The Herpes Simplex Virus-encoded Inhibitor of the Major Histocompatibility Complex (MHC)-encoded Peptide Transporter Associated with Antigen Processing (TAP), Maps to the NH$_2$-terminal 35 Residues

By Begoña Galocha,* Ann Hill,* Barbara C. Barnett,‡ Aidan Dolan,‡ Alejandra Raimondi,* Richard F. Cook,* Joseph Brunner,§ Duncan J. McGeoch,‡ and Hidde L. Ploegh*

From the *Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; ‡Medical Research Council Virology Unit, Institute of Virology, Glasgow G11 5JR, United Kingdom; and the §Institute of Biochemistry, Swiss Federal Institute of Technology Zürich (ETH Z), CH-8092, Zürich, Switzerland

Summary

The herpes simplex virus (HSV) immediate early protein ICP47 inhibits the transporter associated with antigen processing (TAP)-dependent peptide translocation. As a consequence, empty major histocompatibility complex (MHC) class I molecules are retained in the endoplasmic reticulum and recognition of HSV-infected cells by cytotoxic T lymphocytes is abolished. We chemically synthesized full-length ICP47 (sICP47) and show that sICP47 inhibits TAP-dependent peptide translocation in human cells. Its biological activity is indistinguishable from that of recombinant ICP47 (rICP47). By using synthetic peptides, we mapped the core sequence of ICP47 minimally required for TAP inhibition to residues 2–35. This segment is located within the region of the molecule conserved between ICP47 from HSV-1 and HSV-2. Through alanine scanning substitution we identified three segments within this region that are critical for the ability to inhibit TAP function. The interaction of ICP47 with TAP is unlikely to mimic precisely that of the transported peptides, as deduced from differential labeling of the TAP1 and TAP2 subunits using sICP47 fragments with chemical cross-linkers.

The MHC-encoded transporter associated with antigen processing (TAP)$^1$ connects the cytosol with the lumen of the endoplasmic reticulum (ER) to allow loading of MHC class I molecules with cytosolic peptides for presentation to CTL (1–3). This MHC class I-restricted pathway is critical for elimination of most virus infections. TAP, a key component of this pathway, is blocked specifically by the herpes simplex virus (HSV) protein ICP47, a blockade that allows escape from eradication by CTL (4, 5). TAP is a member of the ATP-binding cassette (ABC) family of transporters, which includes the cystic fibrosis transmembrane conductance regulator (CFTR) and the multidrug resistance transporter (MDR) (6). To date, ICP47 is the only known natural inhibitor of a member of the ABC transporter family. A better understanding of the mode of interaction between ICP47 and TAP is relevant not only for learning more about viral evasion strategies, but could also inspire the design of inhibitors for other members of the ABC transporter family.

ICP47 of HSV-1 is an 87-amino acid cytosolic polypeptide, 88 residues if the initiation methionine is included. It binds to the TAP1–TAP2 heterodimer in human but not in mouse cells and prevents transport of peptides through blockade of the peptide binding site of TAP (7, 8). As a consequence, MHC class I molecules fail to be loaded with peptides. The resultant empty class I molecules are retained in the ER and presentation of epitopes to CTL is abolished in HSV-infected human cells (4, 5).

The affinity of the human TAP–ICP47 interaction has been estimated to be around 50 nM (9, 10). The ability of ICP47 to prevent photocross-linking of peptides to TAP indicated that ICP47 prohibited peptide binding to TAP (9). Furthermore, the kinetics of competition between peptide and ICP47 for binding to TAP indicate that ICP47 and peptide may compete for a single binding site (9, 10). While

---

$^1$Abbreviations used in this paper: ABC, ATP-binding cassette; CFTR, cystic fibrosis transmembrane conductance regulator; EGF, epidermal growth factor; ER, endoplasmic reticulum; FPLC, fast performance liquid chromatography; HSV, herpes simplex virus; MDR, multidrug resistance transporter; SLO, streptolysin O; TAP, transporter associated with antigen processing; Tpa, 4'-[Trifluoromethyl–diazirinyl]-phenylalanine.
suggestive, these experiments cannot readily distinguish between a conformational distortion of TAP caused by ICP47, or a direct competition for the binding site.

