Review

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The transporter associated with antigen processing: a key player in adaptive immunity

Abstract: The adaptive immune system co-evolved with sophisticated pathways of antigen processing for efficient clearance of viral infections and malignant transformation. Antigenic peptides are primarily generated by proteasomal degradation and translocated into the lumen of the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP). In the ER, peptides are loaded onto major histocompatibility complex I (MHC I) molecules orchestrated by a multisubunit peptide-loading complex (PLC). Peptide-MHC I complexes are targeted to the cell surface for antigen presentation to cytotoxic T cells, which eventually leads to the elimination of virally infected or malignantly transformed cells. Here, we review MHC I mediated antigen processing with a primary focus on the function and structural organization of the heterodimeric ATP-binding cassette (ABC) transporter TAP1/2. We discuss recent data on the molecular transport mechanism of the antigen translocation complex with respect to structural and biochemical information of other ABC exporters. We further summarize how TAP provides a scaffold for the assembly of the macromolecular PLC, thereby coupling peptide translocation with MHC I loading. TAP inhibition by distinct viral evasins highlights the important role of TAP in adaptive immunity.

Keywords: ABC transporter; antigen processing; membrane proteins; molecular immunology; viral immune evasion.

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Introduction: self-defense against pathogens

Our body is steadily exposed to billions of pathogens such as viruses, parasites, fungi, and bacteria. To neutralize these invaders, an elaborated immune system has evolved, which is subdivided into the innate and adaptive immunity. After primary contact with foreign bodies, the innate immunity immediately responds and activates inflammatory reactions. Within this phase, most infections are already defeated. However, if pathogens successfully evade the innate response, the highly specific adaptive immunity takes over 3–5 days after infection. An adaptive immune response can confer lifetime immunity against recurrent infections. At the very first onset of the adaptive immune response, cell surface antigens are recognized by antibodies, which are secreted by plasma cells during the interconnected humoral defense (Lanzavecchia and Sallusto, 2009). As part of the cell-mediated immune response, professional antigen-presenting cells (B lymphocytes, macrophages, and dendritic cells) internalize and process ‘non-self’ as well as ‘self’ proteins. The resulting antigenic peptides are mainly presented via major histocompatibility complex class II (MHC II) molecules on the cell surface. $CD4^+$ T lymphocytes bind with their T cell receptor (TCR) to antigen-presenting MHC II molecules, and thereby, induce the production of antigen-specific antibodies (Avalos and Ploegh, 2014; Fooksman, 2014).

The cell-mediated immunity constitutes a second arm of the adaptive immune system. In this case, cytotoxic $CD8^+$ T lymphocytes recognize antigens presented on MHC class I (MHC I) molecules on the surface of all nucleated cells (Gromme and Neefjes, 2002; Lehner and Cresswell, 2004), and subsequently eliminate virally infected or malignantly transformed cells. Apoptosis is induced by the binding of the Fas ligand (CD95L, T lymphocyte) with the Fas receptor (CD 95, transformed cell), also called death receptor, and the massive secretion of perforin and granzyme B (Wajant, 2014).

The MHC I restricted antigen processing pathway is composed of four major consecutive events: (i) the generation of antigenic peptides in the cytosol by protein turnover mainly via the proteasome-ubiquitin pathway; (ii) the translocation of these peptides into the lumen of the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP); (iii) antigen loading onto MHC I molecules, peptide editing, and final proofreading; and (iv) the trafficking of MHC I-peptide complexes via the Golgi-network to the cell surface for antigen presentation to cytotoxic T lymphocytes (Figure 1).

Generation of a short-lived pool of peptides

The cytosolic, multicatalytic 26S proteasome complex represents a major pathway of protein degradation (Sledz et al., 2013; Tomko and Hochstrasser, 2013). In its 20S core particle, ubiquitinylated proteins are fragmented into short peptides with a length of 3–22 residues (Ehring et al., 1996; Kisselev et al., 1999). During infection, the replacement of three catalytic subunits, induced by the inflammatory factors interferon-γ and interferon-β, leads to the formation of an immunoproteasome and the synthesis of the proteasome activator complex PA28 (Kloetzel, 2004). The immunoproteasome produces peptides preferably with hydrophobic residues at the C terminus, which are optimally suited for binding to the antigen transporter TAP and to MHC I molecules (Ferrington and Gregerson, 2012). Antigens for MHC I loading have different origins (Farfan-Arribas et al., 2012). Besides the degradation of redundant or otherwise unwanted proteins, they also derive from defective ribosomal products (DRiPs) (Anton and Yewdell, 2014), which consist of prematurely terminated or mistranslated polypeptides. Although the C termini are already processed for ideal MHC I loading, the produced N termini are still disfavored. Additionally, most peptides are too long to meet the favored peptide length of 8–11 residues, presented on MHC I molecules. Peptide editing presumably starts in the cytosol where antigens are shortened by cytosolic aminopeptidases (Weimershaus et al., 2013). However, the relevance of these peptidases in the formation of immunodominant epitopes is not fully confirmed. Actually, the net effect probably results in a destruction of potential MHC I ligands (van Endert, 2011). Antigenic peptides of different length (≥8 residues)

