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Review

ABC transporters in adaptive immunity ☆

Fabian Seyffer [a], Robert Tampé [a,b,*]

[a] Institute of Biochemistry, Biocenter, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany
[b] Cluster of Excellence — Macromolecular Complexes, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

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ABSTRACT

Background: ABC transporters ubiquitously found in all kingdoms of life move a broad range of solutes across membranes. Crystal structures of four distinct types of ABC transport systems have been solved, shedding light on different conformational states within the transport process. Briefly, ATP-dependent flipping between inward- and outward-facing conformations allows directional transport of various solutes.
Scope of review: The heterodimeric transporter associated with antigen processing TAP1/2 (ABCB2/3) is a crucial element of the adaptive immune system. The ABC transport complex shuttles proteasomal degradation products into the endoplasmic reticulum. These antigenic peptides are loaded onto major histocompatibility complex class I molecules and presented on the cell surface. We detail the functional modules of TAP, its ATPase and transport cycle, and its interaction with and modulation by other cellular components. In particular, we emphasize how viral factors inhibit TAP activity and thereby prevent detection of the infected host cell by cytotoxic T-cells.
Major conclusions: Merging functional details on TAP with structural insights from related ABC transporters refines the understanding of solute transport. Although human ABC transporters are extremely diverse, they still may employ conceptually related transport mechanisms. Appropriately, we delineate a working model of the transport cycle and how viral factors arrest TAP in distinct conformations.
General significance: Deciphering the transport cycle of human ABC proteins is the major issue in the field. The defined peptidic substrate, various inhibitory viral factors, and its role in adaptive immunity provide unique tools for the investigation of TAP, making it an ideal model system for ABC transporters in general. This article is part of a Special Issue entitled Structural biochemistry and biophysics of membrane proteins.

1. Introduction

Living cells are separated from the inanimate outside by the plasma membrane, whereas other subcellular membranes play essential roles in the architecture of eukaryotic cells. Cell compartmentalization by membranes, however, demands for systems that mediate the passage of information, ions, and molecules, as for instance transporters that actively or passively shuttle a wide range of solutes across this hydrophobic barrier. ATP-binding cassette (ABC) transporters constitute one of the largest transporter superfamilies in all three kingdoms of life [1]. They are primary active transporters that couple the energy provided by ATP binding and hydrolysis to the directional transport of substrate (solute) molecules. Their modular architecture with two highly variable transmembrane domains (TMDs) associated with two conserved nucleotide-binding domains (NBDs) responsible for ATP turnover allows them to act on myriads of solutes. The five subfamilies of ABC transporters found in humans, for instance, transport antibiotics, toxins, vitamins, drugs, metals, polycarbonates, bile acids, sterols, lipids, chloride ions, peptides, and other molecules [2]. Here, we focus on the transporter associated with antigen processing (TAP) that shuttles peptides into the endoplasmic reticulum (ER) and is found in all nucleated cells of jawed vertebrates [3,4]. As detailed in Section 5, antigenic peptides are subsequently loaded onto major histocompatibility complex class I (MHC I) molecules *via* the macromolecular peptide-loading complex (PLC) and are eventually presented at the cell surface, attributing to TAP an important role in the adaptive immune response. Yet, we primarily aim to shed light on the molecular mechanism that couples ATP binding and hydrolysis with substrate translocation across the membrane.

2. The modular architecture diversifies ABC transporter functions

The histidine importer HisJQMP from *Salmonella typhimurium* and the "energizing" subunit MalK of the maltose importer $MalFGK_2$ from *Escherichia coli* were the first ABC transporters to be sequenced [5–7]. Based on the presence of the Walker A and B motifs characteristic of P-loop NTPases [8,9] as well as the ABC-signature motif LSGGQ (later

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* Corresponding author at: Institute of Biochemistry, Biocenter, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany. Tel.: +49 69 798 29475; fax: +49 69 798 29495.
E-mail address: tampe@em.uni-frankfurt.de (R. Tampé).

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Table 1
Crystal structures of full ABC transporters.

Transporter (TC-#[a])	Organism	Ref.	PDB code (resolution)	Orientation[b] (nucleotide state)	Ligands[c] (used detergents[d])
Type I systems[e] (ABC importer)					
MalFGK$_2$-E (3.A.1.1.1)	*Escherichia coli*	[32]	2R6G (2.80 Å)	Outward, closed (ATP-bound)	Maltose (DDM, UDM)
		[33]	3FH6 (4.50 Å)	Inward, open (apo)	None (DDM, UDM)
		[34]	3PUY (3.10 Å)	Outward, closed (AMPPNP-bound)	Maltose, Mg^{2+}, PGV (DDM, UDM)
			3PUZ (2.90 Å)	Pre-translocation, partially closed (AMPPNP-bound)	Maltose, Mg^{2+}, PGV (DDM, UDM)
			3PV0 (3.10 Å)	Pre-translocation, partially closed (apo)	Maltose, PGV (DDM, UDM)
		[35]	3RLF (2.20 Å)	Outward, closed (AMPPNP-bound)	Maltose, Mg^{2+}, PGV, UDM (DDM, UDM)
			3PUV (2.40 Å)	Outward, closed (ADP-bound)	Maltose, Mg^{2+}, VO_4^{3-}, PGV, UDM (DDM, UDM)
			3PUW (2.30 Å)	Outward, closed (ADP-bound)	Maltose, Mg^{2+}, AlF_4^-, PGV, UDM (DDM, UDM)
			3PUX (2.30 Å)	Outward, closed (ADP-bound)	Maltose, Mg^{2+}, BeF_3^-, PGV, UDM (DDM, UDM)
		[36]	4JBW (3.91 Å)	Inward, open (apo)	Bound to $EIIA^{Glc}$, PGV (DDM)
		[37]	4KHZ (2.90 Å)	Pretranslocation, open (apo)	Maltoheptaose, PGV (DDM, UDM)
			4KI0 (2.38 Å)	Outward, closed (AMPPNP-bound)	Maltohexaose, Mg^{2+}, PGV, UDM (DDM, UDM)
MetN$_2$I$_2$ (3.A.1.24.1)	*Escherichia coli*	[38]	3DHW (3.70 Å)	Inward, open (apo)	None (DDM)
		[39]	3TUI (2.90 Å)	Inward, open (ADP-bound)	None (Cy5)
			3TUJ (4.00 Å)	Inward, open (apo)	None (DM)
			3TUZ (3.40 Å)	Inward, open (ADP-bound)	Se-Met (Cy5)
ModB$_2$C$_2$-A (3.A.1.6.8)	*Archaeoglobus fulgidus*	[40]	2ONK (3.10 Å)	Inward, open (apo)	Mg^{2+}, PO_4^{3-}, WO_4^{2-} ($C_{12}E_8$)
			2ONR (1.60 Å)	Inward, open (apo)	Mg^{2+}, MoO_4^{2-}, NO_3^-($C_{12}E_8$)
			2ONS (1.55 Å)	Inward, open (apo)	Mg^{2+}, NO_3^-, WO_4^{2-}($C_{12}E_8$)
ModB$_2$C$_2$ (3.A.1.8.2)	*Methanosarcina acetivorans*	[41]	3D31 (3.00 Å)	Inward, open (apo)	WO_4^{2-} (DDM, $C_{12}E_8$)
Type II systems[e] (ABC importer)					
BtuC$_2$D$_2$ (3.A.1.13.1)	*Escherichia coli*	[42]	1L7V (3.20 Å)	Outward, closed (apo)	$V_4O_{12}^{4-}$(LDAO)
BtuC$_2$D$_2$-F (3.A.1.13.1)	*Escherichia coli*	[43]	2QI9 (2.60 Å)	Intermediate, open (apo)	PEG400, DEG, PO_4^{3-}, SO_4^{2-}(LDAO, Anapoe-$C_{12}E_8$, $C_{12}E_8$)
		[44]	4DBL (3.49 Å)	Intermediate, open (open)	PO_4^{3-}, SO_4^{2-}(LDAO, $C_{12}E_8$)
		[45]	4FI3 (3.47 Å)	Intermediate, closed (AMPPNP-bound)	Mg^{2+} (LDAO, $C_{12}E_8$)
HI1470/1 (MolB$_2$C$_2$) (3.A.1.14.11)	*Haemophilus influenzae*	[46]	2NQ2 (2.40 Å)	Inward, open (apo)	None (DM)
HmuU$_2$V$_2$ (3.A.1.14.5)	*Yersinia pestis*	[47]	4G1U (3.01 Å)	Outward, open (apo)	PO_4^{3-}(DDM)
Type III systems[e] (ABC exporter)					
ABCB1 (3.A.1.201.1)	*Mus musculus*	[48,49]	3G5U, 4M1M (3.80 Å)	Inward, open (apo)	Hg^{2+} (TX-100, DDM, cholate)
			3G60, 4M2S (4.40 Å)	Inward, open (apo)	QZ59-RRR (TX-100, DDM, cholate)
			3G61, 2M2T (4.35 Å)	Inward, open (apo)	QZ59-SSS (TX-100, DDM, cholate)
		[50]	4KSB (3.80 Å)	Inward, open (apo)	None (TX-100, DDM, cholate)
			4KSC (4.00 Å)	Inward, open (apo)	None (TX-100, DDM, cholate)
			4KSD (4.10 Å)	Inward, open (apo)	Nb592 (TX-100, DDM, cholate)
	Caenorhabditis elegans	[19]	4F4C (3.40 Å)	Inward, open (apo)	UDTM, α-D-mannose,β-D-mannose, GlcNAc, NDG (DDM, UDTM)
	Cyanidioschyzon merolae	[51]	3WME (2.75 Å)	Inward, open (apo)	DM (DM)
			3WMF (2.60 Å)	Inward, open (apo)	aCAP (cyclic peptide), DM (DM)
			3WMG (2.40 Å)	Inward, open (apo)	DM, TRIS (DM)
ABCB10 (3.A.1.201.17)	*Homo sapiens*	[20]	4AYT (2YL4) (2.85 Å)	Inward, open (AMPPCP-bound)	CDL, DDM, glycine, Mg^{2+} (DDM, CDL)
			4AYW (4AA3) (3.30 Å)	Inward, open (AMPPNP-bound)	CHS, DDM (CHS, DDM)
			4AYX (2.90 Å)	Inward, open (AMPPCP-bound)	CDL, DDM, glycine, Mg^{2+} (DDM, CDL)
			3ZDQ (2.85 Å)	Inward, open (apo)	CDL, DDM, glycine (DDM, CDL)
Atm1 (3.A.1.210.1)	*Saccharomyces cerevisiae*	[52]	4MYC[5] (3.06 Å)	Inward, open (apo)	(DDM)
			4MYH[5] (3.38 Å)	Inward, open (apo)	GSH (DDM)
	Novosphingobium aromaticivorans	[53]	4MRN (2.50 Å)	Inward, open (apo)	LDAO, PO_4^{3-} (LDAO, CHAPSO, OG, HEGA-11)
			4MRP (2.50 Å)	Inward, open (apo)	GSH, LDAO, PO_4^{3-} (LDAO, CHAPSO, OG, HEGA-11)
			4MRR (2.97 Å)	Inward, open (apo)	Se-Met, LDAO, PO_4^{3-} (LDAO, CHAPSO, OG, HEGA-11)

Table 1 (*continued*)