Here, we have used chemical synthesis to make full-length ICP47, as well as N H - and C OOH-terminally truncated versions and alanine-substituted peptide analogues. We show that the ability of ICP47 to inhibit TAP lies within the NH - terminal half of the molecule, which is highly conserved between ICP47 from HSV-1 and HSV-2. We present evidence that the mechanism of interaction of ICP47 with the TAP heterodimer likely differs from that of its peptide substrates.

Materials and Methods

Synthesis and Purification of ICP47 and Truncations. The peptides used in this study were synthesized on a multiple peptide synthesizer (model 350; Advanced Chemtech, Louisville, KY) by Fmoc

Figure 1. Synthetic ICP47 has identical biological activity to recombinant ICP47. (A) Elution profile of synthetic ICP47 on a Sephacryl 100 column (48 cm × 1 cm) equilibrated with 150 mM NaHCO , 10 mM NaCl, pH 8.5. The elution profile of sICP47 is shown along with the calibration graph for molecular weight standards (BSA 66,000, OVA 44,000, carbonic anhydrase 30,000, N Ase 13,700 and cytochrome C 12,300). K is plotted against the logarithm of the molecular weight. K = (V - V ) / (V - V ), where V represents the elution volume of the sample, V the void volume, and V the total volume. (B) The human lymphoblastoid cell line Hom2 was permeabilized using SLO and incubated with synthetic or recombinant ICP47 at the concentrations indicated, and with the 125I-labeled peptide library for 10 min at 37°C as described in Materials and Methods. Results represent the amount of radiolabeled, glycosylated peptides recovered with Con A–Sepharose. Synthetic ICP47 (sICP47) (closed symbols) inhibits TAP function with the same efficacy as recombinant ICP47 (rICP47) (open symbols). (C) Hom2 cells were incubated with the 125I-labeled peptide library for 10 min at 37°C for the times indicated and with 5 μM recombinant or synthetic ICP47 either treated with trypsin for 30 min at 37°C or not treated. Radioactivity associated with Con A–Sepharose is counted. Trypsin destroys the biological activity of both synthetic and recombinant ICP47. (D) Hom2 cells were incubated with the 125I-labeled peptide library for 10 min at 37°C and with various concentrations of synthetic ICP47 untreated (open squares) or heated to 95°C for 30 min and then slowly cooled (open circles) or snap-cooled (closed squares). Results in A, B, and C are the mean of duplicates ± SD.
chemicals or on an ABI (Applied Biosystems, Foster City, CA) peptide synthesizer (model 430A) by Boc chemistry and purified by fast performance liquid chromatography (FPLC) on a Sephadex 100 column or by reverse-phase HPLC on a C18 column. Their composition was verified by amino acid analysis and also by mass spectrometry for full-length ICP47. Quantitation was carried out by amino acid analysis or optical density measurement.

4'-[3,5,3-triFluoromethyl-diazeny1]-phenylalanine (Tpa) (11) was coupled to peptide 1–35 (1–35 Tpa) during synthesis by using its Fmoc derivative.

A nantibody. Anti-TAP antisemur was raised against the TAP1 COOH-terminal domain (12) and the anti-ICP47 antisemur against a COOH-terminal peptide of ICP47 (7).

DNA Sequence of the ICP47 Gene from HSV-2. A HindIII–HindIII fragment (8,477 bp) of HSV-2 strain HG52 genomic DNA was cloned into pUC19, and fragments obtained by sonication then subcloned into M13mp8 for sequence determination by chain terminator methods. The sequence comprised adjoining parts of the short unique and short repeat regions of the genome, including the gene for ICP47 (US12), and will be submitted to the EMBL Library as part of the whole genomic sequence of HSV-2 (Dolan, A., and D.J. McGeoch, unpublished observations). The sequence comprised adjoining parts of the short unique and short repeat regions of the genome, including the gene for ICP47 (US12), and will be submitted to the EMBL Library as part of the whole genomic sequence of HSV-2 (Dolan, A., and D.J. McGeoch, unpublished observations).

