Minireview

The transporter associated with antigen processing TAP: structure and function

Brigitte Lankat-Buttgereit, Robert Tampé*

Institut für Physiologische Chemie, Philipps-Universität Marburg, Karl-von-Frisch-Str. 1, 35033 Marburg, Germany

Received 19 October 1999; received in revised form 18 November 1999

Edited by Vladimir Skulachev

Abstract The transport of antigenic peptides from the cytosol to the lumen of the endoplasmic reticulum (ER) is an essential process for presentation to cytotoxic T-lymphocytes. The transporter associated with antigen processing (TAP) is responsible for the intracellular translocation of peptides across the membrane of the ER. Efficient assembly of MHC-peptide complex requires the formation of a macromolecular transport and chaperone complex composed of TAP, tapasin and MHC class I molecules. Therefore, structure and function of TAP is important for the understanding of the immune surveillance.
© 1999 Federation of European Biochemical Societies.

Key words: ABC transporter; Antigen presentation; Transport mechanism; Virus persistence

1. Introduction

Presentation of antigenic peptides by MHC class I molecules to cytotoxic T-lymphocytes (CTL) is an essential process for the cellular immune recognition. These peptides are primarily generated by the proteasome complex, a multisubunit, multicatalytic protease (for review see [1]), and are translocated from the cytosol into the lumen of the endoplasmic reticulum (ER) by a so-called transporter associated with antigen processing (TAP). In the ER, the peptides are loaded onto the newly synthesized MHC class I molecules. Binding of the peptide stabilizes the complex and induces the export to the cell surface for presentation to T cell receptors (Fig. 1A). The class I heterotrimer consists of an MHC encoded heavy chain, β2-microglobulin and peptide and its assembly is well studied (for review see [2,3]). However, less is known of how the peptides are transported into the ER. Some open questions still remain: The amount and quality of peptides generated by the proteasome, the mechanism of the transport as well as the involvement of other factors such as additional chaperones, proteases or transporters. The availability of peptides to the MHC class I molecules could be rate-limiting for antigen presentation, and selectivity of this step could be imprinted onto the pool of dominant and subdominant epitopes. In this re-view, the current knowledge about the mechanisms underlying the transport of the peptides from cytosol to the lumen of the ER is described.

2. Genomic organization of TAP

Studies concerning various cell lines with a strongly reduced level of MHC class I molecules on the cell surface, strengthened the importance of peptide transport into the ER lumen [4]. Peptides exogenously added or introduced into the ER by signal sequences were efficiently presented, but these 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 newly synthesized class I molecules with them. In the following, four groups independently described candidate genes for a factor that would transport peptides across the ER membrane [5–8]. In 1991, a WHO nomenclature committee for factors in the HLA system renamed these genes TAP1 (for RING4, PSF1, mtp1 and HAM1) and TAP2 (for RING11, PSF2, mtp2 and HAM2) [9]. Alignment between the sequences of TAP1 and TAP2 showed the highest homology in a stretch of 200 amino acids located at the C-terminus. This region contains three characteristic motifs. The Walker A and B motifs form a highly conserved ATP-binding cassette. A so-called C-loop, which consists of six to eight conserved amino acids, is located in between the Walker A and B motifs. Therefore, the proteins belong to the superfamily of ATP-binding cassette (ABC) transporters. 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. Functional impact of TAP1 and TAP2 was proven by transfection of defective cell lines with TAP2 and/or TAP1 genes, restoring antigen presenting activity [10,11].

