptide affinity

The last decade has seen greater understanding develop of the balance between the function of the PLC and of the secretory pathway. In the absence of data it had been assumed that only MHC I complexes loaded with high affinity peptides were released from the PLC and consequently exited the ER. Thus the dogma was that the PLC, and by implication tapasin, provided the sole quality-control checkpoint in MHC I antigen presentation. However, this assumption has now been superseded.

In 2007, Garstka et al. analysed the intracellular localisation of MHC I molecules in wild-type or TAP-deficient cells (93). The authors showed that in addition to the ER MHC I complexes can be found in the ER–Golgi intermediate compartment (ERGIC) and the *cis*-Golgi compartments, as had been suggested when MHC I molecules were over-expressed. The mechanism by which MHC I molecules are exported from the ER had long been suspected to be limited by a step subsequent to peptide loading (94, 95). It has now transpired that peptide–MHC I complexes accumulate at specific ER exit sites, and that the MHC I TM domain associates with abundant cargo receptor Bap31, which interacts with COP-II coated vesicles to transport various cargo proteins to the ERGIC (94, 96–98). MHC I export is not however, dependent upon Bap31, suggesting redundancy amongst cargo receptors (96, 97). Intriguingly the function of Bap31 in MHC I export may be regulated by Bap29, which has been implicated in retrograde retrieval of cargo proteins to the ER (97, 98).

Significantly when the contents of purified COP-II vesicles were analysed, peptide–MHC I complexes were shown to be efficiently exported from the ER irrespective of the stability that the peptide confers to the MHC I molecule (93). Crucially, however, only those MHC I molecules loaded with high affinity peptides progress beyond the medial Golgi to the cell surface.

Calreticulin participates in post-ER MHC I quality control

Calreticulin is a soluble chaperone that aids protein folding in the ER. Most substrates are glycosylated proteins recognised by the mono-glucosylated moiety that is transiently displayed in the secretory pathway. However, calreticulin can also interact directly with non-glycosylated proteins through a polypeptide binding site. Calreticulin aids protein folding by: suppressing aggregation; facilitating oxidative folding by recruiting ERp57; directing terminally misfolded proteins towards the ER-based degradation pathway; and retrieving misfolded proteins from post-ER compartments. This ER retrieval mechanism involves:

(1) Calreticulin associating with a misfolded substrate in a post-ER compartment.
(2) Recognition of the C-terminal KDEL (in single letter amino acid code) ER retrieval motif of calreticulin by the KDEL receptor localised in the *cis*-Golgi. This receptor directs calreticulin and its associated substrate to the ER through COP-I-coated vesicles.
(3) Further trimming of the glycan moiety of the misfolded protein occurs in the ER resulting in either the degradation pathway or recognition by the enzyme UDP glucosyl glycoprotein transferase (UGGT). UGGT reattaches the preformed N-linked glycan moiety to non-native substrates thus starting a new folding attempt.

Using a calreticulin–KO cell line, Gao et al. showed that although MHC I molecules are synthesised as usual there is a significant reduction in MHC I surface expression levels and deficient CTL responses to most antigens (99), despite tapasin-mediated peptide editing occurring efficiently (88). Thus although not involved in peptide selection, calreticulin performs an essential quantitative function in MHC I peptide loading. Biochemical analysis has since provided molecular

explanations for the deficiencies apparent in the absence of calreticulin:

Loss of calreticulin impairs PLC stability

There is a slight reduction in the amount of ERp57 and MHC I molecules that can be co-precipitated with the PLC in calreticulin-deficient cells (55, 99, 100). This phenotype supports the molecular glue hypothesis, suggesting that as with ERp57, calreticulin strengthens interactions within the PLC.

MHC I molecules mature at an increased rate without calreticulin

This phenotype is shared by MHC I mutants which do not interact with the PLC and consequently fail to optimise their peptide repertoire (92). The faster maturation rate and knowledge that peptide-receptive MHC I molecules cycle between the ER and *cis*-Golgi suggests that calreticulin may retrieve such complexes from post-ER compartments through the KDEL ER retrieval mechanism. Howe et al. investigated this possibility and made several significant observations (100):

- Although at steady-state calreticulin is retained or retrieved to the ER, the over-expression of substrates can cause calreticulin to co-localise with its substrates in post-ER compartments. Under such conditions, the authors showed co-localisation of calreticulin with sub-optimal peptide–MHC I complexes in the ERGIC and *cis*-Golgi, supporting the possibility that calreticulin may retrieve these molecules to the ER.
- Without the KDEL motif calreticulin is unable to retrieve sub-optimal peptide–MHC complexes to the ER. Consequently, MHC I molecules are transiently expressed at the cell surface before endocytosis and degradation occurs (100).
- Crucially, presentation of a model antigen required the KDEL motif of calreticulin, highlighting the functional significance of the calreticulin-mediated retrieval system.
- Furthermore, calreticulin co-localised with an MHC I mutant which fails to interact with other PLC proteins. Thus calreticulin may retrieve MHC I molecules independently of other PLC members which are also present outside of the ER (101).

Therefore, calreticulin performs two functions in MHC I antigen presentation: strengthening of the interactions within the PLC; and retrieval of inappropriately loaded MHC I molecules to the ER (summarised in Figure 2).

The contribution of other PLC members to MHC I retrieval

Other PLC members can be found outside of the ER, so selective MHC I retrieval may not involve only calreticulin.

