Regulation of Transporter Associated with Antigen Processing by Phosphorylation*

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The ATP-binding cassette transporter associated with antigen processing (TAP) is required for transport of antigenic peptides, generated by proteasome complexes in the cytoplasm, into the lumen of the endoplasmic reticulum where assembly with major histocompatibility complex class I molecules takes place. The TAP transporter is a heterodimer of TAP1 and TAP2. Here we show that both TAP1 and TAP2 are phosphorylated under physiological conditions. Phosphorylation induces formation of high molecular weight TAP complexes that contain TAP1, TAP2, tapasin, and class I heterodimers. In addition, a 43-kDa phosphoprotein, which appears to be a kinase, is contained in the phosphorylated TAP-containing complexes. Phosphorylated TAP complexes are able to bind peptides and ATP; however, they are not capable of transporting peptides. After de-phosphorylation, TAP complexes regain the ability to transport peptides. Interestingly, phosphorylation levels of TAP complexes induced by viral infection inversely correlates with a significant reduction in TAP-dependent peptide transport activity. Enhanced TAP phosphorylation appears to be one of several strategies that viruses have exploited to better escape from host immune surveillance. These results demonstrate that major histocompatibility complex class I antigen processing and presentation is modulated by reversible TAP phosphorylation, and implicate the importance of TAP phosphorylation in the regulation of cytotoxic immune response.

A major function of the immune system is to detect and eliminate the cellular host of invading pathogens (1). Viral-infected cells are identified through the major histocompatibility complex (MHC)† class I molecules that sample the viral antigens synthesized within cells (1–3). Intracellular viral antigens are first ubiquitinylated by the ubiquitination system (4) and then degraded by the multisubunit and multicatalytic protease complexes termed proteasomes (1, 5). The degradation products, 8–10 amino acid peptides, are then translocated by the transporter associated with antigen processing (TAP) across the membrane of the endoplasmic reticulum where they bind to MHC class I molecules stabilizing the assembly of a transmembrane class I heavy chain with a soluble β2-microglobulin. Only after the assembly of the trimolecular complex can these complexes be transported to the cell surface, via the exocytic pathway, for inspection by CD8+ cytotoxic T lymphocytes. The TAP transporter is a heterodimer composed of TAP1 and TAP2 (1, 6), which are encoded in the MHC region and belong to the superfamily of ATP-binding cassette transporters (7) or traffic ATPases (8). The interaction between TAP transporters and MHC class I heterodimers requires the assistance of a MHC-encoded chaperone, tapasin (9, 10). The formation of TAP-class I complexes provides an efficient and effective means to load antigenic peptides from TAP transporters onto MHC class I molecules (1, 11). However, the molecular and biochemical nature of the assembly of TAP-containing complexes as well as the biological importance of the functional regulation of TAP complexes in MHC class I antigen processing and presentation remains to be addressed.

It has previously been observed that cells treated with the phosphatase inhibitor, okadaic acid (OK), display a reduction of surface expression of MHC class I molecules (12), implying a role for phosphorylation in the regulation of MHC class I antigen processing and presentation. Because TAP transporters and proteasomes are known to play essential roles in supplying antigenic peptides to MHC class I molecules (1, 13), they are mostly likely to be targeted by cellular phosphorylation machinery to regulate MHC class I antigen presentation. Although several proteasome subunits have been shown to be phosphorylated (1, 14, 15), phosphorylation does not seem to affect proteasomal generation of class I peptides. On the other hand, it has been well documented that phosphorylation affects the function of members of ATP-binding cassette transporter superfamily (16–19). Interestingly, the cytoplasmic domains of TAP transporters contain several conserved tyrosine, threonine, and serine residues that are potential phosphorylation sites (6, 20). To test the hypothesis that MHC class I antigen presentation is modulated by the phosphorylation of TAP complexes, we performed a systematic analysis to determine the effect of cellular phosphorylation on the formation and function of TAP complexes as well as on the expression of MHC class I molecules. We provide convincing evidence demonstrating that TAP function is reversibly regulated by phosphorylation and suggest that by interfering with antigenic peptide transport, TAP phosphorylation appears to play an important role in developing autoimmune diseases.