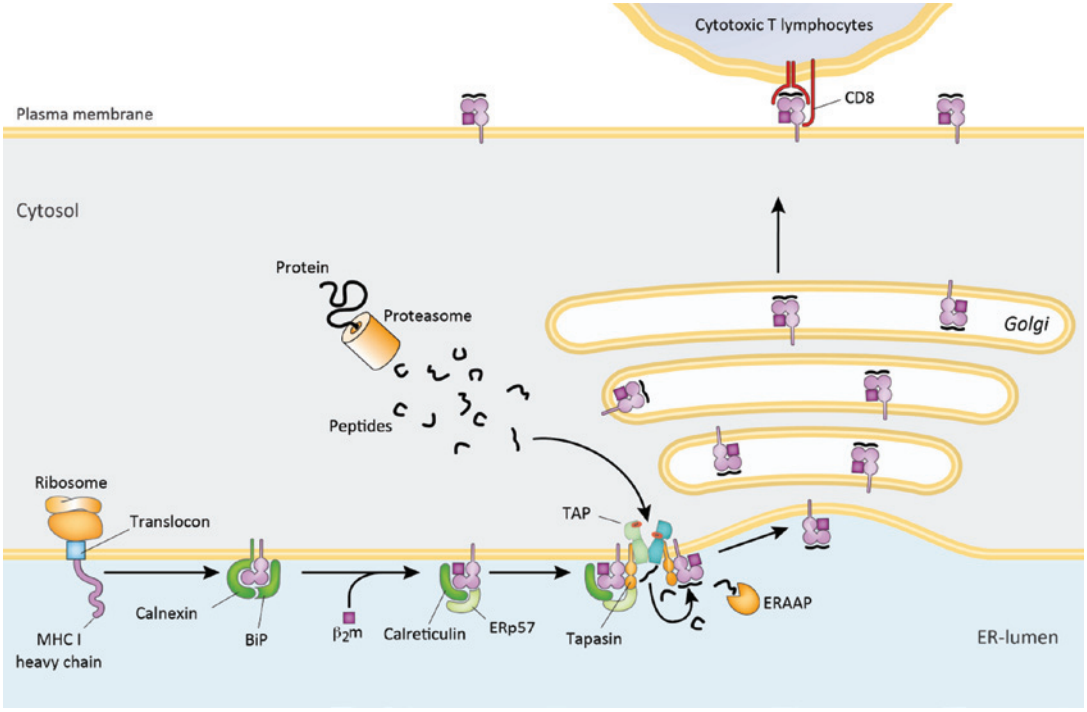

Figure 1: Pathway of major histocompatibility complex I (MHC I) mediated antigen processing.
Antigenic peptides, derived from ubiquitin-proteasome degradation, are translocated from the cytosol into the lumen of the endoplasmic reticulum (ER) by the heterodimeric the transporter associated with antigen processing (TAP)1/TAP2. In the ER lumen, a peptide-loading complex is formed, which stabilizes TAP and MHC I molecules for efficient peptide loading. Stable peptide-MHC I complexes are delivered via the Golgi-network to the cell surface, where they are presented to cytotoxic T lymphocytes [adapted from (Parcej and Tampé, 2010)].

reach their target MHC I complex by translocation from the cytosol into the lumen of the ER via the ATP-binding cassette (ABC) transporter TAP. Once in the ER lumen, antigens are further trimmed N-terminally to their final peptide length by the ER aminopeptidases associated with antigen processing (ERAAP1/2) (Saric et al., 2002; Serwold et al., 2002; Saveanu et al., 2005). Additionally, the ER-membrane protein tapasin, involved in the formation of the peptide-loading complex (PLC), is discussed to play an important role in peptide editing (Kanaseki et al., 2013).

The antigen translocon TAP

The next step in MHC I antigen processing is mediated by the ABC transporter TAP. The translocation machinery preselects antigens suited for MHC I loading and transports them from the cytosol into the ER lumen. A deficiency of the TAP subunits is associated with infectious diseases and tumor development (Lankat-Buttgereit and Tampé, 2002). The heterodimeric TAP1/2 complex belongs to the ABC protein superfamily, which members are found in all three kingdoms of life (Lankat-Buttgereit and Tampé, 2002).

Structural organization and function of TAP

By cycling through rounds of ATP binding and hydrolysis, ABC transporters translocate a broad range of substrates across biological membranes. They execute crucial cellular functions such as multidrug resistance, protein secretion, lipid trafficking, ion homeostasis, signal transduction, or antigen processing (ter Beek et al., 2014). ABC import systems are primarily found in bacteria and archaea, whereas in eukarya ABC transporters act almost exclusively as exporters (Parcej and Tampé, 2010). ABC importers (type I or type II) typically utilize a substrate-binding protein for substrate delivery (Rice et al., 2014). In the human genome, 48 genes encode ABC proteins, which are grouped into seven subfamilies (ABCA to ABCG). Besides the heterodimeric TAP1/2 complex (ABCB2/3), some prominent and physiologically relevant members include the multidrug resistance protein P-glycoprotein (ABCB1), the multidrug resistance-associated protein 1 (MRP1, ABCC1), and the cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7). Subfamilies ABCA to ABCD share the same core architecture forming the minimal functional unit illustrated by the X-ray structure of Sav1866 from *Staphylococcus aureus* (Dawson and Locher, 2006). ABC exporters are composed of two conserved nucleotide-binding domains (NBDs) converting the energy of ATP binding and hydrolysis into conformational changes of two transmembrane domains (TMDs) composed of a 2×6 transmembrane helical region, which executes substrate binding and translocation (Figure 2A). In addition, the antigen translocation complex possesses two N-terminal extra four transmembrane helices (TMD0) fused to each coreTAP subunit (Koch et al., 2004; Schrodt et al., 2006). This auxiliary domain represents a transmembrane interaction hub for the assembly of the PLC. In a simplified working model, peptide and ATP binding to TAP occurs independently of each other, but together induce an allosteric coupling between TMD and NBD. Thereby, the two NBDs dimerize and induce a conformational switch of the TMDs from the inward- to the outward-facing conformation, thus moving peptides from the cytosol into the ER lumen. ATP hydrolysis resets the TAP complex back to the inward-facing, pre-translocation state (Parcej and Tampé, 2010; Seyffer and Tampé, 2015).

