TAPBPR: a new player in the MHC class I presentation pathway

Short title: TAPBPR in MHC class I antigen presentation

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Abstract

In order to provide specificity for T cell responses against pathogens and tumours, MHC class I molecules present high-affinity peptides at the cell surface to T cells. A key player for peptide loading is the MHC class I-dedicated chaperone tapasin. Recently we discovered a second MHC class I-dedicated chaperone, the tapasin-related protein TAPBPR. Here, we review the major steps in the MHC class I pathway and the TAPBPR data. We discuss the potential function of TAPBPR in the MHC class I pathway and the involvement of this previously uncharacterised protein in human health and disease.

Keywords: TAPBPR/TAPBPL, tapasin, antigen processing and presentation, MHC, human, disease association

INTRODUCTION

The presentation of small peptide fragments on MHC class I molecules allows the peptidome of an individual cell to be conveyed to the immune system. In this way, MHC class I molecules act as a critical detection system of both pathogenic infection and cellular transformation. Delineating the mechanisms that underlie peptide selection by MHC class I molecules is of crucial importance for all aspects of immune recognition from infection control and tumour surveillance, to transplant rejection and susceptibility to auto-inflammatory conditions. Over the past 25 years, immense progress has been made in identifying a number of key stages and important players involved in the generation of peptide:MHC complexes. Due to the significant progress already made and the complexity of the pathway, there is perhaps a general misconception that our knowledge of the antigen processing and presentation pathway is complete. This is simply not the case. The molecular mechanisms controlling MHC peptide selection for immune recognition still have many secrets to reveal. In this review we give an overview of the major known molecular players involved in the loading of peptide onto MHC class I molecules, recount our recent identification of TAPBPR as a new MHC class I specific chaperone, then discuss the potential role of TAPBPR in the antigen presentation pathway.

THE MHC I ANTIGEN PROCESSING AND PRESENTATION PATHWAY

The cell biology of MHC class I molecules has already been elegantly and extensively reviewed in this journal in 2010 (1). Thus, we will only give a brief overview here of the major molecular players known to that date (Figure 1).

THE CLASSICAL ROUTE OF ANTIGEN PRESENTATION

Peptide generation and delivery into the ER

Peptides for presentation on MHC class I by the classical presentation pathway are generated by proteasomal degradation of proteins in the cytosol. Although the relative contribution of different peptide sources is still a matter of debate, peptides can originate from the natural degradative turnover of self-proteins, proteins over-expressed as a result of malignant transformation, defective ribosomal products of protein translation and peptides derived from infection by intracellular parasites such as viruses (2-4). A further source of antigenic peptides is the pioneer round of mRNA translation and nuclear translation (5, 6). Furthermore, the cellular machinery may use the unconventional CUG Start Codon to initiate protein synthesis for antigen presentation on MHC class I molecules (7).

Peptides are then transported into the lumen of the ER by the transporter associated with antigen processing (TAP). This complex is a heterodimer composed of TAP1 and TAP2 and belongs to the ATP-binding cassette (ABC) transporter family, responsible for mediating transport of substrates across membranes in an ATP-dependent manner (8-10). The importance of TAP in the MHC class I pathway is seen in TAP-deficient cells, in which MHC class I cell surface expression is severely reduced and degradation of MHC class I molecules is increased (11-13).

Peptide trimming in the ER

After translocation into the lumen of the ER, peptides are typically longer than peptides that finally bind to mature MHC class I molecules and therefore require further trimming. The endoplasmic reticulum aminopeptidase associated with antigen processing (ERAAP in mice, ERAP1/2 in humans) is responsible for trimming N-terminal extended precursor peptides in the ER (14, 15). Recently, it has been shown that ERAP1 and ERAP2 form dimers and this dimerization produces allosteric effects on ERAP1 that enhance peptide trimming efficiency significantly (16). The importance of ERAAP function in the presentation of peptides to T cells is evident from studies using ERAAP depleted cells in which decreased cell surface expression of MHC class I molecules and impaired presentation of antigenic peptides to T cells is observed (14, 15, 17). The peptide repertoire is significantly altered in ERAAP$^{-/-}$ mice, in which both a loss, as well as gain, of new peptide/MHC I complexes is observed (18). An unresolved issue is whether precursor peptides first bind to MHC class I molecules and are then trimmed by ERAAP, or if trimming happens before peptides bind to MHC class I.

Early MHC class I folding events

The type I transmembrane protein calnexin, together with its soluble homologue calreticulin, mediate the folding of nascent glycoproteins in the ER, including the MHC class I heavy chain which is glycosylated at Asn-86 (Figure 1). Both calnexin and calreticulin work together with the protein disulphide isomerase ERp57, which assists with disulphide bond formation within the membrane-proximal α3 domain of the MHC class I heavy chain (19-22). After initial folding, assisted by calnexin, the MHC class I heavy chain associates with β2-microglobulin and subsequently associates with calreticulin (23, 24).

The peptide loading complex and the central role of tapasin

At this point the MHC class I heavy chain is integrated into the peptide loading complex (PLC), a multiprotein complex in which the MHC class I molecules are loaded with peptides. The PLC consists of TAP, the lectin-like chaperone calreticulin, the MHC class I dedicated chaperone tapasin, and the thiol reductase ERp57 (Figure 1). Although protein disulphide isomerase (PDI) has also been reported to associate with the PLC (25), these results remain to be confirmed by other groups (26-28).

Molecular associations governed by tapasin

Tapasin forms the centre-piece of the PLC as it facilitates a number of molecular associations which assemble the multi-protein complex. First, tapasin binds to peptide-receptive MHC class I heterodimers (24). Currently, two distinct associations between the two molecules are thought to occur: a cluster of conserved residues in the N-terminal domain of tapasin contact a loop below the α2-1 helix of the MHC class I, while residues in the membrane proximal Ig-like domain of tapasin associate with a β-strand in the α3 domain of MHC class I (29). Second, tapasin binds to the TAP transporter (24). This interaction, which is dependent on a positively charged residue in tapasin's transmembrane domain, bridges the peptide-receptive MHC class I molecules to the TAP transporter, thus localising the MHC class I heterodimer at the peptide source (24, 30, 31). This association also stabilises TAP, resulting in enhanced TAP levels and increased translocation of peptides into the ER (32). Third, tapasin binds to ERp57 via an intermolecular disulphide bridge between C95 of tapasin and C57 in ERp57 (26, 33). This association is essential for the structural stability of the PLC and therefore is essential for optimal peptide loading of MHC class I molecules (33, 34). Other subsequent

associations known to occur in the PLC are an interaction of ERp57 with the P-domain of calreticulin, which acts as a bridge between calreticulin and tapasin (20), and an association of the monoglucosylated glycan of MHC class I with calreticulin (35). These calreticulin-dependent associations are also essential for PLC activity (36, 37). Thus the molecular associations centred round tapasin provide strength and stability to interactions within the PLC which otherwise would be characteristically weak and transient.

