# The Transporter Associated With Antigen Processing: Function and Implications in Human Diseases

**BRIGITTE LANKAT-BUTTGEREIT AND ROBERT TAMPÉ**

*Institut für Physiologische Chemie, Philipps-Universität Marburg, Marburg, Germany*

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**Lankat-Buttgereit, Brigittte, and Robert Tampé.** The Transporter Associated With Antigen Processing: Function and Implications in Human Diseases. *Physiol Rev* 82: 187–204, 2002; 10.1152/physrev.00025.2001.—The adaptive immune systems have evolved to protect the organism against pathogens encountering the host. Extracellular occurring viruses or bacteria are mainly bound by antibodies from the humoral branch of the immune response, whereas infected or malignant cells are identified and eliminated by the cellular immune system. To enable the recognition, proteins are cleaved into peptides in the cytosol and are presented on the cell surface by class I molecules of the major histocompatibility complex (MHC). The transport of the antigenic peptides into the lumen of the endoplasmic reticulum (ER) and loading onto the MHC class I molecules is an essential process for the presentation to cytotoxic T lymphocytes. The delivery of these peptides is performed by the transporter associated with antigen processing (TAP). TAP is a heterodimer of TAP1 and TAP2, each subunit containing transmembrane domains and an ATP-binding motif. Sequence homology analysis revealed that TAP belongs to the superfamily of ATP-binding cassette transporters. Loss of TAP function leads to a loss of cell surface expression of MHC class I molecules. This may be a strategy for tumors and virus-infected cells to escape immune surveillance. Structure and function of the TAP complex as well as the implications of loss or downregulation of TAP is the topic of this review.
I. INTRODUCTION

A. Overview of Antigen Processing

The adaptive immune system has evolved to protect the organism against pathogens. The recognition and elimination of mutated or infected cells are performed by the cellular immune system, which can be subdivided into the class I major histocompatibility complex (MHC) and the class II MHC pathways. MHC class I molecules are present on the surface of nearly all nucleated cells, whereas class II molecules are restricted to the cells of the immune system. Here, we focus on antigen processing via MHC class I molecules. The peptides needed for antigen presentation are generated in the cytosol by cleavage of endogenous proteins and are displayed on the cell surface by MHC class I molecules (Fig. 1). The chronic presentation of self-protein-derived peptides does not lead to a stimulation of T cells. During the development of the thymus, the T cells with the ability to recognize the self-peptides are deleted. In case of malignant transformation or viral infection of the cells, an additional set of peptides is presented to the cytotoxic T lymphocytes (CTL). The intracellular pathways leading to the generation of peptides, their binding to MHC molecules, and presentation on the cell surface are called antigen processing and presentation. The recognition of MHC class I molecules as “self component” loaded with peptides from “non-self” proteins by CTL leads to apoptosis or lysis of malignant or infected cells. This is an essential process of the cellular immune response (for review, see Refs. 116, 165). The pathway of antigen presentation by the MHC class I complex is constitutively active in all nucleated cells and is upregulated by inflammatory cytokines. Interference with this pathway has evolved as an effective strategy for pathogens to evade immune response, leading to chronic or latent infections. However, human patients deficient in MHC class I antigen presentation do not appear to have a higher incidence of viral infections (33).

In the absence of infection, normal endogenous proteins are degraded in the cytosol, but MHC class I-associated peptides can be also derived from viral antigens in the cytosol (159) as well as from proteins that are artificially introduced into the cytosol (104, 181). Inhibition of protein breakdown in lysosomes did not affect MHC class I presentation (106) and implied that the presented peptides were generated by proteases in an extralysosomal pathway. The major mechanism for degrading proteins in the cytosol is ATP dependent and highly conserved from yeast to mammals (52). The resulting peptides are generated by a proteolytic particle, the 20S/26S proteasome (15, 28, 42, 182). The contribution of the 20S proteasome for peptide generation is shown in Fig. 1. The proteasome is a large, multisubunit complex that consists of at least 28 different subunits, which are arranged in two rings of 11 subunits each (15, 28, 42, 182). The 20S proteasome is the core of the proteasome, and it is responsible for the initial cleavage of proteins into smaller peptides. The 26S proteasome is composed of the 20S proteasome and an additional regulatory particle, the 19S proteasome, which is responsible for targeting proteins to the proteasome and for unfolding the proteins before they are degraded (28, 42, 182).
the generation of antigenic peptides is further strengthened by the observation that proteasomal subunits, LMP2 and LMP7 (low-molecular-mass polypeptides), are encoded within the MHC locus (22, 51, 97). The catalytic core of the proteasome is a 20S (700 kDa) cylindrical particle composed of 28 subunits arranged in four heptameric rings. The outer rings are made up of seven α-subunits; the inner rings are composed of seven β-subunits. Whereas the α-subunits are thought to be primarily responsible for structural and regulatory functions, the β-subunits harbor the catalytic centers (40, 93, 142). Another form of the proteasome is the 26S (1,500 kDa) particle. It contains the 20S complex and additional subunits associated with regulation of its activity (118, 126).

The proteasome produces peptides with a size distribution of 3–30 residues with an optimum of 6–11 residues (39, 77). This size corresponds in part to the size of peptides that is presented by one MHC class I allele. In humans, there are three to six MHC class I alleles. This small set of molecules has to bind the large pool of peptides. Crystal structures of MHC class I molecules provided further insight into this problem. How does a single protein bind many different peptides? The antigenic peptide binds in a groove, which is formed by two α-helices on the rim and eight β-strands on the bottom of the α1- and α2-domain of the heavy chain. The peptide is fixed at the free COOH and NH2 terminus. Anchor residues at position two or three and at the COOH-terminal residue pointing into the groove are also important for binding. The groove accommodates peptides with eight or nine residues in an extended conformation. The side chains between most of these anchor residues point outside the groove. This binding motif explains the large pool of peptides that is presented by one MHC class I allele.