EXPERIMENTAL PROCEDURES

Antibodies and Cells—Antibodies Y3 (21), B22 (21), K270 (22), TAP1 (23, 24), TAP2 (23, 24), tapasin (10), and U56 (25) were titrated and saturating amount of antibodies were used in all experiments. Human foreskin fibroblasts, HeLa, and RMA (23) cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. To increase the expression levels of TAP, cells were cultured for 2–3 days

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† The abbreviations used are: MHC, major histocompatibility complex; HCMV, human cytomegalovirus; OK, okadaic acid; TAP, transporter associated with antigen processing.
in the presence of 2000 or 500 units/ml of human or mouse interferon γ, respectively. Human and murine interferon γ were obtained from Roche Molecular Biochemicals.

**Metabolic Labeling, Immunoprecipitation, Gel Filtration, and Fast Protein Liquid Chromatography**—Metabolic labeling of cells was carried out as described (28). Where necessary, cells were infected with interferon γ, labeled, and subsequently chased in the presence of culture medium for the times indicated in the individual experiments. Cells were solubilized either in 1% Nonidet P-40 in phosphate-buffered saline or 1% digitonin in a buffer (150 mM NaCl and 10 mM Tris, pH 7.8) containing a mixture of phosphatase inhibitors (2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 0.4 mM EDTA, and 10 mM NaF) and protease inhibitors (2 μg/ml aprotinin, 2 μg/ml leupeptin, 2 μg/ml a-antitrypsin, and 1 mM phenylmethylsulfonyl fluoride). Immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and fluorography were performed as described (27). [35S]Metionine- or [32P]orthophosphate-labeled cells were lysed in 1% digitonin lysis buffer. 200 μl of the resulting cell lysates was fractionated by using a gel filtration column (Superose 6, Amersham Pharmacia Biotech) and 0.25 ml/min/fraction was collected (22). Each fraction was divided equally into two aliquots, immunoprecipitated with appropriate antibodies, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Low and high molecular weight gel filtration calibration kits (Amersham Pharmacia Biotech) were used to calibrate the column (22).

**Viral Infection**—Human foreskin fibroblasts were cultured for 48 h in the presence of 2000 units/ml interferon γ and subjected to infection with the Towne strain of human cytomegalovirus (HCMV) at a multiplicity of infection of 20 for 1 h in serum-free medium (28). After washing with phosphate-buffered saline, the cells were cultured either for an additional 96 h and used for microsome preparation, or for 1 additional hour and subjected to starvation for 1 h followed by a 4-h labeling in phosphate-free medium containing 2 mCi/ml [32P]orthophosphate, and an additional 0.5-h incubation with or without 1 μM OK thereafter. The cells were then lysed, immunoprecipitations were performed, and the resulting immunoprecipitated materials were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**In Vitro Kinase and Peptide Transport Assays**—After a 48-h induction by interferon γ, cells were cultured in the presence or absence of OK for an additional 2 h, and lysed in a buffer (150 mM NaCl, 10 mM Tris/HC1, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 2 μg/ml a-antitrypsin, and 1 mM phenylmethylsulfonyl fluoride) containing 1% digitonin. Anti-TAP1 immunoprecipitates were washed 4 times with the lysis buffer and subsequently washed twice with a kinase buffer (10 mM MnCl2, 10 mM MgCl2, 25 mM Tris, 1 mM dithiothreitol, 2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 0.4 mM EDTA, and 10 mM NaF). The resulting immunoprecipitates were then incubated for 25 min at 30 °C in 5 μl of kinase buffer and 50 μCi of [γ-32P]ATP. For microsome preparation, cells were treated with or without OK at 0.1, 1, or 10 μM for 8 h and live cells were collected using Histopak 1080 (Sigma). Crude microsomes that were pretreated with 25 units/ml of a protein phosphatase inhibitor and subsequently washed twice with a kinase buffer (10 mM MnCl2, 10 mM MgCl2, 25 mM Tris, 1 mM dithiothreitol, 2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 0.4 mM EDTA, and 10 mM NaF) and an additional 0.5-h incubation with or without 1 μM OK, were used as a control. The microsomes were purified, adjusted to A280 = 60/μl for protein content, and used with a fluoresceinlabeled reporter peptide (fluorescein isothiocyanate-labeled RYNATRGL) in a peptide transport assay (29).

