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Minireview

Modulation of the antigen transport machinery TAP by friends and enemies

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Abstract The transporter associated with antigen processing (TAP) is a key factor of the major histocompatibility complex (MHC) class I antigen presentation pathway. This ABC transporter translocates peptides derived mainly from proteasomal degradation from the cytosol into the ER lumen for loading onto MHC class I molecules. Manifold mechanisms have evolved to regulate TAP activity. During infection, TAP expression is upregulated by interferon-γ. Furthermore, the assembly and stability of the transport complex is promoted by various auxiliary factors. However, tumors and viruses have developed sophisticated strategies to escape the immune surveillance by suppressing TAP function. The activity of TAP can be impaired on the transcriptional or translational level, by enhanced degradation or by inhibition of peptide translocation. In this review, we briefly summarize existing data concerning the regulation of the TAP complex.

Keywords: ABC transporter; Antigen presentation; Herpes virus; Membrane transport; Peptide-loading complex; Transporter associated with antigen processing; Tumor progression; Viral escape

1. Function of TAP

The transporter associated with antigen processing (TAP) is a central component in the major histocompatibility complex (MHC) class I dependent antigen presentation pathway. TAP translocates peptides derived mainly from proteasomal degradation from the cytosol into the lumen of the endoplasmic reticulum (ER), where these peptides are loaded onto MHC class I molecules (Fig. 1). Stable peptide-MHC complexes are transported to the cell surface to present their antigenic cargo to $CD8^+$-cytotoxic T-lymphocytes. The recognition of viral or tumor antigens leads to an efficient elimination of the infected or malignant cell.

TAP belongs to the family of ATP-binding cassette (ABC) transporters, which translocate a large variety of substrates across membranes driven by ATP hydrolysis [1–3]. Human TAP forms a heterodimer consisting of TAP1 (748 aa) and TAP2 (686 aa) [4]. Both subunits are essential and sufficient for peptide transport [5–7]. TAP is localized in the ER and *cis*-Golgi [8]. Each subunit contains a transmembrane domain (TMD), followed by a cytosolic nucleotide-binding domain (NBD) (Fig. 2). The TMDs comprise the peptide binding pocket and the translocation pathway for the substrate. From hydrophobicity analysis and sequence alignment with P-glycoprotein, 10 and 9 transmembrane helices have been predicted for TAP1 and TAP2, respectively [9]. The peptide-binding pocket is located to a region enclosing the last cytosolic loop and a stretch of 15 residues following the last transmembrane helix of both subunits [10]. Remarkably, TAP1 and TAP2 lacking the first predicated four and three transmembrane helices, respectively, are targeted to the ER membrane and assemble into a fully functional heterodimeric transport complex, demonstrating that the extra N-terminal regions (N-domains) of both subunits are not required for peptide binding and transport [11,12]. These N-terminal regions have been identified to be essential for tapasin binding and the assembly of the peptide-loading complex (see below) [11]. The NBDs containing the highly conserved Walker A/B motifs and the C-loop (ABC-signature) energize peptide transport by ATP binding and hydrolysis.

The transport cycle is a multi-step process composed of ATP and peptide binding, ATP hydrolysis and peptide translocation. Peptide binding follows a two step reaction with a fast association preceding a slow conformational rearrangement, which comprises one-fourth of all residues of TAP [13,14]. A second conformational change seems to occur after ATP binding, since the lateral membrane mobility of TAP decreases drastically in the presence of peptide and ATP [15]. TAP binds and transports most efficiently peptides with a length of 8–16 and 8–12 amino acids, respectively [16,17]. The peptide specificity of TAP is restricted to the three N-terminal residues and the C-terminal residue [18]. The specificity for the C-terminal residue is very similar between the TAP complex, immuno-proteasomes, and MHC class I molecules, suggesting a co-evolution of these factors. However, the length of the peptides as well as the specificity for the N-terminal residue are distinct between TAP, the proteasome

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Abbreviations: ABC, ATP-binding cassette; Ad, adenovirus; BHV, bovine herpes virus; EGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum; HPV, human papillomavirus; HSV, herpes simplex virus; ICP47, infected cell protein 47; IFN-γ, interferon-γ; MHC, major histocompatibility complex; NBD, nucleotide-binding domain; PLC, peptide-loading complex; TAP, transporter associated with antigen processing; TMD, transmembrane domain; UL49.5, unique long region protein 49.5; US6, unique short region protein 6; vhs, virion host shut-off; wt, wild-type