Therefore, tapasin is crucial for the production of a functional PLC (34). In the absence of tapasin, MHC class I assembly with TAP is impaired and MHC class I molecules are inefficiently loaded with peptide. Consequently cell surface expression of conformational MHC class I is severely reduced (24, 30, 38, 39). In tapasin$^{-/-}$ mice cell surface expression of MHC class I molecules such as H2-K^{b} and H2-D^{b} is decreased by at least 100-fold and the mice exhibit severely impaired CD8^{+} T cell immunity compared to wild type animals (38, 39). Consequently, tapasin is crucial for optimal induction of T cell immunity.

The role of tapasin in peptide editing

An important function assigned to tapasin, crucial for the generation of optimal loaded and stable MHC class I-peptide complexes, is peptide optimisation or peptide editing (1, 40-42). This is achieved by promoting the replacement of low-affinity peptides with high-affinity peptides. The precise molecular mechanisms underpinning peptide editing are still unclear. In addition, the nature of the conformational changes in MHC class I molecules upon peptide binding remains unknown, as currently no structure of a peptide-receptive MHC class I molecule exists. However a number of key concepts regarding peptide editing are uncontested and can be stated thus: (i) Peptide editing involves the replacement of sub-

optimal, fast off-rate peptides by higher-affinity, slower off-rate peptides; (ii) Peptide editing is an intrinsic characteristic of MHC class I molecules; (iii) Tapasin is able to enhance this intrinsic characteristic of MHC class I molecules. This quality control for peptide binding is essential for the generation of an optimal T cell response (30) and is in some respects analogous to the HLA-DM molecule in MHC class II antigen presentation (43).

Tapasin dependency of HLA allomorphs

Over the last 18 years, many reports have demonstrated that polymorphism in HLA allomorphs result in MHC class I molecules exhibiting different dependences on tapasin to acquire peptides. First, there is significant variability regarding incorporation of HLA allomorphs into the PLC, with most HLA-A allomorphs displaying efficient incorporation while the majority of HLA-B allomorphs are reported to be inefficiently incorporated (44-46). This phenomenon appears to be strongly influenced by the residue at position 116 in the α2 domain of the MHC class I heavy chain, with additional residues in the surrounding region, such as residue 156, also influencing the interaction. Second, there is significant variability regarding the ability of HLA allomorphs to be stably expressed at the cell surface in the absence of tapasin. At one end of the spectrum are the tapasin-dependent MHC allomorphs, which require tapasin in order to be stably expressed at the cell surface (for example HLA-B*44:02, HLA-A*A01, and B*08:01(47)). At the other end of the spectrum are the tapasin-independent MHC class I allomorphs (for example HLA-B*44:05, HLA-A*02:01 and HLA-B*35:01) whose surface expression is relatively unaffected by the absence of tapasin (30, 48-51). These tapasin-independent MHC molecules are able to load and to modulate peptide cargo over time. Again, tapasin dependency is strongly influenced by the residue at position 116 in MHC class I. The classic example of this is observed with

subtypes of HLA-B44 which only differ at amino acid position 116. HLA-B*44:05 with a tyrosine at position 116 is tapasin-independent while HLA-B*44:02 with an aspartic acid at position 116 is tapasin-dependent (40, 52). The naturally occurring polymorphism in HLA-B44 has been linked to both integration of MHC class I into the PLC and to tapasin-dependent peptide optimisation (40, 52). The residue at position 114 also contributes to tapasin dependency (53). It is important to note that tapasin-independent molecules are still acted upon by tapasin, and their peptide cargo is modulated by the presence of tapasin (54).

Both molecular dynamics simulation studies and mathematical modelling of peptide selection are being used to understand why HLA allomorphs display different dependencies on tapasin for optimal peptide selection (55, 56). Data comparing HLA-B*44:02 and -B*44:05 suggest that tapasin-dependent allomorphs are more open and conformationally flexible than tapasin-independent molecules. In the absence of tapasin, the α2-1 helix of tapasin-dependent MHC class I molecules is predicted to be partially unfolded and therefore unable to bind peptide. As a result, such MHC class I molecules require tapasin to become properly folded and thereby peptide-receptive (55). Therefore, the dependency on tapasin to optimise peptide correlates with the intrinsic ability of different HLA allomorphs to maintain a 'receptive' conformation (1). This concept is further supported by the recent finding that tapasin-dependent HLA-B allotypes exhibited lower refolding ability than tapasin-independent HLA-B allotypes (51).

UGT1 as a post-PLC quality control checkpoint

Upon binding to a high-affinity peptide, the affinity between tapasin and the MHC class I molecule is reduced, releasing the MHC trimer from the PLC (57, 58). However, peptide-

loaded MHC class I molecules do not exit the ER immediately but are retained in the ER once released from TAP (59-61). These findings raised the possibility of an additional MHC class I processing step after the PLC.

In 2011, UDP-glucose glycoprotein glucosyltransferase (UGT1) was identified as an important molecular component of this post-PLC quality control checkpoint (62) (Figure 1). UGT1 is an ER/cis-Golgi resident enzyme which reglucosylates unfolded glycoproteins regenerating the Glc1Man9GlcNA2 moiety (for a review see (63)). This modification in turn sends the glycoprotein back into the calnexin/calreticulin cycle. In UGT1-deficient mouse embryonic fibroblasts surface expression of MHC class I is reduced, MHC class I maturation and assembly is delayed, and peptide selection is impaired (62). Furthermore, UGT1 appears to reglucosylate MHC class I molecules bearing suboptimal peptides and causes their re-engagement with the PLC (36). Thus, UGT1 seems to detect suboptimally loaded MHC class I molecules and sends them back to the PLC. Interestingly, UGT1 serves also as a quality control checkpoint during CD1d assembly and shapes the lipid repertoire of newly synthesized CD1d (64), therefore it has a wider role in presentation of various antigens to immune cells. Precisely how UGT1 recognises conformational anomalies in its substrates is poorly understood (62).

Anterograde trafficking of MHC class I molecule through the secretory pathway

The mechanisms controlling the ER export of MHC class I molecules and their transport through the secretory pathway are rather enigmatic steps in the MHC class I antigen processing and presentation pathway. MHC class I molecules can be selectively sorted into ER exit sites (61). ER exit signals have been identified in the cytoplasmic tail of MHC class

I molecules which sort the molecules into coat complex (COP)II coated vesicles (65-67). In addition, MHC class I molecules have been found to associate with the transport receptor BAP31 which contains an export motif (68-70). The relative contribution of these two mechanisms to the ER export of MHC class I is currently unknown and could reflect alternative fates of defined pools of MHC class I molecules. There also appears to be a further bottleneck in the MHC class I pathway in the Golgi apparatus, supporting the idea that the quality control exerted by the MHC class I antigen processing and presentation pathway extends well beyond the ER (65, 66, 71). Once the MHC class I heterotrimer traverses these various barriers, it travels to the cell surface to be monitored by circulating CTLs and NK cells.