**RESULTS**

**TAP Transporter Is Phosphorylated**—To examine whether TAP1 or TAP2 is phosphorylated, we performed immunoprecipitation analysis of TAP complexes using [32P]orthophosphate-labeled murine RMA or human HeLa cells. As shown in Fig. 1a, both murine and human TAP1 and TAP2 were phosphorylated, suggesting that TAP phosphorylation takes place physiologically. The extent of TAP phosphorylation increased 5-fold when the cells were treated with OK, a serine/threonine phosphatase inhibitor. However, the changes in TAP phosphorylation were not detected with the use of several kinase inhibitors. The finding that the levels of TAP phosphorylation can be preserved and increased in the presence of OK suggests that the de-phosphorylation of TAP by cellular phosphatases takes place rapidly and physiologically. Because the state of cellular protein phosphorylation is known to be affected by viral infection (30, 31), we investigated whether virus-mediated changes in cellular phosphorylation affect the levels of TAP phosphorylation. Compared with mock-infected cells, the phosphorylation level of TAP in HCMV-infected cells increased 4-fold (Fig. 1b). When HCMV-infected cells were treated with OK, the extent of TAP phosphorylation increased by an additional 4-fold. These results suggest that viral infection leads to an increase in TAP phosphorylation. Immunoblot analysis of anti-TAP immunoprecipitates with anti-phosphotyrosine, -serine, or -threonine antibodies demonstrated that both TAP1 and TAP2 were phosphorylated at the serine, threonine, and tyrosine residues, in order of decreasing intensity, indicating that several types of kinases are responsible for TAP phosphorylation. It is conceivable that TAP phosphorylation might influence TAP dimerization, association with class I heterodimers, ATP binding, peptide binding, or peptide transport, resulting in changes in the surface expression levels of MHC class I molecules and affecting the recognition of antigen-presenting cells by CD8+ T lymphocytes.

**MHC Class I Surface Expression Is Abrogated by Phosphorylation**—TAP supplies peptides to MHC class I molecules and promotes MHC class I surface expression (1, 13, 32). If TAP phosphorylation interferes with TAP functions, changes in the surface expression levels of MHC class I molecules are most likely to take place. To investigate whether increased cellular phosphorylation, which leads to an increase in TAP phosphorylation, results in a reduction of MHC class I surface expression, we determined the surface expression levels of class I molecules in cells treated with or without kinase inhibitors or phosphatase inhibitors by flow cytometry. Treatment of cells with several kinase inhibitors did not alter the surface expression levels of MHC class I molecules. When cells were treated with phosphatase inhibitors, either OK or calyculin A, surface expression levels of MHC class I molecules, Db or Kb, decreased by −10- or −20-fold, respectively, compared with the untreated cells (Fig. 2). The surface expression of MHC class I molecules in OK-treated cells was restored upon removal of OK. These results suggest that increased cellular phosphorylation abrogates MHC class I surface expression and that OK-induced cellular phosphorylation does not permanently impair biosynthesis and intracellular transport of MHC class I molecules.

**To firmly rule out the possibility that the observed reduction in MHC class I surface expression is due to an inhibitory effect of OK on the MHC class I biosynthesis, we performed metabolic labeling and pulse-chase experiments under conditions that cells were treated with or without OK. As shown in Fig. 3, the amount of newly synthesized class I molecules in OK-treated

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2 Y. Li and Y. Yang, unpublished observations.
cells was equivalent to those in untreated cells, suggesting that under the conditions used the biogenesis of class I molecules is not affected by OK or by increased cellular phosphorylation. However, these MHC class I molecules were retained in the endoplasmic reticulum of the OK-treated cells as revealed by their faster electrophoretic mobilities as well as their sensitivity to endoglycosidase H digestion (upper panel of Fig. 3). Furthermore, by using a class I thermostability assay (33, 34) we determined that these endoplasmic reticulum-retained MHC class I molecules were "empty," indicating they were void of class I-binding peptides. To examine whether the endoplasmic reticulum-retained MHC class I molecules can be transported intracellularly upon removal of OK, we analyzed the transport kinetics of MHC class I molecules in OK-treated cells by removing the phosphatase inhibitor during the course of intracellular transport. After removal of OK, the endoplasmic reticulum-retained MHC class I molecules regained the ability to be transported to the cell surface and became resistant to endoglycosidase H digestion (lower panel of Fig. 3). Because these MHC class I molecules become transport-competent once the cellular de-phosphorylation event is allowed to take place, the accumulation of empty class I molecules in the endoplasmic reticulum is most likely due to a limited supply of class I-binding peptides imposed by TAP phosphorylation (13).