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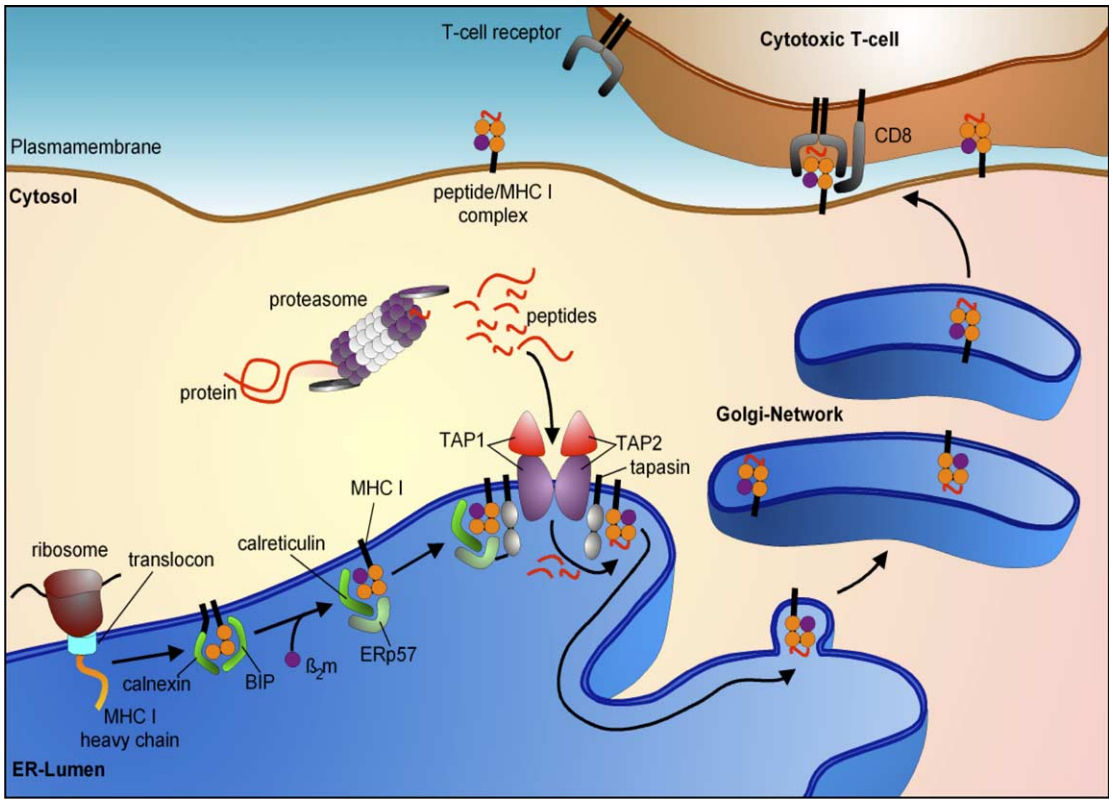

Fig. 1. Antigen presentation pathway via MHC class I molecules. The MHC I heavy chain is co-translationally translocated into the ER, where it folds and assembles with β_2-microglobulin assisted by the immunoglobulin binding protein (BiP) and calnexin. Subsequently, MHC class I molecules are recruited into a macromolecular peptide-loading complex (PLC) composed of calreticulin, ERp57, tapasin, TAP1 and TAP2. Peptides derived mainly by proteasomal degradation in the cytosol are translocated by TAP into the lumen of the ER, where they are loaded onto pre-assembled MHC class I molecules. Kinetically stable peptide-MHC complexes can escape the ER quality control and are transported via the Golgi to the cell surface. Binding of T-cell receptor and CD8 co-receptor to trimeric MHC class I molecules triggers the killing of the target cell (adapted from [99]).