Transporter (TC-#[a])	Organism	Ref.	PDB code (resolution)	Orientation[b] (nucleotide state)	Ligands[c] (used detergents[d])
			4MRS (2.35 Å)	Inward, open (apo)	GSSG, LDAO, PO_4^{3-} (LDAO, CHAPSO, OG, HEGA-11)
			4MRV (2.50 Å)	Inward, open (apo)	GS-Hg, LDAO, PO_4^{3-} (LDAO, CHAPSO, OG, HEGA-11)
McjD (3.A.1.118.1)	*Escherichia coli*	[139]	4PL0 (2.7 Å)	Outward occluded, closed (AMPPNP-bound)	Mg^{2+}, NG (DDM, NG)
MsbA[f] (3.A.1.106.1)	*Escherichia coli*	[54]	3B5W (5.30 Å)	Inward, open (apo)	None (αDDM)
	Vibrio cholerae	[54]	3B5X (5.50 Å)	Inward, closed (apo)	None (DDM)
	Salmonella typhimurium	[54]	3B5Y (4.50 Å)	Outward, closed (AMPPNP-bound)	None (UDM)
			3B5Z (4.20 Å)	Outward, closed (ADP-bound)	VO_4^{3-}(UDM)
			3B60 (3.70 Å)	Outward, closed (AMPPNP-bound)	None (UDM)
Sav1866 (3.A.1.106.2)	*Staphylococcus aureus*	[21]	2HYD (3.00 Å)	Outward, closed (ADP-bound)	Na^+ ($C_{12}E_8$)
		[55]	2ONJ (3.40 Å)	Outward, closed (AMPPNP-bound)	Na^+($C_{12}E_8$)
TM287/288 (3.A.1.135.5)	*Thermotoga maritima*	[56]	3QF4 (2.90 Å)	Inward, partially closed (AMPPNP-bound)	Mg^{2+}(DDM)
Type IV systems (ECF transporters)					
EcfAA'ST (3.A.1.28.2)	*Lactobacillus brevis*	[14]	4HUQ (3.00 Å)	Inward, open (apo)	None (DDM, DM)
		[15]	4HZU (3.53 Å)	Inward, open (apo)	None (DDM, DM)

Abbreviations: aCAP: anti-CmABCB1 peptide; αDDM: n-dodecyl-α-D-maltopyranoside; $C_{12}E_8$: octaethylene glycol monododecyl ether; CDL: cardiolipin; CHAPSO: 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; CHS: cholesterol hemisuccinate; Cy5: cyclohexyl-pentyl-β-D-maltoside; DDM: n-dodecyl-β-D-maltopyranoside; DEG: diethylene glycol; DM: n-decyl-β-D-maltopyranoside; GlcNAc: N-acetyl-D-glucosamine; GS-Hg: S-mercury glutathione; GSH: glutathione; GSSG: oxidized glutathione; HEGA-11: undecanoyl-N-hydroxyethylglucamide; LDAO: dodecyl-*N*,*N*-dimethylamineoxide; Nb592: lama R2 nanobody; NDG: 2-(acetylamino)-2-deoxy-α-D-glucopyranose; OG: n-octyl-β-D-glucopyranoside; PEG400: polyethylene glycol; PGV: phosphatidylglycerol; QZ59-RRR: cyclic-tris-(R)-valineselenazole; QZ59-SSS: cyclic-tris-(S)-valineselenazole; Se-Met: selenomethionine; TRIS: 2-amino-2-hydroxmethyl-propane-1,3-diol; TX-100: Triton® X-100; UDM: n-undecyl-β-D-maltopyranoside; UDTM: n-undecyl-β-D-thiomaltopyranoside.

[a] TC-#: transporter classification number as assigned in the transporter classification database (TCDB, www.tcdb.org)

[b] Outward/inward/pre-translocation refers to the conformation of the TMDs, open/closed refers to the dimerization state of the NBDs.

[c] Excluding nucleotide (which is listed in column to the left).

[d] All detergents that have been used during the purification process are listed, even if they were later washed away. If detergents are also part of the structure, they are listed twice.

[e] System classification as defined in Parcej & Tampé 2010 [4].

[f] An initial structure of MsbA published in 2001 by Chang and Roth has been retracted in 2006, Science 293:1793–800.

also termed C-loop), HisP, MalK, and subsequently identified homologues were classified as ABC proteins [10,11]. NBDs of ABC proteins possess the activity to bind and hydrolyze ATP and, in the case of ABC transporters, are associated with integral membrane domains. The majority of ABC transporters comprise a minimal set of two TMDs and two solvent exposed NBDs, conceptually organized in two halves with one TMD and one NBD each. Considering the two NBDs to be located at the "inside", which may be the cytosol or the matrix/stroma of mitochondria/chloroplasts, ABC transporters exert import or export functions. The four essential domains can be located on individual polypeptide chains, as often found for prokaryotic importers, or are encoded as half- and full-transporters by single genes [12]. As a consequence, the two halves of the functional ABC transporter may be homo- or heterotypic and auxiliary domains may be inserted or terminally fused [13]. However, while even close homologues exhibit this kind of diversity, ABC transporters can be stringently assigned to families based on sequence homology of their core TMDs to importers or exporters with known crystal structures (Table 1). Accordingly, type I ABC systems are characterized by a minimal core of 2 × 5 transmembrane helices (TMs), while type II ABC systems harbor 2 × 10 TMs (Fig. 1A). To date, all known members of those families are importers. The current set of crystallized exporters is structurally distinct from the importers and comprises 2 × 6 TMs. However, as it is not excluded that the identical fold exerts import and export functions in homologous proteins, we prefer to call this family of exporters type III ABC systems [4]. The recently identified structure of the energy-coupling factor (ECF) transporter from *Lactobacillus brevis* establishes a new TMD fold [14,15] (Table 1; Fig. 1A). These type IV ABC–ECF systems found in bacteria, archaea, and some plant organelles translocate essential cofactors and vitamins such as folate, biotin, and thiamine. Worth mentioning, they are different from the other ABC transporters in that they do not have two similar TMDs, but rather one integral membrane protein (EcfT) and an unrelated S component, which acts as high-affinity substrate binding protein embedded in the membrane [16]. Notably, the structure-based classification of ABC systems may not yet be exhaustive as new architectures may be structurally disclosed, and hence there are alternative attempts to comprehensively define ABC transporter superfamilies, as listed in the transporter classification database (TCDB) [17].

In humans, the 48 genes encoding ABC proteins have been grouped into seven subfamilies, termed ABCA to ABCG [2,18] (Fig. 1B). This nomenclature has been partially extended to other eukaryotic ABC transporters, but does not comprehensively include prokaryotic homologues. The human subfamily B, which harbors the TAP transporter (TAP1/2, ABCB2/3) and the multidrug resistance protein 1 (MDR1, ABCB1, also known as P-glycoprotein, P-gp), is evolutionarily most related to the subfamilies C and D, both based on similarity in the NBDs [2] and the TMDs [17]. In the subfamily C, prominent exporters are found such as the multidrug resistance-associated protein 1 (MPR1, ABCC1) and the cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7). As confirmed for the structure of *Caenorhabditis elegans* ABCB1 [19] and human ABCB10 [20], human subfamily B, C, and D exporters are all expected to share the fold of type III ABC systems initially established for the bacterial ABC exporter Sav1866 [21]. Likewise, in all their polypeptide chains the NBDs are downstream of the corresponding TMDs. Despite the common fold, they represent the versatility in gene partitioning, half-transporter similarity, and, most amazingly, substrate specificity typical for ABC transporters: ABCB1 clears cells from hydrophobic drugs and xenobiotics and has implications in cancer therapies, for instance [22]. ABCB1 is encoded by a single gene, where two halves (TMD-NBDs) are connected by a linker region (approx. 60 aa) of unknown function and structure. The peptide transporter TAP is a heterodimer composed of the two half-transporters TAP1 (ABCB2) and TAP2 (ABCB3). CFTR (ABCC7) evolved into an ATP-dependent chloride channel with an extra regulatory domain genetically inserted between the

first NBD and the second TMD. Mutations in CFTR lead to the devastating disease cystic fibrosis [23]. The sulfonylurea receptors SUR1 (ABCC8) and SUR2 (ABCC9) regulate the Kir6.2 potassium channel but have no known inherent transport function [24]. Human ABCA and ABCG proteins are discussed to have TMDs not related to the other human ABC transporters [17]. Intriguingly, ABCA4 has been reported to import N-retinylidene-phosphatidylethanolamine from the lumen to the cytosolic leaflet of disc membranes in retinal photoreceptor cells [25], representing the first and so far only example of a mammalian ABC importer. While in ABCA proteins the TMD precedes the corresponding NBD, a peculiar feature of the ABCG group and many pleiotropic drug transporters in yeast is the reverse domain order, with the TMD following the NBD (Fig. 1B). The one ABCE1 and three ABCF1-3 proteins present in humans are not equipped or associated with a transmembrane domain and play important roles in the control protein translation, ribosome splitting, and mRNA surveillance [26].

While other superfamilies of transporters are defined by their TMD moiety, the corporate feature of ABC transporters is the characteristic ATP-binding cassette, and only then they are diversified based on the TMD. Likewise, the prime target for crystallization was the conserved NBD, clearly also owing to the technical challenge to isolate and crystallize membrane proteins. The first NBD to be crystallized in 1998 was the very same "energizing" HisP subunit from the *S. typhimurium* histidine importer HisJQMP that had previously been the first ABC domain sequenced [27,28]. Some years later, it became clear that NBDs in ABC proteins form dimers in a head-to-tail arrangement, building two sandwiched ATP-binding pockets [29,30] (see Section 2). In many ABC proteins, including TAP1/2, one of the two ATP-binding sites is non-canonic, suggesting asymmetric functions of the two NBDs [4,31]. While the ATPase cycle may turn out to be very similar in ABC proteins, as discussed in Section 3, a proposed polyphyletic origin of TMDs has to be kept in mind when analyzing the transport cycle. In contrast, whether an ABC transporter exerts import, export, or other functions might not be tightly coupled to its phylogenetic clade, as exemplified by the human retinal importer ABCA4, the bidirectional chloride channel CFTR (ABCC7), and the regulatory receptor SUR1/2 (ABCC8/9).

3. Function and structure of the TAP complex

In a process powered by ATP binding and hydrolysis, TAP exports peptides of preferentially 8–16 residues into the ER [57–59]. TAP function essentially depends on heterodimerization of TAP1 and TAP2, which in humans are 748 aa (81 kDa) and 653–703 aa (72–78 kDa, depending on the isoform) in length, respectively [3,4]. Both human TAP1 and TAP2 harbor a specialized N-terminal transmembrane domain (TMD_0), which is then followed by the so-called core module of the TAP half-transporter, composed of one TMD and one NBD (Fig. 2A). Homozygous defects in either of the two TAP genes can lead to a subtype of the bare lymphocyte syndrome type I (BLS I), a disease accompanied by recurrent respiratory bacterial infections and skin lesions [60,61]. It is to note that