The X-ray structures of the nucleotide-free, inward-facing P-glycoprotein and of nucleotide-bound, outward-facing Sav1866 represent two extreme conformations (Dawson and Locher, 2006; Aller et al., 2009). The wide opening and large separation of the two NBD remains controversial considering the dramatic reorganization of lipids required during the conformational changes within the lipid bilayer. As recently demonstrated by the cryo-EM structure of a heterodimeric ABC transporter, the NBDs are separated only by 8 Å in the nucleotide-free state in single particles (Kim et al., 2015). In structures of ABC exporters disclosed so far, the TMs of each subunit are intertwined with the other subunit (Figure 2B). Thereby, TM1 and TM2 of one monomer interconnect with TM3 to TM6 of the other subunit. Another important feature is that all transmembrane segments are connected by large intracellular loops. It is further worth mentioning that the TMDs of TAP exhibit only little sequence homologies with other ABC exporters explaining the variety of substrates. In contrast, the NBDs of all ABC proteins share conserved motifs, which are indispensable for their ATPase activity. Table 1 lists all consensus sequences of TAP1 and TAP2.

The X-ray structure of the soluble TAP1-NBD1 (Gaudet and Wiley, 2001) revealed a typical L-shaped structure with a RecA-like subdomain I composed of Walker A and B sequences (Walker et al., 1982) as well as the switch region (H-loop), the Q-, and D-loops, as well as a smaller α-helical subdomain II containing the signature motif (C-loop) and the X-loop (Figure 2C; Table 1). Upon binding of ATP and substrate, the two NBDs dimerize in a

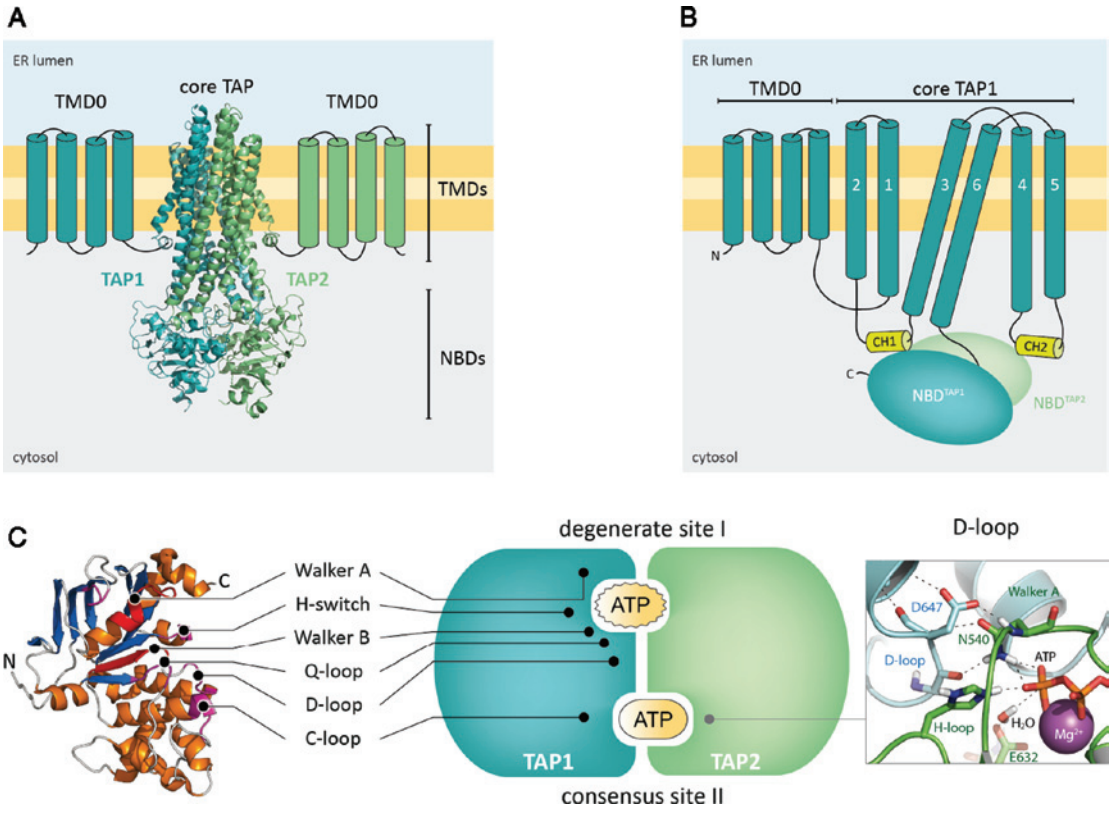

Figure 2: Structural organization of the antigen transport complex TAP.
(A) TAP can be divided in the core complex, responsible for peptide binding and translocation, and two autonomous transmembrane domains (TMD0), contributing in the recruitment of the peptide-loading complex. The core complex is formed by coreTAP1 and coreTAP2, each composed of six transmembrane helices and a cytosolic nucleotide-binding domain (NBD). CoreTAP is modeled on the structure of Sav1866 (Dawson and Locher, 2006) and the TAP1-NBD dimer (Procko et al., 2006; Grossmann et al., 2014). (B) The transmembrane organization is schematically illustrated for TAP1. The TMD is interconnected via its two CH with the NBDs of TAP1 and TAP2 (CH1 in *cis* and *trans*; CH2 only in *trans*) allowing allosteric coupling of peptide binding, transport, and ATP hydrolysis [adopted from (Mayerhofer and Tampé, 2015)]. (C) The conserved NBDs constitute the motor domain for peptide transport and catalyze ATP hydrolysis at two asymmetric ATPase sites. Site II harbors consensus sequence motifs important for ATP binding and hydrolysis, including the D-loop, which is important for NBD dimerization and unidirectional substrate pumping. The enlarged NBD interface shows the electrostatic and hydrogen bond network between the D-loop aspartate and Walker A and B motifs of the opposite NBD. Key residues, ATP, and Mg^{2+} (sphere) are illustrated. The NBD of TAP1 lacks the conserved glutamate in the Walker B motif. All other important motifs are highlighted in the crystal structure of NBD1 (Procko et al., 2006) (adapted from Grossmann et al., 2014 and Seyffer and Tampé, 2015).

Table 1: Conserved sequence motifs in the nucleotide-binding domain (NBD) of ATP-binding cassette transporters.