The genomic structures of the human TAP genes have been established [12]. Each gene is located in the MHC II locus of chromosome 6, comprises about 10 kb and consists of 11 exons (Fig. 1B) [13]. Eight of these exons 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
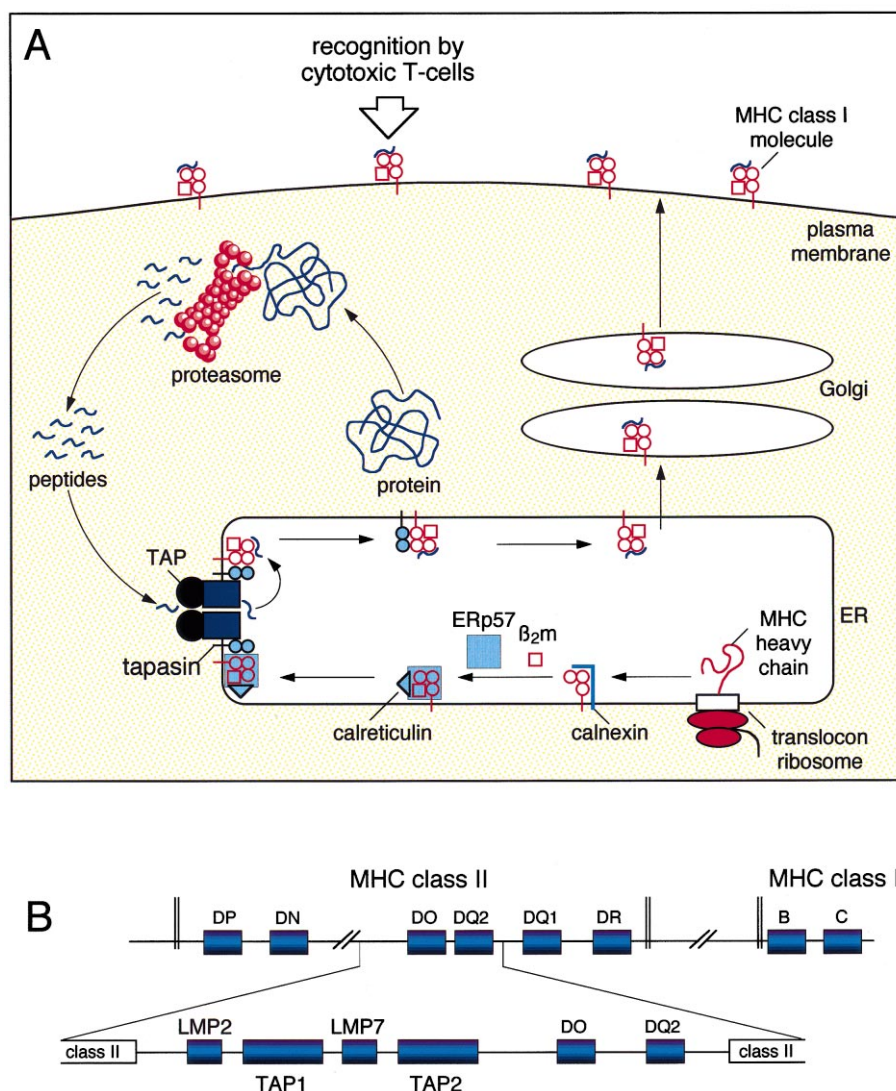
Fig. 1. A: Antigen processing and presentation via MHC class I molecules. Endogenous proteins are degraded by the proteasome. Peptides are transported into the lumen of the ER by the TAP complex. Several molecules are involved in assembly and loading of the MHC class I molecules, including calnexin, calreticulin, tapasin and ERp57. Stable MHC-peptide complexes leave the ER via the Golgi compartment to the cell surface for recognition by cytotoxic T-lymphocytes. Open circles represent folded immunoglobulin domains. B: The location of the TAP genes in the human MHC class II complex (HLA). The scale is intended as a rough guide only.

40% with salmon). The analysis indicates that the degree of relatedness between human TAPs is similar to that between each gene and its homologues in rodents [14]. Although human TAP1 and TAP2 exhibit only 64% homology at the protein level, they have a similar predicted membrane topology. Therefore, it is speculated that the genes have evolved from a common ancestral gene by duplication prior to the development of the adaptive immune system in vertebrates [15].

