Viral modulation of antigen presentation: manipulation of cellular targets in the ER and beyond

Authors’ address
Brendan N. Lilley, Hidde L. Ploegh
Department of Pathology, Harvard Medical School, Boston, MA, USA.

Correspondence to:
Dr Hidde L. Ploegh
Department of Pathology
NRB-836, Harvard Medical School
77 Avenue Louis Pasteur
Boston, MA 02115, USA
Tel.: +1 617 432 4777
Fax: +1 617 432 4778
e-mail: ploegh@hms.harvard.edu

Summary: Viruses that establish long-term infections in their hosts have evolved a number of methods to interfere with the activities of the innate and adaptive immune systems. Control of viral infections is achieved in part through the action of cytotoxic T lymphocytes (CTLs) that recognize cytosolically derived antigenic peptides in the context of class I major histocompatibility complex (MHC) molecules. Viral replication within host cells produces abundant proteinaceous fodder for proteasomal digestion and display by class I MHC products. Tactics that disrupt antigen-presentation pathways and prevent the display of peptides to CD8+ CTLs have been favored during the course of host-virus co-evolution. Viral immunoevasins exploit diverse cellular processes to interfere with host antiviral functions. The study of such viral factors has uncovered novel host proteins that assist these viral factors in their task and that themselves perform important cellular functions. Here, we focus on viral immunoevasins that, together with their cellular targets, interfere with antigen-presentation pathways. In particular, we emphasize the intersection of the cellular quality-control machinery in the endoplasmic reticulum with the herpesvirus proteins that have co-opted it.

Introduction

Given that viruses depend entirely on cells of their hosts for replication, it is axiomatic that cellular functions and proteins that carry out these functions are co-opted by viruses. This manipulation of host factors extends beyond the steps necessary for entry, genome replication, production of viral proteins, packaging of virions, and egress. Viruses that establish long-term infections face a dilemma: how to survive when confronted by a fully primed adaptive arm of the mammalian immune system. Different viruses have evolved their own unique methods to deal with this obstacle to dissemination, but one tactic is the modulation of host-immune recognition (1). The most striking examples of this type of manipulation have revealed themselves through the study of antigen-presentation pathways.

The immune system constantly surveys the cytosolic content of nucleated cells for the presence of foreign invaders. Peptide fragments generated in the cytosol are displayed on
class I major histocompatibility complex (MHC) products at the cell surface of antigen-presenting cells (APCs) or cells infected by a pathogen. Class I MHC–peptide complexes interact with the T-cell receptor (TCR) on CD8+ cytotoxic T lymphocytes (CTLs), and cells displaying peptides recognized as foreign are destroyed (2). Such CTL recognition and destruction of infected cells is crucial for the control of viral replication and dissemination (3). From a simplistic viewpoint, the mere interference with this pathway of antigen presentation should confer a selective advantage on viruses with such manipulative abilities. The dedication by viruses of precious protein-coding capacity to the task of interfering with antigen presentation underscores the likely importance of immune-evasion proteins during the course of infection.

The functional class I MHC antigen-presenting unit at the cell surface consists of a type I membrane glycoprotein heavy chain, a light chain [β2 microglobulin (β2m)], and an 8–10 residue peptide. The α1 and α2 domains of the heavy chain form a groove that displays the peptide, while immuno-globulin (Ig)-like domains of the heavy chain (α3 domain) and the light chain support the peptide-binding cleft (4). Defective proteins synthesized in the cytosol are degraded by the proteasome, resulting in the raw peptide material for display by class I MHC products. Additional oligopeptidases process the proteasomal output into sequences with lengths and residues that bind with high affinity to class I MHC molecules (5). In professional APCs, exogenously acquired proteins are processed also by cytosolic proteasomes, and their peptides can be displayed in the context of class I MHC molecules (6). This process, termed cross-presentation, is important for priming CTLs to foreign antigens (7).

Class I MHC complexes are assembled in the endoplasmic reticulum (ER) through the concerted action of multiple ER-resident proteins. After the heavy and light chains are inserted co-translationally into the ER membrane through the Sec61 complex, a number of chaperones and oxidoreductases assist in the folding of the class I MHC heavy chain and its assembly with β2m (4). Subsequent loading of peptide onto the class I MHC heavy-chain β2m dimer is accomplished by the action of the peptide-loading complex. Tapasin mediates the interaction between class I MHC heavy-chain β2m dimers and the transporter associated with antigen presentation (TAP). Calreticulin and the oxidoreductase ERP57 act in concert with tapasin to load high-affinity peptides onto the class I MHC heterodimer (8). More detailed reviews of the molecular events that lead to the assembly of class I MHC complexes and the generation of antigenic peptides are found elsewhere in this issue. Once the class I MHC complex is formed, it moves to ER exit sites and traffics through the Golgi complex to the cell surface, where it can interact with TCR complexes on CTLs. Each successive step of class I MHC complex biosynthesis is either a known or a potential target of viral immunoevasins. This review focuses on post-translational modulation of antigen presentation, but viruses also interfere with transcriptional regulation of genes that are involved in antigen presentation (1) (Fig. 1).

Viral modulation of antigen presentation: the class I MHC pathway

Manipulation of the endocytic pathway

Display of class I MHC complexes at the cell surface is prevented by viral immunoevasins through a number of different mechanisms and at a number of different locations within the cell (Fig. 1A). Increasing the endocytic rate of class I MHC complexes is a mechanism that has been described for the K3 and K5 immunoevasins of Kaposi’s sarcoma-associated herpesvirus (9). These proteins along with their cellular homologs, the membrane-associated RING-CH (MARCH) proteins, act as E3 ubiquitin ligases that catalyze the ubiquitination of lysine residues in the cytosolic portions of their targets (9, 10). Components of the lysosomal targeting machinery recognize ubiquitinated proteins in the plasma membrane and induce the endocytosis and sorting of the ubiquitin-tagged cargo to lysosomes (11). The activities of these viral E3 ligases are described in detail elsewhere in this issue.

In many instances, the viral immunoevasins that interfere with class I MHC complex trafficking in the secretory pathway act as molecular links between part of the class I MHC complex and cellular factors involved in protein sorting. Targeting of proteins within the endocytic pathway is accomplished, in part, by sorting of cargo proteins into clathrin-coated vesicles. Cargo proteins are targeted to specific compartments via the action of adapter protein (AP) complexes, which are recruited to membranes and interact with sequences in the cytosolic tails of cargo proteins. The AP complexes are heterotetramers that mediate the transport of proteins to different compartments in the cell (11). Each AP complex localizes to a distinct subcellular compartment: AP-1 and AP-3 (which may operate independently of clathrin) localize to the trans-Golgi network (TGN) and endosomes, while AP-2 localizes to the plasma membrane (12). The best characterized sequences that interact with AP complexes are the YXXΦ and the (D/E)XXX(L/I) motifs. The YXXΦ motif interacts with the μ subunits of AP complexes (13), while recent evidence suggests that the
di-leucine motif interacts with the AP-1 γ1-σ1 subunits and with the AP-3 δ-σ3 subunits (14).

The human immunodeficiency virus (HIV) Nef protein is one example of a viral immunoevasin that uses interactions with the endocytic sorting machinery to modulate class I MHC surface expression. Nef interacts directly with the cytosolic regions of human leukocyte antigen-A (HLA-A) and HLA-B locus products (15). Nef possesses sequence motifs important for class I MHC downregulation and class I MHC heavy-chain binding (16, 17). The mechanism of Nef-mediated class I MHC modulation is not clear, but recent findings in primary T cells and in T-cell lines indicate that Nef acts early in the secretory pathway to re-route class I MHC complexes from the TGN to lysosomes (18). Experiments with short-interfering RNAs (siRNAs) indicate that this activity of Nef requires the AP-1A complex but not the AP-3 complex (19). Interestingly, the Nef di-leucine motif, which is required for CD4 downregulation and which interacts with μ subunits of the AP-1, -2, and -3 complexes (20), is not required for class I MHC sorting by Nef or for association of the Nef-class I MHC complex with AP-1 (19). A complete characterization of the sorting signal used by Nef should shed light on the mechanism of protein sorting by AP-1.