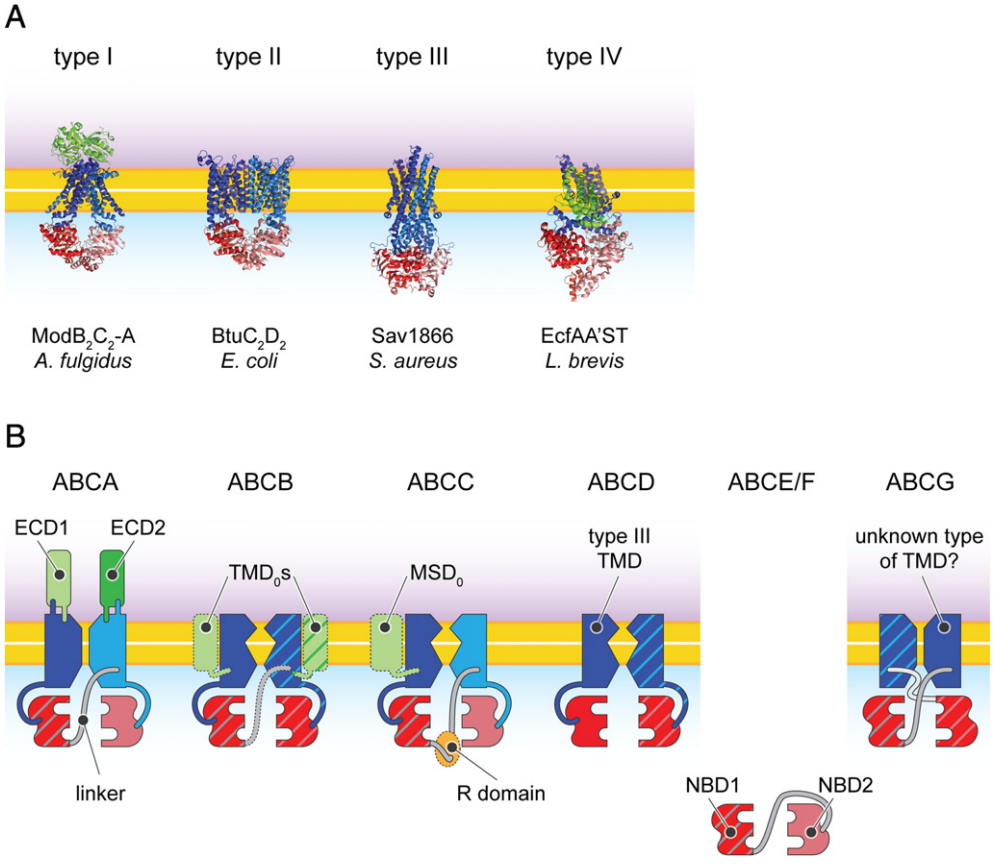

Fig. 1. Structure and domain organization of ABC transporters. A) Four distinct types of transmembrane domains of ABC transporters have been structurally identified. Ribbon representations of the first crystal structure solved for each type are shown. $ModB_2C_2$-A, PDB: 2ONK [40]; $BtuC_2D_2$, PDB: 1L7V [42]; Sav1866, PDB: 2HYD [21]; EcfAA'ST, PDB: 4HUQ [14]. NBDs are colored in red, TMDs in blue, except for the S subunit of the ECF transporter colored in green. The molybdate binding protein ModA is shown in green. Throughout this review, the cytosol is colored in light blue, whereas the "outside" (ER, periplasmic or extracellular space) is colored in magenta. B) Human ABC proteins belong to seven subfamilies with diversity in gene organization and auxiliary domains. NBDs are colored in red, TMDs in blue and auxiliary domains in green and orange. Domains and linkers (gray) with dotted borders exist only in a subset of family members. Half-transporters may be identical (same fill color) or not (differently shaded fill color in right half-transporter), and both variations may be found within one family (hatched fill). Some NBDs may have non-canonic, degenerate ATP-binding sites (gray hatching). The boxes representing the domains are overlapping front to back in correlation to the N- to C-terminal order in the polypeptide, respectively. ABCE/F families consist of cytosolic ABC proteins not associated with any transmembrane moiety and do not exert transport function, but play an important role in translation control and mRNA surveillance. Abbreviations: ECD: extracellular domain; MSD_0: extra N-terminal membrane-spanning domain; NBD: nucleotide-binding domain; R domain: regulatory domain; TMD: transmembrane domain; TMD_0: extra N-terminal transmembrane domain.

only a moderate number of cases have been reported worldwide [60]. The three functional modules of the TAP complex are discussed below.

3.1. TMD_0 — interaction platform for the assembly of the peptide-loading complex (PLC)

Next to TAP and its close homolog TAP-like (TAPL, ABCB9), a couple of other human ABC transporters (ABCB6, ABCC subfamily) have N-terminal extra domains preceding the TMD of type III systems (Fig. 1B). They have little to no sequence homology and are variable in the number of transmembrane helices (TM). In TAP1, TAP2, and TAPL, the TMD_0 is composed of a four-TM bundle, with both termini placed in the cytosol [62,63]. The TMD_0s of TAP are dispensable for integration into the ER membrane as well as for peptide transport [64–67]. In TAPL, which pumps polypeptides into lysosomes, TMD_0 is likewise not required for the transport function, but essential for targeting of the transporter to the lysosomal membrane [68]. While TAPL is still correctly targeted to the lysosomes when its TMD_0 and coreTAPL are encoded on separate genes, it localizes to the plasma membrane in the absence of a functional TMD_0 [68]. In both TAP and TAPL, the TMD_0 plays an essential role by providing an interaction platform for auxiliary proteins that act downstream of the peptide transport. Consistently, at least one of the two TMD_0s has to be present to allow for the incorporation of TAP into the PLC via interaction with the MHC I specific chaperone tapasin [65,67]. In TAPL, interactions of its TMD_0 with lysosome-associated membrane proteins 1 and 2B (LAMP-1/LAMP-2B) have been identified via a proteomics approach [69]. It is not known whether the TMD_0s of TAP and TAPL have further functions, *e.g.* in sensing membrane properties or feedback signaling to the core transporter.

3.2. The TMDs shuttle peptide substrates across the ER membrane

The TMDs are the executive organ of each ABC transporter and are thought to undergo significant structural changes during the transport process [49,73,74] (Fig. 2B). In TAP as well as in other type III ABC transporters, this domain is constituted by six TMs [21]. While the structural similarity of helices 1–3 to the helices 4–6 —with a symmetry axis parallel to the membrane plane— indicates a gene duplication, a computational approach proposes a triplication of a primordial 2 TM segment [17]. N and C termini of the TMD are located in the cytosol, resulting in three small ER-luminal loops and two α-helical extended cytosolic loops. Based on predictions of TMs, the TMD of TAP is connected by a cytosolic loop of roughly 30 residues to its TMD_0. C-terminally, nearly 40 residues link TM6 to the NBD, part of them being organized in the cytosolic extension of TM6. Structural coordinates for TAP were first deduced from a homology model based on the structure of *Staphylococcus aureus* Sav1866 (PDB: 2HYD) [21,71]. In this type III ABC system the TMD starts with a short "elbow" helix snuggling to the cytosolic face of the lipid bilayer. This helix has been consistently found in all type III transporters (Table 1). Its truncation reduces stability and transport activity of coreTAP (unpublished data, Tampé lab). Furthermore, the structure of Sav1866 revealed the presence of short helices connecting the extended TM 2/3 and 4/5 at the tip of the cytosolic loops [21]. These two helices have a parallel orientation relative to the membrane plane and are termed "coupling helices" (CH1 and CH2) due to the contacts they form with the NBDs. The structure confirms genetic and mutational evidence for a prominent contact between CH2 and the NBD of the opposite half-transporter [75,76]. Contacts formed by CH1 depend on the conformational state of the transporter. Sav1866 and the bacterial lipid flippase MsbA were crystallized in an outward-facing conformation with closed, *i.e.* dimerized NBDs, allowing CH1 to form contacts to the NBD of the own half-transporter (*in cis*) as well as to the NBD of the opposite half-transporter (*in trans*) [21,54]. While this conformation is considered to represent a post-export state, with the substrate-binding cavity opened to the outside, the open and closed inward-facing conformation of MsbA, respectively, may illustrate earlier, substrate-receptive steps of the transport cycle [54]. In such inward-facing conformations, which have also been observed in crystal structures of mouse and *C. elegans* P-gp [19,48,50], TM287/288 from *Thermotoga maritima* [56], and human ABCB10 [20], CH1 only contacts the NBD *in cis*, while CH2 remains associated *in trans* with the opposite NBD. In the inward-facing conformation, TM4 and TM5 hook up *in trans* with TM1-3 and TM6. Upon substrate translocation, the helices are assumed to twist into the outward-facing conformation, where they form two wings at the level of the outer membrane leaflet, comprising TM1 and TM2 of one half-transporter and TM3-6 of the opposite half-transporter.

3.3. TAP is specialized on peptide transport

The typical substrate of TAP are oligopeptides (8–16 aa), however, longer, even 40-mer peptides can also be transported, but with lower efficiency [57,77–79]. Facilitated by the hydrophilic nature of oligopeptides, the spectrum and properties of TAP substrates are well explored. Testing various peptide epitopes [80–82] and systematically analyzing combinatorial peptide libraries [78] revealed a strong preference for two basic residues and a subsequent aromatic residue at the three N-terminal positions, while at the C-terminal position a basic or hydrophobic residue is favored. Notably, the C-terminal residue matches the requirements for peptide binding by MHC I molecules (see Section 5). While chemical modifications of the N or C terminus affecting the H-bond network were disfavored [77,83], no restrictions for the remaining residues between positions 1–3 and the C terminus have been found, so even peptides with bulky side chains and fluorophores are transported [78,84,85]. Various strategies have been employed to map peptide-binding regions of TAP. By photo-crosslinking, four regions in the TMD (two each in TAP1 and TAP2) have been identified to be involved in substrate binding (Table 2). They are located to the cytosolic loop 2 (formed between TM4 and TM5) and the cytosolic extension of TM6 [86]. Another strategy, referred to as a *Trojan horse* approach, coupled the peptide substrate with a small chemical protease, cutting the TAP backbone at positions close to the bound peptide [74]. The peptide-binding region in the extended TM6 of TAP1 was confirmed and a fifth site located to the cytosolic loop 1 of TAP1 TMD was identified as a peptide sensor [74].

As demonstrated by pulsed electron double resonance EPR studies, peptides are bound to TAP in an "extended kink" conformation, indicating that TAP harbors two independent recognition sites for the N- and C-terminal residues, which are separated by approximately 2.5 nm [87]. On the basis of biochemical restraints and homology models, potential peptide-binding pockets have recently been proposed [70]. The affinity of substrate is high in the inward-facing conformation of the TMD, but thought to be low in the outward-facing state. A potential "low-affinity" binding site on the ER-luminal face of TAP could allow for trans-inhibition or at least trans-signaling when ER-located substrates are (still) bound. Indeed, albeit TAP is able to pump peptides against a gradient, peptide concentration in reconstituted proteoliposomes does not exceed 16 μM [140].