3. Regulation of TAP genes

MHC class I molecules are expressed at low levels in most cells and are strongly induced by cytokines such as interferons (for review see [16]). Increased expression of MHC class I molecules correlates with increased CTL function. Therefore, it was of special interest to study the gene expression of TAP1 and TAP2. Both TAPs are up-regulated by interferon-γ about 10-fold within 24 h, accompanied by an increased peptide

transport capacity. The TAP genes contain no TATA box motifs in the 5′-flanking sequences, but putative GC-rich elements (Sp1-binding sites, 128 nucleotides upstream the translation start codon for TAP1 and 79 nucleotides upstream for TAP2) [12]. Site directed mutagenesis of the Sp1-binding site leads to a 3-fold reduction of basal promoter activity of TAP1 [17]. 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 low molecular mass polypeptide 2 (LMP2), a β-type proteasomal subunit, β_1. Both genes are induced by TNF-α via an NF-κB element, which is also found in the class I response element [18]. The cytokine induced expression of TAP1 and LMP2 concordantly with class I genes suggest a mechanism to link transporter levels with class I production.

4. Structural organization of the TAP complex

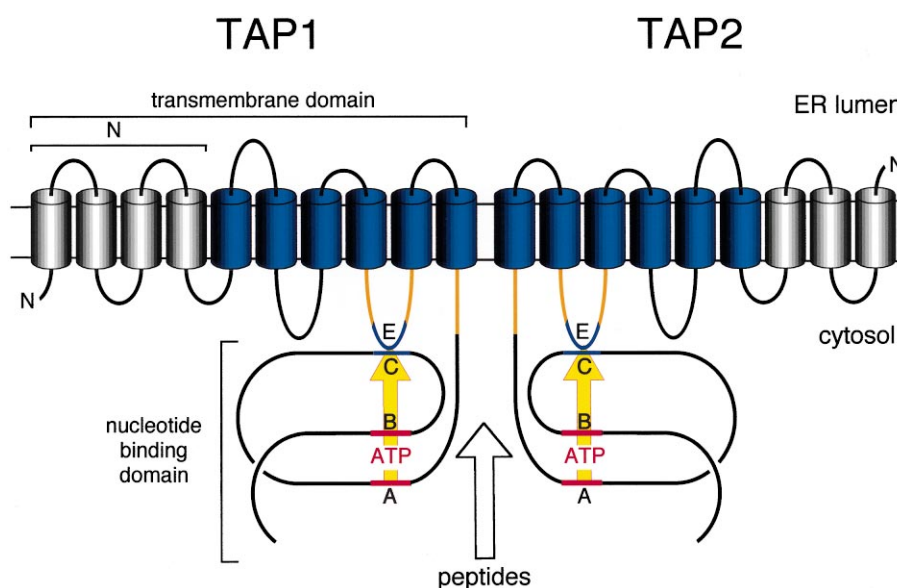As mentioned before, human TAP1 (748 amino acids) and

Fig. 2. Membrane topology of the human TAP complex. The transmembrane helices are predicted from hydrophobicity plots and sequence alignments with other ABC transporters. The N-terminal domain (N) of TAP1 and TAP2 is very hydrophobic, comprising three or four predicted transmembrane helices (gray symbols). The nucleotide-binding domain consists of the Walker A and B motifs (A, B) and the so-called C-loop (C), interacting with a 'EAA'-like motif (E) in the last cytosolic loop of the membrane-spanning domains. The orange lines illustrate binding regions for peptides as identified by photo-crosslinking experiments.

human TAP2 (686 amino acids) belong to the superfamily of ABC transporters, comprising a large number of polytopic membrane proteins transporting a diverse set of molecules across membranes in an ATP-dependent manner (for review see [19,20]). The homology with other members of this family 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 proteins [21]. Comparisons of the phenotypes of different mutant cell lines and heterologous expres-