Peptide Translocation Assay. Peptide translocation was performed essentially as described (7). In brief, cells were washed twice with transport buffer (130 mM KCl, 10 mM NaCl, 1 mM CaCl$_2$, 2 mM EGTA, 2 mM MgCl$_2$, 50 mM Hepes [pH 7.5] with KOH) at 4°C and then permeabilized (10$^4$ cells/ml) in transport buffer containing 2 µM of streptolysin O (SLO) (bioMérieux, Marcy-l'Etoile, France) for 10 min at 37°C. Permeabilization was assessed by Trypan blue exclusion. Permeabilized cells (10$^5$ cells/sample in Eppendorf tubes) were incubated for 10 min at 37°C with 10 µl of the radiolabeled peptide library (13) and 10 µl of ATP generating system (50 µM ATP, 250 µM UTP, 2.5 mM creatine phosphate, and 8 U creatine phosphokinase; Sigma Chemical Co., St. Louis, MO) in a total volume of 100 µl at 37°C. When indicated, synthetic ICP47 was added to the translocation mixture in a volume of 10 µl. Peptide translocation was terminated by adding 1 ml of ice-cold stop buffer (transport buffer plus 10 mM EDTA, 0.02% N a azide). Samples were centrifuged at 14,000 rpm, supernatant was removed, and 1 ml of ice-cold lysis buffer (0.5% NP-40, 5 mM MgCl$_2$, 50 mM Tris-HCl [pH 7.5]) added. After 20 min, debris was removed by centrifugation at 14,000 rpm and the supernatant incubated with gentle agitation for 1 h with 100 µl of Con A–Sepharose beads (Pharmacia, Uppsala, Sweden) at 4°C. Beads were washed three times with transport buffer and radioactivity quantitated by γ counting.

Metabolic Labeling and Immunoprecipitation. TK143 human fibroblasts (American Type Culture Collection, Rockville, MD) and F11 mouse embryonic fibroblasts (gift from L. van Kaer, Howard Hughes Medical Institute, Vanderbilt University School of Medicine, Nashville, TN) were infected with a recombinant vaccinia virus expressing human TAP1 and TAP2 (7) at a multiplicity of infection of 10. 1 h after infection, medium was removed and cells were labeled overnight with $^{35}$S-methionine/cysteine. Cells were lysed in NP-40 lysis buffer (0.5% NP-40, 50 mM Tris-HCl [pH 7.2], 5 mM MgCl$_2$) and immunoprecipitations were performed as described (14). Immune complexes were recovered on Staphylococcus aureus (Staph A) protein A and washed four times with NET buffer (50 mM Tris-HCl [pH 7.4], 0.5% NP-40, 150 mM NaCl, 5 mM EDTA) and analyzed by SDS-PAGE on 10% or 12.5% gels.

Gel Electrophoresis. SDS-PAGE and fluorography were performed as described (15).

Results and Discussion

Biological and Functional Analysis of Synthetic ICP47-1. An 87-amino acid polypeptide corresponding to ICP47 from HSV-1 (strain 17) without the initiating methionine was synthesized on a solid phase support, the substitution grade of which had been reduced to facilitate synthesis of longer peptides. Nor-leucine was substituted for methionine at positions 6 and 14 to avoid oxidation of these residues during HF cleavage and workup of the synthetic product, and lysine was substituted for arginine at the COOH terminus.

Peptide Iodination. Peptides were iodinated by the chloramine T method as described (16).

Cross-linking A says. TK143 cells infected with vaccinia virus expressing human TAP1 and TAP2 in the conditions above described were permeabilized with SLO as previously defined. 5 × 10$^4$ cells were resuspended in 200 µl of transport buffer and incubated with 100 µl of $^{125}$I-labeled 1–35 Tpa or 1–35 polypeptide for 5 min on ice. Cross-linking was induced by irradiation with long-wave UV lamp (Fotodyne, New Berlin, WI) for 5 min on ice (17). Cells were then lysed and immunoprecipitations were performed as described (14).