Murine cytomegalovirus (MCMV) m06/gp48 routes class I MHC complexes to lysosomes using a membrane-proximal di-leucine motif in its cytosolic tail (21). Presumably, this motif interacts with an adapter protein complex, which then directs m06/gp48 and the bound class I MHC molecules to lysosomes for degradation. The human herpesvirus 7 protein U21 also directs class I MHC complexes into lysosomes where they are degraded (22). U21 uses a mechanism that is distinct, however, from the lysosomal targeting pathways that utilize di-leucine-based sorting motifs: the cytosolic portion of U21 is not required for class I MHC routing to lysosomes (23). Defining the pathway manipulated by U21 may reveal an unexpected aspect of protein sorting.

Interfering with TCR recognition
The MCMV protein m04/gp34 binds to class I MHC molecules in the ER, and the m04/gp34-class I MHC complex is then transported to the cell surface (24). The binding of m04/gp34 interferes with CTL recognition of class I MHC molecules, indicating that the conformation of class I MHC
complexes may be altered, or TCR binding may be sterically inhibited by the presence of m04/gp34 (25).

Prevention of class I MHC complex biosynthesis

The ER is the site of action for many viral proteins that interfere with antigen presentation, which is not surprising given that this is the site of class I MHC complex assembly. Inactivation of the TAP peptide transporter is a common tactic used by members of the herpesvirus family. HSV-1 encodes ICP47, a small, cytosolic protein that binds to the TAP1/2 dimer at the cytosolic face of the ER membrane and prevents peptide binding to TAP and transport into the lumen of the ER (26–28). The luminal domain of the human cytomegalovirus (HCMV) US6 protein binds to the core transmembrane domains of the TAP1/2 complex in the lumen of the ER and prevents peptide transport by inhibiting adenosine triphosphate (ATP) binding to the cytosolic adenosine triphosphatase (ATPase) cassettes of TAP1/2 (29, 30). US6 does not affect peptide binding to the transporter (31).

Bovine herpesvirus 1 (BHV-1), pseudorabies virus (PRV), and equine herpesvirus 1 (EHV-1) also inhibit the activity of the TAP transporter (32, 33). The UL49.5 gene product of these varicelloviruses was identified recently as the viral protein that inhibits TAP (34). UL49.5 is a small type I membrane protein that interacts with TAP and blocks peptide transport into the ER. As a result, cells that stably express UL49.5 have significantly reduced levels of class I MHC complexes at the cell surface, and these cells are accordingly impaired in their ability to present antigenic peptides to T cells. The interactions of TAP with ATP and peptide were not affected by UL49.5, which suggests that this 9 kDa viral protein alters the conformation of TAP, such that TAP can no longer transport peptides. Koppers-Lalic et al. (34) also observed a dramatic decrease in the amounts of the TAP subunits when BHV-1 UL49.5 was expressed. TAP could be stabilized with proteasome inhibitors or by eliminating the cytosolic tail of UL49.5 (34). These data indicate that UL49.5 uses its cytosolic tail to catalyze the degradation of TAP via the ubiquitin–proteasome pathway. The mechanisms by which UL49.5 simultaneously engages TAP and a component of the ubiquitin–proteasome pathway (possibly an E3 ligase?) will be a fascinating topic of future research. UL49.5’s ability to inhibit TAP from many different species will also prove to be a useful resource for studies of the consequences of TAP inhibition during viral infection in animal models (34).

Retention of class I MHC complexes in the ER or in associated compartments is another means by which viruses interfere with antigen presentation. The adenovirus E19 protein interacts with certain class I MHC locus products in the ER and retains them via a KKXX ER-retention motif (35, 36). E19 also prevents the tapasin–TAP interaction, thus blocking peptide loading of class I MHC molecules and normal ER egress (36). HCMV US3 is a short-lived type I membrane glycoprotein that causes retention of certain class I MHC locus products in the ER (37, 38). Both the luminal Ig-like domain and the transmembrane domain of US3 are required for interaction and retention of class I MHC complexes (39, 40). A sequence motif in the luminal domain of US3 is necessary for ER retention of US3 itself as well as for ER retention of class I MHC complexes (41). The mechanism for class I MHC retention was thought to involve a transient interaction between class I MHC complexes and ER-retained US3 (42). A recent study of US3 action, however, suggests that US3 may also interfere with peptide loading of class I MHC molecules by inhibiting the function of tapasin (43). A version of a class I MHC heavy chain that requires tapasin for acquisition of high-affinity peptides was affected by US3 expression, whereas versions that are tapasin-independent were not. Interestingly, the US3 ER localization motif was not required for retention of class I MHC molecules in this study (43). It remains possible that US3 retains some tapasin-independent class I MHC products by direct interaction, while tapasin-dependent versions may be retained by both direct interaction with US3 as well as by US3 inhibition of tapasin activity.

Retention in pre-Golgi compartments

MCMV m152 causes the retention of class I MHC molecules in the ER–Golgi intermediate compartment (ERGIC) by an unknown mechanism (44). The luminal domain of m152 is sufficient to cause retention of assembled class I MHC complexes in the ER (45). However, m152 actually traffics through the medial Golgi and is degraded in a lysosomal compartment. Treatment of m152-expressing cells with cycloheximide was used to demonstrate that the continued synthesis of m152 is not required for retention of existing class I MHC molecules (45). This observation suggests that m152 does not need to be physically associated with class I MHC molecules to mediate ERGIC retention. Whether ERGIC-retained class I MHC molecules are properly loaded with peptide is not known. Therefore, it remains possible that m152 inhibits peptide loading. Another intriguing possibility for m152 action is the introduction of a modification onto class I MHC complexes that results in their static retention in the ERGIC (45). Such a hypothetical modification could exclude class I MHC complexes from machinery involved in normal progression through the secretory pathway.
Degradation of class I MHC proteins

Still other viral proteins target components of the class I MHC pathway for destruction. The HCMV proteins US2 and US11 block class I MHC biosynthesis early in the secretory pathway by catalyzing the transport of newly synthesized, membrane-inserted class I MHC heavy chains to the cytosol—a process termed dislocation—where proteasomal degradation ensues (46, 47). The details of class I MHC dislocation mediated by US2 and US11 are discussed below in detail.

The MHV-68 protein mK3 interacts with the components of the peptide-loading complex and uses this interaction as a means to target class I MHC molecules for destruction (48, 49). Like US2 and US11, mK3 also causes class I MHC heavy chains to be removed from the ER and degraded by the proteasome. However, unlike US2 and US11, mK3 contains a cytosolic RING-CH domain that acts as an E3 ubiquitin ligase, the activity of which is essential for class I MHC degradation (48). It is unclear how mK3 initiates class I MHC heavy-chain degradation, as ubiquitination and degradation are independent of the lysine residues in the cytosolic tail of the class I MHC heavy chain (48). Since the bulk of mK3 is located either in the membrane or in the cytosol, exposure of the class I MHC heavy chain luminal domain to the cytosol likely requires additional cellular factors that have yet to be identified. Furthermore, mK3 destabilizes the TAP complex itself, a process dependent on the E3 ubiquitin ligase activity of mK3’s RING-CH domain (50). The proximity of mK3 to TAP may result in a low level of bystander ubiquitination of TAP and its subsequent degradation. Two herpesviruses (BHV-1 and MHV-68) are known to destabilize TAP, but this may be a more common strategy for prevention of class I MHC antigen presentation utilized by other viruses.