Efficient binding of peptides to the MHC class I molecules requires a macromolecular loading complex; the assembly of this complex takes place in the ER and is dependent on different chaperones that ensure correct folding. After cotranslational translocation, the MHC α-chain associates with calnexin, a transmembrane calcium-dependent lectin with chaperone activity, followed by formation of a noncovalent dimer with β2-microglobulin (for review, see Ref. 116). Calnexin is then replaced by calreticulin, another calcium-dependent lectin, and ERP57, a thiol reductase, is added to the complex, but the exact time point of the interaction is unclear. Class I heterodimers, associated with calreticulin and ERP57, form the macromolecular loading complex with tapasin and TAP. The glycoprotein tapasin is required for efficient interaction of TAP and class I molecules (135). Meanwhile, there is evidence that calnexin and ERP57 bind to the TAP-tapasin complex, generating an intermediate which is capable of binding to the MHC class I-β2-microglobulin dimers (37). The formation of this macromolecular complex is accompanied by the loss of calnexin and binding of calreticulin. Furthermore, it could be demonstrated that the association of ERP57 to the complex is tapasin dependent and in concordance with calreticulin, not with calnexin (55). Despite the physical association of TAP and the MHC class I molecules, it was shown by inhibition of binding after antipeptide antibody application that possibly most TAP-transported peptides diffuse through the lumen of the ER before being loaded onto
MHC class I molecules (63). The binding of peptides to class I heterodimers releases the loaded MHC class I molecules from the assembly complex for transport to the cell surface via the Golgi apparatus and the trans-Golgi network. The export from the ER by ER-budding vesicles is the rate-limiting step, and the multitude of ER-to-Golgi transport is independent of peptide loading or dissociation from TAP (151).

During the last years, the comprehension of structure and functions of TAP increased significantly, stressing the importance of this transporter for the adaptive immune response. A growing number of studies deal with the implications of disturbed TAP function in various human diseases from viral infections to tumor development. In this review, the current knowledge about TAP and its implications for diseases are summarized.

II. DISCOVERY, GENOMIC ORGANIZATION, AND HOMOLOGY OF THE TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING GENES

A. Cell Lines With Defects in MHC Class I Presentation

Peptides produced in the cytosol must pass into the lumen of the ER before they can bind to MHC class I molecules. The first indication that this translocation into the ER requires a transporter was based on studies dealing with various cell lines with a strongly reduced level of MHC class I molecules on the cell surface (91, 158). These mutant cell lines exhibit a low cell-surface expression of MHC class I and are deficient in antigen presentation. Although the expression levels of MHC class I heavy chain and β2-microglobulin are normal and peptides exogenously added or introduced into the ER by signal sequences were efficiently presented (8), these defective cell lines were unable to present intracellular antigens on the cell surface. It soon became clear that this defect was due to deletions in the region of the MHC locus and most likely involved a gene or genes that were responsible for delivering peptides to the lumen of the ER and/or loading of these peptides onto newly synthesized class I molecules. These genes were tentatively mapped to the MHC locus by analysis of gene deletions in one of these mutant cells (24). In the following, four groups independently described candidate genes from the MHC region for a factor that would transport peptides across the ER membrane (36, 103, 148, 160). In 1991, a World Health Organization (WHO) nomenclature committee for factors in the HLA system renamed these genes to TAP (155). RING4, PSF1, mtp1, and HAM1 are now TAP1, and RING11, PSP2, mtp2, and HAM2 are renamed TAP2.

B. Characterization

The genes for human TAP1 and TAP2 are located in the MHC II locus of chromosome 6 and comprise 8–12 kb each (Fig. 2) (161). Deletions of either or both of the TAP genes result in greatly reduced surface expression of MHC class I molecules (24, 65, 158). These defects can be partly corrected by addition of exogenously supplied class I-binding peptides (157). The functional impact of TAP1 and TAP2 was proven by transfection of defective cell lines with TAP2 and/or TAP1 genes, restoring antigen-presenting activity (12, 124, 149, 150). These observations implicated that MHC class I molecules are unstable under physiological conditions in the absence of appropriately bound peptides and that the majority of binding peptides are provided by a TAP-mediated transport. Moreover, TAP1-deficient mice created by gene targeting exhibit a phenotype consistent with that observed in TAP-defective cell lines. These mice show a severely reduced class I cell surface expression and fail to present cytosolic antigens to cytotoxic T cells (171).

FIG. 2. The location of the TAP genes in the human MHC class II complex (HLA) in a schematic representation. The scale is intended as a rough guide only. Boxes indicate the approximate position of the genes. The figure underneath the schematic location of the genes illustrates the participation of the gene products in peptide generation and transport.
C. Homologies

The sequences of the TAP1 and TAP2 coding regions were determined, and alignment of TAP1 and TAP2 showed the highest homology in a stretch of 200 amino acids near the COOH terminus. This region contains three characteristic motifs. The Walker A (P loop) and Walker B motifs form a highly conserved ATP-binding cassette (ABC). ATP and other nucleotides bind to these sites and are hydrolyzed in a Mg\(^{2+}\)-dependent manner (107, 134, 177). A so-called C loop, which consists of six to eight conserved amino acids, is located in between the Walker A and B motif. There is genetic evidence that generally a so-called “EAA” sequence found in the last cytosolic loop of the membrane-spanning domain of bacterial ABC transporters interacts with the C loop, thereby coupling ATP hydrolysis to the transmembrane spanning domain (27, 32). Due to the sequence homology, TAP1 and TAP2 belong to the superfamily of ABC transporters and, in more detail, to subfamily B. A conserved nucleotide-binding domain (NBD) and a transmembrane domain (TMD) of about six membrane-spanning segments characterize this protein family. A functional unit comprises two NBDs and two TMDs. ABC transporters are found in all domains of life throughout the animal and plant kingdoms, bacteria, and archea (61). They form the largest and most diverse family of membrane-spanning transport proteins.