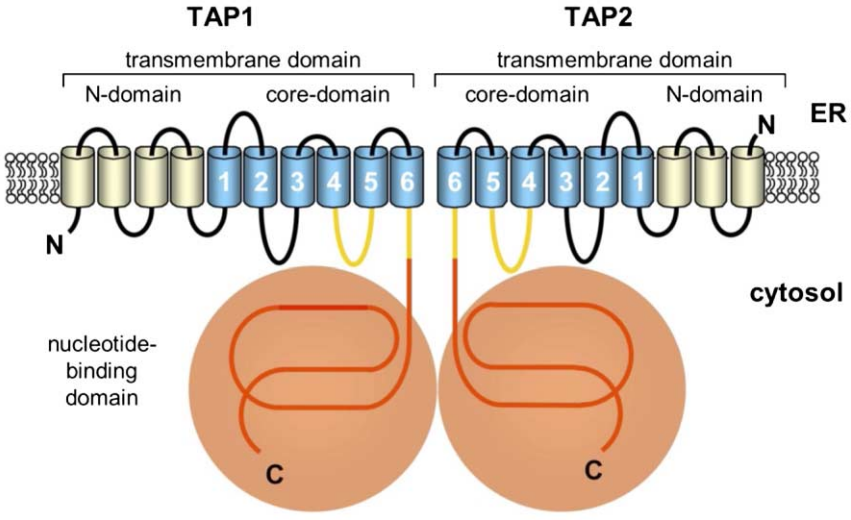

Fig. 2. Schematic model of the TAP complex. TAP forms a heterodimer composed of TAP1 and TAP2. Each subunit comprises an N-terminal transmembrane domain and a C-terminal, cytosolic NBD (red). The transmembrane domain can be subdivided into a six helices containing core domain and an NH_2-terminal extension of four and three helices for TAP1 and TAP2, respectively. Beside the translocation pathway, the TMDs also form the peptide-binding region (orange).

and MHC class I molecules. Peptides transported by TAP are subsequently trimmed by amino exopeptidases in the ER [19–22]. The sequence in between both anchor regions, which is recognized by the T-cell receptor, is highly divers in respect of TAP and MHC class I binding. This kind of clustered promiscuity ensures that one TAP complex in combination with

a limited set of MHC class I alleles can offer an almost unlimited number of peptides to protect the organism against intracellular pathogens and malignant transformation.

Peptides and ATP bind independently from each other to TAP [16,17,23]. However, ATP hydrolysis is directly coupled with peptide transport, since peptide binding correlates with ATPase activity [24]. In this process, a quality control exists, since peptides with bulky side chains, which bind to but are not transported by TAP, do not induce ATP hydrolysis. Although both TAP subunits show an asymmetric functional behavior, both of them are involved in energizing peptide transport in a peptide-dependent manner [25,26].