Viral interference with class II MHC antigen presentation

Assembly of class II MHC complexes

While the class I MHC pathway is the primary method used by the immune system to rid the body of intracellular pathogens, the class II MHC pathway is also important for the generation of antigen-specific CD4⁺ helper T cells that regulate antiviral immune responses and aid in the generation of CD8⁺ CTLs (51). Proteins that enter the endocytic pathway are degraded by endosomal proteases. The resulting peptides are loaded onto class II MHC αβ dimers and are displayed at the cell surface to CD4⁺ T cells. Class II MHC products are most often found on the surface of dendritic cells, macrophages, and B cells, but expression can be induced in other cell types. Class II αβ dimers assemble in the ER and associate with the class II invariant chain (II), which prevents premature peptide loading by occupying the peptide groove. II routes the class II MHC complexes to acidic compartments, where II is removed and peptide loading occurs. The mature class II MHC molecules are transported to the cell surface and interact with the TCR complex on CD4⁺ T cells (52). A schematic representation of the class II MHC pathway and immunoevasins that interfere with it is presented in Fig. 1B.

Blocking recognition of class II MHC products

Epstein–Barr virus (EBV) establishes a latent infection in B cells. The product of the BZLF2 gene, gp42, facilitates infection of class II MHC⁺ cells by binding to the class II MHC product HLA-DR, which serves as a co-receptor for infection (53). Interfering with the display of peptides in the context of class II MHC products may be a strategy beneficial to EBV. gp42 can be proteolytically processed into a secreted form that binds to class II αβ dimers and prevents interactions with TCR complexes on CD4⁺ T cells (54, 55).

Degradation of class II MHC proteins

Several studies have suggested that HCMV US2 can catalyze the destruction of the class II MHC products HLA-DRα and -DMα (56–58). These results were obtained in multiple cell lines that were induced to express class II MHC products by stable transfection with the CIITA transactivator. However, there are conflicting reports on this matter, as US2 was without effect when expressed in human dendritic cells that endogenously express class II MHC molecules (59). The discrepancy may be due to the relative expression levels of US2 and class II MHC proteins achieved in the two different systems. In CIITA-transfected cells, class II MHC levels were also affected by expression of US3 (60). Using cells that stably express the viral gene products, our laboratory has been unable to demonstrate any effect on class II MHC surface levels, despite high rates of class I MHC heavy-chain dislocation (US2) or significant ER retention of class II MHC complexes (US3) (S. Misaghi, F. Ahmad, E. Fiebiger and H.L.P., unpublished observations).

Downregulation of CD4

The CD4 co-receptor is targeted often by viral immunoevasins. In addition to interfering with class I MHC presentation, HIV-1 Nef also routes CD4 to lysosomes using a di-leucine-based sorting motif that interacts with the AP-1 γ1-σ1 subunits and the AP-3 δ-σ3 subunits (14, 20). The precise mechanism(s) involved in Nef-mediated CD4 downregulation...
is still controversial, but recent data suggest that Nef may employ a combination of pathways, the efficiencies of which vary depending on the cell type. Intracellular retention (probably through an interaction between Nef-CD4 and the AP-1 complex at the TGN) followed by targeting to lysosomes is one mechanism (14). An interaction of the di-leucine motif with the AP-2 complex facilitates internalization of CD4 from the plasma membrane (20). Nef also interacts directly with the ARF1 small GTPase, an interaction that may facilitate targeting to endosomes via βCOP (61). The HIV-1 Vpu protein also targets newly synthesized CD4 molecules in the ER for ubiquitination and proteasomal degradation. Vpu recruits the F-box-containing protein β-TrCP to the ER membrane, where it ubiquitimates lysines in the cytosolic tail of CD4 (62, 63). The molecular events that occur after ubiquitination of the CD4 tail are unknown, but the recent recapitulation of Vpu-mediated CD4 degradation in Saccharomyces cerevisiae should allow a detailed analysis of this pathway (64).

Viral immunoevasins target a diverse array of cellular proteins to aid them in their task of interfering with antigen-presentation pathways. Certain viruses, such as HCMV, have confined their efforts largely to the ER, while others more broadly target cellular factors in a number of subcellular compartments. We now discuss how the HCMV proteins US2 and US11 utilize machinery in the ER to catalyze destruction of class I MHC products.

US2 and US11-mediated dislocation: recognition of class I MHC products

Overview
The HCMV glycoproteins US2 and US11 are both made at early times after infection and destabilize newly synthesized class I MHC heavy chains as mentioned above (37). The efficiency of US2 and US11 action varies between different cell lines, but US11 appears to be more efficient at catalyzing class I MHC downregulation (59). Unlike the K3 and K5 proteins, US2 and US11 do not have any cellular homologs, nor do they have any known sequence motifs that would suggest the manner in which they function. In cells that express either US2 or US11, class I MHC heavy chains are inserted into the ER membrane and are glycosylated, but very shortly after completion of synthesis, the class I MHC heavy chains are destroyed (46, 47). The approximate half-life of a class I MHC heavy chain in US11-expressing cells is 2–5 min (47), but it is slightly longer in US2-expressing cells (46). In US2- or US11-expressing cells treated with proteasome inhibitor, the class I MHC heavy chains are stabilized. Treatment with proteasome inhibitors leads to the appearance of de-glycosylated class I MHC heavy chains that have had their N-linked glycan removed by a cytosolic peptide N-glycanase, Png1 (46, 47, 65). The de-glycosylated class I MHC heavy chains are mostly soluble, cytosolic species, although a fraction does remain membrane associated (47). Thus, the class I MHC heavy chains are not degraded in the ER but are transported from the ER membrane into the cytosol (dislocation), where they are degraded by the proteasome. This mechanism for class I MHC heavy chain destruction is not unique to the US2 and US11 systems, but it bears many similarities to the scheme used by the cell to purge the ER of proteins that fail to fold into their proper conformation (66).

US2 structure/function
ER-inserted US2 is a type I membrane protein of 199 amino acids with a single N-linked glycan yielding a protein of 22 kDa (46). Despite lacking any obvious ER-retention motif, US2 does reside in the ER, where it catalyzes class I MHC heavy-chain destruction. US2 is unusual in that it has a non-cleavable signal sequence that directs the protein to the ER. The US2 signal sequence can be appended to other type I membrane proteins and still yield ER insertion without signal peptide cleavage (67). A significant quantity of newly synthesized US2 fails to insert into the ER, is not glycosylated, and is rapidly degraded by the proteasome (67). US2 that does insert into the ER folds into an Ig-like domain of seven β-strands that forms a complex with the luminal domain of class I MHC molecules (68). ER-inserted US2 is degraded in the cytosol by the proteasome and has a half-life of 1 hour (67, 69).

The crystal structure of US2 bound to the complex of the HLA-A2, β2m, and peptide revealed their mode of interaction (68). The luminal domain of US2 binds to the class I MHC molecule in between the peptide-binding region and the α3 domain, a site of interaction on the class I MHC molecule that is not utilized by other class I MHC-binding proteins. The residues in HLA-A2 that contact US2 are present in only certain groups of HLA locus products, notably HLA-A, certain HLA-B products, and HLA-G (70, 71). This heterogeneity in the potential US2 contact residues predicts that US2 should destabilize only class I MHC products that conform to the structural requirements. This appears to be the case, as HLA-B7 and HLA-Cw3 do not have the consensus US2-binding site and are not downregulated, whereas HLA-A and -B alleles that do conform to the consensus are degraded (71). While the interaction between US2 and class I MHC molecules is likely to be a prerequisite for dislocation, the mere interaction between them is not sufficient. Truncated forms of US2
lacking the cytosolic tail are capable of binding to class I MHC molecules, but do not catalyze dislocation (57, 72).