sion in insect cells and yeast suggest that neither TAP1 nor TAP2 can form a functional homodimer, indicating that only a heterodimer is functional [22,23]. Hydrophobicity analysis and sequence alignments with related ABC transporters point to a 2×6 transmembrane helix model of TAP, extended by additional four and three transmembrane helices predicted for the highly diverse N-terminal domain (Fig. 2) [24]. The hydrophobic transmembrane domains 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
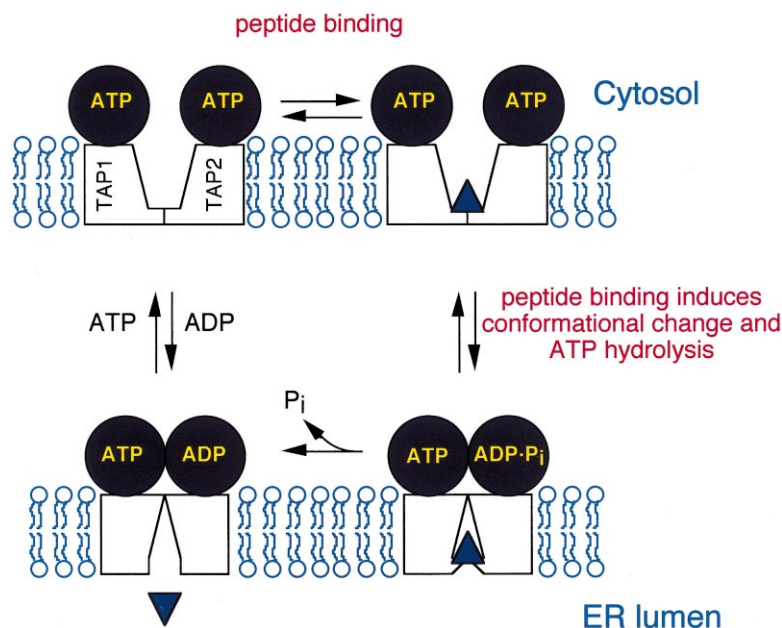

Fig. 3. Model of peptide translocation by the TAP complex. Peptide (dark blue triangles) and ATP/ADP independently bind to TAP in the cytosol. It is unknown, if both NBDs are loaded with ATP. Peptide binding induces a structural reorganization of the TAP complex, triggering ATP hydrolysis and subsequently translocation of the peptide.

model, are located in the cytosol. The C-loop interacts with an 'EAA'-like motif (E) found in the last cytosolic loop of the membrane-spanning domains [25]. The peptide-binding region was mapped by photo-crosslinking of peptides to TAP, digestion by trypsin and/or bromocyan and subsequent immunoprecipitation with antibodies directed against different epitopes of TAP [26]. The analysis of photo-crosslinked fragments revealed a similar binding region for TAP1 and TAP2, comprising the cytosolic loops between TM4 and TM5 and a carboxy-terminal stretch of about 15 amino acids following TM6. So far, the peptide translocation pathway is not known, but it can be speculated that TM5 and TM6 are parts of a translocation channel.

5. Transport mechanism of TAP

Peptide transport by TAP is a multi-step process [27] (Fig. 3). In a fast bimolecular association step, the peptide binds to TAP in an ATP-independent manner [28], followed by a slow isomerization of the TAP complex [29]. It is suggested that this structural reorganization of the molecule triggers ATP hydrolysis and peptide translocation across the membrane. These binding steps primarily determine the selectivity of TAP. The translocation strictly requires hydrolysis of ATP, because non-hydrolyzable ATP analogs do not promote peptide transport. ATP and ADP have similar affinities for TAP [30,31]; therefore, peptide translocation can be inhibited by ADP. 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 (S. Gorbulev and R. Tampé, manuscript in preparation). A structural rearrangement of the NBDs seems to function as a molecular switch to activate the ATPase of TAP. At the moment, there are no data available, if the NBDs are equal in function and if they work in a sequential or synchronic manner, thereby implying whether one or two ATPs are needed for substrate translocation.

6. Substrate specificity of TAP

The peptide specificity was analyzed by experiments based on trapping transported peptides in the ER by glycosylation and on ATP-independent binding assays (for review see [32]). Those peptides are translocated most efficiently into the ER, which are similar or slightly larger in length as expected for MHC class I binding. The contribution of each peptide residue to the affinity for TAP was determined by screening combinatorial peptide libraries [33]. With this method, the average affinity of a randomized peptide mixture with one common residue is compared to a totally randomized peptide mixture. The strongest differences were observed for the carboxy-terminal amino acid and the first three amino-terminal residues. The amino acids in between do not significantly contribute to substrate specificity. This binding motif combines maximal diversity in the epitope where T-cell receptor recognition occurs with maximal binding affinity for antigen processing. The matched preferences of human TAP and class I suggest a coevolution of the genes [32].