Figure 2. Comparison of the ICP47 gene and protein sequences of HSV-1 and HSV-2. (A) Alignment of coding sequences of the HSV-1 and HSV-2 (US12) genes. The ICP47 coding sequences of HSV-1 (top line) and HSV-2 (bottom line) are shown from initiator ATG to translation stop codon (TGA in each case), with identical aligned residues marked by vertical lines. (B) Amino acid sequence comparison of ICP47 from HSV-2 and HSV-1. Regions of identical aligned sequence are boxed.
Use of Synthetic Peptides to Define the Active Site of ICP47
to facilitate future chemical modifications. Synthetic ICP47 (sICP47) eluted as a single major peak in gel chromatography on a Sephacryl 100 matrix, showing a Gaussian distribution with a Stokes radius corresponding to a molecular weight of 19,000 for a globular protein (Fig. 1 A). We conclude that sICP47 (molecular weight 9,598) does not behave as a monomeric globular protein in aqueous solution, but rather like an unfolded polypeptide chain. Fractions were pooled and analyzed by SDS-PAGE on 15% gels. The results are consistent with a single major species of the predicted molecular weight but with lower molecular weight contaminants clearly visible (data not shown).

Mass spectrometry revealed that the major product was of predicted molecular weight, but again, the broad range of masses observed indicated substantial heterogeneity, as expected for an 87-residue synthetic product.

The biological activity of sICP47 was compared with that of recombinant ICP47 (rICP47) in a TAP-dependent peptide transport assay in semi-intact human lymphoblastoid cells (Fig. 1 B). In this assay, the inhibitory activity of sICP47 and rICP47 was indistinguishable. Both preparations inhibit human TAP, with 50% inhibition seen at approximately 0.2 μM. As with rICP47, the activity of sICP47 is destroyed by proteolysis with trypsin (Fig. 1 C) but not by boiling sICP47 for 30 min (Fig. 1 D) (10). We examined the ability of sICP47 to inhibit TAP-dependent peptide transport in mouse cells. As observed for rICP47 (9, 10), sICP47 was unable to block murine TAP at concentrations up to 10 μM (data not shown). We conclude that the biological activity of sICP47 is indeed attributable to the protein itself, and not to some other nonpeptide product that might have persisted after gel filtration.

Solid-phase synthesis of ICP47 is thus a practical proposition, and because the polypeptide is not exposed to proteases during purification, yields a product that is more stable than rICP47. The purity of the resultant product is adequate for quantitative functional studies. Additionally, the ability to synthesize variants of the molecule rapidly makes truncation and mutagenesis studies simple to perform.

Amino Acid Sequence of ICP47 from HSV-2 Reveals Homology Only in the NH₂-terminal Region (Residues 1–54) of the Molecule. Both HSV-1 and HSV-2 inhibit TAP function and cause retention of MHC class I molecules in the
Figure 3. Synthetic peptides map the core region of ICP47-1 responsible for TAP inhibition to a segment between amino acids 2 and 35. Peptide translocation was assayed in the human cell line Hmy2.C1R using the 125I-radiolabeled peptide library as translocated substrate. Results are means of duplicates ± SD. (A) The activity of full-length synthetic ICP47 (open squares) was compared with that of the polypeptides containing residues 1-53 (open circles), 5-53 (closed squares), and 10-53 (closed circles) at the concentrations indicated. (B) Inhibition of peptide translocation by sICP47 was compared with that of synthetic 1-53 polypeptide with progressive truncations from the NH2-terminal residues at 3.3 μM concentration. (C) The ability of sICP47 (open symbols) to block peptide transport was compared with that of peptides with progressive truncations at its COOH terminus (closed symbols) at the concentrations indicated. (D) An alanine scan was performed by replacing each single residue in the polypeptide 1–35 with alanine, except residues 3, 7, and 22 which are alanine in the original ICP47 sequence. The ability of each of these alanine analogues at a concentration of 3.3 μM to inhibit TAP function was tested in a transport assay. Positions conserved between ICP47-1 and ICP47-2 are in bold. Results represent percent peptide transport remaining in the presence of ICP47 alanine analogues. 100% refers to transport in the absence of inhibitors. The amino acid sequence of 1–35 polypeptide is shown. (E) Acetylation of sICP47 has no effect on its biological activity. The ability of 1–53 polypeptide (open symbols) to inhibit TAP function was compared with that of its N-acetylated version (closed symbols) at the concentrations indicated.
Use of Synthetic Peptides to Define the Active Site of IC P47