Initial reports from our laboratory demonstrated the importance of the class I MHC heavy-chain cytosolic tail for US2 degradation (73). U373 cells that stably expressed truncated versions of HLA-A2 were not degraded, whereas their full-length counterparts were. The structural requirements for US2-mediated class I MHC heavy-chain dislocation were examined in murine J26 cells that express human β2m and various versions of HLA locus products. This study demonstrated that the HLA-A2 tail is not required for degradation (71). The apparent discrepancy can be explained by the amounts of class I MHC molecules being synthesized in the two systems. In the U373 system, the total amounts of full-length and tailless class I MHC heavy chains may have exceeded the capacity of the US2-dislocation machinery (73). In a study of US2-mediated degradation in ER-derived microsomes from U373 cells (which are depleted of class I MHC products due to US2 expression), the tailless HLA-A2 introduced by in vitro translation was degraded (74).

The HLA-G protein was previously shown to be refractory to US2 activity, despite having the US2-binding consensus sequence (75). The reason for HLA-G’s resistance to US2 was thought to be a result of HLA-G’s relatively short cytosolic tail. Recent studies using human cells that endogenously express HLA-G, however, reveal that US2 causes a significant decrease in HLA-G surface levels. Examination of HLA-G stability in murine J26 cells indicates that US2 catalyzes dislocation of HLA-G, albeit at a much slower rate than that observed for HLA-A locus products (71).

The cytosolic tail of US2 is required for class I MHC heavy-chain degradation (72). The addition of the US2 tail sequence onto US3 appears to confer upon the fusion protein the ability to catalyze class I MHC heavy-chain degradation (76). In vitro studies of US2 action in ER-derived microsomes have suggested that there are species-specific factors within the ER membrane that assist US2 in the dislocation reaction (72). Identification of such factors will be important for clarifying the mechanism of US2 action and may reveal novel players in the dislocation process.

**US11 structure/function**

US11 also is a type I membrane protein of 215 amino acids with a single N-linked glycan, yielding a protein of 30 kDa (47). US11 is targeted to the ER by a signal sequence, which has the unusual feature of being cleaved post-translationally (77). The rate of signal-sequence cleavage depends on the presence of residues in the transmembrane domain (77, 78).

The lumenal domain of US11 mediates an interaction with the lumenal domain of class I MHC products very shortly after synthesis of the class I MHC heavy chain (78). The US11 lumenal domain is predicted to form an Ig-like fold similar to US2 and US3 (68), but currently there is no structural data to indicate the precise fold or mode of interaction.

Unlike US2, which acts only on properly folded class I MHC complexes (68, 79), US11 interacts with free class I MHC heavy chains or those that are complexed with β2m (B.N.L. and H.L.P., unpublished observations). US11 can catalyze the dislocation of mutant class I molecules that are unable to fold properly (79). A broad range of HLA-A and -B locus products and several murine class I MHC products are affected by US11 (80, 81). The peptide binding α1 and α2 domains of class I MHC heavy chains appear to be the portion of the class I MHC molecule to which US11 binds (81). Thus, US11 seems to be more promiscuous in its binding to class I MHC molecules, both in terms of the alleles that it can bind as well as the structure of the class I MHC molecule targeted. The cytosolic tail of the class I MHC heavy chain is required for US11-mediated degradation. Data from both J26 cells and human U373 cells that express tailless HLA-A2 are consistent in this regard (73, 81). While the specific sequence of the cytosolic tail does not influence dislocation, there does seem to be a length requirement: removal of 10 amino acids from the C-terminus or the addition of green fluorescence protein (GFP) to the C-terminus both block US11-mediated dislocation (81, M. Furman, M. Lorenzo, and H.L.P., unpublished observations).

While the cytosolic portion of US11 is dispensable for activity, the transmembrane domain (TMD) of US11 is essential for catalyzing dislocation. Neither a truncated US11 molecule (lacking the TMD and tail) nor a chimera of the US11 lumenal domain and tail flanking the CD4 TMD are capable of catalyzing dislocation. These US11 mutants form stable complexes with class I MHC products that are retained in the ER (78). US11 lacks a known ER-retention motif, but the lumenal domain is sufficient to localize the protein to the ER (B.N.L. and H.L.P., unpublished observations). In contrast, the lumenal domain of US2, while forming a stable complex with class I MHC complexes in vitro (70), does not have the ability to retain them in the ER. Dislocation-incompetent US2 mutants (that reside in the ER) bind to class I MHC complexes early after synthesis, but this complex dissipates over time as the class I MHC molecules traffic to the cell surface (B.N.L. and H.L.P., unpublished observations). This finding suggests that the association between US2 and the class I MHC complex is dynamic when in the ER.
A glutamine residue at position 192 in the US11 TMD is essential for catalyzing dislocation. Mutation of this residue to leucine (yielding US11\textsubscript{Q192L}) results in a US11 molecule that still binds to class I MHC molecules shortly after they enter the ER but is unable to target them for dislocation (78). The ability of polar residues such as Gln to form interhelical hydrogen bonds within the lipid bilayer has been demonstrated in model systems and for the TMD of the class II MHC invariant chain (82, 83). Our analysis of the US11 TMD indicated that this portion of the protein forms a structure that is in direct contact with a component of the dislocation apparatus. US2 also contains a polar Asn residue within its TMD at position 172, but mutation of this residue to Leu has no effect on US2’s ability to catalyze dislocation (B.N.L. and H.L.P., unpublished observations).

Many proteins that fail to fold properly in the ER are also dislocated to the cytosol and degraded by the proteasome (84). Presumably, the viral proteins catalyze rapid dislocation by bringing class I MHC molecules into contact with the cellular machinery that acts to rid the ER of endogenous misfolded proteins. The recognition events described above likely represent the means by which US2 and US11 specifically recruit the class I MHC molecules for dislocation. The viral factors then employ cellular proteins, which remove defective polypeptides from the ER, to act on class I MHC heavy chains (Fig. 2).

**ER protein quality control and degradation**

A diverse array of proteins enters the secretory pathway at the ER and is subsequently secreted or transported to their final cellular destination. The ER is endowed with a high concentration of chaperones, oxidoreductases, and other enzymes that bring a linear amino acid sequence to a folded three-dimensional structure that includes N-linked glycans, disulfide bonds, and glycosylphosphatidylinositol anchors, among other modifications (85). This protein folding and modification machinery must be highly versatile, as it deals with thousands of topologically and structurally distinct proteins during biosynthesis. The process of protein folding and assembly is far from perfect; many proteins fail to achieve their native conformation. Such misfolded polypeptides are retained in the ER, and if they persist in a misfolded state, the cell targets them for degradation. It is unclear how cells...
recognize what is likely to be a diverse group of misfolded proteins in the ER. Once recognized, one means by which the cell rids the ER of these misfolded polypeptides is to dislocate them from the ER into the cytosol, where they are degraded by the proteasome (84). While other pathways exist to clear the ER of protein waste (86), we focus on dislocation- and proteasome-mediated clearance, because this is the general scheme that is co-opted by US2 and US11 (46, 47).

Although the efficiency of folding in the ER varies widely for different proteins, there is little quantitative data examining the fate of proteins targeted to the ER. In the cytosol, a sizeable portion of all newly synthesized proteins, or defective ribosomal products (DRiPs), are degraded by proteasomes (87). Destruction of proteins on such a large scale provides the raw material for class I MHC antigen presentation (88). Several studies suggest that degradation of misfolded ER proteins can also provide source material for class I MHC presentation (89, 90). The extent to which degradation of misfolded proteins from the ER contributes to the peptide pool displayed by class I MHC antigen presentation is not known.