Although, TAP genes of all species are polymorphic, a functional polymorphism was so far only found for rat TAP [34] and for a human TAP2iso splice variant [35]. Mouse

TAP, rat TAP from the RT1^u strain and human TAP1/2iso were described to be selective for hydrophobic carboxy-termini of the peptide, whereas rat TAP from the RT1^a strain and human TAP seem to be permissive for peptides containing hydrophobic and basic carboxy-termini [36,37].

7. Loading of peptides from TAP onto MHC molecules

The assembly and loading of MHC class I molecules require a number of other proteins (for review see [3]). At least four proteins – calnexin, calreticulin, ERp57 and tapasin – are involved in the assembly of heavy chain and β_2-microglobulin. The MHC class I molecules interact with the TAP complex via an ER-resident type I glycoprotein, named tapasin (Fig. 1A) [38,39]. Tapasin mediates complex formation and the crosstalk of structural information of MHC and TAP and is therefore important for class I assembly and editing. Tapasin has two independent functions: First, it increases the level of TAP, thereby increasing the efficiency of peptide transport and, secondly, it associates with MHC class I molecules, thereby facilitating directly loading and assembly of class I molecules [40,41]. The MHC-peptide complexes, which are kinetically stable, can leave the ER to the cell surface.

8. TAP and human diseases

Some viruses are known to interfere with antigen presentation in infected cells (for review see [42]). For example, the immediate early gene product ICP47 of herpes simplex virus type 1 inhibits peptide transport into the ER by blocking peptide binding to TAP [43–46]. The late expressed ER-resident transmembrane class I glycoprotein US6 from human cytomegalovirus inhibits peptide translocation probably by binding to the ER-luminal part of TAP, because neither peptide binding nor ATP binding to TAP seems to be affected [47–49].

There is little knowledge about human TAP defects. The homozygous TAP2 −/− siblings of one family have reduced expression of MHC class I molecules on the cell surface and reduced amounts of cytotoxic T-lymphocytes. On the other hand, these people do not show an increased susceptibility to viral infections. The antigens seem to be transported into the ER lumen in a TAP-independent manner, although under normal conditions TAP appears to be the dominant pathway [50]. In some tumor tissues, a down-regulation of TAP mRNA by an unknown mechanism or mutation of TAP was observed [51,52]. The suppression of TAP may be a mechanism for tumor cells to escape the immune response.

The TAPs as peptide transporters are a key link between antigen processing and presentation. However, many questions remain open to fully understand the whole process of T-cell immunity. Further analysis will clarify the significance of TAP defects or down-regulation in tumors and, in particular, the role in the interaction of malignant cells with the immune system of the host.

References

[1] Baumeister, W., Walz, J., Zühl, F. and Seemüller, E. (1998) Cell 92, 367–380.
[2] Pamer, E. and Cresswell, P. (1998) Annu. Rev. Immunol. 16, 323–358.