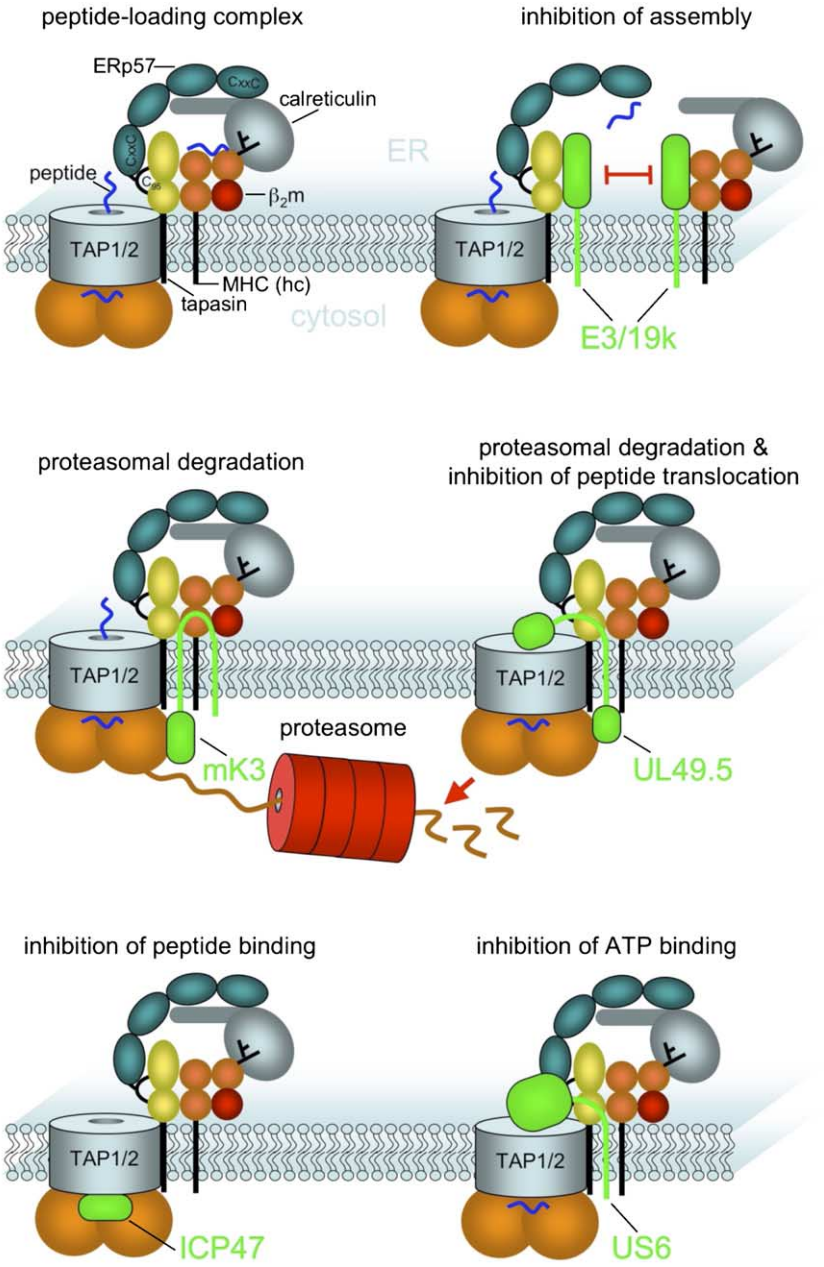

Fig. 3. Modulation of TAP function by viral factors. Upper panel: The structural organization of the peptide-loading complex (PLC) is illustrated as a model. ERp57 is covalently linked to tapasin via a disulfide bond (C95 of tapasin to the CxxC motif of the oxidoreductase). Within the ER quality control, calreticulin recognizes the N-core glycosylation of the MHC I heavy chain (hc) and forms additional contacts with ERp57. For simplicity, only one of four TAP-associated sub-complexes (tapasin, MHC heavy chain, β_2m, ERp57 and calreticulin) is shown. The E19 protein of adenovirus E3 disturbs the interaction of MHC class I molecules with the pre-assembled TAP–tapasin–ERp57 complex which impairs efficient peptide loading of MHC class I. Middle panel: The mK3 protein of murine γ-herpesvirus-68 binds directly to TAP and induces polyubiquitylation and subsequently proteasomal degradation. The interaction of UL49.5 of the bovine herpes virus drives TAP to proteasomal degradation and arrests TAP in a transport-incompetent conformation, in which binding of ATP and peptide is not affected. Lower panel: ICP47 of the herpes simplex virus inhibits peptide binding from the cytosolic site of TAP. The association of US6 of the human cytomegalovirus to the ER-luminal transmembrane core of TAP blocks ATP binding to the cytosolic NBD.

2. Assembly, stabilization and degradation of TAP

TAP alone is sufficient for peptide translocation into the ER lumen [7]. However, within the pathway of MHC class I antigen processing, TAP is part of the multi-component peptide-loading complex (PLC) (Figs. 1 and 3). This macromolecular complex is composed of one TAP heterodimer, together with four tapasin and four MHC class I molecules [27]. In addition, the lectin-like chaperones calreticulin and calnexin, as well as the oxidoreductase ERp57 are associated. Calnexin, calreticulin and ERp57, are involved in the maturation of MHC class I molecules. Additionally, calreticulin and ERp57 seem to assist in the optimization of peptide loading onto MHC class I molecules [28–30]. The type I transmembrane glycoprotein tapasin is an import factor of the PLC and takes over several functions. It recruits the other members of the PLC and connects the peptide translocation machine TAP to the peptide recipient MHC class I [31–33]. The ER-luminal domain of tapasin binds to MHC class I molecules with 1:1 stoichiometry [34,35]. This interaction arrests MHC class I molecules loaded with low affinity peptides in the ER and ensures that only stable peptide-MHC complexes can leave the ER [36]. Mechanistic details of how tapasin may facilitate and control peptide binding to MHC class I molecules are still under discussion [31,36,37]. The transmembrane helix of tapasin binds to the N-domain of the TMDs of TAP subunits [11]. How tapasin is associated with TAP is still puzzling, since the sequence homology between the N-domains of TAP1 and TAP2 is low in comparison to the rest of the protein. By increasing its half-life, tapasin also stabilizes the TAP complex, resulting in a higher steady-state expression level of TAP and, consequently, in a higher peptide transport rate [31,34,38]. This higher TAP level is due to an enhanced thermostability of TAP in the presence of tapasin [39]. In tapasin deficient cells or cells expressing tapasin mutants, which are unable to bind to TAP, the steady-state level of TAP is decreased up to 100-fold [31].