3.4. The NBD is the energizing motor

The NBDs of ABC proteins harbor a number of highly conserved consensus motifs (Table 2, Fig. 2C/D) and dimerize in a characteristic head-to-tail arrangement, much like the yin-and-yang symbol [29,30]. Two sandwiched nucleotide-binding sites are formed, which are equidistant from the membrane plane. Each NBD is composed of three subdomains, with the F1-type ATP-binding core (RecA-like fold) and the ABC specific ABCα and ABCβ subdomains [27,29,30,88]. The Walker A consensus sequence (GxxGxGK(S/T), where x denotes any residue), initially named P-loop, and the Walker B motif (h_4D, where h denotes a hydrophobic residue), responsible for binding of nucleotides and coordination of the essential Mg(II) ion, respectively, are located on the F_1/RecA-type

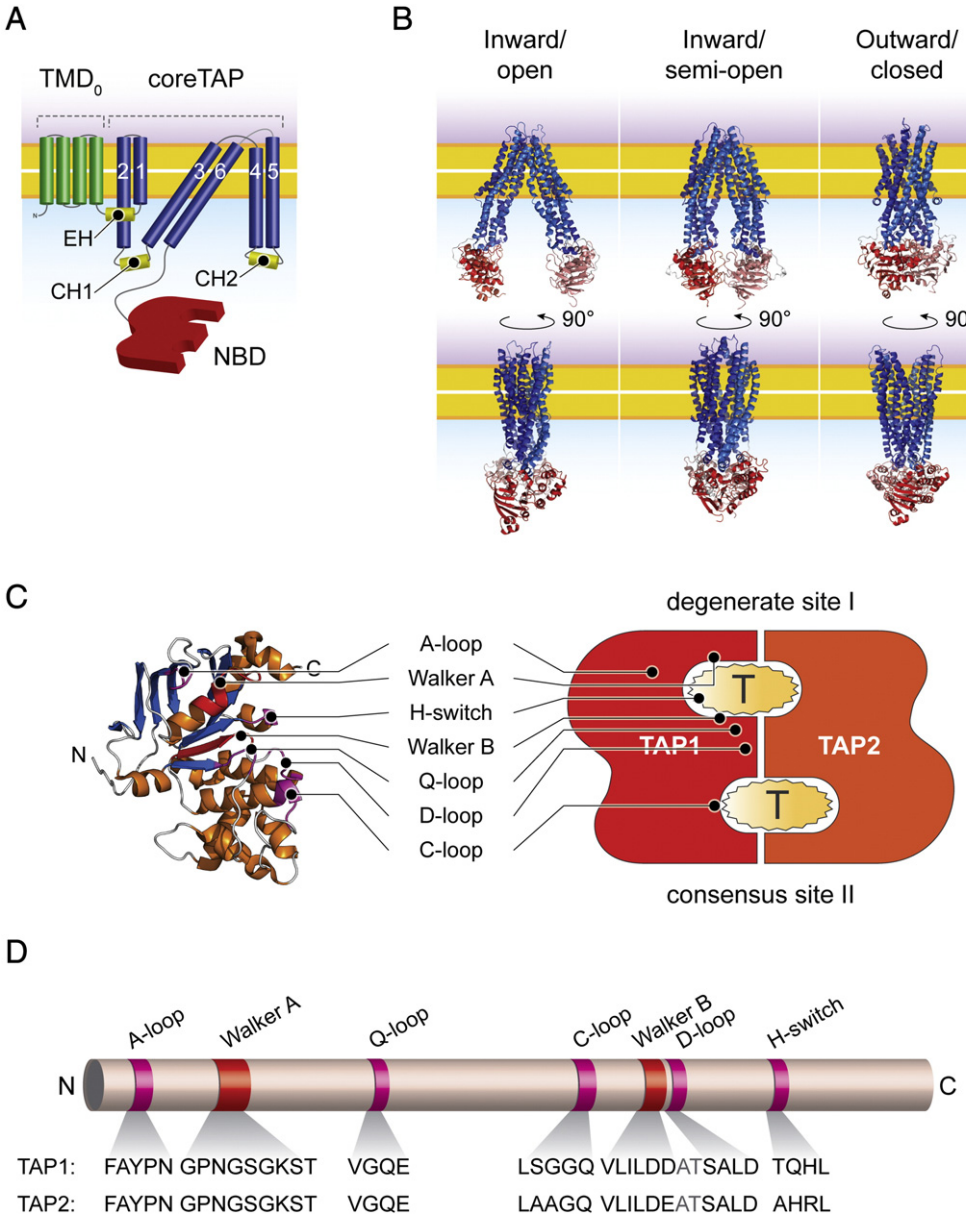

Fig. 2. Domain architecture of TAP and putative conformational rearrangements during the transport cycle. A) The helical architecture of one TAP half-transporter is shown. The transmembrane part of heterodimeric TAP can be divided into two 6 TM core domains, which together with the two NBDs form the coreTAP complex, and two auxiliary N-terminal four-helix bundle domains (TMD_0). Highlighted in yellow are the elbow helix (EH), which connects TMD_0 with coreTAP, and the two coupling helices (CH1, CH2), which make *in cis* and *in trans* contacts with the NBDs. The two nucleotide-binding sites formed by the NBDs are equidistant from the membrane plane. B) Homology models of TAP based on P-gp (PDB: 3G5U), ABCB10 (PDB: 4AYT), and Sav1866 (PDB: 2HYD) visualize the putative conformational rearrangements during the transport cycle. The models were generated as described [70,71]. The structures in the lower panel are rotated by 90° to highlight the twisting motion of the TMs, with the outward opening being orthogonal to the inward opening. C) Correlation between structure and cartoon representation of the TAP NBD. In the crystal structure of TAP1-NBD (PDB: 1JJ7) [72], α-helices are colored in orange and β-sheets in blue. Walker A and B motifs are highlighted in red, all other consensus motifs in magenta. In the cartoon, ATP ("T") is bound to both nucleotide-binding sites. D) Linear arrangement and residues of the canonical elements of the ATP-binding cassettes of TAP1 and TAP2 (listed in Table 2).

ATP-binding core [8,9]. Considered to provide the catalytic base for ATP hydrolysis [29], the conserved glutamate directly adjacent to the Walker B motif is characteristic for RecA-type P-loop NTPases, which are therefore also referred to as Additional Strand Catalytic Glutamate (ASCE) group [89]. A highly conserved tyrosine residue (A-loop) precedes the Walker A motif and makes π–π stacking contacts to the purine base of bound ATP [90]. Again located on the RecA-like subdomain are the ABC protein specific H-switch, which is also discussed to play a prime role in ATP hydrolysis [91,92] and the D-loop, one of the hallmarks of ABC transporters [29,30,72,93]. Notably, the D-loop has significant implications in NBD-TMD communication, ensuring strictly unidirectional transport of peptides across the membrane [140]. Outside the F_1-type ATP-binding core are further the C-loop or signature motif (LSGGQ), completing the ATP-binding site [29,30,94], the Q-loop, interacting with the γ-phosphate of ATP [91] and involved in inter-domain communication [42], and the X-loop, communicating with the TMD coupling helices [21,71].

In TAP, the ATP-binding site I formed by the Walker A and B motifs of TAP1 is non-canonic, as there are mutations in the putative catalytic glutamate next to the Walker B motif, the linchpin histidine of the H-switch [91,97], and the *in trans* C-loop located in TAP2 [94] (Table 2). Such non-equivalent NBDs are found in 28 of the 48 human ABC proteins, including TAP1/2, MRP1/2, CFTR, SUR1/2, and ABCG5/8, suggesting an underlying concept of NBD asymmetry. While it was so far impossible to isolate heterodimers of the NBDs of TAP1 and TAP2, crystal structures of homodimerized TAP1-NBDs are available, engineered in such a way

Table 2
Important sequence motifs of ABC transporters and human TAP.

Motif	Consensus	TAP1	TAP2	Putative function	References
Motifs in NBD					
Common to P-loop NTPases (ASCE-group)					
Walker A	GxxGxGK(S/T)	GPNGSGKST	GPNGSGKST	ATP binding (phosphate)	[8]
Walker B + *catalytic base*	hhhhDE	VLILDD (non-canonic)	VLILDE	Coordinates Mg^{2+}, ATP hydrolysis	[8,89]
Specific for ABC proteins					
A-loop	Y	FAYPN	FAYPN	π–π stacking with adenine of nucleotide	[90]
C-loop (ABC signature)	LSGGQ	LSGGQ	LAAGQ (non-canonic)	Completes the ATP-binding site, ATP hydrolysis	[29,94]
D-loop	SALD	SALD	SALD	Involved in the communication between NBD and TMD	[140]
H-switch	H	TQHL (non-canonic)	AHRL	ATP hydrolysis, catalytic dyad	[91]
Q-loop	h(h/Q)Q(D/E)	VGQE	VGQE	Interaction with γ-phosphate, NBD-TMD communication	[42]
X-loop	TEVGERG	TEVDEAG	TDVGEKG	NBD interaction with TMD coupling helices	[21,71,91]
Motifs in TMD					
Common to type III ABC transporters					
Coupling helix 1		Q271-N279	Q236-T244	TMD interaction with *cis* and *trans* NBDs	[21,71]
Coupling helix 2		A373-N382	Q340-E349	TMD interaction with *trans* NBD	[21,71]
Elbow helix		^{174}RRLLGCL^{180}	^{139}WRLLKLSR^{146}	Unknown	[21]
Specific for TAP					
Peptide sensor		V288		Senses bound peptide	[74]
Binding region		P375-M420 Q453-R487	R354-M389 I414-M433	Contributes to peptide binding pocket	[74,86]
Substrate specificity			T217, M218, A374, R380, C213	Alters the epitope repertoires	[85,95,96]

Abbreviations: x: any residue; h: hydrophobic residue.

that either two entirely non-canonic or two fully functional ATP-binding pockets are formed [31]. Moreover, the TM287/288 transporter from *T. maritima* represents a heterodimeric complex, where one of the NBDs is non-canonic [56]. While X-ray structures of inward-facing MsbA and P-gp showed complete disengagements of the two NBDs when the transporter is in a substrate-receptive conformation, the structure of TM287/288 represents an inward-facing, partially closed conformation, suggesting that cytosolic substrate can bind even when residual contacts between NBDs are maintained.

4. Transport and ATPase cycle of TAP

Crystal structures of type III ABC transporters suggest that the inward-facing conformation of TAP is receptive for binding of cytosolic peptides to a high-affinity binding site. Dynamic conformational rearrangements of TAP then move peptides across the membrane. Once TAP adopts the outward-facing conformation, peptides are released into the ER lumen from a low-affinity binding site. Eventually, the transporter returns to an inward-facing resting state. How such a mechanical cycle of alternating access, *i.e.* access from either the cytosol or the ER lumen, is coupled to ATP binding and hydrolysis as well as the dissociation of inorganic phosphate and ADP is a central question in the field of ABC transporters. When solely looking at the ATPase cycle at the level of the NBD dimer, there are two fundamentally different models discussed, termed “processive clamp” (later called switch-model) and “constant contact”. The processive clamp (switch) model implies NBD dimerization upon binding of two ATP molecules and complete separation after sequential ATP hydrolysis [98,99]. The sequential order is based on the finding that isolated NBD dimers of the mitochondrial ABC transporter Mdl1 were stably arrested either with two ATP, one ATP and one ADP, or two ADP trapped by beryllium fluoride [100]. The two NBDs can only dissociate if both ATP molecules are hydrolyzed. In contrast, the constant contact model is characterized by constant occlusion of at least one of the nucleotide-binding sites [101]. In the constant contact model, ATP would be hydrolyzed in an alternate fashion, with the hallmark that at no time both NBDs are in the same state and, hence, always one nucleotide-binding site is occluded. However, this model should not be mixed up with a constant residual or peripheral interaction between NBDs, as the name could imply. In theory, a third *modus operandi* would be conceivable, where both nucleotide-binding sites bind and hydrolyze ATP truly independently from each other. However, such a purely stochastic mode is rarely discussed for ABC proteins.

A working model of how the ATPase cycle is coupled to transport events in TAP is shown in Fig. 3. By indicating the effect of mutations and viral factors, the figure highlights their value in understanding the molecular mechanism. The ATPase cycle is based on the processive clamp (switch) model, as it is supported by crystal structures (Table 1) and most biochemical data on ABC transporters: Firstly, X-ray structures of ABC transporters exhibit clearly opened ATP-binding sites in the (non-physiological) apo-state. Dissociated NBDs were also observed in the presence of the non-hydrolysable ATP analogs AMPPNP and AMPPCP or — in the case of the methionine transporter MetNI [39] — with bound ADP, indicating that nucleotide binding as such does not necessarily promote NBD closure. In most structures, the open NBD state correlates with an inward-facing conformation of the TMDs. Secondly, outward-facing TMD structures almost invariably feature dimerized NBDs. Those conformations were isolated in the presence of transition-metal-oxide trapped ADP, the non-hydrolysable ATP analog AMPPNP, or ATP, thereby connecting the outward-facing conformation of the TMDs with nucleotide-occluded NBDs. Notably, the outward-facing conformation with closed NBDs was also observed in the presence of ADP or $V_4O_{12}^{4-}$. An interesting intermediate state has been shown for TM287/288. Despite its inward-facing conformation, the NBDs are only partially opened, with AMPPNP bound to the non-canonic nucleotide-binding site and an empty consensus site [56]. Thirdly, biochemical data on ABC transporters and its isolated NBDs are largely discussed in favor of the processive clamp (ATP switch) model [98,99,102]. Worth mentioning, there are substantial variations in this model as to which NBD state —nucleotide binding or occlusion— provides the power stroke for the outward opening of the transporter [92].