[3] Lehner, P.J. and Trowsdale, J. (1998) Curr. Biol. 8, R605–R608.
[4] Ljunggren, H.G., Stam, N.J., Ohlen, C., Neefjes, J.J., Hoglund, P., Heemels, M.T., Bastin, J., Schumacher, T.N., Townsend, A. and Karre, K. et al. (1990) Nature 346, 476–480.
[5] Bahram, S., Arnold, D., Bresnahan, M., Strominger, J.L. and Spies, T. (1991) Proc. Natl. Acad. Sci. USA 88, 10094–10098.
[6] Powis, S.H., Mockridge, I., Kelly, A., Kerr, L.A., Glynne, R., Gileadi, U., Beck, S. and Trowsdale, J. (1992) Proc. Natl. Acad. Sci. USA 89, 1463–1467.
[7] Deverson, E.V., Gow, I.R., Coadwell, W.J., Monaco, J.J., Butcher, G.W. and Howard, J.C. (1990) Nature 348, 738–741.
[8] Monaco, J.J., Cho, S. and Attaya, M. (1990) Science 250, 1723–1726.
[9] The WHO Nomenclature Committee for factors of the HLA system (1991) Immunogenetics 36, 135–148.
[10] Attaya, M., Jameson, S., Martinez, C.K., Hermel, E., Aldrich, C., Forman, J., Lindahl, K.F., Bevan, M.J. and Monaco, J.J. (1992) Nature 355, 647–649.
[11] Spies, T., Cerundolo, V., Colonna, M., Cresswell, P., Townsend, A. and DeMars, R. (1992) Nature 355, 644–646.
[12] Beck, S., Kelly, A., Radley, E., Khurshid, F., Alderton, R.P. and Trowsdale, J. (1992) J. Mol. Biol. 228, 433–441.
[13] Hanson, I.M. and Trowsdale, J. (1991) Immunogenetics 34, 5–11.
[14] Hughes, A.L. (1994) Mol. Biol. Evol. 11, 899–910.
[15] Beck, S., Abdulla, S., Alderton, R., Glynne, P.R., Gut, I.I., Hosking, G.L., Jackson, K.A., Kelly, A., Newell, W., Sanseau, R.P., Radley, E., Thorpe, K. and Trowsdale, J. (1996) J. Mol. Biol. 255, 1–13.
[16] Früh, K. and Yang, Y. (1999) Curr. Opin. Immunol. 11, 76–81.
[17] Wright, K.L., White, L.C., Kelly, A., Beck, S., Trowsdale, J. and Ting, J.P-Y. (1995) J. Exp. Med. 181, 1459–1471.
[18] Israel, A., Le Bail, O., Hatat, D., Piette, J., Kieran, M., Logeat, F., Fellous, M. and Kourilsky, P. (1989) EMBO J. 8, 3793–3800.
[19] Higgins, C.F. (1992) Annu. Rev. Cell Biol. 8, 67–113.
[20] Doige, C.A. and Ames, G.F.L. (1993) Annu. Rev. Microbiol. 47, 291–319.
[21] Kelly, A., Powis, S.H., Kerr, L.A., Mockridge, I., Elliott, T., Bastin, J., Uchanska-Ziegler, B., Ziegler, A., Trowsdale, J. and Townsend, A. (1992) Nature 355, 641–644.
[22] Meyer, T.H., van Endert, P.M., Uebel, S., Ehring, B. and Tampé, R. (1994) FEBS Lett. 35, 443–447.
[23] Urlinger, S., Kuchler, K., Meyer, T.H., Uebel, S. and Tampé, R. (1997) FEBS Lett. 245, 266–272.
[24] Tampé, R., Urlinger, S., Pawlitschko, K. and Uebel, S. (1997) in: Unusual Secretory Pathways: From Bacteria to Man (Kuchler, K., Rubartelli, A. and Holland, B., Eds.), pp. 115–136, Springer, New York.
[25] Cotten, J.F., Ostedgaard, L.S., Carson, M.R. and Welsh, M.J. (1996) J. Biol. Chem. 271, 21279–21284.
[26] Nijenhuis, M. and Hämmerling, G.J. (1996) J. Immunol. 157, 5467–5477.
[27] Van Endert, P.M., Tampé, R., Meyer, T.H., Tisch, R., Bach, J.-F. and McDevitt, H.O. (1994) Immunity 1, 491–500.
[28] Uebel, S., Meyer, T.H., Krass, W., Kienle, S., Jung, G., Wiesmüller, K.-H. and Tampé, R. (1995) J. Biol. Chem. 270, 18512–18516.