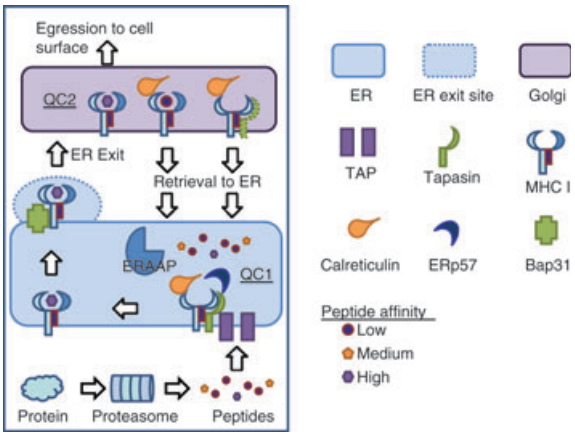

Figure 2 Key steps in MHC I peptide loading.
Peptide production: In the cytoplasm, peptides are produced following the degradation of proteins by the proteasome and other proteases. Some peptides escape complete proteolysis by being transported by TAP into the ER. In the ER peptides can be further processed by ERAAP. Capture of peptides by an MHC I molecule may protect a peptide from over-processing.
Peptide loading in the ER: MHC I molecules are loaded with peptides in the PLC: here tapasin allows MHC I molecules to select peptides according to their off-rates, rather than their concentration in the ER (first quality-control checkpoint, QC1): Peptide–MHC I complexes are released from the PLC loaded predominantly, but not exclusively, with high affinity peptides. Peptide MHC I complexes exit the ER in COP-II coated vesicles via an association with Bap31. No discrimination occurs between peptide MHC I complexes according to peptide affinity.
Second quality-control checkpoint, QC2: Further discrimination of peptide–MHC I complexes occurs in the Golgi complex favouring high affinity peptide–MHC I complexes progressing to the cell surface. MHC I molecules which have lost their peptide cargo, or are loaded with low affinity peptides are returned to the ER in COP-I-coated vesicles. Once returned to the ER MHC I molecules can re-enter the PLC or be degraded. The selection mechanism is unknown, but appears to be orchestrated by calreticulin, although tapasin may also contribute (dotted lines). The increased acidity of the Golgi complex may favour release of low affinity peptides from MHC I, and/or allow novel peptide generation.

Tapasin in particular may participate, on account of its cytoplasmic dilysine motif (KKxx) which can interact with COP-I-coated vesicles to return proteins to the ER (101). Paulsson et al. examined this possibility by expressing a tapasin molecule with a mutated dilysine motif; surprisingly this tapasin mutant remained predominantly in the ER with only a slightly increased representation in the Golgi relative to wild-type cells (102). Thus association with other PLC members may retain the majority of tapasin in the ER. Despite this there were slight deficiencies in MHC I expression which suggest inferior peptide loading occurred. This can be explained in two ways: the slightly greater rate at which tapasin dilysine mutant molecules exited the ER may have decreased peptide-editing efficiency; secondly without the dilysine motif, MHC I molecules did not associate with the

COP-I-coated vesicles that return sub-optimally loaded MHC I molecules to the ER.

While this study highlights the functional significance of MHC I retrieval, the relationship between calreticulin and tapasin requires further clarification. Comparison of the phenotypes of calreticulin-deficient cells or cells that express tapasin dilysine mutants suggest that calreticulin has the most significant impact upon MHC I retrieval. However, it may be that tapasin complements the retrieval function of calreticulin. Similarly the contribution of ERp57, which may be associated with tapasin or calreticulin, to this retrieval mechanism must be ascertained.

Interestingly despite both tapasin and calreticulin participating in MHC I retrieval their interaction with MHC I may differ: while tapasin may sense, or induce, an MHC I conformation when sub-optimal peptides are bound, or that occurs following peptide dissociation; it is not clear how calreticulin recognises MHC I molecules for retrieval. Calreticulin might rely on its lectin-binding site to recognise monoglucosylated MHC I molecules with discrimination between loaded and peptide-free MHC I molecules perhaps being undertaken by UGGT. Alternatively, or additionally, calreticulin might use its polypeptide binding site to monitor MHC I conformation.

Peptide editing beyond the trans-Golgi network?

Recently it has become clear that peptide editing is not achieved in one cellular location at one specific time, with calreticulin-mediated MHC I retrieval providing further opportunity to achieve peptide editing. Other mechanisms may also exist that allow MHC I molecules to optimise their peptide repertoire outside the ER. This is supported by the observation that MHC I peptide editing continues after acquisition of resistance to endoglycosidase-H digestion, which indicates passage through the medial Golgi (50), an observation supported by other studies (103, 104). Knowledge that tapasin, and other PLC members, occur throughout the secretory pathway raises the possibility that these proteins continue to function in other intracellular organelles (which may affect their function). Moreover, peptide editing may not be restricted to the route to the cell surface; the length of time that MHC I molecules are expressed upon the cell surface may be regulated, with MHC I molecules being internalised to enter the endosomal pathway which may ultimately lead to degradation, or recycling to the plasma membrane. This may allow MHC I molecules to exchange their peptide cargo in endosomes.