TAP provides an ideal model system for the analysis of the transport mechanism: first, among the heterodimeric ABC transporters with non-equivalent NBDs, TAP can be best addressed by biochemical and cellular substrate binding and transport assays due to detailed information about its physiologic function, its substrate specificity, and the strict allosteric coupling between solute binding and ATP hydrolysis. Second, at least five different viral factors have been identified so far that abolish TAP function, mostly by interfering with distinct conformational states of the transport cycle [103–105] (Fig. 3).

In principle, both the canonic and the non-canonic site of TAP can hydrolyze ATP [106,107]. Mutating the Walker A motif of TAP2 compromises ATP binding at the consensus site II and is deleterious for TAP function. The appropriate TAP1 mutant, in contrast, still supports residual transport activity [108–110]. Likewise, mutations that allow for ATP

binding but not hydrolysis (Walker B motif, additional glutamate adjacent to Walker B, linchpin histidine in H-loop, C-loop) are tolerated to various extents if located in the non-canonic site I, but largely abrogate transport activity if affecting the consensus site II [94,109,111]. Those findings support the processive clamp (ATP switch) model. Given that ATP remains bound to the non-canonic site I, it either indicates that partial opening of the NBDs is sufficient for inward-opening of the TMDs (Fig. 3, Step 5) or that hydrolysis at the consensus site II is sufficient for full NBD dissociation (shortcut to Step 1b). Next to arrest TAP in states that precede the hydrolytic event, trapping of the catalytic transition state can be mimicked by adding vanadate, aluminum fluoride, or beryllium fluoride in the presence of ATP. Such compounds are *per se* not selective for one of the two TAP subunits, but given a successive order of ATP hydrolysis in the two nucleotide-binding sites, trapping may primarily occur in the nucleotide-binding site that hydrolyzes ATP and releases inorganic phosphate first. Along this line, preferential trapping of the nucleotide-binding site II would explain the increased selective crosslinking of non-hydrolysable 8-azido-ATP[γ]biotin to TAP1, the non-canonic site, in the presence of vanadate [108]. Very likely, such transition metal oxide trapping is dependent on the tight NBD dimerization, referred to as the occluded state [106]. There is strong evidence that assuming the occluded state is sufficient for release of substrate into the ER, as a D-loop mutation in TAP1 supports transport in the presence of ADP [140]. Additionally, chemical or mutational trapping of ABC transporters has been proven useful for crystallization approaches of ABC transporters in general.

The study of viral factors that bind to TAP can further help to identify steps of the transport cycle. The immediate early gene product ICP47 (86–88 aa) encoded by Herpes simplex virus (HSV-1/2) binds from the cytosol to TAP with nanomolar affinity and thereby abrogates peptide binding and transport, while ATP binding is unaffected [112–114]. ATP hydrolysis as well as trapping of the TAP transporter with ATP and beryllium fluoride is blocked [106], suggesting that ICP47 arrests TAP in an inward-facing, NBD open conformation. This is in agreement with the notion that peptide binding is essential for NBD closure and ATP hydrolysis [84]. The early gene product US6 encoded by human cytomegalovirus (HCMV) binds as type I membrane protein to TAP *via* its ER-luminal domain (127 aa) [115–118]. Other than ICP47, US6 does not interfere with peptide binding, but blocks ATP hydrolysis. Neither TAP1 nor TAP2 binds to ATP-agarose in the presence of US6, and binding of 8-azido-ATP[γ]biotin could only be observed for TAP2 [115,116]. Binding to ADP-agarose was not compromised. This suggests that US6 arrests a post-transport state of TAP that just secreted peptide into the ER and is now on its way back to the resting state, where the high-affinity peptide binding site is already accessible, while ADP might not yet be fully dissociated from both NBDs. UL49.5, a type I membrane protein encoded by several varicelloviruses, does neither interfere with ATP nor with peptide binding, but it likely prevents conformational rearrangements towards the outward-facing conformation [119]. The tail-anchored protein BNLF2a from Epstein–Barr-virus (EBV) abrogates both ATP and peptide binding [120,121]. Finally, the recently discovered type II membrane protein CPXV12 from cowpox virus does not prevent peptide binding, but binding of ATP to both NBDs [122]. Noteworthy, the conformational states arrested by viral factors are in part mutually exclusive [123].

In summary, the biochemical phenotype of TAP inhibition by viral factors can best be matched to transport states proclaimed by the processive clamp (ATP switch) model (Fig. 3). Firstly, the apo state with its inward-facing, opened NBDs, is rarely populated and will either be loaded with ATP or, independently, with peptide. In the absence of viral factors, peptide binding may potentially trigger an intermediate conformation of the TMDs, thereby allowing for closure of NBDs. Further conformational rearrangements then lead to the outward-facing conformation. Peptide release could trigger ATP hydrolysis in the canonic nucleotide-binding site II, which would stimulate ATP hydrolysis in the degenerate nucleotide-binding site I. Remarkably, it has thus far not been ruled out that the two hydrolysis events could trigger distinct steps in the transport cycle, with *e.g.* the first hydrolysis leading to partial and the second to complete inward-facing. If partial opening would be sufficient for residual transport activity, this could explain why ATP

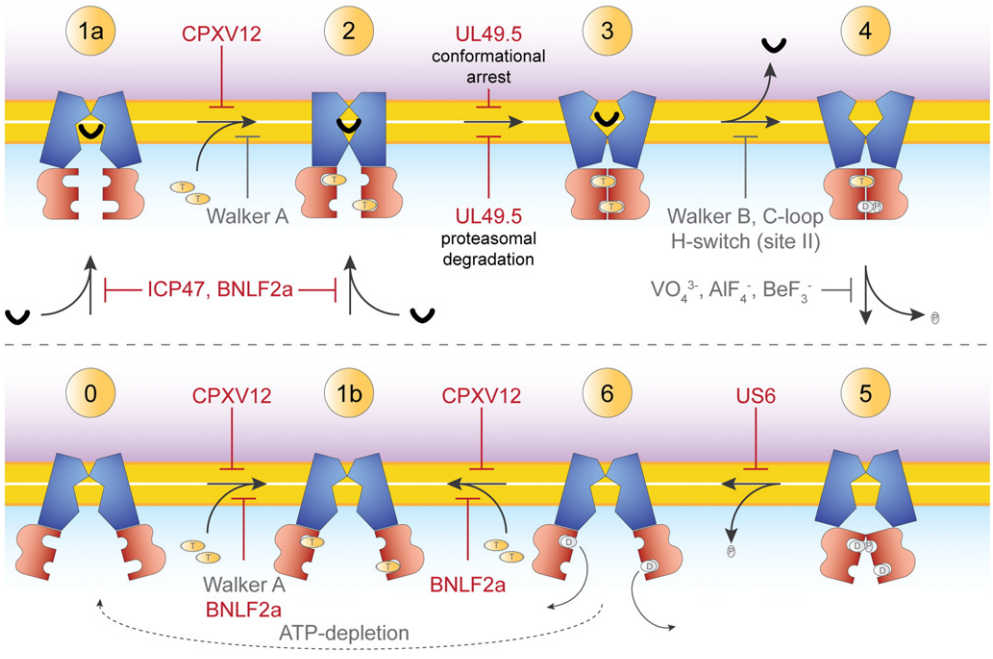

Fig. 3. Transport cycle of TAP. Peptide and ATP ("T") can bind TAP independently of each other (1a/b), inducing initial allosteric structural changes. The apo-state of TAP (0) will be rarely populated under physiological conditions. Binding of both ATP and peptide results in conformational rearrangements of the TMD, which *via* an occluded, pre-translocation state (2) results in the outward opening of the transporter with tightly closed, ATP-sandwiched NBD dimer (3). Peptide release and ATP-hydrolysis in the canonic ATP-binding site II may be coupled events (4). Hydrolysis of ATP and dissociation of inorganic phosphate (P) at only the consensus site are thought to be sufficient to reset the transporter to an inward-facing, substrate receptive conformation (5). Hydrolysis at the degenerate ATP-binding site I and dissociation of P_i and ADP ("D") from all binding sites restore the resting state of the transporter (6), which can be loaded again with ATP and peptide. The indicated effects of viral factors, NBD mutations, and VO_4^{3-}/AlF_4^-/BeF_3^- are discussed and referenced in the main text.

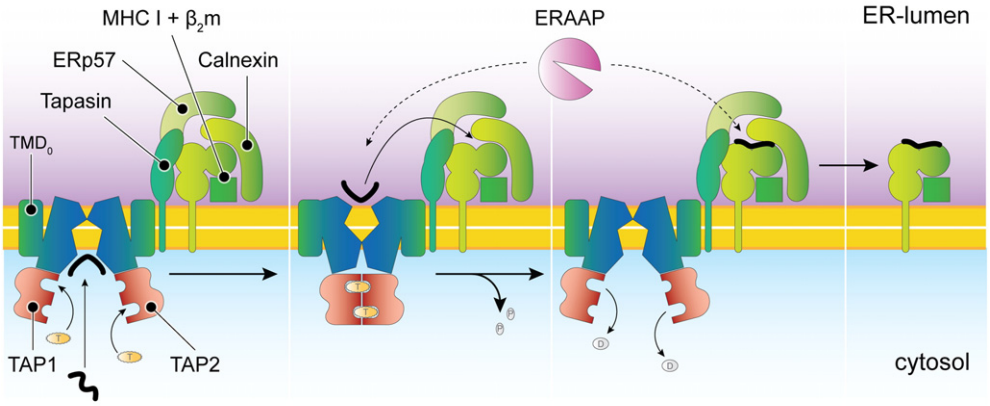

Fig. 4. Processing of antigenic peptides by the PLC. Each TMD_0 of TAP independently serves as a scaffold for PLC assembly by interacting with the TM of tapasin. For clarity, only one PLC per TAP transporter is shown. TAP shuttles antigenic peptides in an ATP-dependent process and forwards them to the associated MHC I molecule. During this process, the peptide is subjected to N-terminal trimming by the aminopeptidase ERAAP. Tapasin proofreads the stable formation of a MHC I-peptide complex, which subsequently traffics *via* the Golgi apparatus to the cellular surface. Abbreviation: β_2m: β_2-microglobulin.

hydrolysis at the non-canonic site is not essential. Finally, ADP dissociation restores the resting state and closes the cycle.