Formation of High Molecular Weight TAP Complexes Is Induced by Phosphorylation—To examine whether the observed increase in TAP phosphorylation interferes with TAP dimerization and/or association with class I heterodimers, we analyzed the assembly of TAP-containing complexes by gel filtration and co-immunoprecipitation using [35S]methionine (Fig. 4a) or [32P]orthophosphate (Fig. 4b) labeled cells that were cultured in the presence or absence of OK. Regardless of whether the cells were treated with OK or not, the contents of TAP complexes, which contain TAP1, TAP2, tapasin, and empty class I heterodimers, were almost identical in [35S]methionine-labeled cells (Fig. 4a). Densitometric analysis of the amounts of TAP complexes present in the fractions (Fig. 4c) revealed that, while the fraction of TAP complexes in untreated cells peaked at fraction 45 (upper panel of Fig. 4a), the peak fraction was reproducibly shifted to fraction 45 in OK-treated cells (middle panel of Fig. 4a), indicating that formation of high molecular weight TAP complexes is induced when the level of cellular phosphorylation increases. Interestingly, a 43-kDa polypeptide, which was barely detected at fraction 45 in untreated samples, seemed to be preserved in TAP-containing complexes of the OK-treated cells (arrows, Fig. 4a). When an aliquot of the same OK-treated cell lysates was treated with a protein phosphatase and then subjected to a gel filtration analysis, we found that the peak fraction of TAP-containing complexes was shifted back to fraction 49 (lower panel of Fig. 4a). These results demonstrate that dynamic assembly and disassembly of high molecular weight TAP complexes is reversibly regulated by phosphorylation.

In [32P]orthophosphate-labeled cells, the peak for [32P]-labeled TAP complexes in untreated cell lysates remained at fraction 49, whereas the peak for TAP complexes in OK-treated cell lysates was reproducibly shifted to fraction 45 (Fig. 4b). The 43-kDa protein was clearly evident in [32P]-labeled TAP complexes of OK-treated cells (arrows, Fig. 4b), but not in untreated cells, indicating that under physiological conditions, the association of this 43-kDa phosphoprotein with TAP complexes is either transient or unstable. These results strongly suggest that phosphorylation induces the formation of high molecular weight TAP complexes that contain phosphorylated TAP1, TAP2, tapasin, the 43-kDa protein, and non-phosphorylated class I molecules.

Presence of a Kinase Activity in TAP Complexes—Because TAP complexes are phosphorylated in vivo, we examined whether a kinase activity is present in TAP-containing complexes. As shown in Fig. 5, anti-TAP1 immunoprecipitate contained TAP1, TAP2, tapasin, class I heterodimers, and the 43-kDa protein. When an aliquot of the same anti-TAP1 immunoprecipitate was subjected to an in vitro kinase reaction with [γ-32P]ATP, we found that TAP and tapasin, but not class I heterodimers, were phosphorylated. These results suggest that TAP-containing complexes contain kinase(s) and that TAP complex-associated kinases selectively phosphorylate tapasin and TAP.

TAP-dependent Peptide Transport Is Impaired by TAP Phosphorylation—To address the question of whether the ability of TAP to bind peptides and/or ATP is affected by phosphorylation, we compared TAP-expressing microsomes that were prepared from untreated or OK-treated cells, with those that were pretreated with protein phosphatase. We made use of a fluorescein-conjugated reporter peptide (29) (fluorescein isothiocyanate-labeled RYNATRGL) and photoaffinity-labeled 8-[azido-γ-32P]ATP to assay for peptide (32) and ATP binding (35),
We found that the ability of TAP to bind peptides or ATP was not measurably affected, suggesting that TAP phosphorylation does not interfere with either peptide or ATP binding to TAP. We next examined whether phosphorylation affects TAP function in peptide transport (Fig. 6a). While TAP-expressing microsomes from untreated cells exhibited high peptide transport activity, the relative peptide transport activity in TAP-expressing microsomes decreased by 50% when 0.1 nM OK was used. When cells were treated with 1 nM OK, peptide transport activity was almost completely abrogated. These results suggest that the degree of TAP inhibition in the peptide transport inversely correlates with the extent of TAP phosphorylation. When the TAP-expressing microsomes from OK-treated cells were pretreated with a protein phosphatase prior to the assay, the TAP activity was substantially restored, suggesting that TAP-mediated peptide transport is reversibly regulated by phosphorylation.