D. Genomic Organization

The genomic structures of the human and mouse TAP genes have been established (17, 160). As mentioned in section II, the human TAP1 and TAP2 genes are located in the MHC II locus of chromosome 6 and are 8–12 kb in size. They consist of 11 exons (54); 8 of them are of the same length, although the intron sizes vary significantly. The remaining three exons 1, 9, and 11 differ in length by 100, 3, and 78 nucleotides, respectively. All 11 intron/exon boundaries are identical in their classes and follow the GT/AG rule. Sequence comparisons from human to salmon TAP1 showed the expected phylogenetic differences (for example, 98.8% homology of human with gorilla TAP1, 69.2% with hamster, and 40% with salmon). The analysis indicates that the degree of relatedness between human TAPs is similar to that between each gene and its homologs in rodents (66). The homology between TAP1 and TAP2 in all species examined up to now is ~35%, although they share a similar predicted membrane topology. Therefore, it is speculated that the genes have evolved from a common ancestral gene by duplication before the development of the adaptive immune system in vertebrates (16).

E. Polymorphism

TAP1 and TAP2 are polymorphic in all species examined, and there is clear evidence that this polymorphism can affect the substrate specificity of the transporter (56, 101, 121, 122, 180). On the other hand, a functional polymorphism was only demonstrated for rat TAP (112) and for the human TAPiso splice variant (180). The rat TAP from the RT1\(^a\) strain as well as human TAPiso most efficiently translocates peptides with hydrophobic COOH-terminal residues, while TAP from the rat RT1\(^a\) strain is permissive for peptides containing hydrophobic and basic COOH termini (56, 101). Human TAP apparently resembles more closely rat TAP from the RT1\(^a\) strain with a broader specificity, whereas mouse TAP has a higher similarity to rat TAP from the RT1\(^a\) strain. The level of structural polymorphism in human and mouse TAP (an average of 2–5 amino acid substitutions between alleles) (26, 123) is reduced compared with that seen in rat (25 positions of allelic variations in the rat TAP2 gene) (121, 125).

Nonetheless, the TAP genes are polymorphic both in mice and humans. Several alleles of human TAP1 and TAP2 have been identified in different populations (133). This suggests that functional differences between alleles exist, even though initial attempts to demonstrate such functional polymorphism have been unsuccessful (31, 114, 141, 168). On the other hand, data concerning polymorphism of the TAP genes in different human populations point to little, if any, functional significance (69, 75, 99, 133).

III. REGULATION OF THE TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING EXPRESSION

A. Induction of TAP by IFN-\(\gamma\) and Other Factors

MHC class I molecules are expressed at low levels in most cells and are strongly induced by cytokines such as IFN-\(\gamma\) (for a review, see Ref. 42). Increased expression of MHC class I molecules correlates with increased CTL function. Therefore, it was of special interest to study the regulation of TAP gene expression. Both TAP1 and TAP2 mRNAs are upregulated ~10- to 20-fold within 12 h by IFN-\(\gamma\), whereas HLA class I heavy chain mRNA is induced more slowly, increasing only 10-fold in 24 h (13, 94). Although TAP1 and TAP2 proteins are also rapidly induced, HLA class I heavy chains and surface expression increase more slowly, accompanied by an increased peptide transport capacity. Comparable to these data, gene transfer of a cDNA encoding IFN-\(\gamma\) into a human renal carcinoma cell line results in a high constitutive expression of TAP, but allospecific CTL reaction was not better stimulated with IFN-\(\gamma\)-transductants than with unmodified tumor cells (138). In some cases, TNF-\(\alpha\) can lead to an increase in TAP1 levels by in vitro treatment.
of tumor samples (109). Another cytokine, interleukin-10, expressed in a murine tumor cell line, has an opposite effect; a reduction of TAP level was observed (136).

Tumor cells may evade tumor surveillance by acquiring mutations that inhibit antigen processing via MHC class I. Because more than 50% of human tumors have a dysfunctional p53, this mutation may diminish or abrogate the host tumor surveillance. Therefore, the effect of p53 on TAP1 expression was investigated (185). The authors observed that p53 as well as DNA-damaging agents could increase TAP1 levels and subsequent MHC class I cell surface expression, indicating that a dysfunctional p53 in human tumor cells would not induce TAP1 after genotoxic stress. These results suggest that tumor surveillance may be a mechanism by which p53 acts as a tumor suppressor. Furthermore, several breast cancer cell lines showed lower expression levels of TAP mRNA compared with normal breast epithelial cells and a cell cycle-dependent regulation (6).

B. Promoter of the TAP1 Gene

The human TAP genes contain no TATA box motifs in the 5′-flanking sequences but putative GC-rich elements (Sp1 binding sites, 128 nucleotides upstream of the translation start codon for TAP1 and 79 nucleotides upstream in the case of TAP2) (17). Site-directed mutagenesis of the Sp1 binding site leads to a threefold reduction of basal promoter activity of the TAP1 gene (179). The promoter is induced by several IFN-γ-responsive elements and a p53-responsive element, which act independently from each other (185). However, the activity of the p53-responsive element was proven in vivo only in context with a heterologous minimal promoter, and no data are available concerning the significance of this element in the homologous promoter region. It should be noted that the TAP1 gene is coordinately regulated by a bidirectional promoter of 593 bp, also directing the divergently transcribed gene of LMP2, the transcriptional promoter of 593 bp, also directing the divergently from each other (185). However, the activity of the p53-responsive element was proven in vivo only in context with a heterologous minimal promoter, and no data are available concerning the significance of this element in the homologous promoter region. It should be noted that the TAP1 gene is coordinately regulated by a bidirectional promoter of 593 bp, also directing the divergently transcribed gene of LMP2, the p53-dependent regulation (6).