ALTERNATIVE PATHWAYS OF MHC CLASS I ANTIGEN PRESENTATION

In addition to the classical MHC class I antigen processing and presentation pathway described above, alternative mechanisms can also contribute to the peptide repertoire presented to the immune system. These pathways have been discovered by characterisation of TAP-deficient cells, on which a pool of peptide epitopes named 'T-cell Epitopes associated with Impaired Peptide Processing' are presented by MHC class I molecules (72-75). These alternative peptides appear to stem from a variety of sources. A classic example is signal sequences liberated by signal peptidase and signal sequence peptidase (75, 76). The mechanism of peptide loading in the absence of TAP currently remains unknown. One possibility is the existence of a second PLC, distinct from the tapasin/TAP complex. Alternatively, TAP-independent peptide loading of MHC class I could involve components of the MHC class II pathway such as the invariant chain, which exhibits a more prominent

association with MHC class I molecules in TAP deficient cells (77, 78). Further research is needed to elucidate these alternative pathways of antigen presentation in detail.

CROSS-PRESENTATION

Cross-presentation is a second MHC I presentation pathway that occurs in Dendritic cells (DCs) involving the presentation of peptides from exogenous proteins, a process that is essential for triggering T-lymphocyte responses against infectious agents and tumours (79). The molecular mechanisms underlying cross-presentation remain elusive and the subject of intense investigation (for a comprehensive review see (80)). In general, three major pathways for cross-presentation have thus far been suggested, namely a cytosolic, a vacuolar and a phago-ER pathway. In the cytosolic pathway antigens are produced in the endosomes and transported into the cytosol via SEC transporters. These antigens can then enter the classical route of antigen presentation via TAP. The vacuolar pathway involves recycling of MHC class I from the cell surface. MHC class I molecules can enter this pathway through endosomal compartments or possibly through the MIIC compartment where they are loaded with exogenous peptides. The process is guided by the tyrosine internalisation signal on the cytoplasmic tail of MHC class I molecules. It has been suggested that the MHC class II chaperone invariant chain (or CD74) is also involved in delivering a proportion of MHC class I molecules to such compartments for cross-presentation (77, 78, 81). Basha and colleagues have further highlighted the critical role of CD74 in cross-presentation of MHC class I molecules *in vivo* (82). The vacuolar pathway is known to occur *in vivo* for certain antigens. Another suggested route for cross-presentation is the 'phago-ER' (endoplasmic reticulum–mediated phagocytosis) model, which was considered a dominant pathway of cross-presentation. This pathway involves a fusion event between the ER and a phagosome. This

fusion event would allow enzymatic machinery, which is required for the release of phagosomal proteins into the cytosol to traffic to phagosomes. The validity of this route has been questioned.

TAPBPR: AN ADDITIONAL MHC CLASS I SPECIFIC CHAPERONE IN THE ANTIGEN PROCESSING AND PRESENTATION PATHWAY

In 2000, a human tapasin related gene (TAPBPL/TAPBPR) was identified on chromosome position 12p13.3 near an MHC paralogous locus (83). Like tapasin, TAPBPR is an IFN-γ inducible, type I transmembrane protein consisting of three extracellular domains, a unique membrane distal domain, IgSF V domain and IgC1 domain (84-86)(Figure 2). In 2013, we showed that human TAPBPR shared tapasin's ability to bind to MHC class I, a finding that was initially somewhat surprising, given that the two related molecules only share approximately 22% sequence identity (86). Both tapasin and TAPBPR appear to recognise a roughly similar form of MHC class I; an intracellular heavy chain/β2m heterodimer which is peptide-receptive. Through amino acid sequence alignment and homology modelling, we showed that the MHC class I binding site is conserved between tapasin and TAPBPR (Figure 2), explaining the shared ligand, despite the large variability between the two proteins (29).

TAPBPR is not simply a tapasin analogue. A number of functional disparities are clearly evident between of the two proteins even when simply comparing their amino acid sequence (Figure 2). For example, the charged residue in the TMD of tapasin, which is essential for its association with the TAP transporter (30, 31), is absent in TAPBPR. In addition, the ER retention motif in the cytoplasmic tail of tapasin (87) is not found in the tail of TAPBPR. In agreement with these observations, we found that, in contrast to tapasin, TAPBPR is not an

integral component of the peptide loading complex and its expression is not restricted to the ER (86). Furthermore, TAPBPR does not bind to ERp57, despite having an unpaired cysteine residue in its extracellular domain (86). The differences between tapasin and TAPBPR are strikingly obvious when expression of the two related molecules is altered. TAPBPR over-expression in HeLa cells leads to a down-regulation of surface expression of conformational MHC class I, a phenotype resembling that of a tapasin-deficient cell (24, 30, 38, 39, 86). Therefore, it seems that, in these experiments at least, TAPBPR has an opposing function to that of tapasin. The lack of analogous function on MHC class I between tapasin and TAPBPR is further supported in TAPBPR deficient cells, in which no obvious phenotypic effect on conformational MHC class I expression is observed (86). This suggests that TAPBPR is not required for the initial peptide loading event, or is certainly not as crucial as tapasin for peptide loading.

POTENTIAL FUNCTIONS OF TAPBPR IN THE MHC CLASS I PATHWAY

For the past two decades tapasin was the only known MHC class I dedicated chaperone in the MHC class I antigen processing and presentation pathway. The existence of TAPBPR has prompted a radical review of the whole process and some aspects of tapasin function will need to be re-evaluated. But what is the actual function of TAPBPR in the MHC class I pathway? Although this is currently unknown, below we speculate on some possibilities and examine the current evidence for and against each suggestion (Figure 3).

1. **TAPBPR could represent a tapasin-independent pathway of antigen presentation in the secretory pathway**

The first possibility to consider is that TAPBPR represents an independent folding pathway for MHC class I which runs alongside the classical antigen presentation pathway (Figure 3A). As highlighted above, TAPBPR is not simply a tapasin analogue, particularly since it is not an integral component of the PLC. However, given the orientation of TAPBPR on MHC class I, it may share some functional properties of tapasin. The positioning of tapasin on MHC class I is thought to be of critical importance for tapasin functioning as a peptide loader and editor. As TAPBPR is orientated on MHC class I in a similar manner to tapasin, it is reasonable to suspect that it could also be involved in peptide loading and/or peptide editing on MHC class I.

The orientation of TAPBPR on MHC class I in a similar way to tapasin may shed light on an old observation that has never been satisfactorily explained, namely why some MHC class I molecules can load peptides in a tapasin-independent manner. This tapasin-independence is likely to be highly influenced by the intrinsic characteristic of the specific MHC class I allomorph. That said, TAPBPR may also significantly influence the ability of certain MHC molecules (such as HLA-A2 and –B4405) to load peptides in the absence of tapasin. In this respect, we would imagine that TAPBPR would need to share tapasin's ability to load peptides. But as TAPBPR is not an integral component of the PLC, what would be the nature of these TAPBPR-dependent peptides be? Could TAPBPR be responsible for the TAP-independent presentation pathway, loading the alternative peptide sources such as the 'T-cell Epitopes associated with Impaired Peptide Processing' described above? (74, 75). This idea may be supported by the fact that we found TAPBPR to bind more strongly, or to exhibit a more prolonged association, with HLA-A molecules such as HLA-A2 and HLA-A68, which are capable of loading signal sequences peptides, than with HLA-B molecules (29, 86). In

what context would these TAPBPR-dependent sources of peptides be loaded onto MHC class I? As an isolated entity, or in complex with other molecules which constitute an alternative PLC in the ER? Intriguingly, TAPBPR has been identified as a leukocyte nuclear envelope transmembrane protein, which influences the positioning of genes/chromosomes in the nucleus (88). An interesting speculation is that TAPBPR might be involved in antigenic peptide presentation from products generated directly in the nucleus, such as pioneer translational products (5, 6).