The effect of virus-induced TAP phosphorylation on TAP function was investigated. We prepared microsomes from HCMV-infected cells under the experimental infection conditions that the expression of US6, the only known TAP inhibitor respectively. We found that the ability of TAP to bind peptides or ATP was not measurably affected, suggesting that TAP phosphorylation does not interfere with either peptide or ATP binding to TAP. We next examined whether phosphorylation affects TAP function in peptide transport (Fig. 6a). While TAP-expressing microsomes from untreated cells exhibited high peptide transport activity, the relative peptide transport activity in TAP-expressing microsomes decreased by 50% when 0.1 nM OK was used. When cells were treated with 1 nM OK, peptide transport activity was almost completely abrogated. These results suggest that the degree of TAP inhibition in the peptide transport inversely correlates with the extent of TAP phosphorylation. When the TAP-expressing microsomes from OK-treated cells were pretreated with a protein phosphatase prior to the assay, the TAP activity was substantially restored, suggesting that TAP-mediated peptide transport is reversibly regulated by phosphorylation.

The effect of virus-induced TAP phosphorylation on TAP function was investigated. We prepared microsomes from HCMV-infected cells under the experimental infection conditions that the expression of US6, the only known TAP inhibitor
in the HCMV genome (2, 25), was not detected. We found that the peptide transport activity of TAP-expressing microsomes from HCMV-infected cells was reduced by 25%, compared with microsomes from mock-infected cells (Fig. 6b). When the microsomes from HCMV-infected cells were pretreated with a protein phosphatase prior to the assays, the peptide transport activity was almost completely restored, suggesting that TAP inhibition imposed by HCMV infection can be reversed by the removal of protein phosphorylation. Because US6 is not expressed in HCMV-infected cells and because HCMV infection enhances TAP phosphorylation, it can be concluded that the difference in the extent of TAP inhibition between HCMV-infected and mock-infected cells is most likely attributed to the virus-induced TAP phosphorylation. These results strongly suggest that virus has exploited this regulatory TAP phosphorylation mechanism to evade host immune surveillance.

DISCUSSION

Phosphorylation is known to modulate functions of members of the ATP-binding cassette transporter family (7, 16—19). A typical example is the cystic fibrosis transmembrane regulator (18), which has been demonstrated to interact with Na+ and Cl− channels in airway epithelium as well as to modulate the function of renal K+ channels by altering the phosphorylation state of either renal K+ channels, associated proteina, or itself (36). TAP as an ATP-binding cassette transporter is no exception. TAP appears to be phosphorylated at several evolutionarily conserved residues present in its cytoplasmic domains (6, 20). As a consequence of phosphorylation-induced formation of high molecular weight TAP-containing complexes, peptide transport activity of TAP is altered. The recent finding that TAP activity inversely correlates with its lateral mobility on the membrane (37) leads us to suggest that TAP’s mobility on the endoplasmic reticulum membrane might be the result of a dynamic assembly and disassembly of TAP complexes, which appear to be regulated by phosphorylation.

We have carefully calibrated the gel filtration column used in the study and determined that the estimated molecular masses for soluble proteins at fractions 45 and 49 are ≈2200 and ≈750 kDa, respectively. However, molecules present in TAP-containing complexes are not soluble but transmembrane proteins, making the estimated molecular weight values for fractions 45 and 49 meaningless. In addition, the presence of additional TAP complex-associated factors, which may not be preserved with our immunoprecipitation protocol, may lead us to draw incorrect conclusions. Nevertheless, we have estimated that, with respect to TAP1, the phosphorylation intensity ratio of TAP1, TAP2, and tapasin is 1:1:1 at fraction 49, whereas at fraction 45 the ratio was changed to 1:0:1.15 (see Fig. 4b). Because the phosphorylation level of tapasin seems to markedly increase in the TAP-containing complexes, it is possible that hyperphosphorylated tapasin plays a critical role in mediating the assembly of high molecular weight TAP complexes.