B. The TAP Complex

Immunoelectron and immunofluorescence microscopy analyses have localized TAP1 and TAP2 in the ER and the cis-Golgi (78, 98), retarded by a so far uncharacterized ER retention signal. This subcellular distribution is consistent with the location where MHC molecules bind peptides. The homology with other ABC transporters points to a functional TAP protein either as a homodimer of TAP1 or TAP2 or a heterodimer. Indeed, immunoprecipitations with antisera directed against TAP1 coprecipitated TAP1 and TAP2 (76). Comparisons of the phenotypes of different mutant cell lines suggest, and heterologous coexpression of TAP1 and TAP2 in insect cells and yeast demonstrate, that neither TAP1 nor TAP2 forms a functional homodimer (98, 166). Furthermore, no additional factors of the immune system are required for TAP function. TAP1 and TAP2 are found to be nonglycosylated, although TAP1 has three putative glycosylation sites; however, two are facing the cytosol, and one is placed in an ER loop too short for effective glycosylation (98).

Hydropathy analysis and sequence alignments of TAP proteins with related ABC transporters point to a two × six transmembrane helix model of TAP, extended by additional four and three transmembrane helices predicted for the highly diverse NH2-terminal domain (Fig. 3) (152). Up to now, the function of these NH2-terminal hydrophobic extensions is unclear. As also found for other ABC transporters located in the ER, both TAP molecules lack a known NH2-terminal signal sequence for the import into the ER, indicating that an internal signal sequence may exist. Experiments with truncated proteins suggest that TAP1 and TAP2 contain multiple ER reten-
tion signals in the transmembrane regions, including the first transmembrane segment (176). The hydrophobic transmembrane regions are linked to the nucleotide binding domains, which contain the conserved Walker A and B motifs for ATP-binding and hydrolysis and, based on this model, are located in the cytosol. According to this model, the complex is highly asymmetric with respect to the cytosolic and ER-luminal regions; large cytosolic loops and both NBDs are located in the cytosol, but only a small portion (<10%) reaches toward the ER lumen (152). It was further proposed that the pore-forming domains of the TAP1 and TAP2 heterodimer are aligned in a head-head/tail-tail orientation (175), which however contradicts the TMD organization of P-glycoprotein (92).

Peptides are photo-cross-linked to TAP1 and TAP2, suggesting that both subunits are involved in peptide binding (10, 11). More recently, the peptide-binding region was mapped after peptide photo-cross-linking, digestion of TAP by trypsin and/or bromocyan, and subsequent immunoprecipitation with antibodies directed against different epitopes of TAP (113). This analysis revealed a similar binding region for TAP1 and TAP2, comprising the cytosolic loops between TM4 and TM5 and a COOH-terminal stretch of ~15 amino acids after TM6 (Fig. 3). Deletion of some of these potential peptide-binding sites between residues 366 and 405 of TAP1 resulted in defective peptide transport (130). Due to the topological model of TAP (1, 152), all these regions are expected to be exposed to the cytosol. Recently, a slightly different model of the TAP complex was presented (176). With a set of COOH-terminal deletions for the TAP1 and TAP2 subunits, the topology of TAP was investigated. Eight and seven transmembrane segments were identified for TAP1 and TAP2, respectively. Due to this model, no evidence for membrane integration was obtained for the two hydrophobic regions adjacent to the nucleotide binding domains for both subunits. However, this model is in contrast to the established membrane topology of the ABC transporter P-glycoprotein (92). Because of the discrepancies in the models, further studies are needed to clarify the structural organization and membrane topology of TAP.

V. SUBSTRATE SPECIFICITY OF THE TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING

A. Peptides Optimal for Transport

The characterization of antigenic epitopes is very important for the knowledge about the immunologic response and the development of vaccination strategies. Therefore, the substrate specificity of TAP is well studied. The first results were obtained by trapping transported peptides in the ER via glycosylation (110). The comparison of glycosylated peptides differing in length or amino acid composition provided information about length and sequence preferences of the transported peptides (for review, see Refs. 10, 165). Peptides with a length of 8–16 amino acids bind to TAP with equal affinity (170); translocation into the ER was most efficient for peptides of 8–12 amino acids (83). Transport of peptides with even 40 amino acids was observed, but with lower efficiency. Therefore, TAP transports preferentially peptides with a similar or slightly larger size suitable for MHC class I binding. Another approach to study TAP selectivity was realized determining the equilibrium binding affinity constant (164, 170). Equal binding affinities were found for peptides ranging from 8 to 16 amino acids (170). The only discrepancies exist in the absolute values between binding affinity and transport efficiency (10, 164, 169). The glycosylation method includes several side reactions such as peptide transport, glycosylation, degradation, and export and, therefore, does not necessarily reflect transport rates. Thus the binding assay seems to be more accurate for the analysis of substrate specificity.
The contribution of each peptide residue to the affinity for TAP was determined systemically by screening combinatorial peptide libraries (163) (Fig. 4). With this method, the average affinity of a randomized peptide mixture with one defined residue is compared with a totally randomized peptide library. Therefore, the influence of each peptide residue was analyzed independent of a given sequence context on the affinity of TAP. The effect of each amino acid residue on the stabilization of peptide binding to TAP was determined. The strongest differences were observed for the COOH-terminal amino acid and the first three NH$_2$-terminal residues. The amino acids in between do not significantly contribute to substrate specificity of TAP but are necessary for recognition of a MHC class I-bound peptide by the T-cell receptor. This binding motif combines two advantages: 1) maximal diversity of residues 5–8 in the antigenic peptide where T-cell receptor recognition and no selection by TAP occurs, and 2) maximal binding affinity at the termini for TAP. The matched preferences of human TAP and MHC class I suggest a coevolution of the genes (165) as it was also proposed for rat TAP and MHC class I molecules (72).