3. **ICP47-1–35 most likely to determine biological activity.** Fig. 3 shows the ability of these peptides to inhibit peptide transport. Three regions (A, 8–12; B, 17–24; and C, 28–31) appear important for the ability to inhibit TAP, with segment 17–24 showing the least tolerance of alanine substitution at any residue, with the exception of position 20.

Finally, we note that transport of peptides by TAP cannot be competed by NH2-terminal acetylation of the peptide substrate (20). Because of the importance of the NH2-terminal region of ICP47 to its ability to inhibit TAP, and because ICP47 and transportable peptides compete for the same binding site (9, 10), we tested whether N-acetylation affects transport of peptide substrates (20), but not the ability of ICP47 to prevent transport; and (d) the inability of ICP47 to inhibit mouse TAP (9, 10) all argue for distinct modes of action.

The TAP Complex Coimmunoprecipitates with Synthetic ICP47. The TAP1–TAP2 heterodimer coprecipitates with ICP47 in cells infected with a recombinant adenovirus expressing ICP47 (7) or stably transfected with ICP47 (8). To determine whether the TAP complex could be coprecipitated with sICP47, sICP47 was introduced into semi-intact mouse fibroblasts that had been infected with a vaccinia vector expressing human TAP1–TAP2 for 1 h and then labeled with [35S]methionine/cysteine overnight. SLO-permeabilized cells were incubated for 10 min with synthetic ICP47 either at 4°C or 37°C, lysed, and immunoprecipitated either with an anti-ICP47 or with an anti-ICP47 antiserum. Samples were analyzed by 12.5% SDS-PAGE and fluorography. The anti-ICP47 antiserum nonspecifically precipitates a protein with a mobility identical to that of the TAP1 subunit. (B) Cross-linking with 1–35 Tpa results in labeling both TAP subunits, whereas only TAP1 subunit is labeled with 1–35 polypeptide labeled at Tyr 21. TK143 human fibroblasts were infected with a recombinant vaccinia virus expressing human TAP1–TAP2. Cells were permeabilized with SLO and incubated for 5 min on ice either with 125I-radiolabeled 1–35 Tpa (lane 2) or with 125I-radiolabeled Tyr21 1–35 polypeptide (lane 3). Cross-linking was induced by exposure to long wave UV light for 5 min on ice. After lysis in 0.5% NP-40 lysis buffer, samples were immunoprecipitated with an anti-TAP1 antiserum. As a control, TK143 cells infected with vaccinia virus expressing TAP1+2 were metabolically labeled with [35S]methionine/cysteine and immunoprecipitated with the same anti-TAP1 antiserum (lane 1). Samples were analyzed by 10% SDS-PAGE and fluorography. The position of migration of TAP1 and TAP2 was established based on the mobility of [35S]methionine/cysteine-labeled samples. The increase in size observed for the cross-linked products is due to the covalent attachment of the 125I-labeled 1–35 fragment.

(C) Model of ICP47–TAP interaction. The NH2 terminus of ICP47 contacts both TAP subunits, whereas residue 21 (Y21) binds only to TAP1 subunit. Likely, the COOH-terminal half of ICP47 does not interact with TAP.