Endocytic targeting is achieved by conjugation of ubiquitin to the cytoplasmic portion of various cell surface glycoproteins including MHC I (105–108). The proteins that conjugate ubiquitin to the substrate protein are viral or cellular orthologues of E3 ubiquitin ligases. With regard to the internalisation of MHC I, the best characterised pathways involve viral E3 ligases that allow virally infected cells to escape CTL-induced lysis via internalisation and lysosome-mediated degradation of MHC I. Unlike most cellular E3 ligases, viral E3 ligases are integral membrane proteins whose dual TM regions confer substrate recognition, with membrane proximal cytoplasmic lysine residues of MHC I being polyubiquitinylated (105, 107–109). Mammalian orthologues of E3 ligases (membrane associated RING-CH proteins, or MARCH) have been identified, some of which (MARCH IV and IX) ubiquitinylate MHC I molecules leading to destruction (108). Other studies implicate a conserved cytoplasmic MHC I tyrosine motif in the endosomal trafficking of MHC I molecules in the cross-presentation pathway (110). Less is known about recycling MHC I molecules from endosomes back to the plasma membrane; a process that might allow peptide exchange to occur outside of the ER, with the lower pH of the endo/lysosomal compartment likely to favour dissociation of MHC-bound peptides and generation of transiently peptide-receptive MHC I molecules. Moreover, novel peptide epitopes may be generated in such conditions via proteases localised in such vesicles; indeed Leonhardt et al. recently showed the proprotein convertase PC7 to be indispensable to the post-ER optimisation of sub-optimal peptide–MHC complexes (103). The authors showed PC7, but not other proprotein convertases, to participate in the generation of novel peptide epitopes, thus restoring stable peptide–MHC I complex surface expression levels (presumably) following vesicular peptide exchange. It is likely that the post-ER peptide optimisation ‘rescue’ mechanism may be highly relevant for successful immune responses to occur in virally infected cells or tumours, where PLC functionality is frequently impaired.

MHC I peptide editing in the context of an immune response

When a pathogen infects a cell it is likely that there will be many pathogen-derived epitopes capable of binding to MHC I and potentially inducing a CTL response. However, immune responses frequently focus on only a few epitopes (a phenomenon called immunodominance). Many factors contribute to immunodominance including: generation of pathogen-derived peptides; successful MHC I peptide loading; the length of time that peptide–MHC I complexes are expressed at the cell surface; and many factors affecting T-cell receptor specificity and functionality which are beyond the scope of this review. While all of these are significant, one of the most important concerns the duration that peptide–MHC I complexes exist at the surface of the cells which prime a CD8 T-cell response (111–113). Intuitively it follows that the focusing of a CD8 T-cell response correlates with the MHC I peptide repertoire favoured by tapasin: successful immune responses may require peptide–MHC I complexes to persist at the surface of antigen-presenting cells (APCs) during transit from the infection site to the secondary lymphoid organs

where immune responses are initiated. That this is the case has recently been confirmed.

Thirdborough et al. compared CD8 T-cell responses in wild-type or tapasin-deficient mice individually administered with DNA vaccines encoding one of a series of peptide variants (89). These peptide variants had previously been shown to be presented through tapasin-meditated peptide loading in a hierarchy according to the stability that the peptide conferred to the peptide–MHC I complex (88). Thirdborough et al. showed that following direct presentation in wild-type APCs, a CD8 T-cell response hierarchy was established which correlated with peptide–MHC I stability (89). This was also the case if the peptides were cross-presented by APCs, providing there was transient over-expression of the peptide. Prolonged over-expression led to comparable responses against all peptides, even for a fast off-rate peptide. As peptide loading occurring without tapasin is likely to be dictated by peptide availability, the continual high abundance of any peptide in the ER may lead to significant expression of peptide–MHC I complexes at the cell surface which, even if the peptide–MHC I complexes are unstable, may initiate an immune response. Therefore a direct correlation exists between CD8 T-cell response magnitude and the length of time that peptide–MHC I complexes persist at the cell surface. As tapasin favours the presentation of peptides conferring greatest stability to MHC I, tapasin can have a significant impact upon immunodominance. Additionally, the continual high abundance of a peptide can also affect CD8 T-cell response magnitude irrespective of peptide affinity.

Boulanger et al. analysed CD8 T-cell responses in a more complex system: where distinct viral epitopes were simultaneously expressed in wild-type or tapasin-deficient mice, with the peptides conferring differing stability to MHC I and being recognised by different T cells (90). The key finding was consistent; in wild-type mice the CD8 T-cell response hierarchy favours slow off-rate peptide–MHC I complexes. Without tapasin this hierarchy was altered, with greater responses to sub-optimal peptide–MHC I complexes. That the altered T-cell hierarchy resulted from defective peptide editing in tapasin-deficient APCs and not from the altered CD8 T-cell repertoire in tapasin-deficient mice (7, 8) was shown using bone marrow chimeric mice.