B. Polymorphism and Substrate Specificity of TAP

The strongest differences in peptide binding affinity to TAP were detected at the COOH terminus of peptides (101, 103, 141, 163). As mentioned in section II, a limited degree of sequence polymorphism has been found for human and mouse TAP genes by restriction length polymorphism and sequence analysis, but no measurable alteration in function of these gene products was observed (133, 141). However, it has been shown that in rats, TAP polymorphism has an influence on peptide selectivity. TAP from the rat strain RT1$^a$ as well as human TAP translocates peptides with a broad specificity (hydrophobic or basic amino acids at the COOH terminus), whereas TAP from the rat strain RT1$^u$ and mouse TAP prefers peptides with hydrophobic COOH termini. This pattern correlates with the predominant peptide binding profiles of human and mouse MHC class I molecules.

Although human TAP genes are less allelically diverse than rat genes, another possibility to generate different substrate specificity in the human transporter was recently reported (180). An alternative splicing variant of the human TAP2 gene was described termed TAP2iso. It lacks exon 11, a part of the coding region and the original 3'-untranslated region, but contains the newly identified exon 12. TAP2iso mRNA was normally coexpressed with TAP2 mRNA in all human lymphocyte cell lines examined. TAP1-TAP2iso transporters exhibited distinct and opposing influences on peptide selectivity, more resembling the TAP complex from rat strain RT1$^a$ and mouse TAP. The common expression of an alternative splice product of the TAP2 gene may contribute to a broadened immune diversity.

VI. TRANSPORT MECHANISM OF THE TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING

A. Binding of Peptides

Peptide transport by TAP is a multistep process (170). The peptide-binding pocket is formed by TAP1 and
TAP2. Peptides associate with TAP in an ATP-independent manner at low temperatures (164). So far, no indication for a second binding site was found by direct peptide binding or competition assays as well as by photo-cross-linking experiments. With the use of fluorescence-labeled peptides, the association pathway to TAP could be kinetically dissected in real time (112). In a fast bimolecular association step, peptide binds to TAP, followed by a slow isomerization of the TAP complex. It is suggested that this structural reorganization of the molecule triggers ATP hydrolysis and peptide translocation across the membrane. The binding step determines the selectivity of TAP (112). The conformational change of TAP also influences its lateral mobility, shown by tagging the TAP1 subunit with green fluorescent protein (127). The mobility of the TAP complex increases when it is inactive and decreases during peptide translocation.

B. Translocation of Peptides

The translocation strictly requires hydrolysis of ATP, because nonhydrolyzable ATP analogs do not promote peptide transport. In addition to ATP, UTP, CTP, and GTP (9) can energize peptide translocation. Direct binding of nucleotides was demonstrated by 8-azido-ATP photo-cross-linking experiments (107, 134, 176). ATP and ADP have similar affinities for TAP; therefore, ADP and other nucleoside diphosphates competing for ATP binding inhibit peptide translocation. The ATPase activity of TAP is substrate specific and tightly coupled to peptide binding, indicating that peptide binding is a prerequisite for ATP hydrolysis, thereby possibly preventing waste of ATP without transport of peptides (53). ATP hydrolysis follows Michaelis-Menten kinetics with a turnover number $k_{\text{cat}} \approx 5$ ATP/s. This turnover rate is sufficient to account for the essential role of TAP in the overall process of antigen processing. Interestingly, sterically restricted peptides that bind to but that are not transported by TAP do not stimulate the ATPase activity of TAP (53). Both nucleotide-binding domains of TAP interact with ATP, even if expressed separately (107, 176). A structural rearrangement of the NBDs by peptide binding seems to function as a molecular switch to activate the ATPase of TAP, since the NBDs alone are unable to hydrolyze ATP (107, 177). It is known that both NBDs are essential for TAP function, since mutation of one NBD leads to a loss of transport action (25). It may be that hydrolysis at one NBD starts the peptide transport, whereas hydrolysis at the second NBD is necessary for completion of the translocation cycle and perhaps for the reconversion of the initial substrate-binding site. A model of the peptide translocation by the TAP complex is presented in Figure 5. Lysine mutation of the Walker A motif of TAP1 and TAP2 points to distinct functional properties of both NBDs (84, 137). Even if the data concerning nucleotide and peptide binding after mutation of human TAP1 or TAP2 are somehow contradictory, it seems that ATP binding to both subunits is tightly regulated and transport cycle dependent. Results with glycine mutations in the Walker A motif of rat TAP1 or TAP2 indicate that binding of ATP to TAP1 is the first step in energizing the transport cycle and induces ATP binding to TAP2 (4). Future investigations are needed to clarify these conflicting observations.

TAP only transports peptides longer than 7 residues; the efficiency drops off with peptides longer than 12 amino acids, but there is no clear limit (9, 22, 101, 145). A complicating factor for analyzing TAP-dependent transport is the presence of an efficient ATP-dependent peptide export system out of the ER and into the cytoplasm (82, 132, 140). Peptides with low affinities may not be rapidly bound to the MHC class I molecules and are therefore available for export; this may be another mechanism to select for high-affinity peptides binding to the MHC class I molecules.