However, the lack of any obvious phenotype of cell surface expression of MHC class I in TAPBPR depleted cells argues against a crucial role for the protein in the initial step of loading peptide onto MHC class I. This lack of phenotype may occur due to redundancy in the MHC class I pathway; in the absence of TAPBPR, tapasin would load peptides onto MHC class I molecules, therefore there would be no obvious phenotype in respect to total MHC class I expression in a TAPBPR-depleted cell. Nevertheless, the fact that, in cells over-expressing TAPBPR, surface expression of MHC class I is reduced, further argues against TAPBPR as a peptide loader. But as the loading of high-affinity peptide onto MHC class I is such a crucial event in mounting an efficient immune response, it seems intuitively unlikely that the single protein tapasin would assume this role. Other proteins could be envisaged to operate under conditions such as viral infection and cellular transformation, in which components of the antigen processing and presentation pathway are down-regulated in the course of immune evasion.

2. TAPBPR could represent a novel sequential step in the classical antigen presentation pathway

A second possibility to consider is that TAPBPR represents a sequential step in the classical MHC class I secretory pathway i.e. tapasin and TAPBPR represent consecutive steps in the same pathway (Figure 3B). The strongest data supporting this idea is our finding that TAPBPR prolongs the association of MHC class I with the PLC, despite TAPBPR itself not associating with the PLC (86). This is consistent with an influence of TAPBPR directly on the classical antigen presentation pathway, as opposed to some novel independent pathway. Furthermore, TAPBPR slows down the ER export rate of MHC class I, supporting a role for it in the conventional secretory pathway for MHC class I (86). In addition, an increased association between MHC class I and TAPBPR is observed in tapasin deficient cells, suggesting that TAPBPR and tapasin function are somehow intertwined (29).

Our current data intriguingly suggests that TAPBPR may antagonise the function of tapasin. First, over-expression of TAPBPR reduces cell surface expression of MHC class I, resembling the MHC class I phenotype of a tapasin deficient cell (86). Second, as mentioned above, tapasin and TAPBPR share the same binding site on MHC class I, raising the possibility of competition between the two related chaperones (29). However, the relationship between tapasin and TAPBPR in the MHC class I pathway appears to be far more complex than simple competition, as although the association between TAPBPR and MHC class I is increased in the absence of tapasin, no such effect on the association between tapasin and MHC I is observed in the absence of TAPBPR (29). Therefore, much has still to be revealed regarding the relationship between tapasin and TAPBPR. Speculation naturally turns to the possibility that parallel systems have evolved in the MHC class I and class II system, with the function of tapasin and HLA-DM, being regulated by TAPBPR and HLA-DO, respectively (89-91).

Our data is not particularly supportive of a role of TAPBPR in peptide loading, especially not for the majority of the MHC class I population of molecules. Most of the data are more consistent with TAPBPR antagonising peptide loading. Thus, could TAPBPR function as a peptide editor or peptide restrictor, somehow monitoring the quality of peptides loaded on MHC class I by tapasin? The reduction in the anterograde trafficking of MHC class I is supportive of this, as is the fact that association between TAPBPR and MHC class I is enhanced in cells lacking a functional PLC (29, 86). In this regard, TAPBPR would represent a previously uncharacterised quality control step in the MHC class I pathway (Figure 3B). Where in the MHC class I pathway would this quality control step be positioned? The fact that TAPBPR binds to a MHC class I heavy chain/ β2m heterodimer in a TAP and tapasin independent manner could fit with a role before the PLC. However, given that TAPBPR appears to stay associated with MHC class I through the medial Golgi, TAPBPR may functions beyond the ER and cis-Golgi (86). In addition to this, the association between TAPBPR and MHC class I is enhanced in cells lacking a functional PLC. Thus, we favour a location after the PLC, analogous to that identified recently for UGT1 (Figure 3B).

3. TAPBPR could function in the MHC class I cross-presentation pathway

A third possibility to consider is that TAPBPR plays a role in the MHC class I cross-presentation pathway (Figure 3C). If this were the case, we would envisage that TAPBPR could function as a delivery system, trafficking peptide-receptive MHC class I through the secretory pathway and into the endosomal system for peptide loading in endosomes or the MHC class II compartment (MIIC). Thus, TAPBPR would be analogous to the invariant chain, but for MHC class I molecules.

Three essential features of the invariant chain permit its function in the MHC class II pathway:1) It blocks MHC class II molecules from picking up peptide in the secretory pathway by inserting a segment of itself, the so called class II associated invariant chain peptide (CLIP), into the peptide-binding groove of MHC class II (92, 93). 2) It contains trafficking motifs in its cytoplasmic tail, which assist in the trafficking of the MHC class II molecules into the endosomal system (94-96). 3) In the endosomal environment, it is released from the MHC class II molecules, freeing the peptide-receptive MHC class II molecules (97, 98). For TAPBPR to be considered analogous to the invariant chain then it should share these three properties. Is TAPBPR capable of blocking the peptide-binding groove of MHC class I molecules in the ER? Our current data suggests that TAPBPR may stabilise a peptide-receptive form of MHC class I but whether TAPBPR is capable of inserting a segment of itself into the peptide-binding groove of MHC class I remains to be determined. Does TAPBPR contain endosomal trafficking motifs in its cytoplasmic tail? As mentioned above, TAPBPR is not restricted to the ER but transports with MHC class I through the secretory pathway (86). Furthermore, when TAPBPR is over-expressed in HeLa cells it can traffic to the cell surface and is internalised into endosomes which contain MHC class I (86). Although classical internalisation motifs such as [D/E]xxxL[L/I], YxxΦ, and FxNPxY are not obvious in the cytoplasmic tail of TAPBPR, the repertoire of endocytosis motifs is more extensive than these three motifs (99). Thus, TAPBPR could contain an as yet uncharacterised trafficking motif or use an alternative mechanism to gain access into the endosomal environment.

One argument against TAPBPR being a missing component in the cross-presentation pathway is the fact that it is widely expressed and not exclusively found in cells which cross-

present, such as DCs. However, the existence of alternative TAPBPR transcripts containing an additional exon in the cytoplasmic region and the finding that these transcripts are abundant in dendritic cells as well as peripheral blood mononuclear cells raise the possibility that, by producing alternative isoforms, TAPBPR may have access to other subcellular compartment in specific cell types (100).

The importance of TAPBPR in human health and disease?