Motif	Consensus sequence	Sequence in TAP1	Sequence in TAP2	Putative function
Walker A	GxxGxGK(S/T)	GPNGSGKST	GPNGSGKST	Interacts with α- and β-phosphate of ATP or ADP
Walker B	ϕϕϕϕDE	VLILDD	VLILDE	Coordinates of catalytic water and Mg^{2+}, ends with catalytic glutamate
Catalytic glutamate	E	D	E	Polarizes a water molecule for the hydrolysis of ATP
Signature (C-loop)	LSGGQ	LSGGQ	LAAGQ	Involved in ATP binding
Switch(H-loop)	H	TQHL	AHRL	ATP hydrolysis
D-loop	SALD	SALD	SALD	Contact site within NBD dimer, aligning the ATP-binding site
Q-loop	ϕϕ/QQD/E	VGQE	VGQE	γ-phosphate sensor and communication with TMD
X-loop	TEVGERG	TEVDEAG	TDVGEKG	Communication with TMD and peptide sensing

TMD, transmembrane helices; x represents any amino acid; ϕ reflects hydrophobic residues.

head-to-tail orientation with two nucleotides sandwiched in their interface (Smith et al., 2002; Procko et al., 2006). Thereby, the Walker A and Walker B motifs of one NBD and the C-loop of the opposing NBD constitute one ATP-binding site (Jones and George, 1999; Smith et al., 2002) (Figure 2C). During a transport cycle, the largest conformational changes within the rigid body movement of the RecA-like subdomain towards the α-helical subdomain are found in the Q-, X-, and D-loop. The D-loop is important for the dimerization of NBDs (Grossmann et al., 2014; Hohl et al., 2014). So far, scant attention was drawn to the D-loop and its role in the transport mechanism of ABC exporters remained elusive. Upon ATP binding, the conserved aspartate forms hydrogen bonds and an electrostatic network to the H-loop of the opposite NBD (Zaitseva et al., 2005) and the Walker A motif (Grossmann et al., 2014; Hohl et al., 2014), whereas the two D-loop backbones interact with each other (Smith et al., 2002). Together with the Walker B motif, the D-loop positions and activates the putative attacking water for ATP hydrolysis (Smith et al., 2002; Procko et al., 2006) (Figure 2C). Interestingly, a single point mutation of the consensus D-loop aspartate to an alanine in TAP1 abolishes ATP hydrolysis, whereas transport activity is preserved. Astonishingly, transport is still nucleotide-dependent, but does not necessarily require ATP (Grossmann et al., 2014). A mutation of the D-loop aspartate in the degenerate ATP-binding site I does not affect the transport activity, whereas the doubled D-to-A D-loop mutant is transport inactive (Grossmann et al., 2014). Investigations on the D-loop mutation in heterodimeric ABCG5/8 confirm a preserved transport activity also *in vivo* (Wang et al., 2011). Notably, in homodimers, the D-loop mutation affects both ATPase sites and therefore, cause different effects than in heterodimers, where only one ATPase site is affected. The data strongly indicate an involvement of the D-loop in allosteric control of transport and ATP hydrolysis and the directionality of substrate transport by TAP (Grossmann et al., 2014). Wild-type TAP accumulates peptides in the lumen of TAP-containing proteoliposomes due to an unidirectional transport against the concentration gradient, whereas the D-to-A D-loop mutant of TAP1 facilitates a bidirectional passive flux down the concentration gradient (Grossmann et al., 2014). Thus, a mutation in the D-loop turns an active unidirectional uphill transporter into a nucleotide-dependent facilitator by disrupting the allosteric coupling between peptide transport and ATPase activity. In conclusion, the conserved D-loop plays an important role for the directionality and energetics of ABC exporters.

Structural rearrangements within the NBDs are transmitted via the coupling helices (CH1 and CH2) at the tip of the large intracellular loops (coupling loops, CL) to the TMDs (Figure 2B; Table 2). This conformational coupling allows energy transduction and hence, substrate transport (Dawson and Locher, 2006; Damas et al., 2011). Thereby, the coupling helices connecting TM2 and TM3 (CH2) and TM4 and TM5 (CH1) form a transmission interface (Table 2). CH1 is coupled in *cis* and *trans* to the Q-loops of both NBDs, whereas CH2 solely contacts the opposite NBD in *trans* (Dalmas et al., 2005; Dawson and Locher, 2006; He et al., 2008; Serohijos et al., 2008; Oancea et al., 2009) (Figure 2B). In TAP and other ABC exporters, the X-loop also contributes to the inter-domain crosstalk by interacting with CHs of TAP1 and TAP2 (Oancea et al., 2009; Becker et al., 2010; Damas et al., 2011).

Heterodimeric ABC exporters, including TAP1/2, display asymmetric ATP-binding sites (Figure 2C). ATP-binding site II forms a canonical site, aligned by Walker A and B of TAP2 and the C-loop of TAP1, whereas ATP-binding site I is degenerate, lacking both the catalytic glutamate adjacent to the Walker B motif (TAP1) and the histidine in the H-loop (TAP1), as well as displaying mutations in the C-loop (TAP2). Despite these differences, both nucleotide-binding sites bind and hydrolyze ATP. However, the degenerate site I has a very low ATP hydrolysis rate (Chen et al., 2003). Similar effects were observed for other heterodimeric ABC exporters such as TmrAB from *Thermus thermophilus* (Zutz et al., 2011), LmrCD from *Lactococcus*

Table 2: Essential sequences for peptide transport in antigen processing (TAP)1 and TAP2 next to ATP-binding cassette motifs.