C. Protein Machinery for Peptide Recognition, Transport, and Loading of MHC Class I Molecules

The view that TAP plays an important role in class I assembly derives from the observation that it not only
delivers peptides to the ER, but also is involved in the formation of a large loading complex critical for class I maturation (Fig. 6). A number of other proteins are required for the assembly and loading of MHC class I molecules, and several chaperone-like proteins have been identified (for review, see Refs. 29, 87). At least four proteins, calnexin, calreticulin, ERp57, and tapasin, are involved in the assembly of heavy chain and β2-microglobulin. The dominant chaperones associated with newly synthesized class I heavy chains are calnexin and calreticulin (67, 135). Calreticulin is a soluble homolog to calnexin and shares its specificity for monoglycosylated N-linked glycans. The mechanism how calnexin/calreticulin facilitates folding is not understood, but it is suggested to be involved in a binding and release cycle. If the association of these chaperones is disturbed by glucosidase inhibitors, the folding and subsequent surface expression of MHC class I molecules are dramatically reduced (153, 172). An additional housekeeping molecule, called ERp57, is part of the large multisubunit TAP-tapasin-MHC complex (67, 90, 105). ERp57 is a member of the thioredoxin family of enzymes and also binds to the complex in a very early step of the maturation, probably supporting the correct formation of disulfide bridges.

The MHC class I molecules interact with the TAP complex via an ER-resident type I glycoprotein, named tapasin for TAP-associated glycoprotein (88, 115). Tapasin is a transmembrane protein that, like MHC molecules, is a member of the immunoglobulin superfamily. It has an apparent molecular mass of 48 kDa. Tapasin mediates complex formation and the cross-talk of structural information of MHC and TAP and is therefore important for class I assembly and editing (135). In its absence, the assembly in the ER is impaired, and MHC class I surface expression is reduced. Tapasin has two independent functions: 1) it increases the level of TAP, thereby increasing the efficiency of peptide transport, and 2) it associates with MHC class I molecules, thereby facilitating directly loading and assembly of class I molecules (14, 86). Approximately four MHC class I molecules seem to be linked via four tapasin to one TAP complex (115). Truncated tapasin missing the transmembrane region and the cytosolic tail appears to be not associated with TAP, but still rescues MHC class I expression (86).

Peptide binding to MHC class I molecules is a prerequisite for dissociation of TAP-MHC complexes. This peptide-mediated detachment depends on conformational signals from the TAP complex induced by ATP binding (80). Recent data suggest that release of peptide-loaded MHC class I molecules is predominantly dependent on the conformation of TAP1 (4). Therefore, the dynamic activity of the TAP-MHC class I complex is synchronized with the peptide binding and translocation cycle of TAP.

VII. HUMAN DISEASES ASSOCIATED WITH THE TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING

A. Viral Infections and Virus Persistence

CTL recognize and eliminate virus-infected cells. This requires the presentation of viral peptide fragments by MHC class I molecules at the cell surface. Persistent viruses, such as the large DNA-containing herpes viruses, have been forced to evolve different strategies to evade the immune defense, which means especially by interfering with antigen processing and presentation (for review, see Refs. 59, 120).

The immediate early protein ICP47 encoded by herpes simplex virus type 1 (HSV-1) was identified to be responsible for the downregulation of the MHC class I surface expression in human fibroblasts (183). Therefore, HSV-infected cells are masked for immune recognition by CTL. ICP47 inhibits the TAP-mediated peptide transport into the ER by binding to the TAP complex (41, 62). It was demonstrated that ICP47 binds with high affinity at least in part to the peptide-binding site of TAP, thereby blocking the first and essential step in the translocation pathway (3, 156). Recently, a similar downregulation of peptide transport activity was observed in bovine epithelial cells infected with bovine herpesvirus-1 (64). The interaction of human ICP47 and the TAP complex is species specific, since ICP47 has a 100-fold higher affinity for human than for murine TAP (3, 156). Meanwhile, the critical amino acids of ICP47, which are necessary to inhibit TAP function, have been identified (111, 159). These amino acids comprise the NH2-terminal region of ICP47 that is responsible for the binding to TAP. The interaction of ICP47 with TAP requires a footprint of approximately 30 amino acids on TAP that overlaps with the peptide-binding site.

FIG. 6. The macromolecular transport and chaperone complex. The de novo-synthesized MHC-encoded heavy chains (left) assemble with calnexin and β2-microglobulin. After binding to ERp57 and calreticulin, the MHC class I molecules can associate via tapasin to the TAP complex. Peptides are translocated into the lumen of the ER by TAP and loaded onto the MHC class I complex, which leaves the ER to the cell surface.
from residue 3 to 34 (111). This active domain of ICP47 appears to be mainly unstructured in aqueous solution (18). In the presence of lipidlike environment, an α-helical structure is induced, which is composed of two helical regions extending from residues 4–15 and 22–32 connected by a flexible loop (18, 119). On the basis of these results, therapeutic drugs could be designed that are potent immune suppressors or that are applicable in novel vaccination strategies against HSV, restoring the ability of the immune system to recognize the infected cells.

Human cytomegalovirus (HCMV) encodes at least four proteins that decrease the cell surface expression of MHC class I molecules (for review, see Ref. 59). An ER-resident glycoprotein, called gpUS6, inhibits the TAP-mediated peptide transport. The mechanism is probably due to binding of gpUS6 to the ER-luminal part of the TAP complex (2, 58, 85). Peptide binding is not affected by gpUS6; also, the association of tapasin, calreticulin, and MHC class I molecules to TAP appears normal. Recent findings indicate that gpUS6 binds to TAP and stabilizes a TAP1 conformation, which is unable to bind ATP, and subsequently, peptide translocation is inhibited (60). Overexpression of TAP with IFN-γ induction overrides the inhibition of peptide transport by gpUS6, but there are other proteins of HCMV, expressed in the early phase of infection, which interfere in a different way with the MHC class I pathway of antigen presentation (57; for a review, see Ref. 178).

Another herpes virus that hampers the MHC class I immune response is the porcine pseudorabies virus (PrV). It causes a highly infectious disease of pigs and can exist in a latent form in several tissues, but mainly in the trigeminal ganglia. PrV infects epithelial cells of mucosal surfaces and spreads by cell-to-cell contact. It could be shown that inhibition of peptide transport activity of the TAP complex is one of the mechanisms by which an early PrV protein downregulates MHC class I expression (7).