The connection between misfolded ER proteins and proteasomal degradation was made in the US11-class I MHC heavy-chain system (vide supra) in other mammalian systems as well as in yeast. The identification of the factors that participate in the clearance of misfolded proteins from the ER, however, has been accomplished largely using genetic screens in yeast. The results from two different genetic screens identified many of the players involved in ER protein degradation. The 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGR) is a multispanning ER-membrane protein that is degraded in response to flux in the mevalonate pathway of sterol synthesis (91). A screen for mutants that were defective in this regulated degradation yielded a number of genes (HRD for HMG CoA reductase degradation) (92). Another screen was performed using the steady-state level of a soluble, misfolded, mutant carboxypeptidase Y protein (CPY*) as a read-out (93). Mutants that were unable to degrade the CPY* protein were identified and called DER genes for ‘degradation in the ER’. Even though these screens examined very different types of substrates, their results revealed the existence of a conserved group of factors that degrade proteins that misfold in the ER. Subsequent to these initial screens, the power of the yeast genetic toolkit has uncovered even more factors (94). The mechanisms underlying ER protein degradation seem to be largely conserved throughout eukaryotic organisms, as are the proteins that carry out the process. Both US2 and US11 seem to have co-opted this cellular pathway to catalyze destruction of class I MHC heavy chains, but the identification of the factors that interact with US2 and US11 has proven difficult.

US11-mediated dislocation from the ER to the cytosol: molecular mechanisms

Overview

The large quantity of substrate that is dislocated per unit time makes the US11-class I MHC heavy-chain system a valuable model system for the study of the biochemical events that occur during dislocation. Compared to misfolded cellular proteins, which typically have half-lives between 30 min and several hours, the half-life of the class I MHC heavy chain in cells that express US2 or US11 is much shorter (2–5 min) (46, 47). For certain cellular proteins, the rate-limiting step in degradation is the recognition step that distinguishes the misfolded protein from others undergoing productive folding (95). We propose that US2 and US11 cause class I MHC heavy chains to bypass the normal machinery that recognizes misfolded proteins and target the class I MHC heavy chain directly to the dislocation machinery. Once class I MHC heavy chains are targeted for dislocation, subsequent events are mostly similar to those that occur during the dislocation of other misfolded proteins.

Proteasomal inhibition and de-glycosylated class I MHC heavy chains

In cells that express US2 and US11, the class I MHC heavy chain is completely dislocated to the cytosol when the proteasome is inhibited (46, 47). There is compelling evidence for only a few other substrates for complete removal from the ER membrane (i.e. soluble deglycosylated form) (96, 97). Other substrates arrest in the ER or in a partially cytosolically exposed, membrane-associated state when the proteasome is inhibited [mammals (40, 98)] or when genes affecting dislocation are mutated [i.e. NPL4 or UFD1 mutants in yeast (99, 100)]. These observations suggest tight coupling between dislocation and degradation. A feedback mechanism may be at work: when proteolysis is blocked, inhibition of dislocation to the cytosol is the result.

The observation of the de-glycosylated form of the class I MHC heavy chain may not simply be the result of rapid kinetics of dislocation induced by US2 and US11. In cells that lack β2m, the class I MHC heavy chain is dislocated to the cytosol, and de-glycosylated class I MHC heavy chains are observed when the proteasome is blocked (96). The ease with which the de-glycosylated form of the class I MHC heavy chain can be observed may reflect a general biochemical
property of the protein. It may also be indicative, however, of underlying features of the machinery used to dispose of different types of proteins. Perhaps the proteins that eliminate class I MHC heavy chains favor the complete dislocation and de-glycosylation of substrates upon proteasomal inhibition, whereas other pathways may be more sensitive to the effects of the inhibitors.

**Dislocation: crossing the membrane**

The means by which ER proteins actually cross the membrane and gain exposure to the cytosol remains one of the most pressing questions in the field. It has been hypothesized that there is a channel in the ER membrane that allows the exit of proteins that reside mostly or entirely within the ER lumen (66). The initial data from mammalian cells suggested that Sec61 participates in the removal of proteins from the ER; these findings are supported by genetic data from yeast (101–103). The data in support of Sec61’s involvement in mammalian cells came from studies that examined associations of dislocated proteins with Sec61β by coimmunoprecipitation (46, 104, 105). These data, however, do not provide conclusive evidence for the Sec61-mediated removal of proteins from the ER.

A model for the open state of the channel formed by the archaeal version of the Sec61 complex indicates a very narrow opening (approximately/≈10–12 Å) that accommodates polypeptide chains (106). Structures that are known to be completely dislocated to the cytosol would have great difficulty moving through a channel with such dimensions. For instance, when Png1 activity is blocked either by siRNA or by the inhibitor Z-VAD-fmk, class I MHC heavy chains with an attached high-mannose N-linked glycan can be retrieved readily from the cytosol (40, 107). Furthermore, the addition of GFP and DHFR domains onto the luminal region of the class I MHC heavy chain fails to inhibit dislocation of these chimeras to the cytosol (108, 109). When proteasome inhibitors were added, dislocated class I MHC heavy chains attached to GFP and folded DHFR could be recovered from the cytosol. While it is not possible to rule out that these domains unfold and then refold once in the cytosol, it seems likely that such relatively compact domains may pass across the ER membrane in a partially folded conformation. These findings again indicate a channel with properties that are inconsistent with the observed dimensions of the Sec61 protein-conducting channel. Do additional factors act to alter the conformation of Sec61 to increase the size of the channel? Does dislocation occur via another channel independently of Sec61?

Examination of US11-mediated degradation of the class I MHC heavy chain has uncovered new players in the dislocation process that are likely to be involved in movement of proteins across the ER membrane.

**Dislocation of class I MHC molecules by US2 and US11: some disassembly required?**

The mechanisms proposed above for the action of US2 and US11 raise further questions. Both viral proteins form complexes with class I MHC heavy chains after insertion into the ER (73, 78). In the case of US2, this complex is highly stable in vitro (70). The instability and proteasomal degradation of US2 suggested the possibility that US2 might accompany the class I MHC heavy chain out of the ER (46). The wholesale dislocation of a folded US2-class I MHC heavy-chain complex would require a channel of considerable size. The reduction of disulfide bonds in the class I MHC heavy chain occurs, however, prior to de-glycosylation; this observation indicates unfolding prior to or during dislocation (110). Thus, it seems likely that complexes between the viral proteins and the class I MHC heavy chain disassemble immediately prior to movement of the class I MHC heavy chain across the ER membrane. Proteins involved in such a reaction remain to be identified. There is evidence for protein disulfide isomerase (PDI)-mediated unfolding in the ER of the cholera toxin A subunit – a protein that is also dislocated (111). While unfolding of substrates with larger domains may occur prior to dislocation, it is unknown whether there is a strict requirement for such molecular rearrangement.

**Initiating dislocation**

For entirely luminal proteins, dislocation must be initiated within the ER. Proteins that recognize the substrates may be membrane associated and thus may recruit the substrate to the channel and position it for dislocation. For transmembrane proteins, such as the class I MHC heavy chain, the steps involved in the initiation of dislocation could originate on either side of the membrane. Since only a small portion of the class I MHC heavy chain resides in the cytosol, it seems more probable that dislocation begins in the lumen. After recognition takes place, there are at least two possibilities for how the protein could be moved into the cytosol. The luminal domain could be mobilized to the cytosol through the channel, followed by extraction of the TMD from the lipid bilayer. Alternatively, the TMD could be partitioned out of the lipid bilayer into the channel, followed by movement of the polypeptide chain across the membrane. Removal of amino acid sequences from the cytosolic tail of the class I MHC heavy chain blocks US11-mediated degradation (73, 81), but adding
an entire folded domain to the N-terminus has no effect on dislocation (108, 109). These observations suggest, in the case of US11-mediated dislocation of the class I MHC heavy chain, that dislocation may be initiated by first removing the TMD from the lipid environment, followed by movement into the cytosol. The proteins that initiate movement across the ER membrane from the luminal side remain to be described.