[29] Neumann, L. and Tampé, R. (1999) J. Mol. Biol. 294, 1203–1213.
[30] Müller, K.M., Ebensperger, C. and Tampé, R. (1994) J. Biol. Chem. 269, 14032–14037.
[31] Russ, G., Esquivel, F., Yewdell, J.W., Cresswell, P., Spies, T. and Bennink, J.R. (1995) J. Biol. Chem. 270, 21312–21318.
[32] Uebel, S. and Tampé, R. (1999) Curr. Opin. Immunol. 11, 203–208.
[33] Uebel, S., Kraas, W., Kienle, S., Wiesmüller, K.-H., Jung, G. and Tampé, R. (1997) Proc. Natl. Acad. Sci. USA 94, 8976–8981.
[34] Powis, S.J., Deverson, E.V., Coadwell, W.J., Ciruela, A., Huskisson, N.S., Smith, H., Butcher, G.W. and Howard, J.C. (1992) Nature 357, 211–215.
[35] Yan, G., Shi, L. and Faustman, D. (1999) J. Immunol. 162, 852–859.
[36] Heemels, M.T., Schumacher, T.N., Wonigeit, K. and Ploegh, H.L. (1993) Science 262, 2059–2063.
[37] Momburg, F., Roelse, J., Howard, J.C., Butcher, G.W., Hämmerling, G.J. and Neefjes, J.J. (1994) Nature 367, 648–651.
[38] Ortmann, B., Copeman, J., Lehner, P.J., Sadasivan, B., Herberg, J.A., Grandea, A.G., Riddell, S.R., Tampé, R., Spies, T. and Trowsdale, J. (1997) Science 277, 1306–1309.
[39] Li, S.L., Sjogren, H.O., Hellman, U., Pettersson, R.F. and Wang, P. (1997) Proc. Natl. Acad. Sci. USA 94, 8708–8713.
[40] Lehner, P.J., Surman, M.J. and Cresswell, P. (1998) Immunity 8, 221–231.
[41] Bangia, N., Lehner, P.J., Hughes, E.A., Surman, M. and Cresswell, P. (1999) Eur. J. Immunol. 29, 1858–1870.
[42] Ploegh, H.L. (1998) Science 280, 248–253.
[43] Hill, A., Jugovic, P., York, I., Russ, G., Bennink, J., Yewdell, J., Ploegh, H. and Johnson, D. (1995) Nature 375, 411–415.
[44] Früh, K., Ahn, K., Djaballah, H., Sempé, P., van Endert, P.M., Tampé, R., Peterson, P.A. and Yang, Y. (1995) Nature 375, 415–418.
[45] Ahn, K., Meyer, T.H., Uebel, S., Sempé, P., Djaballah, H., Yang, Y., Peterson, P.A., Früh, K. and Tampé, R. (1996) EMBO J. 15, 3247–3255.
[46] Tomazin, R., Hill, A.B., Jugovic, P., York, I., van Endert, P., Ploegh, H.L., Andrews, D.W. and Johnson, D.C. (1996) EMBO J. 15, 3256–3266.
[47] Hengel, H., Koopmann, J.O., Flohr, T., Muranyi, W., Goulmy, E., Hämmerling, G.J., Koszinowski, U.H. and Momburg, F. (1997) Immunity 6, 623–632.
[48] Ahn, K., Gruhler, A., Galocha, B., Jones, T.R., Wiertz, E.J.H.J., Ploegh, H.L., Peterson, P.A., Yang, Y. and Früh, K. (1997) Immunity 6, 613–621.
[49] Lehner, P.J., Karttunen, J.T., Wilkinson, G.W.G. and Cresswell, P. (1997) Proc. Natl. Acad. Sci. USA 94, 6904–6909.
[50] de la Salle, H., Houssaint, E., Peyrat, M.A., Arnold, D., Salamero, J., Pinczon, D., Stevanovic, S., Bausinger, H., Fricker, D. and Gomard, E. (1997) J. Immunol. 158, 4555–4563.
[51] Seliger, B., Hohne, A., Jung, D., Kallfelz, M., Knuth, A., Jaeger, E., Bernhard, H., Momburg, F., Tampé, R. and Huber, C. (1997) Exp. Hematol. 25, 608–614.
[52] Chen, H.L., Gabrilovich, D., Tampé, R., Girgis, K.R., Nadaf, S. and Carbone, D.P. (1996) Nat. Genet. 13, 210–213.