![Figure 4](image.png)

**Figure 4.** Synthetic ICP47 forms a complex with the TAP1 and TAP2 heterodimer. (A) Mouse F1 fibroblasts were infected with a recombinant vaccinia virus expressing human TAP1+2 for 1 h and then labeled with [35S]methionine/cysteine overnight. SLO-permeabilized cells were incubated for 10 min with synthetic ICP47 either at 4°C or 37°C, lysed, and immunoprecipitated either with an anti-ICP47 or with an anti-ICP47 antiserum. Samples were analyzed by 12.5% SDS-PAGE and fluorography. The anti-ICP47 antiserum nonspecifically precipitates a protein with a mobility identical to that of the TAP1 subunit. (B) Cross-linking with 1–35 Tpa results in labeling both TAP subunits, whereas only TAP1 subunit is labeled with 1–35 polypeptide labeled at Tyr 21. TK143 human fibroblasts were infected with a recombinant vaccinia virus expressing human TAP1–TAP2. Cells were permeabilized with SLO and incubated for 5 min on ice either with 125I-radiolabeled 1–35 Tpa (lane 2) or with 125I-radiolabeled Tyr21 1–35 polypeptide (lane 3). Cross-linking was induced by exposure to long wave UV light for 5 min on ice. After lysis in 0.5% NP-40 lysis buffer, samples were immunoprecipitated with an anti-TAP1 antiserum. As a control, TK143 cells infected with vaccinia virus expressing TAP1+2 were metabolically labeled with [35S]methionine/cysteine and immunoprecipitated with the same anti-TAP1 antiserum (lane 1). Samples were analyzed by 10% SDS-PAGE and fluorography. The position of migration of TAP1 and TAP2 was established based on the mobility of [35S]methionine/cysteine-labeled samples. The increase in size observed for the cross-linked products is due to the covalent attachment of the 125I-labeled 1–35 fragment. (C) Model of ICP47–TAP interaction. The NH2 terminus of ICP47 contacts both TAP subunits, whereas residue 21 (Y21) binds only to TAP1 subunit. Likely, the COOH-terminal half of ICP47 does not interact with TAP.
Photocross-linking of ICP47-1–35 to TAP. To determine whether the 1–35 polypeptide has the same ability as the full-length sICP47 to interact with the TAP complex, a cross-linkable 1–35-mer polypeptide was synthesized by coupling the UV photoactivatable phenylalanine analogue Tpa to its NH$_2$ terminus (1–35 Tpa). The ability to assign the length of the active core of ICP47 to residues 1–35 makes the synthesis of the photoactivatable analogue feasible. This analogue also blocks TAP function, although less efficiently than the unmodified 1–35 polypeptide (data not shown). Although peptide substrates containing Tpa are translocated normally by TAP (17), it is likely, given the importance of the NH$_2$-terminal region of ICP47 to its function, that Tpa modification renders the molecule less effective in blocking TAP.

Permeabilized human TK143 fibroblasts infected with a recombinant vaccinia virus expressing human TAP1–2 were incubated with $^{125}$I-labeled 1–35 Tpa. Both TAP1 and TAP2 subunits were photolabeled with 1–35 Tpa cross-linkable polypeptide with similar efficacy (Fig. 4 B).

Small polypeptides labeled with $^{125}$I at Tyr residues can often be cross-linked to its receptor. While the chemistry underlying this reaction is not known, it is a highly effective way of cross-linking epidermal growth factor (EGF) to its receptor (23). We explored a similar strategy for $^{125}$I-Tyr$_{21}$ labeled 1–35 polypeptide. We observed that only TAP1 crosslinks with this $^{125}$I-labeled Tyr$_{21}$ 1–35 polypeptide (Fig. 4 B).

ICP47 inhibits TAP function by binding to a site in TAP that includes both subunits and overlaps the peptide binding domain (9, 10). Because the NH$_2$ terminus of ICP47 interacts with both TAP1 and TAP2 as deduced from cross-linking with 1–35 Tpa, we suggest that ICP47 must contact spatially close cytosolic domains in both subunits to allow residue Y21 in ICP47, where $^{125}$I-labeled moiety is attached, to interact with TAP1 subunit (Fig. 4 C). Residue 21 and adjoining residues (17–24) are particularly intolerant of alanine substitution (see Fig. 3 D), emphasizing the involvement of this region of ICP47 in binding and inhibition of TAP function.