Feature	TAP1	TAP2	Putative function
Coupling helix 1	Q271-N279	Q236-T244	TMD interaction with NBD in *cis* and *trans* (Q-loop, only *trans* X-loop)
Coupling helix 2	A373-N382	Q340-E349	TMD interaction with NBD in *trans* (Q- and X-loop)
Peptide binding region	P375-M420, Q453-R487	R354-M389, I414-M433	Peptide binding
Peptide sensor	V288	–	Peptide sensing of bound peptide
Peptide specificity	–	T217, M218, A374, R380, C213[a]	Altered peptide specificity

TMD, transmembrane helices; NBD, nucleotide-binding domain. [a]Armandola et al., 1996; Momburg et al., 1996; Baldauf et al., 2010.

lactis (Lubelski et al., 2006), ABCG5/ABCG8 (Berge et al., 2000), and many others. Apparently, the degenerate site does not actively participate in the transport cycle but rather contributes in NBD dimer formation and regulation of the ATP hydrolysis cycle (Yang et al., 2003; Berger et al., 2005; Zutz et al., 2011). Therefore, the consensus ATPase site II is the 'principle motor' of substrate transport by TAP (Chen et al., 2004; Procko et al., 2006). Such asymmetry can be retraced by the X-ray structure of a heterodimeric ABC transporter TM287/288 from *Thermotoga maritima* in its inward-facing conformation with one AMP-PNP bound (Hohl et al., 2012). TM287/288 deviates from the consensus sequence at the same position as TAP1/2. In contrast to structures of other ABC exporters (Ward et al., 2007; Aller et al., 2009; Jin et al., 2012), the NBDs only partially separate and might form an intermediate conformation. Pulsed electron-electron double resonance studies on BmrCD from *Bacillus subtilis* suggest a mechanistic role of these intermediates in the presence of substrate and under physiological conditions of ATP turnover (Mishra et al., 2014). Combining this information with the nucleotide-free, inward-facing cryo-EM structure of heterodimeric TmrAB (Kim et al., 2015), the following mechanism can be derived for heterodimeric ABC transporters. In the resting state, the two NBDs complementing two ATP-binding sites are separated and positioned via their C-terminal helices. In the absence of substrate, the two Walker A regions are opposite to each other. Independent binding of two ATP molecules and one substrate leads to a NBD sliding and rotation against each other occurs, leading to a tight NBD dimer and a transition from the inward- to the outward-facing conformation of the TMDs. Under physiological conditions, two ATP molecules are bound: one at the canonic and one at the degenerate nucleotide-binding site. A hydrolysis-competent configuration is induced by a substrate-induced change from the inward-facing, NBD open to the outward-facing, NDB closed conformation. In the latter conformation, the bound substrate can escape into the ER lumen. Release of inorganic phosphate and ADP from the consensus site prior to the slow hydrolysis products at the degenerate site enables multiple asymmetric ATPase cycles without large separation of the NBDs as anticipated from the cryo-EM structure of TmrAB (Kim et al., 2015).

Substrate promiscuity

ATP and peptide bind independently to the TAP complex, but together induce an allosteric coupling between transport and ATP hydrolysis (Gorbulev et al., 2001). TAP preferentially translocates peptides with a length of 8–12 residues, although the binding affinity for peptides with 8–16 amino acids is similar (Androlewicz et al., 1993; Neefjes et al., 1993; van Endert et al., 1994). It is worth mentioning that only one peptide binds with high-affinity to the TAP complex at the same time (Herget et al., 2009). Both subunits contribute to the peptide-binding site (van Endert et al., 1994), which was mapped to CL2 and a region following TM6 (Nijenhuis and Hämmerling, 1996) (Table 2). Strikingly, a single cysteinyl residue in TAP2 was found to be essential for peptide binding and translocation. A deletion of this critical cysteine results in an altered epitope repertoire translocated by TAP (Baldauf et al., 2010).

By using combinatory peptide libraries, the substrate recognition principle of human TAP was deciphered. Favored and disfavored residues at each peptide position were scored (Uebel et al., 1997). The three N- and the C-terminal residues are critical for binding, with positively charged or aromatic residues at the N terminus and a hydrophobic amino acid at the C terminus. Residues in between do not largely contributed to substrate recognition. Strikingly, even peptides of 40 aa in length or peptides with bulky side chains, such as fluorophores, chemical proteases, or polylysine chains can be transported, thus pointing to a structural flexibility of the peptide-binding pocket (Koopmann et al., 1996; Gorbulev et al., 2001; Herget et al., 2007). The high mobility at the center and tight binding at the anchor positions (1, 2, 3, and C-terminus) of bound peptides was demonstrated by electron paramagnetic resonance studies (Herget et al., 2011). Notably, the distance between N and C terminus is 2.1 nm, independent from peptide length, suggesting that longer peptides bind in a kinked conformation (Herget et al., 2011). The favored anchor positions, especially at the C terminus, and the flexibility point to a co-evolution of the major players in the antigen processing pathway including the immunoproteasome, TAP, MHC I, and the recognition principle of T lymphocytes.

Peptide binding occurs in two steps, with a fast association phase followed by a slow structural rearrangement, involving high activation energies and approximately 25% of all TAP residues (Neumann and Tampé, 1999). Interestingly, a residue within the CL2 of TAP1 was determined to function as a peptide sensor (Herget et al., 2007). As this loop keeps contact with the NBDs upon peptide and ATP binding, the importance of an inter-domain communication is again emphasized. Beside the cytosolic (high-affinity) peptide-binding pocket, a low-affinity ER-lumenal binding site is proposed (Grossmann et al.,

2014). Independent from the number of TAP complexes reconstituted in proteoliposomes, peptide translocation stops at a critical lumenal peptide concentration of 16 μM (Grossmann et al., 2014). Therefore, high ER-lumenal peptide concentrations might saturate a low-affinity binding site. Thereby, further peptide translocation is inhibited in *trans* by preventing the back-switch from the outward- to the inward-facing conformation and thus ATP hydrolysis. This negative feedback mechanism or trans-inhibition might prevent a peptide overloading in the ER and hence an unfolded protein response, a signal for ER stress. It is worth mentioning that all previous studies refer on *in vitro* assays using non-physiologically high peptide concentrations in the micromolar range (1–10 μM). However, recently, antigen compartmentalization in scarce primary immune cell subsets was followed by an ultrasensitive single-cell based transport assay at physiological peptide concentrations of 1–10 nM, reflecting the short-lived peptidome (Fischbach et al., 2015).