5. Cooperation of TAP within the peptide-loading complex (PLC)

The final destination of peptides shuttled into the ER by TAP is the cell surface. To assure efficient peptide loading, *i.e.* formation of kinetically stable MHC I-peptide complexes with a half-life of 5–7 days, TAP is organized in the PLC. Next to TAP and the MHC I heavy-chain/β_2-microglobulin complex, the PLC comprises the MHC I-specific chaperone tapasin, the oxidoreductase ERp57, and calreticulin (Fig. 4). The architecture of the PLC and the dynamic interplay of its components have been recently reviewed in great detail [124]. Essentially, TAP interacts with tapasin *via* its TMD_0 [64–66], allowing that maximal two tapasin molecules interact with one TAP transporter, resulting in two platforms for PLC assembly [63,67,125]. While the interaction between TAP and tapasin is located to the transmembrane helices [126–128], interaction between tapasin and the other components is mediated by multiple ER-lumenal contacts. Spatial clustering of the components within the PLC is essential because the replacement of tapasin by a soluble truncation mutant causes a significant decrease in MHC I surface expression [67,126]. Moreover, in the absence of tapasin, TAP is destabilized and prone to proteasomal degradation [129–131]. Although mutationally arrested TAP or imperfectly loaded MHC I increases the stability of the PLC [132] (unpublished data, Tampé lab), there is little evidence for a general feedback signaling within the PLC. In contrast, the essential function of the PLC in proofreading peptide-MHC I complexes for highest stability is well established [124,133]. While TAP transports peptides with 8–16 aa in length [57] (see Section 3.3), MHC I can accommodate peptides of 9–10 aa [134]. Notably, the TAP specificity for a basic or hydrophobic C-terminal residue of translocated peptides fits well to the preferences of the MHC I-binding pocket, suggesting that this specificity is already reflected by degradation products generated by the immunoproteasome [135]. The N terminus, in contrast, is subject to trimming, which is performed by the ER aminopeptidase associated with antigen processing (ERAAP) [136]. However, it is unclear how ERAAP interacts with peptides in-between transport and loading, as there are neither any known physical interactions with components of the PLC nor is there evidence for interdependence of ERAAP and the PLC [133]. It is proposed that while ERAAP edits the N-terminal end of peptides dedicated to MHC I presentation, tapasin proofreads the peptides for their contribution to MHC I stability. Only stably loaded MHC I-peptide complexes are then allowed to migrate to the cell surface, where they are presented to the cells of the adaptive immune system.

6. Quo vadis?

TAP is a highly specialized cogwheel in the large machinery of the adaptive immunity, but at the same time it can serve as a blueprint for the structure and function of ABC exporters in general. In both aspects, it will be the major challenge to correlate the increasing number of biochemical and cellular information with structural coordinates.

First, further refining of the ABC transport cycle may be aided by the study of viral factors that target TAP, as discussed. With its negligible ATPase activity in the absence of peptide substrates and its receptiveness for peptides of different length, TAP can furthermore contribute to the unresolved question of how much ATP is consumed by ABC transporters per transport event. Moreover, the effect of "*trans*-inhibition", *i.e.* blocking of the transport activity by accumulated substrates in the ER, has to be further investigated to complete the biochemical dataset.

Second, the structural identification of different steps in the transport cycle would be an important milestone. The challenge of synchronously arresting TAP in different conformations could be facilitated by the use of nanobodies, as exemplified for P-gp [50], by the use of DARPins, as exemplified for AcrB [137], or — again — by the use of viral factors, as illustrated for TAP. An interesting aspect when determining the X-ray structure of TAP are the unique TMD_0s, as they connect TAP to the PLC but so far lack homologous structures.

Third, the structure of the PLC itself is poorly understood. While for most of the soluble domains of its components the X-ray structure has been solved, detailed knowledge about the overall architecture of the PLC and about the interaction sites between the components is lacking. Here, integrative structural approaches might be most suitable, making use of, for instance, high-resolution cryo-electron microscopy, crosslinking combined with mass spectrometry, and *in silico* molecular dynamics simulations, as exemplified for the nuclear pore complex [138]. Summarized, structural information is essential for a precise mapping of the route of antigenic peptides, from their pickup in the cytosol *via* their trimming by aminopeptidases to their proofread loading onto MHC I molecules. Having in hand a precise map will then facilitate the understanding of host-viral interactions and potentially allows targeted clinical interference with antigen presentation.

Acknowledgments

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References

[1] W. Busch, M.H. Saier Jr., The transporter classification (TC) system, 2002, Crit. Rev. Biochem. Mol. Biol. 37 (2002) 287–337.
[2] M. Dean, A. Rzhetsky, R. Allikmets, The human ATP-binding cassette (ABC) transporter superfamily, Genome Res. 11 (2001) 1156–1166.
[3] B. Lankat-Buttgereit, R. Tampé, The transporter associated with antigen processing: function and implications in human diseases, Physiol. Rev. 82 (2002) 187–204.
[4] D. Parcej, R. Tampé, ABC proteins in antigen translocation and viral inhibition, Nat. Chem. Biol. 6 (2010) 572–580.
[5] C.F. Higgins, P.D. Haag, K. Nikaido, F. Ardeshir, G. Garcia, G.F. Ames, Complete nucleotide sequence and identification of membrane components of the histidine transport operon of *S. typhimurium*, Nature 298 (1982) 723–727.
[6] E. Gilson, C.F. Higgins, M. Hofnung, G. Ferro-Luzzi Ames, H. Nikaido, Extensive homology between membrane-associated components of histidine and maltose transport systems of *Salmonella typhimurium* and *Escherichia coli*, J. Biol. Chem. 257 (1982) 9915–9918.
[7] E. Gilson, H. Nikaido, M. Hofnung, Sequence of the malK gene in *E. coli* K12, Nucleic Acids Res. 10 (1982) 7449–7458.
[8] J.E. Walker, M. Saraste, M.J. Runswick, N.J. Gay, Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold, EMBO J. 1 (1982) 945–951.
[9] M. Saraste, P.R. Sibbald, A. Wittinghofer, The P-loop—a common motif in ATP- and GTP-binding proteins, Trends Biochem. Sci. 15 (1990) 430–434.
[10] C.F. Higgins, I.D. Hiles, G.P. Salmond, D.R. Gill, J.A. Downie, I.J. Evans, I.B. Holland, L. Gray, S.D. Buckel, A.W. Bell, et al., A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria, Nature 323 (1986) 448–450.
[11] S.C. Hyde, P. Emsley, M.J. Hartshorn, M.M. Mimmack, U. Gileadi, S.R. Pearce, M.P. Gallagher, D.R. Gill, R.E. Hubbard, C.F. Higgins, Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport, Nature 346 (1990) 362–365.
[12] K. Hollenstein, R.J. Dawson, K.P. Locher, Structure and mechanism of ABC transporter proteins, Curr. Opin. Struct. Biol. 17 (2007) 412–418.
[13] E. Biemans-Oldehinkel, M.K. Doeven, B. Poolman, ABC transporter architecture and regulatory roles of accessory domains, FEBS Lett. 580 (2006) 1023–1035.
[14] K. Xu, M. Zhang, Q. Zhao, F. Yu, H. Guo, C. Wang, F. He, J. Ding, P. Zhang, Crystal structure of a folate energy-coupling factor transporter from *Lactobacillus brevis*, Nature 497 (2013) 268–271.
[15] T. Wang, G. Fu, X. Pan, J. Wu, X. Gong, J. Wang, Y. Shi, Structure of a bacterial energy-coupling factor transporter, Nature 497 (2013) 272–276.
[16] D.J. Slotboom, Structural and mechanistic insights into prokaryotic energy-coupling factor transporters, Nat. Rev. Microbiol. 12 (2014) 79–87.
[17] B. Wang, M. Dukarevich, E.I. Sun, M.R. Yen, M.H. Saier Jr., Membrane porters of ATP-binding cassette transport systems are polyphyletic, J. Membr. Biol. 231 (2009) 1–10.
[18] R. Allikmets, B. Gerrard, A. Hutchinson, M. Dean, Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database, Hum. Mol. Genet. 5 (1996) 1649–1655.
[19] M.S. Jin, M.L. Oldham, Q. Zhang, J. Chen, Crystal structure of the multidrug transporter P-glycoprotein from *Caenorhabditis elegans*, Nature 490 (2012) 566–569.
[20] C.A. Shintre, A.C. Pike, Q. Li, J.I. Kim, A.J. Barr, S. Goubin, L. Shrestha, J. Yang, G. Berridge, J. Ross, P.J. Stansfeld, M.S. Sansom, A.M. Edwards, C. Bountra, B.D. Marsden, F. von Delft, A.N. Bullock, O. Gileadi, N.A. Burgess-Brown, E.P. Carpenter, Structures of ABCB10, a human ATP-binding cassette transporter in apo- and nucleotide-bound states, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 9710–9715.
[21] R.J. Dawson, K.P. Locher, Structure of a bacterial multidrug ABC transporter, Nature 443 (2006) 180–185.
[22] F.J. Sharom, The P-glycoprotein multidrug transporter, Essays Biochem. 50 (2011) 161–178.
[23] J.R. Riordan, The cystic-fibrosis transmembrane conductance regulator, Annu. Rev. Physiol. 55 (1993) 609–630.
[24] L. Aguilar-Bryan, J. Bryan, Molecular biology of adenosine triphosphate-sensitive potassium channels, Endocr. Rev. 20 (1999) 101–135.
[25] F. Quazi, S. Lenevich, R.S. Molday, ABCA4 is an N-retinylidene-phosphatidylethanolamine and phosphatidylethanolamine importer, Nat. Commun. 3 (2012) 925.
[26] E. Nürenberg, R. Tampé, Tying up loose ends: ribosome recycling in eukaryotes and archaea, Trends Biochem. Sci. 38 (2013) 64–74.
[27] L.W. Hung, I.X. Wang, K. Nikaido, P.Q. Liu, G.F. Ames, S.H. Kim, Crystal structure of the ATP-binding subunit of an ABC transporter, Nature 396 (1998) 703–707.
[28] L. Schmitt, R. Tampé, Structure and mechanism of ABC transporters, Curr. Opin. Struct. Biol. 12 (2002) 754–760.
[29] P.C. Smith, N. Karpowich, L. Millen, J.E. Moody, J. Rosen, P.J. Thomas, J.F. Hunt, ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer, Mol. Cell 10 (2002) 139–149.
[30] K.P. Hopfner, A. Karcher, D.S. Shin, L. Craig, L.M. Arthur, J.P. Carney, J.A. Tainer, Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily, Cell 101 (2000) 789–800.
[31] E. Procko, I. Ferrin-O'Connell, S.L. Ng, R. Gaudet, Distinct structural and functional properties of the ATPase sites in an asymmetric ABC transporter, Mol. Cell 24 (2006) 51–62.
[32] M.L. Oldham, D. Khare, F.A. Quiocho, A.L. Davidson, J. Chen, Crystal structure of a catalytic intermediate of the maltose transporter, Nature 450 (2007) 515–521.
[33] D. Khare, M.L. Oldham, C. Orelle, A.L. Davidson, J. Chen, Alternating access in maltose transporter mediated by rigid-body rotations, Mol. Cell 33 (2009) 528–536.
[34] M.L. Oldham, J. Chen, Crystal structure of the maltose transporter in a pretranslocation intermediate state, Science 332 (2011) 1202–1205.
[35] M.L. Oldham, J. Chen, Snapshots of the maltose transporter during ATP hydrolysis, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 15152–15156.
[36] S. Chen, M.L. Oldham, A.L. Davidson, J. Chen, Carbon catabolite repression of the maltose transporter revealed by X-ray crystallography, Nature 499 (2013) 364–368.
[37] M.L. Oldham, S. Chen, J. Chen, Structural basis for substrate specificity in the *Escherichia coli* maltose transport system, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 18132–18137.
[38] N.S. Kadaba, J.T. Kaiser, E. Johnson, A. Lee, D.C. Rees, The high-affinity *E. coli* methionine ABC transporter: structure and allosteric regulation, Science 321 (2008) 250–253.
[39] E. Johnson, P.T. Nguyen, T.O. Yeates, D.C. Rees, Inward facing conformations of the MetNI methionine ABC transporter: implications for the mechanism of transinhibition, Protein Sci. 21 (2012) 84–96.
[40] K. Hollenstein, D.C. Frei, K.P. Locher, Structure of an ABC transporter in complex with its binding protein, Nature 446 (2007) 213–216.
[41] S. Gerber, M. Comellas-Bigler, B.A. Goetz, K.P. Locher, Structural basis of transinhibition in a molybdate/tungstate ABC transporter, Science 321 (2008) 246–250.
[42] K.P. Locher, A.T. Lee, D.C. Rees, The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism, Science 296 (2002) 1091–1098.
[43] R.N. Hvorup, B.A. Goetz, M. Niederer, K. Hollenstein, E. Perozo, K.P. Locher, Asymmetry in the structure of the ABC transporter-binding protein complex BtuCD–BtuF, Science 317 (2007) 1387–1390.
[44] V.M. Korkhov, S.A. Mireku, R.N. Hvorup, K.P. Locher, Asymmetric states of vitamin B(1)(2) transporter BtuCD are not discriminated by its cognate substrate binding protein BtuF, FEBS Lett. 586 (2012) 972–976.
[45] V.M. Korkhov, S.A. Mireku, K.P. Locher, Structure of AMP-PNP-bound vitamin B12 transporter BtuCD-F, Nature 490 (2012) 367–372.
[46] H.W. Pinkett, A.T. Lee, P. Lum, K.P. Locher, D.C. Rees, An inward-facing conformation of a putative metal-chelate-type ABC transporter, Science 315 (2007) 373–377.
[47] J.S. Woo, A. Zeltina, B.A. Goetz, K.P. Locher, X-ray structure of the *Yersinia pestis* heme transporter HmuUV, Nat. Struct. Mol. Biol. 19 (2012) 1310–1315.
[48] S.G. Aller, J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch, G. Chang, Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding, Science 323 (2009) 1718–1722.
[49] J. Li, K.F. Jaimes, S.G. Aller, Refined structures of mouse P-glycoprotein, Protein Sci. 23 (2014) 34–46.
[50] A.B. Ward, P. Szewczyk, V. Grimard, C.W. Lee, L. Martinez, R. Doshi, A. Caya, M. Villaluz, E. Pardon, C. Cregger, D.J. Swartz, P.G. Falson, I.L. Urbatsch, C. Govaerts, J. Steyaert, G. Chang, Structures of P-glycoprotein reveal its conformational flexibility and an epitope on the nucleotide-binding domain, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 13386–13391.
[51] A. Kodan, T. Yamaguchi, T. Nakatsu, K. Sakiyama, C.J. Hipolito, A. Fujioka, R. Hirokane, K. Ikeguchi, B. Watanabe, J. Hiratake, Y. Kimura, H. Suga, K. Ueda, H. Kato, Structural basis for gating mechanisms of a eukaryotic P-glycoprotein homolog, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 4049–4054.
[52] V. Srinivasan, A.J. Pierik, R. Lill, Crystal structures of nucleotide-free and glutathione-bound mitochondrial ABC transporter Atm1, Science 343 (2014) 1137–1140.
[53] J.Y. Lee, J.G. Yang, D. Zhitnitsky, O. Lewinson, D.C. Rees, Structural basis for heavy metal detoxification by an Atm1-type ABC exporter, Science 343 (2014) 1133–1136.
[54] A. Ward, C.L. Reyes, J. Yu, C.B. Roth, G. Chang, Flexibility in the ABC transporter MsbA: alternating access with a twist, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19005–19010.
[55] R.J. Dawson, K.P. Locher, Structure of the multidrug ABC transporter Sav 1866 from *Staphylococcus aureus* in complex with AMP-PNP, FEBS Lett. 581 (2007) 935–938.
[56] M. Hohl, C. Briand, M.G. Grutter, M.A. Seeger, Crystal structure of a heterodimeric ABC transporter in its inward-facing conformation, Nat. Struct. Mol. Biol. 19 (2012) 395–402.
[57] P.M. van Endert, R. Tampé, T.H. Meyer, R. Tisch, J.F. Bach, H.O. McDevitt, A sequential model for peptide binding and transport by the transporters associated with antigen processing, Immunity 1 (1994) 491–500.
[58] J.J. Neefjes, F. Momburg, G.J. Hammerling, Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter, Science 261 (1993) 769–771.
[59] T.H. Meyer, P.M. van Endert, S. Uebel, B. Ehring, R. Tampé, Functional expression and purification of the ABC transporter complex associated with antigen processing (TAP) in insect cells, FEBS Lett. 351 (1994) 443–447.
[60] H. de la Salle, D. Hanau, D. Fricker, A. Urlacher, A. Kelly, J. Salamero, S.H. Powis, L. Donato, H. Bausinger, M. Laforet, Homozygous human tap peptide transporter mutation in HLA class I deficiency, Science 265 (1994) 237–241.
[61] S.D. Gadola, H.T. Moins-Teisserenc, J. Trowsdale, W.L. Gross, V. Cerundolo, TAP deficiency syndrome, Clin. Exp. Immunol. 121 (2000) 173–178.
[62] S. Heintke, M. Chen, U. Ritz, B. Lankat-Buttgereit, J. Koch, R. Abele, B. Seliger, R. Tampé, Functional cysteine-less subunits of the transporter associated with antigen processing (TAP1 and TAP2) by de novo gene assembly, FEBS Lett. 533 (2003) 42–46.
[63] S. Hulpke, M. Tomioka, E. Kremmer, K. Ueda, R. Abele, R. Tampé, Direct evidence that the N-terminal extensions of the TAP complex act as autonomous interaction scaffolds for the assembly of the MHC I peptide-loading complex, Cell. Mol. Life Sci. 69 (2012) 3317–3327.
[64] J. Koch, R. Guntrum, R. Tampé, Exploring the minimal functional unit of the transporter associated with antigen processing, FEBS Lett. 579 (2005) 4413–4416.