While exposure of luminal domains to the cytosol likely requires a conduit within the ER membrane, proteins that are largely of cytosolic disposition may be less dependent on a channel for removal of membrane-anchoring segments (112). In the cases of AAA proteases in bacteria and mitochondria, it has been suggested that a dedicated channel is not necessary to remove the hydrophobic regions from the lipid bilayer for proteolysis (113, 114). In either case, the removal of hydrophobic TMDs from the lipid bilayer incurs a large energetic cost. ATP hydrolysis is an essential requirement for multiple steps of dislocation (46, 115). Even a rough estimate of the number of ATP molecules hydrolyzed per substrate dislocated will be difficult to generate without a defined in vitro system. The enumeration of all ATP-dependent steps required for dislocation, even ones that occur in the ER lumen (116), is clearly a task important for deciphering the mechanism.

Ubiquitination of class I MHC heavy chains during dislocation
In addition to its role in proteasomal proteolysis, polyubiquitination through K48 linkages plays an important role in movement of class I MHC heavy chains from the ER membrane to the cytosol (117). As class I MHC heavy chains are exposed to the cytosol in either US2- or US11-expressing cells, they are ubiquitinated by an as yet unknown E3 ligase. The majority of polyubiquitinated class I MHC heavy chains are soluble, although a fraction can be found associated with the membrane (118). When the proteasome is inhibited, the majority of class I MHC heavy chains found in the cytosol lack any ubiquitin modification (47, 118). If all class I MHC heavy chains were modified with polyubiquitin chains as they left the ER, then removal via a de-ubiquitinating enzyme may also happen soon after dislocation (115). Three of the many known de-ubiquitinating enzymes are known to be components of the 19S proteasome cap structure (119–121); removal of polyubiquitin chains from substrate precedes proteolysis. The tight coupling between dislocation and degradation suggests that 26S proteasomes may be in close proximity to the site of removal from the ER.

When ubiquitin conjugation is blocked in a permeabilized cell system by depletion of ubiquitin or in a cell line with a temperature-sensitive E1 ubiquitin-activating enzyme, class I MHC heavy chains remain in the ER membrane; their luminal domains are entirely resistant to added protease (117, 122). A similar situation is observed in yeast that are deficient in ER-associated ubiquitin-conjugating enzymes or ligases (100, 123). The question of whether direct ubiquitination of the class I MHC heavy chain is required for dislocation remains unanswered. Nearly all misfolded ER proteins that are degraded by the proteasome can be detected as ubiquitinated species, either in soluble or in membrane-associated form. Thus, there is a strong correlation between substrate dislocation to the cytosol and polyubiquitination of these substrate proteins. A TCR$\beta$ mutant that lacks all lysine residues carried only 10% as much polyubiquitin as the wildtype version, but it was still degraded with normal kinetics (124). Could the introduction of polyubiquitin onto a protein other than the substrate be the actual requirement for dislocation? Can polyubiquitination of the N-terminus of the substrate protein, in the absence of other lysine residues, be sufficient for dislocation?

While ubiquitination is necessary for dislocation, it is not the initiating step. In the case of class I MHC heavy chains in both US2- and US11-expressing cells, cytosolic lyses are not required for dislocation or ubiquitination (79, 118). US2 and US11 may be responsible, either in isolation or in concert with other factors, for initiating the movement of the class I MHC heavy chain into the cytosol. US2 and US11 may facilitate the partitioning of the class I MHC heavy chain TMD from the lipid bilayer into a channel, followed by movement of luminal regions into the cytosol where they are ubiquitinated. The situation for proteins with large cytosolic domains may be different, due to the fact that there is no membrane that separates the ubiquitin-conjugation machinery from the target. In this case, recognition of the misfolded domain could be followed by ubiquitination, which may lead to removal from the ER. HIV-1 Vpu recruits a cytosolic ubiquitin ligase that introduces ubiquitin onto lysine residues of the cytosolic tail of CD4 (62, 63). The mere introduction of these modifications may be sufficient to divert CD4 to degradation, by promoting recognition by downstream effectors (see below). The examination of Vpu-induced CD4 degradation in yeast will reveal whether this pathway intersects with components that deal more generally with the removal of ubiquitinated proteins from the ER (64).

Removal from the membrane: the p97 ATPase and its cofactors
Complete dislocation to the cytosol requires more than polyubiquitination at the ER membrane. US11-expressing cells
treated with the non-hydrolyzable ATP analog AMP-PNP, which allows ubiquitin conjugation to proceed but blocks other ATP-dependent processes, caused the arrest of poly-
ubiquitinated class I MHC heavy chains at the membrane in a partially dislocated state (115). This observation suggested that an additional ATP-dependent step is required to liberate class I MHC heavy chains. A number of groups identified the AAA ATPase Cdc48p (yeast) or p97 [mammals, also known as valosin-containing protein (VCP)] as a factor required for degradation (99, 100, 125, 126). Cdc48/p97 is an abundant cytosolic protein involved in many cellular processes, including homotypic membrane fusion, nuclear envelope assembly, and spindle disassembly after mitosis (127). Cdc48/p97 forms hexamers; each subunit contains two AAA domains with Walker A and B motifs. A portion of Cdc48/p97 associates with membranes, an interaction mediated by the N-terminal domain (128). During cycles of ATP hydrolysis, the domains of p97 undergo dramatic conformational changes that may be responsible for the protein’s disassembly or ‘segregase’ activity (129–131). Cdc48/p97 functions with a number of cofactors that are specific to certain cellular functions (127). The Npl4 and Ufd1 cofactors are important for degradation of ER proteins (99, 125, 132).

Introduction of p97 mutants incapable of hydrolyzing ATP results in a dominant-negative effect on US11-mediated dislocation: ubiquitinated class I MHC heavy chains become trapped at the membrane and are sensitive to added protease (125). This phenotype resembles that of yeast-bearing mutations in either of the Cdc48p cofactors Npl4p or Ufd1p (99, 100). Inducible expression of ATPase-deficient mutants of p97 in mammalian cells also results in the accumulation of ubiquitinated proteins at the ER membrane (133). Collectively, these phenotypes indicate that the Cdc48/p97-Npl4-Ufd1 complex mobilizes polyubiquitinated proteins from the ER membrane prior to proteasomal degradation.

The Npl4 and Ufd1 cofactors mediate binding of polyubiquitin chains by the Cdc48/p97-Npl4-Ufd1 complex (134). The mammalian Npl4 protein has a zinc-finger domain (NZF domain) at its C-terminus that can interact with polyubiquitin chains (134). This domain is not required, however, for US11-mediated dislocation of class I MHC heavy chains (128). The Ufd1 cofactor also binds to K48-linked polyubiquitin chains through its N-terminal UT3 domain. When introduced into a permeabilized cell system, Ufd1 that lacks its UT3 domain acts in a dominant-negative fashion to inhibit degradation of class I MHC heavy chains catalyzed by US11 (128). In this case, polyubiquitinated class I MHC heavy chains are arrested on the membrane. Thus, the UT3 domain of Ufd1 may mediate the interaction of polyubiquitinated class I MHC heavy chains with the p97-Npl4-Ufd1 complex (128).

p97 can also interact with class I MHC heavy chains that have not been ubiquitinated, an interaction dependent upon ATP binding to the D1 domain of p97 (128). This experiment used the permeabilized cell system in which the cytosol had been depleted of ubiquitin. When ubiquitin was depleted from the cytosol, only the cytosolic tail of the class I MHC heavy chains was sensitive to added protease (117), consistent with retention of the luminal region within the ER membrane. When considered together, these results suggest that p97 binds to the cytosolic tail of the class I MHC heavy chain when ubiquitin conjugation is blocked. In the case of US11-mediated dislocation, the tail of the class I MHC heavy chain is required for dislocation (73, 81). Perhaps, US11 recruits the class I MHC heavy chain to a channel in the ER membrane that is in close proximity to p97 and associated factors, allowing for the direct interaction of p97 with the tail of the class I MHC heavy chain. An important test of this hypothesis for the initiation of US11-mediated dislocation will be to examine whether tailless mutants of the class I MHC heavy chain still interact with p97. Interestingly, mitochondrial AAA proteases, which contain both an AAA ATPase domain and a protease domain, act in a similar way to what we propose for the class I MHC heavy chain and p97. Their AAA ATPase and protease domains reside in the matrix and inner membrane space in mitochondria and degrade inner membrane inserted proteins (113). These proteins are capable of pulling unfolded membrane proteins across the membrane for complete proteolysis of the polypeptide chain, but they require a certain length of polypeptide to extend into the compartment where the protease resides (135).