Co-immunoprecipitation with anti-TAPI, -TAP2, or -tapasin antibodies demonstrated that the 43-kDa protein is physically associated with the TAP complexes2 (Figs. 4 and 5). The finding that the main difference between the protein compositions of anti-TAPI immunoprecipitates prepared from OK-treated and untreated cells was the amount of the 43-kDa protein (Fig. 4), in conjunction with the observation that the immunoprecipitates from cells without OK treatment had lower kinase activity (Fig. 5), suggests that the 43-kDa phosphoprotein is a TAP complex-associated kinase. Because TAP phosphorylation requires tyrosine, serine, and threonine kinases, the 43-kDa phosphoprotein appears to be one of several TAP complex-specific kinases. The observation that the amount of the 43-kDa protein in anti-TAPI immunoprecipitates was greater than those in anti-TAP2 or -tapasin immunoprecipitates suggests that the 43-kDa phosphoprotein might directly interact with TAP1. Thus, in vivo the increased level of TAP1 phosphorylation could be due to this preferred association of the 43-kDa phosphoprotein with TAP1 (Fig. 4). Because the peptide transport activity of TAP in the 43-kDa protein-containing TAP complexes is severely altered, the 43-kDa protein-containing TAP complexes might represent a subset of non-functional TAP complexes, whose formation is promoted and/or preserved by phosphorylation.

The finding that dephosphorylation of TAP complexes restores TAP peptide transport activity suggests that via phosphorylation TAP function is reversibly regulated by the dynamic assembly and disassembly of TAP complexes. It is conceivable that TAP phosphorylation is a regulatory mechanism that has evolved to control MHC class I antigen presentation in a tissue and/or cell-type specific manner. Because dephosphorylation of TAP occurs rapidly under physiological conditions, a role for TAP-specific phosphatases in regulating MHC class I antigen presentation can be envisaged. Under interferon induction TAP dephosphorylation takes place at a rate faster than its kinase-dependent phosphorylation, indicating that TAP-specific phosphatase activity is not a rate-limiting step in the MHC antigen presentation pathway. It is possible that, in antigen-presenting or viral-infected cells, expression of TAP-specific phosphatases is up-regulated by interferon. As a result of an increase in the activity of TAP-specific phosphatase, a net increase in TAP activity and an increase in the surface level of MHC class I expression can be achieved during cytotoxic immune response.

At least two types of viruses, HCMV and herpes simplex virus, have evolved to have gene products, US6 (25) and ICP47 (24), respectively, which physically bind to, and inhibit TAP function, making TAP a proven target that has been exploited by viruses to escape host immune detection. The present study demonstrates that virus-induced changes in the phosphorylation of cellular proteins (30, 31) increase the phosphorylation level of TAP complexes, thus affecting MHC class I antigen presentation. Because the kinase activity present in anti-TAPI immunoprecipitates of HCMV-infected cells was greater than that in non-infected cells,2 TAP phosphorylation could be directly and/or indirectly enhanced by virus-encoded kinases (38–40). One likely viral kinase candidate is the HCMV-encoded serine/threonine kinase pp65 (39), which has been shown to selectively block antigen processing and presentation of its immediate-early gene product (38). Alternatively, viruses might recruit host cellular kinases (31) to catalyze TAP phosphorylation. Enhancing TAP phosphorylation represents a newly discovered mechanism that has been exploited by viruses to evade immune surveillance, further confirming that TAP is a target for virus-driven evolution to evade the immune system.

Functional polymorphisms in rat and Syrian hamster TAP are known to change the peptide pool available for binding and presentation by MHC class I alleles (41–43). Similarly, human TAP polymorphism has been shown to influence in vivo antigenic peptide presentation (44). Because several TAP polymorphic residues, such as those at codon positions 374 (Ser-Ala), 565 (Ala-Thr), and 665 (Ala-Thr) of TAP2 (6), are potential sites of phosphorylation (20), TAP phosphorylation at those sites could interfere with TAP-dependent transport of certain antigenic peptides. Indeed, a single amino acid substitution at position 374 in human TAP2 has been demonstrated to change the preference of transported peptides (45). It is therefore conceivable that abnormal TAP phosphorylation might lead to dangerous cytotoxic immune responses, perhaps developing
into certain human autoimmune diseases, such as Graves’ disease, diffuse panbronchiolitis, and Reiter’s syndrome, all of which are known to have strong associations with TAP polymorphism (6, 46, 47). In light of the finding that viral infection changes the hosts cellular protein content and phosphorylation state, which in turn affects the TAP phosphorylation state, the issue of whether virus-mediated TAP phosphorylation contributes to the development of autoimmune diseases should be re-addressed.

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