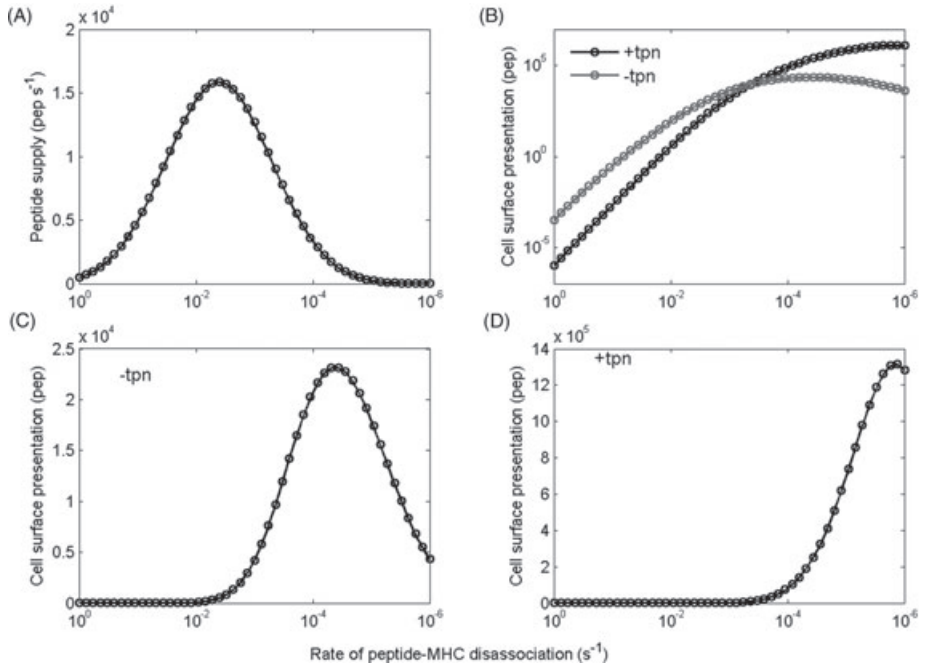

Figure 3 The relationship between peptide abundance and peptide selection. Simulated cell surface presentation of hepatitis B virus (HBV) peptides by MHC I in the presence or absence of tapasin illustrates preferential loading of slow off-rate peptides in the presence of tapasin. (A) The amino acid sequences of proteins expressed by HBV (GenBank X04615) were used to predict peptide dissociation rates from HLA-B*4403 using the BIMAS algorithm [Ref (114), http://www-bimas.cit.nih.gov/molbio/hla_bind]. The abundance of the expressed proteins is assumed to be such that S protein is 100× more abundant than X protein, polymerase and core antigen. A log-normal distribution was fitted to this distribution, and sampled by altering peptide supply rates for a range of uniformly spaced off-rates. (B) Simulated absolute steady-state presentation for B*4403 at the cell surface in the presence (black lines) or absence (grey lines) of tapasin, generated using computational models in which tapasin functions to uniformly accelerate peptide dissociation when bound to MHC I in the ER (unpublished data). This illustrates how tapasin allows presentation of a greater number of higher affinity peptide–MHC I complexes than is evident in the absence of tapasin. (C) and (D) Simulated absolute steady-state presentation for B*4403 at the cell surface in the absence of tapasin (C), or presence of tapasin (D). Note the difference in scales of the y axis (cell surface presentation), illustrating the quantitative and qualitative improvement tapasin affords in cell surface MHC I peptide presentation.

That efficient immune responses still generally occur in tapasin-deficient mice, even if the responses are against different epitopes, leads us to question what evolutionary advantage there is to focusing an immune response upon the stable peptide–MHC I complexes favoured by tapasin. It is possible that tapasin does not generally affect the ability to respond to pathogens (with most pathogens likely to produce a variety of potentially antigenic peptides), but instead by focusing the repertoire of peptides presented by MHC I upon slow off-rate peptides tapasin effectively decreases the diversity of T cells permitted to respond to a pathogen, thus lowering the likelihood of a cross-reactive autoimmune response. This raises the issue as to how peptide abundance in the ER (be it from high levels of source protein, abundant defective ribosomal products, efficient proteolysis or peptide transport) modulates the editing process. In modelling experiments we have shown that in the absence of tapasin the repertoire of peptides presented by MHC I molecules at the cell surface is determined to a large extent by the abundance of peptides that are available to assembling MHC I molecules in the ER, with peptide off-rate having a lesser influence (Figure 3). However, in the presence of tapasin, the repertoire of MHC-presented peptides is determined principally by off-rate, with preferential selection of slow off-rate peptides even if they have very low abundance in the ER with a massive excess of competing fast off-rate peptides.

In summary, the last decade has seen great advances in our knowledge of MHC I peptide loading. We have gained greater appreciation of the molecular architecture of the PLC and of the mechanistic contribution of constituent proteins to successful MHC I peptide loading. Specifically, we are more aware of the mechanism by which tapasin, the lynchpin of peptide selection, allows MHC I to select high affinity peptides against a prevalence of competing lower affinity peptides. Through this act, tapasin has a significant impact upon which peptide epitopes immune responses are frequently focused upon. The future is likely to see us refining our knowledge of peptide selection and attempting to manipulate peptide selection to allow clinically desirable immune responses. For example, as there are plentiful situations where tapasin expression is lost: in tumour cells (115–119); or following viral infection (84, 85) it follows that in such cells immune responses are focused against sub-dominant peptide–MHC I complexes. Therefore, vaccination strategies targeting tapasin-deficient tumours or virus infected cells that are designed to induce responses against sub-dominant epitopes might lead to better prognosis. Alternatively it might be possible to make therapeutic tapasin-deficient APCs which might be transfused into patients in order to induce responses against otherwise sub-dominant epitopes.