[65] J. Koch, R. Guntrum, S. Heintke, C. Kyritsis, R. Tampé, Functional dissection of the transmembrane domains of the transporter associated with antigen processing (TAP), J. Biol. Chem. 279 (2004) 10142–10147.
[66] E. Procko, G. Raghuraman, D.C. Wiley, M. Raghavan, R. Gaudet, Identification of domain boundaries within the N-termini of TAP1 and TAP2 and their importance in tapasin binding and tapasin-mediated increase in peptide loading of MHC class I, Immunol. Cell Biol. 83 (2005) 475–482.
[67] S. Hulpke, C. Baldauf, R. Tampé, Molecular architecture of the MHC I peptide-loading complex: one tapasin molecule is essential and sufficient for antigen processing, FASEB J. 26 (2012) 5071–5080.
[68] O. Demirel, I. Bangert, R. Tampé, R. Abele, Tuning the cellular trafficking of the lysosomal peptide transporter TAPL by its N-terminal domain, Traffic 11 (2010) 383–393.
[69] O. Demirel, I. Jan, D. Wolters, J. Blanz, P. Saftig, R. Tampé, R. Abele, The lysosomal polypeptide transporter TAPL is stabilized by interaction with LAMP-1 and LAMP-2, J. Cell Sci. 125 (2012) 4230–4240.
[70] V. Corradi, G. Singh, D.P. Tieleman, The human transporter associated with antigen processing: molecular models to describe peptide binding competent states, J. Biol. Chem. 287 (2012) 28099–28111.
[71] G. Oancea, M.L. O'Mara, W.F. Bennett, D.P. Tieleman, R. Abele, R. Tampé, Structural arrangement of the transmission interface in the antigen ABC transport complex TAP, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5551–5556.
[72] R. Gaudet, D.C. Wiley, Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing, EMBO J. 20 (2001) 4964–4972.
[73] M. Moradi, E. Tajkhorshid, Mechanistic picture for conformational transition of a membrane transporter at atomic resolution, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 18916–18921.
[74] M. Herget, G. Oancea, S. Schrodt, M. Karas, R. Tampé, R. Abele, Mechanism of substrate sensing and signal transmission within an ABC transporter: use of a Trojan horse strategy, J. Biol. Chem. 282 (2007) 3871–3880.
[75] J.F. Cotten, L.S. Ostedgaard, M.R. Carson, M.J. Welsh, Effect of cystic fibrosis-associated mutations in the fourth intracellular loop of cystic fibrosis transmembrane conductance regulator, J. Biol. Chem. 271 (1996) 21279–21284.
[76] S.J. Currier, S.E. Kane, M.C. Willingham, C.O. Cardarelli, I. Pastan, M.M. Gottesman, Identification of residues in the first cytoplasmic loop of P-glycoprotein involved in the function of chimeric human MDR1-MDR2 transporters, J. Biol. Chem. 267 (1992) 25153–25159.
[77] S. Uebel, T.H. Meyer, W. Kraas, S. Kienle, G. Jung, K.H. Wiesmüller, R. Tampé, Requirements for peptide binding to the human transporter associated with antigen processing revealed by peptide scans and complex peptide libraries, J. Biol. Chem. 270 (1995) 18512–18516.
[78] S. Uebel, W. Kraas, S. Kienle, K.H. Wiesmüller, G. Jung, R. Tampé, Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 8976–8981.
[79] J.O. Koopmann, M. Post, J.J. Neefjes, G.J. Hämmerling, F. Momburg, Translocation of long peptides by transporters associated with antigen processing (TAP), Eur. J. Immunol. 26 (1996) 1720–1728.
[80] F. Momburg, J. Roelse, J.C. Howard, G.W. Butcher, G.J. Hämmerling, J.J. Neefjes, Selectivity of MHC-encoded peptide transporters from human, mouse and rat, Nature 367 (1994) 648–651.
[81] P.M. van Endert, D. Riganelli, G. Greco, K. Fleischhauer, J. Sidney, A. Sette, J.F. Bach, The peptide-binding motif for the human transporter associated with antigen-processing, J. Exp. Med. 182 (1995) 1883–1895.
[82] M.T. Heemels, H.L. Ploegh, Substrate specificity of allelic variants of the TAP peptide transporter, Immunity 1 (1994) 775–784.
[83] M.J. Androlewicz, P. Cresswell, Human transporters associated with antigen processing possess a promiscuous peptide-binding site, Immunity 1 (1994) 7–14.
[84] S. Gorbulev, R. Abele, R. Tampé, Allosteric crosstalk between peptide-binding, transport, and ATP hydrolysis of the ABC transporter TAP, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3732–3737.
[85] C. Baldauf, S. Schrodt, M. Herget, J. Koch, R. Tampé, Single residue within the antigen translocation complex TAP controls the epitope repertoire by stabilizing a receptive conformation, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 9135–9140.
[86] M. Nijenhuis, G.J. Hämmerling, Multiple regions of the transporter associated with antigen processing (TAP) contribute to its peptide binding site, J. Immunol. 157 (1996) 5467–5477.
[87] M. Herget, C. Baldauf, C. Scholz, D. Parcej, K.H. Wiesmuller, R. Tampé, R. Abele, E. Bordignon, Conformation of peptides bound to the transporter associated with antigen processing (TAP), Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 1349–1354.
[88] N. Karpowich, O. Martsinkevich, L. Millen, Y.R. Yuan, P.L. Dai, K. MacVey, P.J. Thomas, J.F. Hunt, Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter, Structure (Camb) 9 (2001) 571–586.
[89] D.D. Leipe, E.V. Koonin, L. Aravind, Evolution and classification of P-loop kinases and related proteins, J. Mol. Biol. 333 (2003) 781–815.
[90] I.W. Kim, X.H. Peng, Z.E. Sauna, P.C. FitzGerald, D. Xia, M. Muller, K. Nandigama, S.V. Ambudkar, The conserved tyrosine residues 401 and 1044 in ATP sites of human P-glycoprotein are critical for ATP binding and hydrolysis: evidence for a conserved subdomain, the A-loop in the ATP-binding cassette, Biochemistry 45 (2006) 7605–7616.
[91] J. Zaitseva, S. Jenewein, T. Jumpertz, I.B. Holland, L. Schmitt, H662 is the linchpin of ATP hydrolysis in the nucleotide-binding domain of the ABC transporter HlyB, EMBO J. 24 (2005) 1901–1910.
[92] M.A. Seeger, H.W. van Veen, Molecular basis of multidrug transport by ABC transporters, Biochim. Biophys. Acta 1794 (2009) 725–737.
[93] K. Nikaido, G.F. Ames, One intact ATP-binding subunit is sufficient to support ATP hydrolysis and translocation in an ABC transporter, the histidine permease, J. Biol. Chem. 274 (1999) 26727–26735.
[94] M. Chen, R. Abele, R. Tampé, Functional non-equivalence of ATP-binding cassette signature motifs in the transporter associated with antigen processing (TAP), J. Biol. Chem. 279 (2004) 46073–46081.
[95] E.A. Armandola, F. Momburg, M. Nijenhuis, N. Bulbuc, K. Fruh, G.J. Hammerling, A point mutation in the human transporter associated with antigen processing (TAP2) alters the peptide transport specificity, Eur. J. Immunol. 26 (1996) 1748–1755.
[96] F. Momburg, E.A. Armandola, M. Post, G.J. Hammerling, Residues in TAP2 peptide transporters controlling substrate specificity, J. Immunol. 156 (1996) 1756–1763.
[97] R. Ernst, J. Koch, C. Horn, R. Tampé, L. Schmitt, Engineering ATPase activity in the isolated ABC cassette of human TAP1, J. Biol. Chem. 281 (2006) 27471–27480.
[98] C. van der Does, R. Tampé, How do ABC transporters drive transport? Biol. Chem. 385 (2004) 927–933.
[99] R. Abele, R. Tampé, The ABCs of immunology: structure and function of TAP, the transporter associated with antigen processing, Physiology (Bethesda) 19 (2004) 216–224.
[100] E. Janas, M. Hofacker, M. Chen, S. Gompf, C. van der Does, R. Tampé, The ATP hydrolysis cycle of the nucleotide-binding domain of the mitochondrial ATP-binding cassette transporter Mdl1p, J. Biol. Chem. 278 (2003) 26862–26869.
[101] P.M. Jones, A.M. George, Mechanism of the ABC transporter ATPase domains: catalytic models and the biochemical and biophysical record, Crit. Rev. Biochem. Mol. Biol. 48 (2013) 39–50.
[102] C.F. Higgins, K.J. Linton, The ATP switch model for ABC transporters, Nat. Struct. Mol. Biol. 11 (2004) 918–926.
[103] M.E. Ressing, R.D. Luteijn, D. Horst, E.J. Wiertz, Viral interference with antigen presentation: trapping TAP, Mol. Immunol. 55 (2013) 139–142.
[104] S. Loch, R. Tampé, Viral evasion of the MHC class I antigen-processing machinery, Pflugers Arch. 451 (2005) 409–417.
[105] T.H. Hansen, M. Bouvier, MHC class I antigen presentation: learning from viral evasion strategies, Nat. Rev. Immunol. 9 (2009) 503–513.
[106] M. Chen, R. Abele, R. Tampé, Peptides induce ATP hydrolysis at both subunits of the transporter associated with antigen processing, J. Biol. Chem. 278 (2003) 29686–29692.
[107] E. Procko, R. Gaudet, Functionally important interactions between the nucleotide-binding domains of an antigenic peptide transporter, Biochemistry 47 (2008) 5699–5708.
[108] J.T. Karttunen, P.J. Lehner, S.S. Gupta, E.W. Hewitt, P. Cresswell, Distinct functions and cooperative interaction of the subunits of the transporter associated with antigen processing (TAP), Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 7431–7436.
[109] L. Saveanu, S. Daniel, P.M. van Endert, Distinct functions of the ATP binding cassettes of transporters associated with antigen processing: a mutational analysis of Walker A and B sequences, J. Biol. Chem. 276 (2001) 22107–22113.
[110] P.E. Lapinski, R.R. Neubig, M. Raghavan, Walker A lysine mutations of TAP1 and TAP2 interfere with peptide translocation but not peptide binding, J. Biol. Chem. 276 (2001) 7526–7533.
[111] C.L. Perria, V. Rajamanickam, P.E. Lapinski, M. Raghavan, Catalytic site modifications of TAP1 and TAP2 and their functional consequences, J. Biol. Chem. 281 (2006) 39839–39851.
[112] K. Früh, K. Ahn, H. Djaballah, P. Sempé, P.M. van Endert, R. Tampé, P.A. Peterson, Y. Yang, A viral inhibitor of peptide transporters for antigen presentation, Nature 375 (1995) 415–418.
[113] A. Hill, P. Jugovic, I. York, G. Russ, J. Bennink, J. Yewdell, H. Ploegh, D. Johnson, Herpes simplex virus turns off the TAP to evade host immunity, Nature 375 (1995) 411–415.
[114] K. Ahn, T.H. Meyer, S. Uebel, P. Sempé, H. Djaballah, Y. Yang, P.A. Peterson, K. Früh, R. Tampé, Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus ICP47, EMBO J. 15 (1996) 3247–3255.
[115] C. Kyritsis, S. Gorbulev, S. Hutschenreiter, K. Pawlitschko, R. Abele, R. Tampé, Molecular mechanism and structural aspects of transporter associated with antigen processing inhibition by the cytomegalovirus protein US6, J. Biol. Chem. 276 (2001) 48031–48039.
[116] E.W. Hewitt, S.S. Gupta, P.J. Lehner, The human cytomegalovirus gene product US6 inhibits ATP binding by TAP, EMBO J. 20 (2001) 387–396.
[117] K. Ahn, A. Gruhler, B. Galocha, T.R. Jones, E.J. Wiertz, H.L. Ploegh, P.A. Peterson, Y. Yang, K. Früh, The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP, Immunity 6 (1997) 613–621.
[118] H. Hengel, J.O. Koopmann, T. Flohr, W. Muranyi, E. Goulmy, G.J. Hammerling, U.H. Koszinowski, F. Momburg, A viral ER-resident glycoprotein inactivates the MHC-encoded peptide transporter, Immunity 6 (1997) 623–632.
[119] D. Koppers-Lalic, E.A. Reits, M.E. Ressing, A.D. Lipinska, R. Abele, J. Koch, M. Marcondes Rezende, P. Admiraal, D. van Leeuwen, K. Bienkowska-Szewczyk, T.C. Mettenleiter, F.A. Rijsewijk, R. Tampé, J. Neefjes, E.J. Wiertz, Varicelloviruses avoid T cell recognition by UL49.5-mediated inactivation of the transporter associated with antigen processing, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 5144–5149.
[120] M.E. Ressing, S.E. Keating, D. van Leeuwen, D. Koppers-Lalic, I.Y. Pappworth, E.J. Wiertz, M. Rowe, Impaired transporter associated with antigen processing-dependent peptide transport during productive EBV infection, J. Immunol. 174 (2005) 6829–6838.
[121] S. Loch, F. Klauschies, C. Schölz, M.C. Verweij, E.J. Wiertz, J. Koch, R. Tampé, Signaling of a varicelloviral factor across the endoplasmic reticulum membrane induces destruction of the peptide-loading complex and immune evasion, J. Biol. Chem. 283 (2008) 13428–13436.