Remarkably, the stability of the TAP complex does not only rely on the interaction with tapasin, but also on the interaction between both subunits. TAP1 alone can be expressed in TAP-deficient T2 cells [40]. However, expression of single TAP2 is not possible in this cell line. Furthermore, expression of TAP2 is not observed in cells isolated from Bare Lymphocytes Syndrome (BLS) type I patients lacking the expression of TAP1 [41,42]. In the human melanoma cell line buf1280, the TAP1 gene has a deletion mutation at position 1489, leading to a frame shift and to an early stop codon [43]. In these cells, mRNA of TAP1 and TAP2 is present, however, neither TAP1 nor TAP2 can be detected on the protein level. After introducing TAP1 wild-type (wt) in this cell line, normal TAP1 and TAP2 expression as well as peptide transport were restored. Therefore, it appears that TAP2 is only stable in the presence of TAP1 and single TAP2 is immediately degraded, possibly by the proteasomal degradation pathway. Interestingly, interferon-γ, which is released during an infection, enhances the expression of MHC class I heavy chain, β_2m, tapasin, TAP1, and TAP2. Promoter studies have shown that TAP1 and TAP2 expression is regulated by partially different second messenger pathways and transcription factors [44,45].

3. Tumor escape by downregulation of TAP

A variety of ABC transporters are associated with cancer in human. P-glycoprotein (ABCB1), the members of the multidrug resistance related protein (MRP) family, and ABCG2 transport anticancer drugs out of the cell, thus conferring tumor cells resistance to chemotherapy. In addition, a surface downregulation of MHC class I molecules on tumor cell is often observed, which facilitates these cells to escape immune surveillance by suppression of the presentation of tumor-specific antigens. A deficient MHC class I cell surface expression can be caused by defects in expression or function of factors of the MHC class I antigen presentation pathway [46,47]. Frequently, MHC class I downregulation is associated with TAP malfunction [48]. The low or missing expression of TAP in tumor cells impairs the formation of the peptide–MHC I complexes in the ER. A higher frequency of deletion of the TAP complex was observed in metastatic lesions than in primary lesions [48]. The importance of TAP in the immune surveillance was highlighted in studies using a TAP1 deficient mouse lung carcinoma cell line (CMT.64). Restoration of TAP expression by introducing exogenous TAP1 or by stimulation with interferon-γ (IFN-γ) could correct the MHC class I deficiency [49,50]. After TAP restoration, the tumor cell line was recognized by cytotoxic T-cells in vitro. A decrease in tumor growth and incidence was observed in vivo, by treating mice bearing CMT.64 tumors with vaccinia or replication deficient adenovirus coding for TAP1 [51,52]. Different mechanisms exist for the downregulation of TAP in malignant cells. First, mutations in the TAP1 gene were observed. One case is reported, where a deletion of one nucleotide in the gene of TAP1 results in an early stop codon. This truncated subunit cannot form a functional TAP complex [43]. In a small cell lung cancer, an amino acid exchange of TAP1 (R659Q) is found, which leads to a nonfunctional TAP complex [53]. More often, the mRNA level of TAP is decreased in malignant cells. In these cases, TAP expression can be restored by IFN-γ treatment [54]. By chromatin immunoprecipitation assays, the transcription rate of the TAP1 gene was analyzed in different murine carcinoma cell lines [54]. By comparing the RNA polymerase II level at the 3′ end of the coding region and the promoter region of the TAP1 gene, it became obvious that the reduced transcriptional rate is depending on impaired initiation of transcription. Surprisingly, the TAP1 promoter shows no mutation in the CMT.64 cell line. The analysis by TAP1-promoter driven enhanced green fluorescent protein (EGFP) expression demonstrated that the promoter activity could be restored by IFN-γ, indicating that the promoter contains *cis*-acting elements, conferring the relatively low activity in TAP1 deficient cells. In addition to *cis*-acting elements, *trans*-acting factors are likely to be involved in regulating the TAP1 promoter activity, since fusion of TAP1 deficient carcinoma cells and wt fibroblasts restored TAP deficiency, at least partially. The TAP deficiency in these carcinoma cell lines is not solely due to inhibition of transcription, since a decreased but significant promoter activity could be detected. Again, in fusions of wt fibroblasts with TAP1 deficient carcinoma cell lines, it could be shown that the stability of especially TAP1 mRNA, but not of unrelated mRNA, was decreased by an unknown mechanism. Accelerated degradation of TAP1 mRNA was also found in the human SK-MEL-19 melanoma cell line bearing a single-nucleotide deletion at position 1489 [55]. Despite an active