References

1. Williams A, Peh CA, Elliott T. The cell biology of MHC class I antigen presentation. *Tissue Antigens* 2002: **59**: 3–17.
2. Ortmann B, Androlewicz MJ, Cresswell P. MHC class I/beta 2-microglobulin complexes associate with TAP transporters before peptide binding. *Nature* 1994: **368**: 864–7.
3. Suh WK, Cohen-Doyle MF, Fruh K, Wang K, Peterson PA, Williams DB. Interaction of MHC class I molecules with the transporter associated with antigen processing. *Science* 1994: **264**: 1322–6.
4. 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**: 103–14.
5. Grandea AG 3rd, Androlewicz MJ, Athwal RS, Geraghty DE, Spies T. Dependence of peptide binding by MHC class I molecules on their interaction with TAP. *Science* 1995: **270**: 105–8.
6. Greenwood R, Shimizu Y, Sekhon GS, DeMars R. Novel allele-specific, post-translational reduction in HLA class I surface expression in a mutant human B cell line. *J Immunol* 1994: **153**: 5525–36.
7. Grandea AG 3rd, Golovina TN, Hamilton SE et al. Impaired assembly yet normal trafficking of MHC class I molecules in Tapasin mutant mice. *Immunity* 2000: **13**: 213–22.
8. Garbi N, Tan P, Diehl AD et al. Impaired immune responses and altered peptide repertoire in tapasin-deficient mice. *Nat Immunol* 2000: **1**: 234–8.
9. Li S, Sjogren HO, Hellman U, Pettersson RF, Wang P. Cloning and functional characterization of a subunit of the transporter associated with antigen processing. *Proc Natl Acad Sci U S A* 1997: **94**: 8708–13.
10. Tan P, Kropshofer H, Mandelboim O, Bulbuc N, Hammerling GJ, Momburg F. Recruitment of MHC class I molecules by tapasin into the transporter associated with antigen processing-associated complex is essential for optimal peptide loading. *J Immunol* 2002: **168**: 1950–60.
11. Kulig K, Nandi D, Bacik I, Monaco JJ, Vukmanovic S. Physical and functional association of the major histocompatibility complex class I heavy chain alpha3 domain with the transporter associated with antigen processing. *J Exp Med* 1998: **187**: 865–74.
12. Suh WK, Derby MA, Cohen-Doyle MF et al. 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**: 1530–40.
13. Harris MR, Lybarger L, Myers NB et al. Interactions of HLA-B27 with the peptide loading complex as revealed by heavy chain mutations. *Int Immunol* 2001: **13**: 1275–82.
14. 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**: 873–83.
15. Peace-Brewer AL, Tussey LG, Matsui M, Li G, Quinn DG, Frelinger JA. A point mutation in HLA-A*0201 results in failure to bind the TAP complex and to present virus-derived peptides to CTL. *Immunity* 1996: **4**: 505–14.
16. 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**: 4427–33.
17. Bangia N, Lehner PJ, Hughes EA, Surman M, Cresswell P. The N-terminal region of tapasin is required to stabilize the

MHC class I loading complex. *Eur J Immunol* 1999: **29**: 1858–70.
18. 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**: 221–31.
19. Schoenhals GJ, Krishna RM, Grandea AG 3rd et al. Retention of empty MHC class I molecules by tapasin is essential to reconstitute antigen presentation in invertebrate cells. *EMBO J* 1999: **18**: 743–53.
20. Barnden MJ, Purcell AW, Gorman JJ, McCluskey J. Tapasin-mediated retention and optimization of peptide ligands during the assembly of class I molecules. *J Immunol* 2000: **165**: 322–30.
21. Tian G, Xiang S, Noiva R, Lennarz WJ, Schindelin H. The crystal structure of yeast protein disulfide isomerase suggests cooperativity between its active sites. *Cell* 2006: **124**: 61–73.
22. Zhang Y, Baig E, Williams DB. Functions of ERp57 in the folding and assembly of major histocompatibility complex class I molecules. *J Biol Chem* 2006: **281**: 14622–31.
23. Garbi N, Tanaka S, Momburg F, Hammerling GJ. Impaired assembly of the major histocompatibility complex class I peptide-loading complex in mice deficient in the oxidoreductase ERp57. *Nat Immunol* 2006: **7**: 93–102.
24. 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.
25. Kienast A, Preuss M, Winkler M, Dick TP. Redox regulation of peptide receptivity of major histocompatibility complex class I molecules by ERp57 and tapasin. *Nat Immunol* 2007: **8**: 864–72.
26. Peaper DR, Wearsch PA, Cresswell P. Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. *EMBO J* 2005.