[122] J. Lin, S. Schmidt, S. Hank, R. Wieneke, P.U. Mayerhofer, R. Tampé, A trans-inhibition Modulator of Antigen Translocation Evolved From a Frameshift in the Cowpox Virus Genome, PLoS Pathogens, (in press).
[123] A.I. Wycisk, J. Lin, S. Loch, K. Hobohm, J. Funke, R. Wieneke, J. Koch, W.R. Skach, P.U. Mayerhofer, R. Tampé, Epstein–Barr viral BNLF2a protein hijacks the tail-anchored protein insertion machinery to block antigen processing by the transport complex TAP, J. Biol. Chem. 286 (2011) 41402–41412.
[124] S. Hulpke, R. Tampé, The MHC I loading complex: a multitasking machinery in adaptive immunity, Trends Biochem. Sci. 38 (2013) 412–420.
[125] M.S. Panter, A. Jain, R.M. Leonhardt, T. Ha, P. Cresswell, Dynamics of major histocompatibility complex class I association with the human peptide-loading complex, J. Biol. Chem. 287 (2012) 31172–31184.
[126] P. Tan, H. Kropshofer, O. Mandelboim, N. Bulbuc, G.J. Hammerling, F. Momburg, Recruitment of MHC class I molecules by tapasin into the transporter associated with antigen processing-associated complex is essential for optimal peptide loading, J. Immunol. 168 (2002) 1950–1960.
[127] J.L. Petersen, H.D. Hickman-Miller, M.M. McIlhaney, S.E. Vargas, A.W. Purcell, W.H. Hildebrand, J.C. Solheim, A charged amino acid residue in the transmembrane/cytoplasmic region of tapasin influences MHC class I assembly and maturation, J. Immunol. 174 (2005) 962–969.
[128] J. Koch, R. Guntrum, R. Tampé, The first N-terminal transmembrane helix of each subunit of the antigenic peptide transporter TAP is essential for independent tapasin binding, FEBS Lett. 580 (2006) 4091–4096.
[129] M. Papadopoulos, F. Momburg, Multiple residues in the transmembrane helix and connecting peptide of mouse tapasin stabilize the transporter associated with the antigen-processing TAP2 subunit, J. Biol. Chem. 282 (2007) 9401–9410.
[130] N. Garbi, N. Tiwari, F. Momburg, G.J. Hammerling, A major role for tapasin as a stabilizer of the TAP peptide transporter and consequences for MHC class I expression, Eur. J. Immunol. 33 (2003) 264–273.
[131] G. Raghuraman, P.E. Lapinski, M. Raghavan, Tapasin interacts with the membrane-spanning domains of both TAP subunits and enhances the structural stability of TAP1 × TAP2 Complexes, J. Biol. Chem. 277 (2002) 41786–41794.
[132] M.R. Knittler, P. Alberts, E.V. Deverson, J.C. Howard, Nucleotide binding by TAP mediates association with peptide and release of assembled MHC class I molecules, Curr. Biol. 9 (1999) 999–1008.
[133] T. Kanaseki, N. Shastri, Biochemical analysis of naturally processed antigenic peptides presented by MHC class I molecules, Methods Mol. Biol. 960 (2013) 179–185.
[134] D.R. Madden, The three-dimensional structure of peptide-MHC complexes, Annu. Rev. Immunol. 13 (1995) 587–622.
[135] M. Basler, C.J. Kirk, M. Groettrup, The immunoproteasome in antigen processing and other immunological functions, Curr. Opin. Immunol. 25 (2013) 74–80.
[136] N.A. Nagarajan, N. Shastri, Immune surveillance for ERAAP dysfunction, Mol. Immunol. 55 (2013) 120–122.
[137] N. Monroe, G. Sennhauser, M.A. Seeger, C. Briand, M.G. Grutter, Designed ankyrin repeat protein binders for the crystallization of AcrB: plasticity of the dominant interface, J. Struct. Biol. 174 (2011) 269–281.
[138] J. Fernandez-Martinez, M.P. Rout, A jumbo problem: mapping the structure and functions of the nuclear pore complex, Curr. Opin. Cell Biol. 24 (2012) 92–99.
[139] H.G. Choudhury, Z. Tong, I. Mathavan, Y. Li, S. Iwata, S. Zirah, S. Rebuffat, H.W. van Veen, K. Beis, Structure of an antibacterial peptide ATP-binding cassette transporter in a novel outward occluded state, Proc. Natl. Acad. Sci. U. S. A. 111 (25) (2014) 9145–9150.
[140] N. Grossmann, A.S. Vakkasoglu, S. Hulpke, R. Abele, R. Gaudet, R. Tampé, Mechansitic determinants of the directionality and energetics of active export by a heterodimeric ABC transporter, Nature Commun. (in press), http://dx.doi.org/10.1038/ncomms6419.