Two viruses that do not belong to the herpes virus family but interfere with the MHC class I pathway and TAP function are the adenovirus and the human papilloma virus (HPV) (20, 167). Adenoviruses encode a protein, E3/19K, that directly binds MHC class I molecules, trapping them in the ER (20). Moreover, E3/19K seems to bind to TAP and inhibits the tapasin action, thereby preventing MHC class I/TAP association (20). HPV causes recurrent papillomatosis that is characterized by a variable clinical course and that can include significant morbidity and occasional mortality. Complete eradication of HPV is rare with repeated surgical excision. The knowledge about the mechanism responsible for the persistence of latent HPV would be valuable for treatment. In laryngeal papilloma tissue biopsies and in cell culture of primary explants, a downregulation of TAP1 was observed (167). More significantly, the amount of TAP1 protein expression correlated inversely with the frequency of disease recurrence. These findings suggest that HPV may evade immune recognition by downregulating MHC class I cell surface expression via decreased TAP1 levels. The expression of TAP1 could be used for prognostic evaluation of disease severity. Moreover, a similar mechanism with decreased TAP1 and MHC class I protein was described in other malignancies induced by HPV, for example, HPV-16- and HPV-18-infected carcinomas of the cervix (30). Similar to HPV, the Epstein-Barr virus (EBV) has evolved a strategy to avoid immune surveillance by downregulation of TAP1 (184). EBV expresses a protein, vIL-10, that is similar in sequence to human interleukin-10. vIL-10 downregulates the expression of the TAP1 gene, but not of the TAP2 gene, thereby affecting the transport of peptides into the ER. This mechanism might contribute to the persistence of EBV-infected cells in the human host.

B. Genetic Diseases

There is little knowledge about human genetic TAP defects. Bare lymphocyte syndrome (BLS) is known to be characterized by a severe downregulation or deficiency of MHC class I and/or MHC class II molecules. In type 1 BLS, the defects are assigned to the MHC class I, whereas in type 2, the MHC class II molecules are affected. The characterization of 22 patients with type 1 BLS revealed the existence of 3 clinically and immunologically distinct subsets (for review, see Ref. 45). Two groups include patients with a defective transcription of MHC class I molecules and β2-microglobulin. The third group is the best-characterized subset of type 1 BLS and comprises 15 patients from different families. The reduced MHC class I cell surface expression manifests with necrotizing granulomatous skin lesions and recurrent bacterial infections. Tissue typing of 10 patients indicated that a defect TAP complex is responsible for the downregulation of MHC class I molecules. Studies of two families revealed that the disease is caused by deficiency of TAP2, which is mainly due to a premature stop codon resulting in a nonfunctional TAP complex (33, 154). The homozygous TAP2+/− siblings of one family have reduced expression of MHC class I molecules on the cell surface and reduced amounts of CTL. On the other hand, these people do not show an increased susceptibility to viral infections. A fraction of antigenic peptides seems to be transported into the ER lumen in a TAP-independent manner, although TAP appears to be the dominant pathway under normal conditions (34).

Five adults with a syndrome resembling Wegener’s granulomatosis were also found to exhibit low surface expression of MHC class I molecules and defective expression of the TAP complex (100). In two patients, a mutation in the TAP2 gene was identified, responsible for this defective expression of the TAP complex.
In genetic diseases, not only TAP2, but also TAP1 may be involved. In one patient suffering type 1 BLS, a splice site mutation in the TAP1 gene was described (44). Interestingly, TAP2 protein was undetectable. Two other patients, displaying not only the typical lung syndrome of MHC class I deficiency but also skin lesions, were found to be deficient of TAP1 (35). In both cases, mutations introducing a frame shift into the first third of the coding sequence were observed. The syndromes associated with TAP1 and TAP2 deficiency are similar and are characterized by the development of chronic inflammation of the respiratory tract in late childhood and vasculitis at variable stages, but deficiency of TAP is compatible with life. In later stages, recurrent bacterial infections may lead to progressive lung damage, respiratory failure, and death. Early recognition, treatment of the respiratory infections, and cleansing of skin ulcers of TAP-deficient patients are the major aims of therapy. Surgical intervention for chronic sinusitis seems to promote progression of nasal disease leading to bronchial infections; immunosuppressive and immunomodulatory treatments are associated with progression of skin lesions (45). Due to the ubiquitous tissue distribution of MHC class I molecules, a gene therapy of TAP-deficiency syndrome may not be practicable.

C. Autoimmune Disease and Transplantation

The participation of TAP in autoimmune diseases is discussed controversially. For several patients with diverse MHC-linked autoimmune diseases, such as type I diabetes, Sjogren’s syndrome, Graves’ disease, and Hashimoto’s disease, a downregulation of mRNA levels for TAP1 and TAP2 was found in varying degrees (43). These data suggest that defective transcription of TAP genes can contribute to reduced MHC class I cell surface expression in autoimmune diseases. Cells lacking MHC class I molecules might then become targets for natural killer cells. Recently, the regulation of TAP by phosphorylation was examined (89). Phosphorylated TAP complexes can bind ATP and peptides but are not capable of transporting peptides. Several TAP polymorphic residues are potential phosphorylation sites. Therefore, it was suggested that abnormal TAP phosphorylation leads to cytotoxic immune responses and subsequent development of certain autoimmune diseases, which are known to have associations with TAP polymorphism.