27. Dong G, Wearsch PA, Peaper DR, Cresswell P, Reinisch KM. Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thiol oxidoreductase heterodimer. *Immunity* 2009: **30**: 21–32.
28. Peaper DR, Cresswell P. The redox activity of ERp57 is not essential for its functions in MHC class I peptide loading. *Proc Natl Acad Sci U S A* 2008: **105**: 10477–82.
29. Zhang Y, Kozlov G, Pocanschi CL et al. ERp57 does not require interactions with calnexin and calreticulin to promote assembly of class I histocompatibility molecules, and it enhances peptide loading independently of its redox activity. *J Biol Chem* 2009: **284**: 10160–73.
30. Wearsch PA, Cresswell P. Selective loading of high-affinity peptides onto major histocompatibility complex class I molecules by the tapasin-ERp57 heterodimer. *Nat Immunol* 2007: **8**: 873–81.
31. Del Cid N, Jeffery E, Rizvi SM et al. Modes of calreticulin recruitment to the major histocompatibility complex class I assembly pathway. *J Biol Chem* 2010: **285**: 4520–35.
32. Wearsch PA, Jakob CA, Vallin A, Dwek RA, Rudd PM, Cresswell P. Major histocompatibility complex class I molecules expressed with monoglucosylated N-linked glycans bind calreticulin independently of their assembly status. *J Biol Chem* 2004: **279**: 25112–21.
33. Chen M, Bouvier M. Analysis of interactions in a tapasin/class I complex provides a mechanism for peptide selection. *EMBO J* 2007: **26**: 1681–90.
34. Park B, Lee S, Kim E et al. Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. *Cell* 2006: **127**: 369–82.
35. Lee S, Park B, Kang K, Ahn K. Redox-regulated export of the major histocompatibility complex class I-peptide complexes from the endoplasmic reticulum. *Mol Biol Cell* 2009: **20**: 3285–94.
36. Rizvi SM, Raghavan M. Mechanisms of function of tapasin, a critical major histocompatibility complex class I assembly factor. *Traffic* 2010: **11**: 332–47.
37. Ortmann B, Copeman J, Lehner PJ et al. A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. *Science* 1997: **277**: 1306–9.
38. Roder G, Geironson L, Darabi A et al. The outermost N-terminal region of tapasin facilitates folding of major histocompatibility complex class I. *Eur J Immunol* 2009: **39**: 2682–94.
39. Dominguez C, Boelens R, Bonvin AM. HADDOCK: a protein-protein docking approach based on biochemical or biophysical information. *J Am Chem Soc* 2003: **125**: 1731–7.
40. Reid SW, McAdam S, Smith KJ et al. Antagonist HIV-1 Gag peptides induce structural changes in HLA B8. *J Exp Med* 1996: **184**: 2279–86.
41. Case DA, Darden TA, Cheatham TE III, Simmerling CL et al. *AMBER 10*, *San Francisco*: *University of California*, *2008*.
42. Eswar N, Webb B, Marti-Renom MA et al. Comparative protein structure modeling using MODELLER. *Curr Protoc Protein Sci* 2007: Chapter 2: Unit 2.9.
43. Pettersen EF, Goddard TD, Huang CC et al. UCSF chimera - A visualization system for exploratory research and analysis. *J Comput Chem* 2004: **25**: 1605–12.
44. Sieker F, Springer S, Zacharias M. Comparative molecular dynamics analysis of tapasin-dependent and -independent MHC class I alleles. *Protein Sci* 2007: **16**: 299–308.
45. Bouvier M, Wiley DC. Structural characterization of a soluble and partially folded class I major histocompatibility heavy chain/beta 2m heterodimer. *Nat Struct Biol* 1998: **5**: 377–84.
46. Zacharias M, Springer S. Conformational flexibility of the MHC class I alpha1-alpha2 domain in peptide bound and free states: a molecular dynamics simulation study. *Biophys J* 2004: **87**: 2203–14.
47. Elliott T. How does TAP associate with MHC class I molecules? *Immunol Today* 1997: **18**: 375–9.
48. Olson R, Huey-Tubman KE, Dulac C, Bjorkman PJ. Structure of a pheromone receptor-associated MHC molecule with an open and empty groove. *PLoS Biol* 2005: **3**: e257.
49. Zernich D, Purcell AW, Macdonald WA et al. Natural HLA class I polymorphism controls the pathway of antigen presentation and susceptibility to viral evasion. *J Exp Med* 2004: **200**: 13–24.
50. 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**: 509–20.
51. Yaneva R, Springer S, Zacharias M. Flexibility of the MHC class II peptide binding cleft in the bound, partially filled, and