The results presented here show that (a) residues 2–35 contain the core region responsible for TAP inhibition and that (b) within this sequence, subregions are rather intolerant of amino acid substitutions, suggesting that local conformation or specific local interactions may be necessary for TAP inhibition. Thus, the distal frameshift between ICP47 coding sequences of ICP47 from HSV-1 and HSV-2 is tolerated, presumably because the COOH-terminal one-third of the molecule is not functionally important. Of interest is the fact that the sequence most highly conserved between ICP47-1 and ICP47-2, between residues 33 and 47, lies largely outside the region we have mapped as essential for blockade of TAP. We assume that this region has been conserved because it is important perhaps for the stability of ICP47 inside the cell. Alternatively, this region may interact with TAP outside the peptide binding area and account for the prolonged association between TAP and ICP47 (10). As our assay involves the near simultaneous addition of ICP47 and reporter peptide, we would not have detected an alteration in the stability of the ICP47–TAP interaction. However, we do note that the interaction of peptide substrate with TAP has two distinct phases: binding occurs regardless of the presence of ATP. Binding of ATP to TAP then results in either transport of peptide or its rapid dissociation from TAP (24). ICP47 is not transported and avoids this ATP-induced dissociation (9, 10). We suggest that interactions with TAP outside the peptide binding site may be important for this stability. Mapping these sites should facilitate the design of better inhibitors for TAP and might perhaps be useful for identification or design of inhibitors of other members of the ABC transporter family.

We thank Dr. D.C. Johnson for providing recombinant ICP47 of HSV-2, and Dr. J. Yewdell for making available recombinant vaccinia virus expressing human TAP1–2.

This work was supported by the National Institutes of Health (NIH; Grant No. ROIAI33456). Begoña Galocha is fellow of Ministerio de Educación y Ciencia, Spain.

Address correspondence to Hidde L. Ploegh, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, 40 Ames Street, Cambridge, Massachusetts 02139. The current address for Ann Hill is the Department of Molecular Microbiology and Immunology, O.H.S.U., L220, 3181, SW Sam Jackson Park Road, Portland, Oregon 97201-3098. The current address for Alejandra Raimondi is Chemogenics Pharmaceuticals, One Kendall Square, Building 300, Cambridge, Massachusetts 02139.

Received for publication 20 December 1996 and in revised form 25 February 1997.