In the cytosol: de-glycosylation and degradation

Removal of N-linked glycans from class I MHC heavy chains happens shortly after exposure to the cytosol (46, 47). De-glycosylated forms can be observed only when the proteasome is inhibited. Thus, de-glycosylation and degradation are closely coupled. The Png1 enzyme removes high mannose oligosaccharides from misfolded proteins via a transglutaminase-like catalytic domain that is inhibited by the pan-caspase inhibitor Z-VAD-fmk (40, 65, 107, 136). While Png1 activity, at first glance, should facilitate proteasomal proteolysis by removing the bulky carbohydrate, Png1 is not required for dislocation or degradation of glycosylated proteins in mammals (40, 107). Png1p activity may increase the rate of glycoprotein degradation in yeast (137). An interaction
between Png1 and a human homolog of yeast Rad23 has been reported (138). Together with the involvement of Rad23 and Dsk2 in mediating interactions between ubiquitinated proteins and the proteasome (139), these data suggest that Png1 may be part of a complex that deglycosylates ubiquitinated proteins after dislocation but prior to proteolysis. Png1 may be important for efficient proteasomal proteolysis of proteins that have large numbers of N-linked glycans or when large quantities of glycosylated proteins are being dislocated.

After removal from the ER membrane, polyubiquitinated proteins are ready targets for the 26S proteasome. The tight coupling of dislocation with proteasomal degradation suggests spatial coordination of these events as well. Although 26S proteasomes reportedly interact with membranes (140), the existence of a specific membrane receptor has not been demonstrated. The Herp protein is localized to the ER and contains a ubiquitin-like domain (141), a module that binds to subunits of the 19S cap of the proteasome (142). Whether the ubiquitin-like domain of Herp or other ER-localized ubiquitin-like proteins recruit proteasomes to the ER membrane is unknown.

The interaction of US11 with the dislocation machinery

Identifying US11’s partner

Mutations that remove critical residues in the US11 TMD result in dislocation-incompetent proteins that are unable to expose the class I MHC heavy chain to the cytosolic environment (78). These results suggest that US11 interacts with a component of the dislocation machinery through residues in its TMD. In order to identify these hypothetical factors, we utilized an affinity-purification approach to identify proteins that associated with wildtype US11 and with the inactive US11Q192L with the idea that proteins that aid US11 in catalyzing dislocation would be accessible only to the wildtype version. A number of ER-resident proteins associate with both versions of US11: BiP, calnexin (CNX), and subunits of the oligosaccharyltransferase complex (OST48, Ribophorins I and II) (69). Because the US11 proteins were expressed at high levels, these associations may reflect mere chaperone–client interactions due to incomplete folding of US11. It is also possible, however, that US11 uses BiP and/or CNX to alter the structure of the class I MHC heavy chain luminal domain prior to dislocation. Another possibility is that the stable interaction of US11 with proteins that participate in class I MHC complex biosynthesis allows US11 to intercept the class I MHC heavy chain early after insertion into the ER.

Identification of US11 lumenal mutants that fail to interact with any one of these proteins could reveal events that occur in the lumen during dislocation.

The one protein that was associated with wildtype US11 and not with the mutant form was a human homolog of yeast Dfm1p (93), which is called Der1-like protein 1 (Derlin-1) (69, 143). This protein is so named because it contains a series of highly conserved residues that conform to the Der1-like domain. Der1p is a yeast protein that is required for degradation of proteins, either soluble or membrane spanning, that have luminal domains defective in folding (93). Only three substrates (CPY*, KHN, and KWW) are known to require Der1p for function (93, 144, 145), but it is likely that there are others. The precise mechanism of Der1p action is unknown, but the deletion of Der1p results in the accumulation of CPY* inside the ER membrane in a ubiquitin-free form (123), suggesting that Der1p is somehow involved in movement of substrates into the cytosol.

Derlin proteins are present in all eukaryotic species and are all relatively small (20–30 kDa) and hydrophobic. Derlins share a common topology of four TMDs with both the N- and C-terminus in the cytosol. Each eukaryotic genome contains at least two Derlins [mammals have three Derlins: Derlin-1, -2, and -3 (69)]. The other Der1p-like protein in yeast is called Dfm1p (Der1p family member 1), and while not involved in CPY* degradation (146), it is an ER membrane protein strongly induced by the accumulation of misfolded proteins in the ER (the unfolded protein response (UPR)) (146, 147). The requirement for Dfm1p in the degradation of additional substrates has not been reported.

In cells that express wildtype US11, Derlin-1 interacts with the class I MHC heavy chain during dislocation (69, 143). An interaction between Derlin-1 and the class I MHC heavy chain is not seen in cells that express US11Q192L. Thus, US11 uses its TMD to recruit class I MHC heavy chains into a complex with Derlin-1. In US2-expressing cells, the class I MHC heavy chain does not associate with Derlin-1 (69), providing further evidence for the use of distinct dislocation pathways by US2 and US11 (Fig. 3A). Both ER-inserted glycosylated and cytosolically exposed de-glycosylated forms of the class I MHC heavy chain can be recovered in a complex with Derlin-1, when the proteasome is inhibited in US11-expressing cells. The interaction between Derlin-1 and the de-glycosylated class I MHC heavy chain is specific, because no de-glycosylated class I MHC heavy chain is recovered with Derlin-1 in cells that express US2, despite amounts of the de-glycosylated forms that are equivalent to those seen in US11-expressing cells (69).
Addition of GFP to the C-terminus of Derlin-1 results in a fusion protein that acts in a dominant-negative fashion to inhibit dislocation of class I MHC heavy chains mediated by US11 (69) (Fig. 3B). The expression of the related Derlin proteins, Derlin-2 and -3, in GFP-tagged form was without effect (69, B.N.L., B. Mueller, and H.L.P., unpublished results). The expression of the Derlin-1 dominant-negative caused class I MHC heavy chains to arrest in the ER in a complex with US11 (69). The above observations, together with Derlin-1’s integral membrane disposition, suggest that Derlin-1 participates in the movement of class I MHC heavy chains into the cytosol during dislocation.

The Derlin-1 dominant-negative was without effect on US2-mediated dislocation, and no interaction between class I MHC heavy chains and Derlin-1 was observed in US2-expressing cells (69). These observations provide definitive evidence that US2 utilizes a pathway for class I MHC heavy-chain dislocation distinct from the one used by US11 (Fig. 3). An approach similar to that used for US11 should reveal interacting partners that assist US2 in its function (Fig. 3).

Using the p97 ATPase as bait, Ye et al. (143) searched for ER membrane proteins that bind to the ATPase and identified Derlin-1 and another protein called VIMP (VCP-interacting membrane protein). Association of Derlin-1 with US11 and the class I MHC heavy chain was also shown. Both Derlin-1 and VIMP associate with polyubiquitinated proteins as they exit the ER membrane. RNAi of the Derlin-1 ortholog in Caenorhabditis elegans induced the UPR, which suggests that Derlin-1 plays a more general role in the clearance of misfolded proteins from the ER. Thus, Derlin-1 represents a likely target for viruses that seek to destroy, early during biosynthesis, proteins that enter the secretory pathway.

The role of VIMP in dislocation is not clear. The cytosolic domain of VIMP interacts with p97, which suggested that VIMP might be an ER membrane receptor for p97 (143). Only mammalian genomes possess VIMP, however, which was previously identified as a selenoprotein (SelS) (148). It is unknown whether the presence of a selenocysteine residue in VIMP affects the function of the protein, nor is it known whether VIMP is required for dislocation.