empty states: a molecular dynamics simulation study. *Biopolymers* 2009: **91**: 14–27.
52. Gupta S, Hopner S, Rupp B et al. Anchor side chains of short peptide fragments trigger ligand-exchange of class II MHC molecules. *PLoS One* 2008: **3**: e1814.
53. Painter CA, Cruz A, Lopez GE, Stern LJ, Zavala-Ruiz Z. Model for the peptide-free conformation of class II MHC proteins. *PLoS One* 2008: **3**: e2403.
54. Teng MS, Stephens R, Du Pasquier L, Freeman T, Lindquist JA, Trowsdale J. A human TAPBP (TAPASIN)-related gene, TAPBP-R. *Eur J Immunol* 2002: **32**: 1059–68.
55. Ireland BS, Brockmeier U, Howe CM, Elliott T, Williams DB. Lectin-deficient calreticulin retains full functionality as a chaperone for class I histocompatibility molecules. *Mol Biol Cell* 2008: **19**: 2413–23.
56. Rufer E, Leonhardt RM, Knittler MR. Molecular architecture of the TAP-associated MHC class I peptide-loading complex. *J Immunol* 2007: **179**: 5717–27.
57. Koch J, Guntrum R, Heintke S, Kyritsis C, Tampe R. Functional dissection of the transmembrane domains of the transporter associated with antigen processing (TAP). *J Biol Chem* 2004: **279**: 10142–7.
58. Papadopoulos M, Momburg F. Multiple residues in the transmembrane helix and connecting peptide of mouse tapasin stabilize the transporter associated with the antigen-processing TAP2 subunit. *J Biol Chem* 2007: **282**: 9401–10.
59. Procko E, Raghuraman G, Wiley DC, Raghavan M, Gaudet R. Identification of domain boundaries within the N-termini of TAP1 and TAP2 and their importance in tapasin binding and tapasin-mediated increase in peptide loading of MHC class I. *Immunol Cell Biol* 2005: **83**: 475–82.
60. Walker BA, van Hateren A, Milne S, Beck S, Kaufman J. Chicken TAP genes differ from their human orthologues in locus organisation, size, sequence features and polymorphism. *Immunogenetics* 2005: **57**: 232–47.
61. Elliott T, Willis A, Cerundolo V, Townsend A. Processing of major histocompatibility class I-restricted antigens in the endoplasmic reticulum. *J Exp Med* 1995: **181**: 1481–91.
62. Snyder HL, Yewdell JW, Bennink JR. Trimming of antigenic peptides in an early secretory compartment. *J Exp Med* 1994: **180**: 2389–94.
63. Saric T, Chang SC, Hattori A et al. An IFN-gamma-induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. *Nat Immunol* 2002: **3**: 1169–76.
64. Serwold T, Gonzalez F, Kim J, Jacob R, Shastri N. ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum. *Nature* 2002: **419**: 480–3.
65. York IA, Chang SC, Saric T et al. The ER aminopeptidase ERAP1 enhances or limits antigen presentation by trimming epitopes to 8-9 residues. *Nat Immunol* 2002: **3**: 1177–84.
66. Neisig A, Roelse J, Sijts AJ et al. Major differences in transporter associated with antigen presentation (TAP)-dependent translocation of MHC class I-presentable peptides and the effect of flanking sequences. *J Immunol* 1995: **154**: 1273–9.
67. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* 1999: **50**: 213–9.
68. van Endert PM, Riganelli D, Greco G et al. The peptide-binding motif for the human transporter associated with antigen processing. *J Exp Med* 1995: **182**: 1883–95.
69. Tanioka T, Hattori A, Masuda S et al. Human leukocyte-derived arginine aminopeptidase. The third member of the oxytocinase subfamily of aminopeptidases. *J Biol Chem* 2003: **278**: 32275–83.
70. Chang SC, Momburg F, Bhutani N, Goldberg AL. The ER. aminopeptidase, ERAP1, trims precursors to lengths of MHC class I peptides by a "molecular ruler" mechanism. *Proc Natl Acad Sci U S A* 2005: **102**: 17107–12.
71. Saveanu L, Carroll O, Lindo V et al. Concerted peptide trimming by human ERAP1 and ERAP2 aminopeptidase complexes in the endoplasmic reticulum. *Nat Immunol* 2005: **6**: 689–97.
72. Fruci D, Ferracuti S, Limongi MZ et al. Expression of endoplasmic reticulum aminopeptidases in EBV-B cell lines from healthy donors and in leukemia/lymphoma, carcinoma, and melanoma cell lines. *J Immunol* 2006: **176**: 4869–79.
73. Firat E, Saveanu L, Aichele P et al. The role of endoplasmic reticulum-associated aminopeptidase 1 in immunity to infection and in cross-presentation. *J Immunol* 2007: **178**: 2241–8.
74. Hammer GE, Gonzalez F, Champsaur M, Cado D, Shastri N. The aminopeptidase ERAAP shapes the peptide repertoire displayed by major histocompatibility complex class I molecules. *Nat Immunol* 2006: **7**: 103–12.
75. Yan J, Parekh VV, Mendez-Fernandez Y et al. In vivo role of ER-associated peptidase activity in tailoring peptides for presentation by MHC class Ia and class Ib molecules. *J Exp Med* 2006: **203**: 647–59.
76. York IA, Brehm MA, Zendzian S, Towne CF, Rock KL. Endoplasmic reticulum aminopeptidase 1 (ERAP1) trims MHC class I-presented peptides in vivo and plays an important role in immunodominance. *Proc Natl Acad Sci U S A* 2006: **103**: 9202–7.
77. Blanchard N, Gonzalez F, Schaeffer M et al Immunodominant, protective response to the parasite Toxoplasma gondii requires antigen processing in the endoplasmic reticulum. *Nat Immunol* 2008: **9**: 937–44.
78. Hammer GE, Gonzalez F, James E, Nolla H, Shastri N. In the absence of aminopeptidase ERAAP, MHC class I molecules present many unstable and highly immunogenic peptides. *Nat Immunol* 2007: **8**: 101–8.
79. Kanaseki T, Blanchard N, Hammer GE, Gonzalez F, Shastri N. ERAAP synergizes with MHC class I molecules to make the final cut in the antigenic peptide precursors in the endoplasmic reticulum. *Immunity* 2006: **25**: 795–806.
80. Burton PR, Clayton DG, Cardon LR et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007: **39**: 1329–37.
81. Allen RL, Bowness P, McMichael A. The role of HLA-B27 in spondyloarthritis. *Immunogenetics* 1999: **50**: 220–7.
82. Edwards JC, Bowness P, Archer JR. Jekyll and Hyde: the transformation of HLA-B27. *Immunol Today* 2000: **21**: 256–60.
83. Bird LA, Peh CA, Kollnberger S, Elliott T, McMichael AJ, Bowness P. Lymphoblastoid cells express HLA-B27 homodimers both intracellularly and at the cell surface