Transport mechanism for TAP

By combining recent structural and biochemical information, we propose the following transport cycle of TAP (Figure 3). In the inward-facing conformation, the TMs of TAP1 and TAP2 seal the pathway to the ER lumen and enable independent cytosolic binding of two ATPs to the open ATP-binding sites and one peptide. Simultaneous peptide and ATP binding cause conformational changes allowing an NBD-TMD crosstalk. A slight rotation of the RecA-like arm I towards the α-helical arm II of the NBD followed by a sliding of both NBDs towards each other results in an optimal interface for NBD dimerization (nucleotide-occluded state). A tight NBD dimerization drives the switch from the inward-facing to the outward-facing conformation and a peptide transport event. Peptide release into the ER lumen may trigger ATP hydrolysis at the consensus ATP-binding site, which subsequently destabilizes the dimer interface. This event causes a back-switch of the TMDs to the inward-facing open conformation. The degenerate ATP-binding site is most likely occupied by ATP and it hardly contributes to the ATPase activity. At high peptide concentration in the ER lumen (16 μM), TAP is presumably locked in the outward-facing conformation, caused by a trans-inhibition mechanism.

Despite the wealth of biochemical data, both the transport process and the conformational dynamics within the TAP complex during peptide translocation across the ER membrane are not well characterized. To

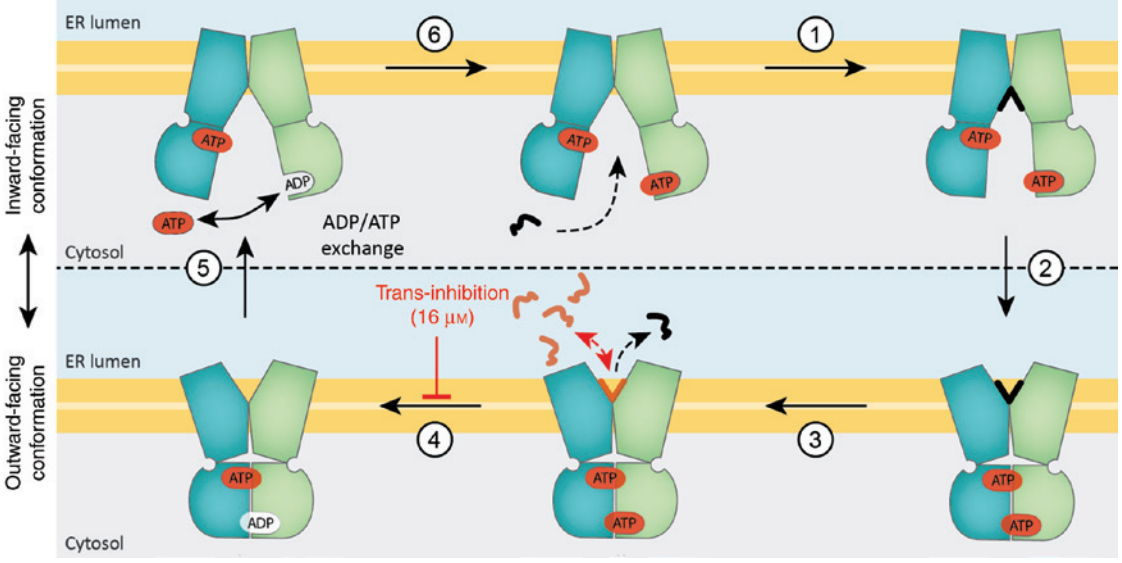

Figure 3: Current model of antigen translocation by the transporter associated with antigen processing (TAP).
Upon independent binding of one peptide and two ATPs to TAP in its inward-cytosolic-facing conformation (1), nucleotide-binding domains (NBDs) dimerize and structural rearrangements switch the TAP complex to the outward-facing state (2), thereby releasing the peptide into the lumen of the endoplasmic reticulum (ER) (3). Next, ATP hydrolysis (4) switches the transmembrane domains (TMDs) back and leads to an opening of the NBD dimer (5). ADP and P_i release enable binding of new ATP, and thus, another round of transport (6). The degenerate ATP-binding site displays a very low ATPase activity, thus likely carrying an ATP-bound intermediate into the next cycle. A high local ER-lumenal peptide concentration inhibits further peptide transport by a negative feedback mechanism (trans-inhibition) and arrests the TAP complex in an outward-occluded ATP hydrolysis-inactive conformation (adapted from Grossmann et al., 2014).

gain a deeper mechanistic insight, the functional reconstitution of the TAP complex in a membrane environment is critical. A substantial impact of lipids on the function of TAP was reported. Lipidomic approaches determined that phosphatidylinositol and phosphatidylethanolamine are important for TAP function (Schölz et al., 2011). Notably, only a very limited number of detergents maintain this crucial lipid environment (Herget et al., 2009; Schölz et al., 2011). As the lipid environment has a strong impact on TAP function, the analysis of the transporter reconstituted in membrane systems became indispensable. The successful insertion of TAP and other ABC transporters into nanodiscs provides new options to study the transport mechanism in a membrane-embedded environment (Kawai et al., 2011; Ritchie et al., 2011; Bao and Duong, 2014; Eggensperger et al., 2014; Nasr and Singh, 2014). For TAP in nanodiscs, an annular lipid belt was found to be sufficient to maintain TAP function (Eggensperger et al., 2014). Peptide-stimulated ATP hydrolysis was preserved, reflecting the allosteric coupling between peptide binding and ATP hydrolysis. Furthermore, the rate of ATP hydrolysis and peptide translocation is reduced by longer peptides (18 and 27 aa). Thus, the peptide-stimulated ATPase activity indirectly reports on the translocation process of TAP in nanodiscs (Eggensperger et al., 2014). In conclusion, nanodiscs are a promising tool to dissect the molecular mechanism, lipid interaction, and modulation of the translocation machinery.