References
1. Androlewicz, M. J., K. S. Anderson, and P. Cresswell. 1993. Evidence that transporters associated with antigen processing translocate a major histocompatibility complex class I-binding peptide into the endoplasmic reticulum in an ATP-dependent manner. Proc Natl Acad Sci U S A. 90:9130–9134.
2. Neefjes, J. J., F. Momburg, and G. J. Hämmerling. 1993. Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter. Science (Wash. D. C.). 261:769–771.
3. Shepherd, J.C., T.N.M. Schumacher, P.G. Ashton-Rickardt, S. Imaeda, H.L. Ploegh, C.A. Janeway, and S. Tonegawa. 1993. TAP1-dependent peptide translocation in vitro is ATP-dependent and selective. Cell. 74:577–584.
4. York, I.A., C. Roop, D.W. Andrews, S.R. Riddell, F.L. Graham, and D.C. Johnson. 1994. A cytosolic Herpes simplex protein inhibits antigen presentation to CD8+ T lymphocytes. Cell. 77:525–535.
5. Hill, A.B., B.C. Barnett, A.J. McMichael, and D.J. McGeoch. 1994. HLA class I molecules are not transported to the cell surface in cells infected with Herpes simplex virus types 1 and 2. J. Immunol. 152:2736–2741.
6. Higgins, C.F. 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. 8:67–113.
7. Hill, A., P. Jugovic, I. York, G. Ruus, J. Bennink, J. Yewdell, H. Ploegh, and D. Johnson. 1995. Herpes simplex virus turns off the TAP to evade host immunity. Nature (Lond.). 375:411–415.
8. Früh, K., K. Ahn, H. Djaballah, P. Sempé, P.M. van Endert, R. Tampé, P.A. Peterson, and Y. Yang. 1995. A viral inhibitor of peptide transporters for antigen presentation. Nature (Lond.). 375:415–418.
9. Ahn, K., T.H. Meyer, S. Uebel, P. Sempé, H. Djaballah, Y. Yang, P.A. Peterson, K. Früh, and R. Tampé. 1996. Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus protein IC P47. EMBO (Eur. Mol. Biol. Organ.) J. 15:3247–3256.
10. Tomazin, R., A.B. Hill, P. Jugovic, I. York, P. van Endert, H.L. Ploegh, D.W. Andrews, and D.C. Johnson. 1996. Stable binding of the herpes simplex virus IC P47 protein to the peptide binding site of TAP. EMBO (Eur. Mol. Biol. Organ.) J. 15:3256–3266.
11. Brunner, J. 1993. New photoimaging and crosslinking methods. Annu. Rev. Biochem. 62:483–514.
12. Cromme, F.V., J. Airey, M.-T. Heemels, H.L. Ploegh, P.J. Keating, P.L. Stern, C.J.L.M. Miejer, and J.M.M. Van Boomers. 1994. Loss of transporter protein, encoded by the TAP-1 gene, is highly correlated with loss of HLA expression in cervical carcinomas. J. Exp. Med. 179:335–340.
13. Schumacher, T.N.M., D.V. Kantesaria, D.V. Serreze, D.C. Roppeanen, and H.L. Ploegh. 1994. Transporters from H-2, H-2, H-2, and H-2 (NOD/Lt) haplotypes translocate similar sets of peptides. Proc. Natl. Acad. Sci. USA. 91:13004–13008.
14. Beersma, M.F.C., M.J.E. Bijlmaekers, and H.L. Ploegh. 1993. Human cytomegalovirus down-regulates HLA class I expression by reducing the stability of class I H chains. J. Immunol. 151:4455–4464.
15. Ploegh, H.L. 1995. One-dimensional isoelectric focusing of proteins in slab gels. In Current Protocols in Protein Science. J.E. Coligan, B.M. Dunn, H.L. Ploegh, D.W. Speicher, and P.T. Wingfield, editors. John Wiley & Sons, New York. 10.2.1–10.2.8.
16. Hunter, W.M., and F.C. Greenwood. 1962. Preparation of iodine-131 labelled growth hormone of high specific activity. Nature (Lond.). 194:495–496.
17. Nijenhuis, M., S. Schmitt, E.A. Armendola, R. Obst, J. Brunner, and G.J. Hammerling. 1996. Identification of a contact region for peptide on the TAP1 chain of the transporter associated with antigen processing. J. Immunol. 156:2186–2195.
18. Rixon, F.J., and D.J. McGeoch. 1984. A 3' co-terminal family of mRNAs from the herpes simplex virus type 1 short region: two overlapping reading frames encode unrelated polypeptides one of which has a highly reiterated amino acid sequence. Nucleic Acids Res. 12:2473–2487.
19. McGeoch, D.J., A. Dolan, S. Donald, and F.J. Rixon. 1985. Sequence determination of genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. 181:1–13.
20. Schumacher, T.N.M., D.V. Kantesaria, M.-T. Heemels, P.G. Ashton-Rickardt, J.C. Shepherd, K. Früh, Y. Yang, P.A. Peterson, S. Tonegawa, and H.L. Ploegh. 1994. Peptide length and sequence specificity of the mouse TAP1/TAP2 translocator. J. Exp. Med. 179:533–540.
21. van Endert, P.M., R. Tampé, T.H. Meyer, R. Tisch, J.-F. Bach, and H.O. McDevitt. 1994. A sequential model for peptide binding and transport by the transporters associated with antigen processing. Immunity. 1:491–500.
22. Androlewicz, M.J., and P. Cresswell. 1994. Human transporters associated with antigen processing possess a promiscuous peptide-binding site. Immunity. 1:7–14.
23. Verheijden, G.F., I. Verlaan, M.J. van Iersel, and W.H. Moolenaar. 1990. Second messenger modulation of epidermal growth factor receptor function does not occur at the level of receptor dimerization. Biochem. J. 271:215–221.
24. Heemels, M.-T., and H.L. Ploegh. 1995. Generation, translocation, and presentation of HLA class I-restricted peptides. Annu. Rev. Biochem. 64:463–491.