MHC class I molecules are not only critical mediators in host defense against intracellular pathogens, but also can stimulate strong alloreactions against transplanted tissue. The incidence and severity of acute rejection after renal transplantations seem to be influenced by TAP2 gene polymorphism (81). It appears that donor’s antigen presenting cells with the TAP2*0103 allele have attenuated efficacy in the presentation of allospecific antigens to the recipient’s T cells, but further investigations are necessary to evaluate the significance of the TAP complex in rejection after transplantations.

D. TAP Deficiency and Tumor Development

Many tumors escape recognition by CTLs. In some cancers, including melanomas, this has been associated with ineffective antigen processing and presentation of tumor-specific peptides because of low levels of MHC class I molecules (144, 146). In tumor cell lines from breast cancer (5) or human small cell lung cancer (144), low to nondetectable levels of TAP1 and/or TAP2 mRNA were found, which can be restored by IFN-γ. In murine as well as human cancers, a downregulation of TAP1 expression by an unknown mechanism or a mutation of TAP resulting in a loss or decrease in class I surface expression has been demonstrated (25, 128, 143). In some malignant tumor cells, the reduced levels of TAP mRNA could be restored by IFN-γ stimulation (143). One type of human tumor characterized by more than one group of investigators is breast carcinomas. TAP1 downregulation was found in 13 and 44% of the lesions (73, 174). The variations in the results may be determined by characteristics of the patients, the tumor type, or the proliferative status. The frequency of TAP2 downregulation correlates with that of TAP1 but was rarely examined. The mRNA levels of TAP1 and TAP2 can be restored by treatment with IFN-γ. Recently, it was observed in metastatic melanoma cells that, despite an increase in TAP1 and TAP2 mRNA levels by IFN-γ, a similar increase in cytotoxicity did not occur (108).

The downregulation of TAP in several tumor cell lines can be corrected by transfection with the wild-type form or the treatment with several cytokines, such as IFN-γ and TNF-α. The phenotypic changes are accompanied by an increase in MHC class I expression. Recently, in a small cell lung carcinoma cell line the potential of providing exogenous TAP in cancer therapies was tested (5). With vaccinia virus containing the TAP1 gene, it could be shown that this virus could reduce tumors in mice and, therefore, TAP should be considered for inclusion in cancer therapies. In contrast to the above-mentioned cytokines, interleukin-10 leads to TAP downregulation and subsequently to a reduced cell surface expression of class I antigens (184). This fact may be of clinical relevance, since a large number of human tumors secrete interleukin-10.

Downregulation of TAP expression and, thereby, loss of surface HLA class I molecules, can be a tumor escape mechanism, but also mutations in the TAP genes, as shown for example for TAP1 in a small cell lung cancer (25). This mutation introduces an amino acid exchange at position 659 in TAP1, resulting in a nonfunctional TAP1...
protein. MHC class I molecule expression on the cell surface could be restored by expression of wild-type TAP1 or addition of exogenous peptides. The mutation of TAP1 is located between the C loop and the Walker B motif, indicating that this mutation might affect ATP binding or hydrolysis. Interestingly, mutations that affect TAP1 occur frequently in a variety of human tumors, for example, in primary breast cancer (73, 174). The expression of TAP1 in carcinoma lesions was significantly associated with tumor grading and reduced survival. Like normal mammary tissue, low-grade breast carcinoma lesions showed no reduced TAP1 protein (174). A similar situation was found for colorectal carcinomas (74), whereas adenomas and normal mucose tissue always exhibit no loss of TAP1.

Even if some studies correlate the presence of deficiencies in the MHC class I antigen processing pathway with tumor progression (73, 144, 174), it is still unclear whether these deficits have any consequence for tumor development. To clarify this point, matched panels of TAP1-positive and TAP1-negative tumor cell lines were generated (71). Inoculation of mice with TAP1-negative cells produced large and persistent tumors, whereas in contrast, TAP1-positive cells did not generate lasting tumors. In athymic mice, both cell lines produced tumors, confirming that TAP-dependent differences in tumorigenicity were due to T cell-dependent immune responses. It can be concluded that loss of TAP function allows some tumor cells to escape immune recognition.

There is evidence that downregulation of MHC class I cell surface expression is associated with malignant transformation, and each step in the antigen-processing pathway can be affected. The target and frequency of the mutations vary markedly among different types of tumors and, thereby, the clinical impact of such deficiencies will vary from one type of tumor to another and needs further investigations.

VIII. CONCLUDING REMARKS

The TAP peptide transporters are a key link between antigen processing and presentation. The evaluation of its molecular structure and function will contribute to the understanding of (patho)physiological processes in which the TAP complex may be involved. However, many questions remain open to fully understand the whole process of T-cell immunity, especially regarding the mechanisms governing peptide binding to MHC class I molecules in the ER. On one level, the process seems to represent a complex folding event; on another level it is a highly regulated system ensuring the surface expression of high-affinity MHC class I-peptide complexes. These complexes are essential for CD8+ T cell-mediated immunity. The elucidation of all steps in the antigen presentation pathway is essential for many new therapeutic approaches in different diseases. Knowledge about the molecular mechanisms of how viruses evade the immune defense will identify novel pathogenic factors and will probably allow the development of new vaccination strategies. Moreover, studies have revealed that loss of TAP function occurs more often in metastatic than in primary tumors. The ability of tumor cells to evade T-cell responses has important implications for the understanding of tumorigenesis. The potential efficacy of immunotherapy for cancer is dependent on our knowledge of the underlying mechanisms. If mutations in TAP function allow tumor cells to escape immune surveillance, this may have implications in the therapeutic strategies. Further analysis will clarify the interaction of malignant cells with the immune system of the host and the significance of the loss of TAP function.

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Address for reprint requests and other correspondence: R. Tampé, Institut für Biochemie, Biozentrum Frankfurt, Goethe-Universität Frankfurt, Marie-Curie-Str. 9, D-60439 Frankfurt a.M., Germany (E-mail: tampe@em.uni-frankfurt.de).

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