As TAPBPR is a novel component of the MHC class I antigen processing and presentation pathway, and given that it is up-regulated by IFN-γ, it seems very likely that TAPBPR contributes to pathogen control, particularly to viral infections. There is also the intriguing possibility that TAPBPR might influence immune recognition of tumours and a recent study has suggested there is a strong association between TAPBPR expression and glioblastoma multiforme patient survival (101). In expression quantitative trait locus (eQTL) studies a significant increase in TAPBPR expression level was observed in tumour samples compared to organ specific controls and high expression of TAPBPR appeared to be associated with a poor outcome (101).

It is worth noting that TAPBPR is polymorphic. We have currently identified 6 alleles that produce nonsynonymous amino acid changes to TAPBPR (Figure 4). Polymorphisms both in MHC class I molecules themselves and in specific components of the MHC class I pathway are known to strongly influence an individual's susceptibility to disease. The autoinflammatory disease Ankylosing Spondylitis (AS) serves as a classic example of polymorphism affecting disease susceptibility. Not only is AS strongly associated with inheritance of a specific HLA-B allomorph, HLA-B27, but polymorphisms in ERAP1, the

ER aminopeptidase which trims the peptides presented by MHC class I molecules, have been strongly associated with AS (102-104). Naturally occurring variants of ERAP1 have been shown to directly alter protein function, producing molecules that over-trim or under-trim peptides (105, 106). Thus peptide handling by HLA-B27 is likely to be important in susceptibility to AS (105). Polymorphism in MHC class I is also associated with time to onset of AIDS (107). Whether polymorphisms in TAPBPR influence TAPBPR function or are influential in susceptibility to autoinflammatory or pathogenic diseases remains to be determined. Intriguingly SNPs on chromosome 12p13, adjacent to the *TAPBPL* gene, are associated with AS (105).

CONCLUSIONS

Although the precise function of TAPBPR in MHC class I pathway remains to be determined, its discovery calls for a re-evaluation of the MHC class I antigen processing and presentation pathway. Furthermore, since MHC class I polymorphism is associated with clinical illnesses including infectious diseases, autoinflammatory conditions and cancer, it is worth considering whether variation in TAPBPR is associated with disease.

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REFERENCES

1. Van Hateren A, James E, Bailey A, Phillips A, Dalchau N, Elliott T. The cell biology of major histocompatibility complex class I assembly: towards a molecular understanding. *Tissue Antigens* 2010: **76**:259-75.

2. Yewdell JW, Anton LC, Bennink JR. Defective ribosomal products (DRiPs): a major source of antigenic peptides for MHC class I molecules? *J Immunol* 1996: **157**:1823-6.

3. Eisenlohr LC, Huang L, Golovina TN. Rethinking peptide supply to MHC class I molecules. *Nat Rev Immunol* 2007: **7**:403-10.

4. Saunders PM, van Endert P. Running the gauntlet: from peptide generation to antigen presentation by MHC class I. *Tissue Antigens* 2011: **78**:161-70.

5. Apcher S, Daskalogianni C, Lejeune F, et al. Major source of antigenic peptides for the MHC class I pathway is produced during the pioneer round of mRNA translation. *Proc Natl Acad Sci U S A* 2011: **108**:11572-7.

6. Apcher S, Millot G, Daskalogianni C, Scherl A, Manoury B, Fahraeus R. Translation of pre-spliced RNAs in the nuclear compartment generates peptides for the MHC class I pathway. *Proc Natl Acad Sci U S A* 2013: **110**:17951-6.

7. Starck SR, Jiang V, Pavon-Eternod M, et al. Leucine-tRNA initiates at CUG start codons for protein synthesis and presentation by MHC class I. *Science* 2012: **336**:1719-23.

8. Deverson EV, Gow IR, Coadwell WJ, Monaco JJ, Butcher GW, Howard JC. MHC class II region encoding proteins related to the multidrug resistance family of transmembrane transporters. *Nature* 1990: **348**:738-41.

9. Trowsdale J, Hanson I, Mockridge I, Beck S, Townsend A, Kelly A. Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. *Nature* 1990: **348**:741-4.

10. Spies T, Bresnahan M, Bahram S, et al. A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. *Nature* 1990: **348**:744-7.

11. Salter RD, Cresswell P. Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. *EMBO J* 1986: **5**:943-9.

12. Spies T, DeMars R. Restored expression of major histocompatibility class I molecules by gene transfer of a putative peptide transporter. *Nature* 1991: **351**:323-4.

13. Spies T, Cerundolo V, Colonna M, Cresswell P, Townsend A, DeMars R. Presentation of viral antigen by MHC class I molecules is dependent on a putative peptide transporter heterodimer. *Nature* 1992: **355**:644-6.

14. 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.

15. 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.

16. Evnouchidou I, Weimershaus M, Saveanu L, van Endert P. ERAP1-ERAP2 dimerization increases peptide-trimming efficiency. *J Immunol* 2014: **193**:901-8.

17. 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.

18. Kanaseki T, Lind KC, Escobar H, et al. ERAAP and tapasin independently edit the amino and carboxyl termini of MHC class I peptides. *J Immunol* 2013: **191**:1547-55.

19. Ellgaard L, Bettendorff P, Braun D, et al. NMR structures of 36 and 73-residue fragments of the calreticulin P-domain. *J Mol Biol* 2002: **322**:773-84.

20. 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 U S A* 2002: **99**:1954-9.

21. Leach MR, Cohen-Doyle MF, Thomas DY, Williams DB. Localization of the lectin, ERp57 binding, and polypeptide binding sites of calnexin and calreticulin. *J Biol Chem* 2002: **277**:29686-97.

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. Degen E, Cohen-Doyle MF, Williams DB. Efficient dissociation of the p88 chaperone from major histocompatibility complex class I molecules requires both beta 2-microglobulin and peptide. *J Exp Med* 1992: **175**:1653-61.

24. 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.

25. 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.

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: **24**:3613-23.

27. 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.

28. Rufer E, Leonhardt RM, Knittler MR. Molecular architecture of the TAP-associated MHC class I peptide-loading complex. *J Immunol* 2007: **179**:5717-27.

29. Hermann C, Strittmatter LM, Deane JE, Boyle LH. The binding of TAPBPR and Tapasin to MHC class I is mutually exclusive. *J Immunol* 2013: **191**:5743-50.

30. 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.

31. Petersen JL, Hickman-Miller HD, McIlhaney MM, et al. A charged amino acid residue in the transmembrane/cytoplasmic region of tapasin influences MHC class I assembly and maturation. *J Immunol* 2005: **174**:962-9.

32. 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.

33. 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.

34. 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.

35. 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.

36. Wearsch PA, Peaper DR, Cresswell P. Essential glycan-dependent interactions optimize MHC class I peptide loading. *Proc Natl Acad Sci U S A* 2011: **108**:4950-5.

37. 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.

38. 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.

39. 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.

40. 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.

41. 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.

42. Purcell AW, Gorman JJ, Garcia-Peydro M, et al. Quantitative and qualitative influences of tapasin on the class I peptide repertoire. *J Immunol* 2001: **166**:1016-27.

43. Brocke P, Garbi N, Momburg F, Hammerling GJ. HLA-DM, HLA-DO and tapasin: functional similarities and differences. *Curr Opin Immunol* 2002: **14**:22-9.