following endosomal recycling. *Eur J Immunol* 2003: **33**: 748–59.
84. Park B, Kim Y, Shin J et al. Human cytomegalovirus inhibits tapasin-dependent peptide loading and optimization of the MHC class I peptide cargo for immune evasion. *Immunity* 2004: **20**: 71–85.
85. Bennett EM, Bennink JR, Yewdell JW, Brodsky FM. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. *J Immunol* 1999: **162**: 5049–52.
86. Romero JM, Jimenez P, Cabrera T et al. Coordinated downregulation of the antigen presentation machinery and HLA class I/beta2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer. *Int J Cancer* 2005: **113**: 605–10.
87. Albers A, Abe K, Hunt J et al. Antitumor activity of human papillomavirus type 16 E7-specific T cells against virally infected squamous cell carcinoma of the head and neck. *Cancer Res* 2005: **65**: 11146–55.
88. Howarth M, Williams A, Tolstrup AB, Elliott T. Tapasin enhances MHC class I peptide presentation according to peptide half-life. *Proc Natl Acad Sci U S A* 2004: **101**: 11737–42.
89. Thirdborough SM, Roddick JS, Radcliffe JN, Howarth M, Stevenson FK, Elliott T. Tapasin shapes immunodominance hierarchies according to the kinetic stability of peptide-MHC class I complexes. *Eur J Immunol* 2008: **38**: 364–9.
90. Boulanger DS, Oliveira R, Ayers L et al. Absence of tapasin alters immunodominance against a lymphocytic choriomeningitis virus polytope. *J Immunol* 2009.
91. Sadegh-Nasseri S, Chen M, Narayan K, Bouvier M. The convergent roles of tapasin and HLA-DM in antigen presentation. *Trends Immunol* 2008: **29**: 141–7.
92. Lewis JW, Elliott T. Evidence for successive peptide binding and quality control stages during MHC class I assembly. *Curr Biol* 1998: **8**: 717–20.
93. Garstka M, Borchert B, Al-Balushi M et al. Peptide-receptive major histocompatibility complex class I molecules cycle between endoplasmic reticulum and cis-Golgi in wild-type lymphocytes. *J Biol Chem* 2007: **282**: 30680–90.
94. 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**: 841–51.
95. Marguet D, Spiliotis ET, Pentcheva T, Lebowitz M, Schneck J, Edidin M. Lateral diffusion of GFP-tagged H2Ld molecules and of GFP-TAP1 reports on the assembly and retention of these molecules in the endoplasmic reticulum. *Immunity* 1999: **11**: 231–40.
96. Paquet ME, Cohen-Doyle M, Shore GC, Williams DB. Bap29/31 influences the intracellular traffic of MHC class I molecules. *J Immunol* 2004: **172**: 7548–55.
97. Ladasky JJ, Boyle S, Seth M et al. Bap31 enhances the endoplasmic reticulum export and quality control of human class I MHC molecules. *J Immunol* 2006: **177**: 6172–81.
98. Abe F, Van Prooyen N, Ladasky JJ, Edidin M. Interaction of Bap31 and MHC class I molecules and their traffic out of the endoplasmic reticulum. *J Immunol* 2009: **182**: 4776–83.
99. Gao B, Adhikari R, Howarth M et al. Assembly and antigen-presenting function of MHC class I molecules in cells lacking the ER chaperone calreticulin. *Immunity* 2002: **16**: 99–109.
100. Howe C, Garstka M, Al-Balushi M et al. Calreticulin-dependent recycling in the early secretory pathway mediates optimal peptide loading of MHC class I molecules. *EMBO J* 2009: **28**: 3730–44.
101. Paulsson KM, Kleijmeer MJ, Griffith J et al. Association of tapasin and COPI provides a mechanism for the retrograde transport of major histocompatibility complex (MHC) class I molecules from the Golgi complex to the endoplasmic reticulum. *J Biol Chem* 2002: **277**: 18266–71.
102. Paulsson KM, Jevon M, Wang JW, Li S, Wang P. The double lysine motif of tapasin is a retrieval signal for retention of unstable MHC class I molecules in the endoplasmic reticulum. *J Immunol* 2006: **176**: 7482–8.
103. Leonhardt RM, Fiegl D, Rufer E, Karger A, Bettin B, Knittler MR. Post-endoplasmic reticulum rescue of unstable MHC class I requires proprotein convertase PC7. *J Immunol* 2010: **184**: 2985–98.
104. Leonhardt RM, Keusekotten K, Bekpen C, Knittler MR. Critical Role for the Tapasin-Docking Site of TAP2 in the Functional Integrity of the MHC Class I-Peptide-Loading Complex. *J Immunol* 2005: **175**: 5104–14.
105. Duncan LM, Piper S, Dodd RB et al. Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. *EMBO J* 2006: **25**: 1635–45.
106. Hewitt EW, Duncan L, Mufti D, Baker J, Stevenson PG, Lehner PJ. Ubiquitylation of MHC class I by the K3 viral protein signals internalization and TSG101-dependent degradation. *EMBO J* 2002: **21**: 2418–29.
107. Lehner PJ, Hoer S, Dodd R, Duncan LM. Downregulation of cell surface receptors by the K3 family of viral and cellular ubiquitin E3 ligases. *Immunol Rev* 2005: **207**: 112–25.
108. Bartee E, Mansouri M, Hovey Nerenberg BT, Gouveia K, Fruh K. Downregulation of major histocompatibility complex class I by human ubiquitin ligases related to viral immune evasion proteins. *J Virol* 2004: **78**: 1109–20.
109. Boname JM, de Lima BD, Lehner PJ, Stevenson PG. Viral degradation of the MHC class I peptide loading complex. *Immunity* 2004: **20**: 305–17.
110. Lizee G, Basha G, Tiong J et al. Control of dendritic cell cross-presentation by the major histocompatibility complex class I cytoplasmic domain. *Nat Immunol* 2003: **4**: 1065–73.
111. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. *Annu Rev Immunol* 1999: **17**: 51–88.
112. Busch DH, Pamer EG. MHC class I/peptide stability: implications for immunodominance, in vitro proliferation, and diversity of responding CTL. *J Immunol* 1998: **160**: 4441–8.
113. van der Burg SH, Visseren MJ, Brandt RM, Kast WM, Melief CJ. Immunogenicity of peptides bound to MHC class I molecules depends on the MHC-peptide complex stability. *J Immunol* 1996: **156**: 3308–14.
114. Parker KC, Bednarek MA, Coligan JE. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. *J Immunol* 1994: **152**: 163–75.

115. Atkins D, Breuckmann A, Schmahl GE et al. MHC class I antigen processing pathway defects, ras mutations and disease stage in colorectal carcinoma. *Int J Cancer* 2004: **109**: 265–73.
116. Dissemond J, Kothen T, Mors J et al. Downregulation of tapasin expression in progressive human malignant melanoma. *Arch Dermatol Res* 2003.
117. Facoetti A, Nano R, Zelini P et al. Human leukocyte antigen and antigen processing machinery component defects in astrocytic tumors. *Clin Cancer Res* 2005: **11**: 8304–11.
118. Ferris RL, Whiteside TL, Ferrone S. Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. *Clin Cancer Res* 2006: **12**: 3890–5.
119. Ogino T, Bandoh N, Hayashi T, Miyokawa N, Harabuchi Y, Ferrone S. Association of tapasin and HLA class I antigen down-regulation in primary maxillary sinus squamous cell carcinoma lesions with reduced survival of patients. *Clin Cancer Res* 2003: **9**: 4043–51.