transcription of TAP1 gene, even after INF-γ treatment, TAP1 mRNA was not detectable. The mechanism of the degradation is not known, but it is not affected by nonsense-mediated mRNA decay, because deletions of two additional nucleotides in the region, which corrected the nonsense mutation, did not restore TAP1 mRNA stability. In contrast to IFN-γ, interleukin-10 (IL-10) leads to downregulation of TAP and, subsequently, to reduced surface expression of MHC class I molecules [56]. This fact may be of clinical relevance, since a large number of human tumors secrete IL-10.

4. Viral immune evasion by blocking TAP function

Many viruses, in particular slow replicating DNA viruses, have developed elaborated mechanisms to evade immune surveillance. These strategies prevent or delay immune recognition of infected cells to enable the virus to replicate, colonize the host, and transmit to other individuals. Frequently, the viral factors block the function of the MHC class I antigen presentation pathway and, hence, prevent the recognition and elimination by cytotoxic T-lymphocytes [57,58]. Especially, persistent viruses rely on this mechanism to circumvent T-cell recognition. In addition to MHC class I molecules, TAP, and factors of the peptide-loading complex may be an important target of the viral attack. Peptide transport into the ER can be impaired by different mechanisms, including downregulation of TAP expression, enhanced degradation mediated by viral factors, and blocking peptide transport by direct interaction.

For cells infected with the oncogenic human papillomavirus (HPV) type-18 and the adenovirus (Ad) type-12, downregulation of TAP1 transcription was observed. The E7 protein of HPV-18 and the E1A protein of Ad-12 repress the promoter activity of *tap1*. Although both DNA viruses belong to different virus classes, the two factors are similar in structure and function.

The virion host shut-off (vhs) mechanism, best studied on the subfamily of *Alphaherpesvirinae* comprising herpes simplex virus-1 (HSV1) and 2, varicella-zoster virus, and bovine herpesvirus (BHV), is a general strategy to suppress the expression of host genes [59,60]. As a part of the infection virion, the vhs protein accelerates the degradation of host mRNA and supports immune evasion in the early state of infection.

The mK3 protein of the murine γ-herpesvirus-68 and the E19 protein of the adenovirus E3 inhibit the antigen presenting machinery by interacting with TAP without affecting peptide transport (Fig. 3). The mK3 protein is a type III transmembrane protein containing two transmembrane helices. mK3 binds via its C-terminal tail to tapasin and TAP [61,62], thereby positioning the N-terminal RING-finger motif with an E3 ubiquitin ligase activity to the cytoplasmic tail of MHC class I for subsequent ubiquitinylation and proteasomal degradation [63,64]. However, mK3 also induces in a MHC I independent mechanism the degradation of TAP subunits and tapasin [65,66]. For degradation of TAP, interaction of mK3 with tapasin lacking the ER-luminal domain is sufficient; while, for association of mK3 with and degradation of MHC class I, the interaction with full-length tapasin is mandatory [66]. The ER-resident type I transmembrane glycoprotein E19 (19 kDa) protein of adenovirus E3 suppresses antigen presentation by MHC class I molecules [67,68]. E3/19k binds via its ER-luminal domain to MHC class I molecules [69] and retains these in the ER by a di-lysine motif localized in the cytosolic domain of E3/19k [70]. Remarkably, E3/19k binds to TAP and prevents assembly of MHC I-loading complex by disturbing the MHC–tapasin interaction [71].