44. 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**:3196-206.

45. Turnquist HR, Thomas HJ, Prilliman KR, Lutz CT, Hildebrand WH, Solheim JC. HLA-B polymorphism affects interactions with multiple endoplasmic reticulum proteins. *Eur J Immunol* 2000: **30**:3021-8.

46. Turnquist HR, Schenk EL, McIlhaney MM, Hickman HD, Hildebrand WH, Solheim JC. Disparate binding of chaperone proteins by HLA-A subtypes. *Immunogenetics* 2002: **53**:830-4.

47. Lewis JW, Sewell A, Price D, Elliott T. HLA-A*0201 presents TAP-dependent peptide epitopes to cytotoxic T lymphocytes in the absence of tapasin. *Eur J Immunol* 1998: **28**:3214-20.

48. 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.

49. 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.

50. Peh CA, Burrows SR, Barnden M, et al. HLA-B27-restricted antigen presentation in the absence of tapasin reveals polymorphism in mechanisms of HLA class I peptide loading. *Immunity* 1998: **8**:531-42.

51. Rizvi SM, Salam N, Geng J, et al. Distinct assembly profiles of HLA-B molecules. *J Immunol* 2014: **192**:4967-76.

52. 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.

53. Park B, Lee S, Kim E, Ahn K. A single polymorphic residue within the peptide-binding cleft of MHC class I molecules determines spectrum of tapasin dependence. *J Immunol* 2003: **170**:961-8.

54. Elliott T, Williams A. The optimization of peptide cargo bound to MHC class I molecules by the peptide-loading complex. *Immunol Rev* 2005: **207**:89-99.

55. Garstka MA, Fritzsche S, Lenart I, et al. Tapasin dependence of major histocompatibility complex class I molecules correlates with their conformational flexibility. *FASEB J* 2011: **25**:3989-98.

56. Dalchau N, Phillips A, Goldstein LD, et al. A peptide filtering relation quantifies MHC class I peptide optimization. *PLoS computational biology* 2011: **7**:e1002144.

57. 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.

58. 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.

59. Suh WK, Mitchell EK, Yang Y, Peterson PA, Waneck GL, Williams DB. MHC class I molecules form ternary complexes with calnexin and TAP and undergo peptide-regulated interaction with TAP via their extracellular domains. *J Exp Med* 1996: **184**:337-48.

60. 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.

61. 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.

62. Zhang W, Wearsch PA, Zhu Y, Leonhardt RM, Cresswell P. A role for UDP-glucose glycoprotein glucosyltransferase in expression and quality control of MHC class I molecules. *Proc Natl Acad Sci U S A* 2011: **108**:4956-61.

63. D'Alessio C, Caramelo JJ, Parodi AJ. UDP-GlC:glycoprotein glucosyltransferase-glucosidase II, the ying-yang of the ER quality control. *Semin Cell Dev Biol* 2010: **21**:491-9.

64. Kunte A, Zhang W, Paduraru C, et al. Endoplasmic reticulum glycoprotein quality control regulates CD1d assembly and CD1d-mediated antigen presentation. *J Biol Chem* 2013: **288**:16391-402.

65. Boyle LH, Gillingham AK, Munro S, Trowsdale J. Selective export of HLA-F by its cytoplasmic tail. *J Immunol* 2006: **176**:6464-72.

66. 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.

67. Cho S, Ryoo J, Jun Y, Ahn K. Receptor-mediated ER export of human MHC class I molecules is regulated by the C-terminal single amino acid. *Traffic* 2011: **12**:42-55.

68. 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.

69. 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.

70. 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.

71. Park B, Lee S, Kim E, Chang S, Jin M, Ahn K. The truncated cytoplasmic tail of HLA-G serves a quality-control function in post-ER compartments. *Immunity* 2001: **15**:213-24.

72. van Hall T, Wolpert EZ, van Veelen P, et al. Selective cytotoxic T-lymphocyte targeting of tumor immune escape variants. *Nat Med* 2006: **12**:417-24.

73. Lampen MH, Hassan C, Sluijter M, et al. Alternative peptide repertoire of HLA-E reveals a binding motif that is strikingly similar to HLA-A2. *Mol Immunol* 2013: **53**:126-31.

74. Oliveira CC, van Hall T. Importance of TAP-independent processing pathways. *Mol Immunol* 2013: **55**:113-6.

75. Del Val M, Lazaro S, Ramos M, Anton LC. Are membrane proteins favored over cytosolic proteins in TAP-independent processing pathways? *Mol Immunol* 2013: **55**:117-9.

76. Wei ML, Cresswell P. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. *Nature* 1992: **356**:443-6.

77. Sugita M, Brenner MB. Association of the invariant chain with major histocompatibility complex class I molecules directs trafficking to endocytic compartments. *J Biol Chem* 1995: **270**:1443-8.

78. Vigna JL, Smith KD, Lutz CT. Invariant chain association with MHC class I: preference for HLA class I/beta 2-microglobulin heterodimers, specificity, and influence of the MHC peptide-binding groove. *J Immunol* 1996: **157**:4503-10.

79. Bevan MJ. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J Exp Med* 1976: **143**:1283-8.

80. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. *Nat Rev Immunol* 2012: **12**:557-69.

81. Kleijmeer MJ, Escola JM, UytdeHaag FG, et al. Antigen loading of MHC class I molecules in the endocytic tract. *Traffic* 2001: **2**:124-37.

82. Basha G, Omilusik K, Chavez-Steenbock A, et al. A CD74-dependent MHC class I endolysosomal cross-presentation pathway. *Nat Immunol* 2012: **13**:237-45.

83. Du Pasquier L. The phylogenetic origin of antigen-specific receptors. *Curr Top Microbiol Immunol* 2000: **248**:160-85.

84. 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.

85. Landis ED, Palti Y, Dekoning J, Drew R, Phillips RB, Hansen JD. Identification and regulatory analysis of rainbow trout tapasin and tapasin-related genes. *Immunogenetics* 2006: **58**:56-69.

86. Boyle LH, Hermann C, Boname JM, et al. Tapasin-related protein TAPBPR is an additional component of the MHC class I presentation pathway. *Proc Natl Acad Sci U S A* 2013: **110**:3465-70.

87. 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.

88. Korfali N, Wilkie GS, Swanson SK, et al. The leukocyte nuclear envelope proteome varies with cell activation and contains novel transmembrane proteins that affect genome architecture. *Molecular & cellular proteomics : MCP* 2010: **9**:2571-85.

89. Denzin LK, Sant'Angelo DB, Hammond C, Surman MJ, Cresswell P. Negative regulation by HLA-DO of MHC class II-restricted antigen processing. *Science* 1997: **278**:106-9.

90. van Ham SM, Tjin EP, Lillemeier BF, et al. HLA-DO is a negative modulator of HLA-DM-mediated MHC class II peptide loading. *Curr Biol* 1997: **7**:950-7.

91. Denzin LK, Fallas JL, Prendes M, Yi W. Right place, right time, right peptide: DO keeps DM focused. *Immunol Rev* 2005: **207**:279-92.