Function of the MHC I peptide-loading complex (PLC)

In the ER membrane, a dynamic multisubunit PLC is formed, providing an optimal platform for efficient MHC I assembly and peptide loading (Hulpke and Tampé, 2013) (Figure 1). The transporter TAP acts as the peptide supplier and is the central element of the PLC. Each of the N-terminal extensions (TMD0s) of TAP1 and TAP2 harbors one binding site for the membrane glycoprotein tapasin (Hulpke et al., 2012b), which itself interacts with MHC I molecules. Hence, tapasin bridges the peptide donor (TAP) with the peptide acceptor (MHC I), providing a close proximity for optimal peptide loading (Hulpke et al., 2012a). Although tapasin-TAP interaction sites have not been revealed yet, some potential interaction sites within the TM of tapasin were mapped (Tan et al., 2002; Petersen et al., 2005). Notably, the tapasin-TAP interaction enhances the stability of the TAP complex (Garbi et al., 2003; Oliveira and van Hall, 2013). Besides the function as an adaptor chaperone, tapasin is involved in peptide editing and proofreading. The binding to MHC I heavy chain presumably supports an opening of the peptide-binding groove, acceleration of low-affinity peptide dissociation and binding of high-affinity peptides (Chen and Bouvier, 2007; Wearsch and Cresswell, 2007; Sieker et al., 2008; Praveen et al., 2010). Two accessory chaperones, ERp57 and calreticulin, complete the PLC. ERp57 is a thiol oxidoreductase forming an intermolecular disulfide bond to tapasin (Dick et al., 2002; Peaper et al., 2005). Its major function is the stabilization of the entire PLC (Stepensky et al., 2007; Peaper and Cresswell, 2008; Zhang et al., 2009), as well as peptide editing and proofreading in complex with tapasin (Wearsch et al., 2011). An X-ray structure of tapasin in complex with ERp57 revealed a 1:1 stoichiometry (Dong et al., 2009). The lectin-like chaperone calreticulin interacts with ERp57 via its β-strand hairpin domain (Oliver et al., 1999; Frickel et al., 2002) and MHC I molecules via their glycans (Rizvi et al., 2011). Already early in biogenesis, *de novo* synthesized MHC I heavy chains are recognized by the binding immunoglobulin protein (BiP) and the lectin-like chaperone calnexin. Upon binding of β_2-microglobulin, calreticulin replaces calnexin and escorts MHC I/β_2-microglobulin dimers to the PLC (Chapman and Williams, 2010). After peptide loading, removal of a single glucose residue triggers dissociation of kinetically stable peptide-MHC I complexes from the PLC and their targeting to the plasma membrane via the secretory pathway for antigen presentation. If a suboptimal peptide was loaded, a re-glycosylation and peptide exchange takes place (Zhang et al., 2011). On the way to the cell surface, quality control is still incomplete and suboptimal loaded MHC I-peptide complexes will be recycled to the ER (Howe et al., 2009). Despite the biochemical characterization, a detailed understanding of the structural arrangement within the PLC is still missing. However, the stoichiometry between TAP, tapasin and MHC I was recently solved. Although one TAP complex is able to recruit two tapasin molecules, the occupation of one single TMD0 or both depends on peptide concentration and the MHC I allele involved (Panter et al., 2012). According to this, one tapasin and one MHC I molecule are sufficient for MHC I mediated antigen surface presentation (Hulpke et al., 2012a).

Viral immune evasion

The abundance of immune evasion strategies evolved by viruses to overcome antigen presentation underlines

the importance of the MHC I restricted antigen processing. In particular, members of the *Herpesviridae* encode gene products, which interfere with this pathway (Griffin et al., 2010). From degradation of host mRNA by the ribonuclease UL41 (Everly et al., 2002), the prevention of synthesis and assembly of the immunoproteasome (Khan et al., 2004), and ER-associated degradation of MHC I by US2 and US11 (Wiertz et al., 1996; Tomazin et al., 1999) to the induction of endocytosis and lysosomal degradation of surface-exposed peptide-loaded MHC I by BILF1 (Zuo et al., 2011) (and many more), almost all steps in antigen processing are targeted. Amongst these, the peptide transporter TAP is a very prominent target and antigen translocation into the ER is affected at any step in the transport cycle (Figure 4).

The type I membrane protein UL49.5 encoded by several varicelloviruses arrests TAP in a transport inactive conformation by an ER-lumenal TAP interaction (Koppers-Lalic et al., 2008) and blocks either ATP binding to TAP (equine Herpes virus 1 and 4) or induces degradation of TAP by its cytosolic C-terminal residues (bovine Herpes virus 1 and 4) (Loch et al., 2008; Verweij et al., 2011). Furthermore, the early gene product US6 from human cytomegalovirus interacts with ER-lumenal loops of TAP1 and TAP2 (Halenius et al., 2006) and locks the peptide translocation machinery in a post-transport intermediate state, while preventing ATP binding (Ahn et al., 1997; Hewitt et al., 2001; Kyritsis et al., 2001). In contrast, the tail-anchored protein BNLF2a from Epstein-Barr-virus inhibits ATP and peptide binding to the TAP complex *via* its N-terminal cytosolic region (Horst et al., 2011; Wycisk et al., 2011).

In the following two viral inhibitors are discussed in more detail. ICP47 (infected cell protein 47) from Herpes simplex virus (HSV, type 1 and 2) competes for high-affinity peptide binding to TAP (Früh et al., 1995; Hill et al., 1995; Ahn et al., 1996; Tomazin et al., 1996). In contrast, CPXV12, a gene product from the *Orthopoxviridae* cowpox virus, presumably interferes with a low-affinity, trans-inhibition site in the ER lumen. Thus, both viral proteins target peptide-binding sites with distinct strategies. ICP47 of HSV-1 is a cytosolic polypeptide (88 aa) with its active domain mapped to the N-terminal region ($ICP47_{3-34}$) (Galocha et al., 1997; Neumann et al., 1997). The ability to block peptide binding is restricted to human TAP with a 100-fold higher affinity (K_D=50 nM) compared to murine TAP (Ahn et al., 1996; Tomazin et al., 1996). Strikingly, ICP47 displays a 100-fold lower affinity to TAP solubilized in digitonin as reconstituted in membranes or membrane mimics (Aisenbrey et al., 2006; Eggensperger et al., 2014). When ICP47 binds to negatively charged lipid bilayers or detergent micelles, a transition of a mostly unstructured to a helix-loop-helix conformation is induced (Beinert et al., 1997; Pfänder et al., 1999). An important impact constitutes the TAP-lipid interface. The active domain of $ICP47_{3-34}$ is oriented almost parallel to the membrane surface, with the N-terminal helix immerging into the bilayer, which might serve as a membrane anchor (Aisenbrey