The human herpes simplex virus-1 and 2, the human cytomegalovirus, and the bovine herpes virus code for factors inhibiting TAP-dependent peptide transport into the ER (Fig. 3). Strikingly, herpesviruses have developed distinct strategies [58]. The immediate early gene product of HSV-1 (IE12; infected cell protein 47 (ICP47)) encodes an 88 amino acids long cytosolic protein, which interferes with TAP function [72,73]. By binding with high affinity to human TAP, ICP47 inhibits peptide binding to and thereby ATP hydrolysis of TAP [24,74,75]. However, ICP47 does not only compete with peptide binding, but also induces a conformational change of TAP, as shown by chemical cross-linking [76]. ICP47 is highly species specific, since it inhibits with high affinity peptide binding to human TAP (IC_{50} = 50 nM), but not to TAP from mice, rabbit or guinea pig [74,75,77,78]. The active domain of ICP47 was determined by truncation studies to residues 3–34 [13]. The function of the C-terminal domain of ICP47 is not yet understood. The active domain undergoes a conformational change during membrane association. In aqueous solution, ICP47 is unstructured, whereas it adopts an α-helical conformation after membrane association [79]. As resolved by solution NMR in membrane mimicking environment, the active domain of ICP47 contains two α-helices connected by a flexible loop [80]. By an alanine scanning approach and site-directed mutagenesis, residues critical for TAP inhibition were deciphered [13,81]. These residues are clustered in three regions, which cover both α-helices and the connecting loop. Which region of TAP interacts with ICP47 is, however, still a puzzling question.

The human cytomegalovirus has evolved several strategies to downregulate MHC class I surface expression [82,83]. One mechanism is the inhibition of peptide transport by the 23 kDa type I transmembrane glycoprotein unique short region protein 6 (US6) of the unique short (US) region [84–86]. US6 inhibits TAP via its ER-luminal domain (Fig. 3) [84]. Binding of US6 to TAP in the ER lumen prevents ATP binding to the NBDs localized in the cytosol, whereas peptide binding is not affected by US6 [87,88]. US6 seems to induce a long ranging conformational rearrangement in TAP. Like ICP47, US6 prevents conformational rearrangements induced by peptide binding [76,87]. TAP denatures rapidly in vitro at physiological temperature, which is reflected in decreased peptide binding and transport, as well as decreased immunodetection by antibodies [89]. The stability is drastically increased in the presence of ADP or ATP. US6 seems to induce similar structural effects as nucleotides, since it also stabilizes TAP against thermal denaturation [87]. By using the isolated, soluble ER-luminal domain of US6, an IC_{50} value of 1 μM for TAP inhibition was determined [88]. The binding sites of US6 are mapped to the inner 2×6 transmembrane helices, as N-terminal truncated TAP complexes are inhibited by US6 [11].

The bovine herpesvirus is a member of the subfamily of the *Alphaherpesvirinae* persisting in its host for life after a short acute phase of infection. Interestingly, BHV can also infect human cells and interferes with antigen processing and presentation in human cells [90]. Gene products from the early phase of

infection inhibit peptide transport in bovine and human cells [91,92]. In a proteomic approach, the viral factor unique long region protein 49.5 (UL49.5) was identified to be associated with human TAP in the human melanoma cell line Mel JuSo [90]. The type I transmembrane glycoprotein (9 kDa) UL49.5, also known as glycoprotein N (gN) [93], is found as monomer, homodimer and heterodimer with the viral glycoprotein M (gM) in infected cells and virion envelopes [94,95]. This factor has two independent mechanisms to downregulate peptide supply into the ER lumen (Fig. 3). The association of UL49.5 with TAP induces the proteasomal degradation of TAP as well as UL49.5. For this process, the C-terminal, cytoplasmic tail of UL49.5 is essential. Additionally, UL49.5 arrests TAP in a peptide translocation-incompetent conformation, in which peptide and ATP binding are not affected. By tagging TAP1 C-terminally with GFP, the UL49.5 mediated degradation, but neither the association of nor the transport inhibition by UL49.5 could be inhibited. Although homologues of UL49.5 are found in genomes of all herpesviruses sequenced to date [96], only UL49.5 homologues from pseudorabies virus and from equine herpesvirus-1, but not from HSV1 and HSV2, Epstein–Barr virus and varicella-zoster virus interfered with TAP function [90].

Obviously, further viral strategies to inhibit TAP function do exist, since cells infected by human immune-deficiency virus type 1 (HIV-1) or Epstein–Barr virus (EBV) show impaired peptide transport into the ER lumen [97,98]. However, the factors and molecular mechanisms of TAP mechanism still have to be elucidated.

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