92. Roche PA, Cresswell P. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 1990: **345**:615-8.

93. Riberdy JM, Newcomb JR, Surman MJ, Barbosa JA, Cresswell P. HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides. *Nature* 1992: **360**:474-7.

94. Bakke O, Dobberstein B. MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. *Cell* 1990: **63**:707-16.

95. Lotteau V, Teyton L, Peleraux A, et al. Intracellular transport of class II MHC molecules directed by invariant chain. *Nature* 1990: **348**:600-5.

96. Romagnoli P, Layet C, Yewdell J, Bakke O, Germain RN. Relationship between invariant chain expression and major histocompatibility complex class II transport into early and late endocytic compartments. *J Exp Med* 1993: **177**:583-96.

97. Roche PA, Cresswell P. Proteolysis of the class II-associated invariant chain generates a peptide binding site in intracellular HLA-DR molecules. *Proc Natl Acad Sci U S A* 1991: **88**:3150-4.

98. Maric MA, Taylor MD, Blum JS. Endosomal aspartic proteinases are required for invariant-chain processing. *Proc Natl Acad Sci U S A* 1994: **91**:2171-5.

99. Kozik P, Francis RW, Seaman MN, Robinson MS. A screen for endocytic motifs. *Traffic* 2010: **11**:843-55.

100. Porter KM, Hermann C, Traherne JA, Boyle LH. TAPBPR isoforms exhibit altered association with MHC class I. *Immunology* 2014: **142**:289-99.

101. Chen QR, Hu Y, Yan C, Buetow K, Meerzaman D. Systematic genetic analysis identifies Cis-eQTL target genes associated with glioblastoma patient survival. *PLoS One* 2014: **9**:e105393.

102. Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. *Lancet* 1973: **1**:904-7.

103. Schlosstein L, Terasaki PI, Bluestone R, Pearson CM. High association of an HL-A antigen, W27, with ankylosing spondylitis. *The New England journal of medicine* 1973: **288**:704-6.

104. Wellcome Trust Case Control C, Australo-Anglo-American Spondylitis C, Burton PR, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007: **39**:1329-37.

105. Evans DM, Spencer CC, Pointon JJ, et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat Genet* 2011: **43**:761-7.

106. Reeves E, Edwards CJ, Elliott T, James E. Naturally occurring ERAP1 haplotypes encode functionally distinct alleles with fine substrate specificity. *J Immunol* 2013: **191**:35-43.

107. Goulder PJ, Walker BD. HIV and HLA class I: an evolving relationship. *Immunity* 2012: **37**:426-40.

Figure legends

Figure 1: The classical MHC class I antigen processing and presentation pathway

Antigenic peptides (coloured triangles) generated in the cytosol by the proteasome are transported into the lumen of the ER by the TAP transporters. Further peptide trimming is performed by ERAP1/2. MHC class I heavy chain and β2m are assembled into heterodimers by the ER resident chaperones calnexin and calreticulin and then loaded with peptide when incorporated into PLC, composed of TAP, tapasin, ERp57 and calreticulin. UGT1 reglucosylates MHC I molecules associated with sub-optimal peptide (light blue), thereby permitting PLC re-engagement of MHC class I molecules. After loading high affinity peptide (dark blue), the MHC class I heterotrimer travel to the cell surface where it is monitored by $CD8^+$ T cells.

Figure 2: Comparison of functional domains in tapasin and TAPBPR

A. Structure of tapasin (PDB ID 3F8U) with residues involved in MHC class I binding highlighted in red and C95 highlighted in yellow. B. Homology model of TAPBPR with residues involved in MHC class I binding highlighted in red. C. Comparison of transmembrane domain and cytoplasmic tail of tapasin and TAPBPR with Lysine 408 and ER retention motif KK in tapasin highlighted in red.

Figure 3: Potential function of TAPBPR in the MHC class I antigen presentation pathway

A. TAPBPR could be part of a tapasin-independent folding pathway for MHC class I running in parallel to the classical antigen presentation pathway. B. TAPBPR could be a novel sequential step in the classical MHC class I antigen presentation pathway, possibly after the

PLC in a location analogous to UGT1. C. TAPBPR could play a role in the MHC class I cross-presentation, possibly delivering peptide-receptive MHC class I into the endosomal system.

Figure 4: Polymorphisms identified in TAPBPR

A. Amino acid sequence of TAPBPR (NP_060479.3) with polymorphisms highlighted in yellow boxes. B. Homology model of TAPBPR with polymorphic residues highlighted in yellow.

Figure 1

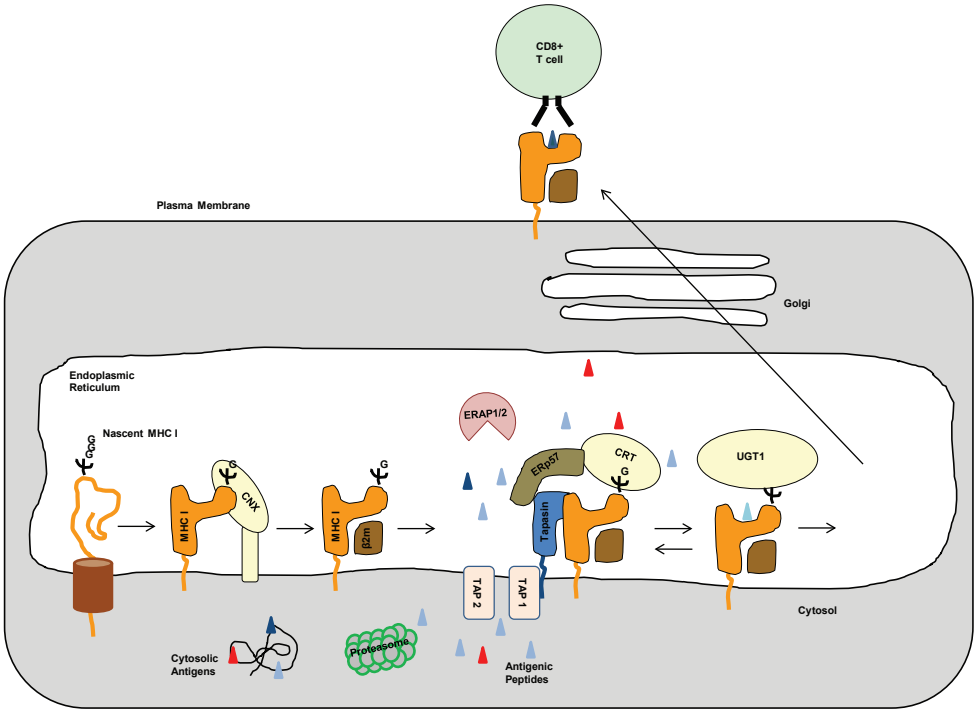
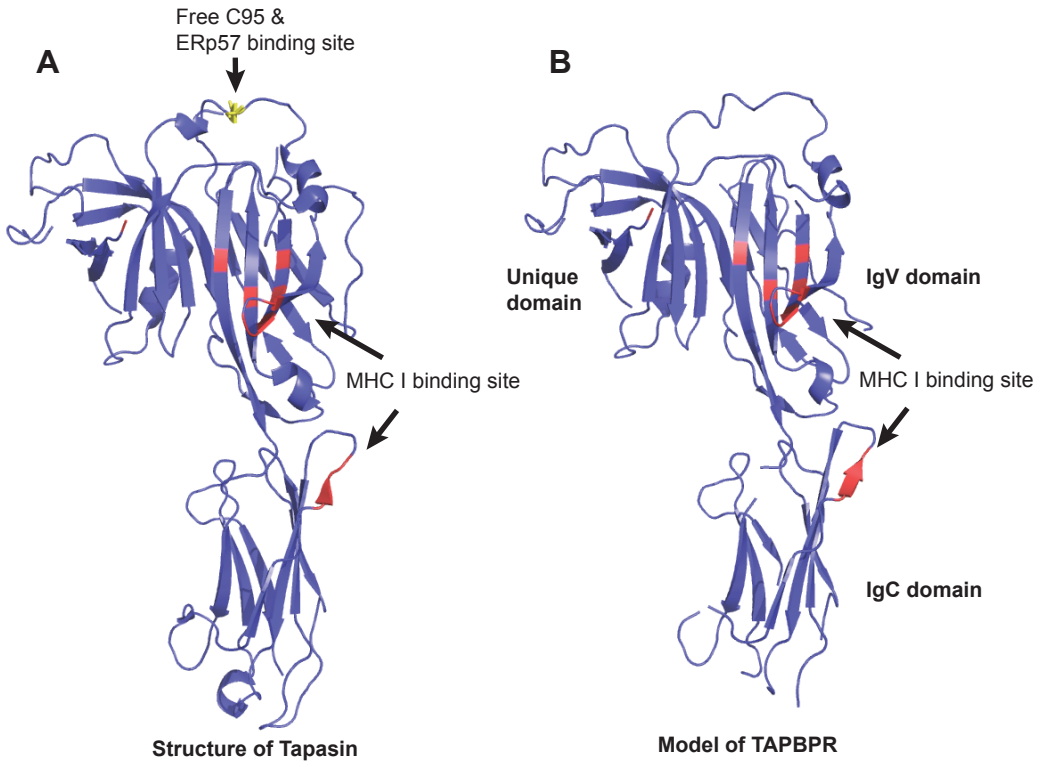