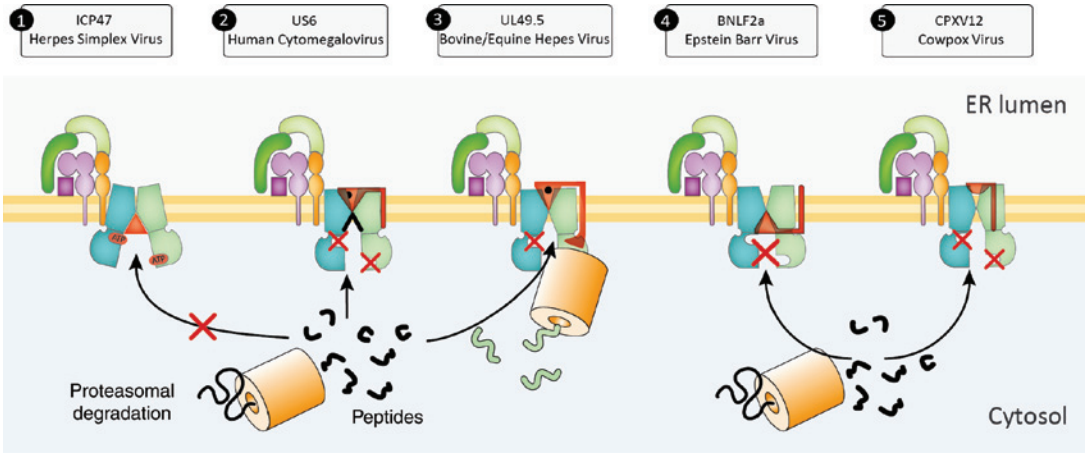

Figure 4: Inhibition mechanisms of viral immune evasions targeting peptide translocation mediated by the transporter associated with antigen processing (TAP).
Soluble ICP47 blocks cytosolic peptide binding in a competitive manner. In contrast, the membrane resident CPXV012 prevents ATP binding by an endoplasmic reticulum (ER)-lumenal interaction with a putative low-affinity peptide-binding site with its C-terminal tail. Depending on the species, UL49.5 arrests the TAP complex in a transport-incompetent state by blocking ATP binding to TAP or initiates proteasomal degradation of TAP. The viral factor US6 interferes with TAP from the ER lumen and also prevents ATP binding. Finally, the tail-anchored viral factor BNLF2a inhibits peptide and ATP binding with its cytosolic domain, exploiting a new inhibition mechanism.

et al., 2006). In our current model, ICP47 competes with cytosolic peptide binding by interacting with both TAP subunits (Ahn et al., 1996; Tomazin et al., 1996) at the subunit-membrane interface within the lipid head group region (Aisenbrey et al., 2006).

In contrast, the recently discovered and so far only known poxviral TAP inhibitor CPXV012 (Alzhanova et al., 2009; Byun et al., 2009) inhibits peptide transport by preventing ATP binding to TAP1 and TAP2 but not high-affinity peptide binding (Lin et al., 2014; Luteijn et al., 2014). CPXV012 is a 69-aa type II membrane protein consisting of a short N-terminal cytosolic region, a transmembrane helix, and a short ER-lumenal tail. The C-terminal region is sufficient for TAP inhibition; however, the transmembrane and cytosolic regions increase the inhibition effect by providing an additional interaction site for TAP (Lin et al., 2014; Luteijn et al., 2014). Notably, the C-terminal region of CPXV012 evolved from a genomic frameshift mutation in the extended ER-lumenal tail of homologous inactive CPXV012 variants as detected for D10L (Alzhanova et al., 2009; Carroll et al., 2011). This rearrangement results in a short missense ER-lumenal region of 25 aa. Using systematic truncations, the active region was narrowed down to the last ten amino acids (Lin et al., 2014). These residues exploit all features of an optimal TAP substrate. Therefore, CPXV012 is suggested to mimic a high-affinity TAP peptide, which binds to the TAP complex in *trans*. Thus, CPXV012 might abuse the ER-lumenal negative feedback peptide sensing by TAP at the putative low-affinity peptide binding site for its own molecular inhibition mechanism (Lin et al., 2014). In conclusion, two viral inhibitors interfere with peptide-binding sites and block peptide translocation with completely different molecular mechanisms.

Final remarks

TAP and other ABC transporters take over important cellular functions and their absence or dysfunction is related to frequent and rare diseases. Here, the crucial role of TAP is highlighted by the fact that viruses escape immune surveillance by targeting the antigen translocation complex using sophisticated strategies. Thus, a detailed mechanistic understanding of the translocation cycle of TAP and its inhibition by viral proteins is essential to develop successful approaches in controlling the host-pathogen interactions and targeting immune therapies against tumor progression and infectious diseases. The combination of biochemical, biophysical and structural data allowed us to propose a model of how TAP translocates peptides into the ER for loading of MHC I. However, there are still plenty of questions to be answered: What are the conformational dynamics in each step of the translocation cycle? What is the exact role of the degenerate ATP-binding site, and what is the stoichiometry of ATP hydrolysis per transport event? Where is the low-affinity binding site located and how is its specificity? The *tour-de-force* on the purification and functional reconstitution in liposomes, and recently also in nanodiscs, provides optimal tools to address these questions. Finally, a three-dimensional structure enlightened by either X-ray crystallography or single particle cryo-EM, most desirable in distinct intermediate states, in combination with pulsed EPR, solid-state NMR, and single-molecule FRET, would enhance our understanding on substrate translocation by TAP and ABC transporters in general.

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