CD8+
T cell
Plasma Membrane
Golgi
Endoplasmic
Reticulum
Nascent MHC I
MHC I
CNX
MHC I
β2m
ERAP1/2
ER57
CRT
Tapasin
UGT1
TAP 2
TAP 1
Cytosol
Cytosolic
Antigens
Proteasome
Antigenic
Peptides

Figure 2



C

	Transmembrane Domain	Cytoplasmic Tail
TAPBPR	TALGVIFASSLFLLALMFLGL	QRRQAPTGLGLLQAERWETTSCADTQSSHLHEDRTARVSQPS
Tapasin	GLFLSAFLLLGLFKALGWAAV	YLSTCKDSKKKAE

TAP association

ER retention motif

Figure 3

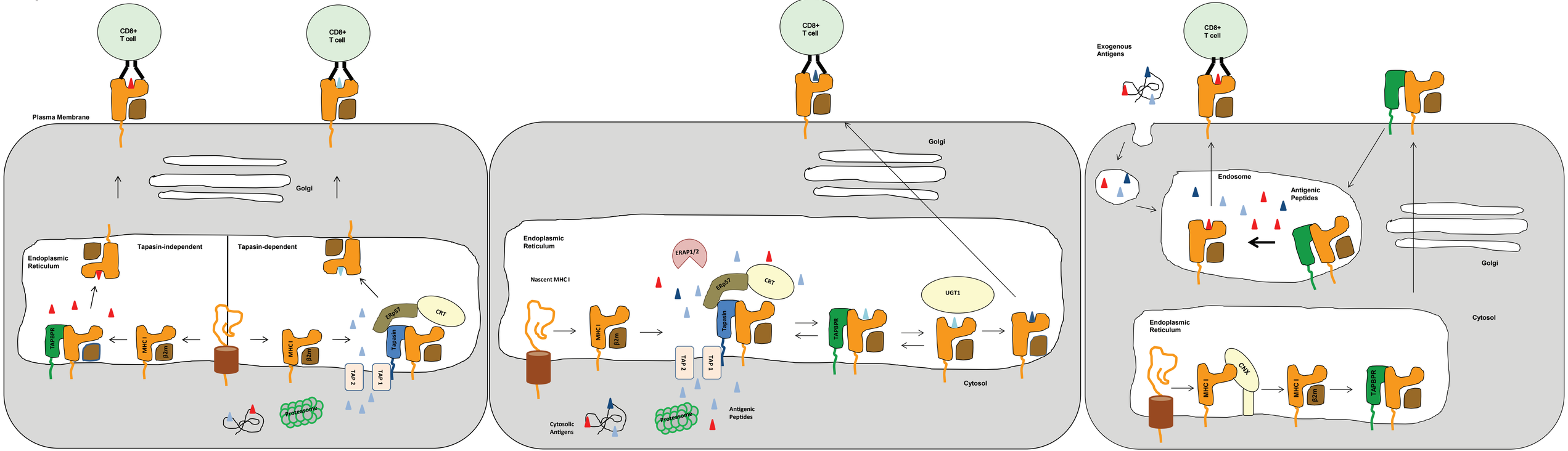

CD8+
T cell
CD8+
T cell
Plasma Membrane
Golgi
Endoplasmic
Reticulum
Tapasin-independent
Tapasin-dependent
TAPBPR
MHC I
β2m
MHC I
β2m
ERp57
CRT
Tapasin
TAP 2
TAP 1
Proteasome
A Tapasin-independent Pathway
CD8+
T cell
Golgi
Endoplasmic
Reticulum
Nascent MHC I
ERAP1/2
ERp57
CRT
Tapasin
MHC I
β2m
TAP 2
TAP 1
TAPBPR
UGT1
Cytosol
Cytosolic
Antigens
Proteasome
Antigenic
Peptides
B Classical Pathway
Exogenous
Antigens
CD8+
T cell
Endosome
Antigenic
Peptides
Golgi
Cytosol
Endoplasmic
Reticulum
MHC I
CNX
MHC I
β2m
TAPBPR
C Cross-presentation Pathway

Figure 4

A

```
                                                 V
MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLAKDGAHRGALASSEDRARA

SLVLKQVPVLDDGSLEDFTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEV

          V    R                A   V
TCEISRYFLQMTETTVKTAAWFMANMQVSGGGPSISLVMKTPRVTKNEALWHPTLNLPLS

PQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQHKGRGQLVYS

WTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNI

                                  M
QASPKVRLSLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVA

GTYSISSSLTAEPGSAGATYTCQVTHISLEEPLGASTQVVPPERRTALGVIFASSLFLLA

LMFLGLQRRQAPTGLGLLQAERWETTSCADTQSSHLHEDRTARVSQPS
```

B