The data from our report as well as from Ye et al. (143) establish Derlin-1 as a central factor within the ER lipid bilayer that links US11 and the class I MHC heavy chain to downstream proteins that participate in the removal of proteins from the ER. There is no definitive evidence to indicate how Derlin-1 might participate in dislocation. One possibility that has been proposed is the formation of a channel by Derlin-1. Because Derlin-1 only possesses four TMDs, the formation of a channel that could accommodate intact proteins would likely require oligomerization or association with other factors (69, 143). This hypothesis is attractive, but it requires further investigation. Consistent with this idea, we observe both homo- and hetero-oligomerization...
by Derlin proteins as well as association of Derlins with a number of other factors (149).

We propose a model that is depicted schematically in Fig. 4, which accounts for the observations that have been made this far. US11 exists in a stable complex with Derlin-1, an interaction mediated by the TMD of US11 with residues of Derlin-1 that remain to be identified (B.N.L. and H.L.P., unpublished observations). After insertion into the ER, class I MHC heavy chains are recruited into the US11-Derlin-1 complex, which is mediated by the interaction of the US11 and class I MHC heavy-chain lumenal domains. Once this complex is formed, Derlin-1 participates in the movement of the heavy chain across the ER membrane and mediates an interaction between the class I MHC heavy chain and the p97 ATPase, which results in movement into the cytosol. During extraction, unknown ubiquitin ligases act on the heavy chain. Polyubiquitinated class I heavy chains then interact with the p97 cofactors Ufd1 and Npl4 and are completely extracted from the membrane. Once in the cytosol, the Png1 enzyme removes the glycan, and de-ubiquitinating enzymes remove the polyubiquitin tree prior to proteasomal proteolysis. All of these events are tightly coupled and likely occur in close proximity to one another (Fig. 4).

Concluding remarks

Viral immunoevasins manipulate cellular proteins in order to modulate immune recognition of virally infected cells. In many cases, the cellular factors used by the viral proteins to modulate class I MHC expression remain unknown. Two more well-understood immunoevasins are the HCMV proteins US2 and US11. Their ability to catalyze class I MHC heavy-chain destruction has been well described. The domains of both the class I MHC heavy chain and of the US2 and US11 proteins that are required for dislocation have also been thoroughly characterized. While it had long been hypothesized that US2 and US11 co-opt cellular machinery involved in ER protein degradation, direct interactions between the viral proteins and cellular factors had not been demonstrated. The identification of Derlin-1 as the factor that US11 co-opts to catalyze class I MHC heavy-chain destruction is an excellent illustration of the molecular interactions between viral and host proteins involved in immune evasion. The mode of action of many immunoevasins remains unknown as are the cellular proteins that are co-opted by the viral modulators. Further dissection of the mechanisms by which antigen-presentation pathways are subverted will reveal the elegant mechanisms that have arisen during the course of host-virus co-evolution, and it will also uncover novel cellular proteins that are involved in protein transport and degradation pathways. A more refined analysis of the molecular interactions between viral immunoevasins and their cellular targets should allow for the development of methods to interfere with such interactions, which may represent a useful strategy to prevent dissemination of viral pathogens that cause persistent infections.

Note added in proof

It was recently shown that the Sec 61 channel acts as an ER membrane receptor for proteasomes (150).
References


24. Kleijnen MF, et al. A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC molecules in the ER, which is not retained but is transported to the cell surface. EMBO J 1997; 16: 685–694.


36. Kleijnen MF, et al. A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC molecules in the ER, which is not retained but is transported to the cell surface. EMBO J 1997; 16: 685–694.
75. Schust DJ, Tortorella D, Seebach J, Phan C, Ploegh HL. Trophoblast class I major histo-
compatibility complex (MHC) products are resistant to rapid degradation imposed by the
76. Chevalier MS, Johnson DC. Human cytome-
galgovirus US3 chimeras containing US2 cytosolic residues acquire major histo-
77. Rehn A, Stern P, Ploegh HL, Tortorella D. Signal peptide cleavage of a type I
membrane protein, HCMV US11, is dependent on its membrane anchor. EMBO J
78. Lilley BN, Tortorella D, Ploegh HL. Dislocation of a type I membrane protein
requires interactions between membrane-spanning segments within the lipid bilayer.
79. Furman MH, Loureiro J, Ploegh HL, Tortorella D. Ubiquitylation of the cyto-
solic domain of a type I membrane protein is not required to initiate its dislocation
80. Machold RP, Wiertz EJ, Jones TR, Ploegh HL. The HCMV gene products US11 and
US2 differ in their ability to attack allelic forms of murine major histocompatibility
81. Barel MT, Pizzato N, van Leeuwen D, Bouteiller PL, Wiertz EJ, Lenfant F. Amino
acid composition of alpha1/alpha2 domains and cytoplasmic tail of MHC class I molecules
determine their susceptibility to human cyto-
algrovirus US11-mediated down-regula-
82. Pootp RN, Engelman DM. Helical membrane protein folding, stability, and evolution.
83. Kukol A, Torres J, Arkin LT. A structure for the trimeric MHC class II-associated invar-
84. Hirsch C, Jarosch E, Sommer T, Wolf DH. Endoplasmic reticulum-associated protein
85. Ellgaard L, Helenius A. Quality control in
86. Schmitz A, Herzog V. Endoplasmic reticu-
87. Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation
88. Princiotta MF, et al. Quantitating protein
89. Holttappels R, Thomas D, Podlech J, Geginat G, Steffens HP, Reddelheze MJ. The
putative natural killer decylo early gene m04 (gp34) of murine cytomegalovirus encodes
90. Ostankovitch M, Robila V, Engelhard VH. Regulated folding of tyrosinase in the
endoplasmic reticulum demonstrates that misfolded full-length proteins are efficient
substrates for class I processing and presen-
91. Hampton RY, Rine J. Regulated degradation
of HMG-CoA reductase, an integral membrane protein of the endoplasmic reticulum,
92. Hampton RY, Gardiner RG, Rine J. Role of
26S proteasome and HRD genes in the
degradation of 3-hydroxy-3-methylglutaryl-
CoA reductase, an integral endoplasmic reticulum membrane protein. Mol Biol Cell
93. Knop M, Finger A, Braun T, Hellmuth K, Wolf DH. Der1, a novel protein specifically
94. Ahner A, Brodsky JL. Checkpoints in
ER-associated degradation: excuse me, which way to the proteasome? Trends Cell
95. Molinari M, Calanca V, Galli C, Lucca P, Pagantini P. Role of EDEM in the release of
96. Hughes EA, Hammond C, Cresswell P. Misfolded major histocompatibility complex
class I heavy chains are translocated into the
cytoplasm and degraded by the proteasome.
97. Kitzmuller C, et al. Processing of N-linked
glycans during endoplasmic-recticum-associated degradation of a short-lived var-
98. Elbazey T, Shaipra I, Ravishakovich E, Bar-Nun S. Distinct steps in dislocation of
luminal endoplasmic reticulum-associated degradation substrates: roles of endoplasmic
reticulum-bound p97/Cdc48p and protea-
NPL4 is required for the proteasomal
processing of ubiquitinated ER proteins. Mol
100. Jarosch E, et al. Protein dislocation from the
ER requires polyubiquitination and the
101. Plemper RR, Bohmier S, Bordallo J,
Sommer T, Wolf DH. Mutant analysis links
the translocon and BiP to retrograde protein
102. Plemper RR, Bordallo J, Deak PM, Taxis C,
Hirt R, Wolf DH. Genetic interactions of
Hrd3p and Der3p/Hrd1p with Sec61p sug-
gest a retro-translocation complex mediat-
ing protein transport for ER degradation. J
103. Zhou M, Schekman R. The engagement of
Sec61p in the ER dislocation process. Mol
104. Bebok Z, Mazooci C, King SA, Hong JS,
Sorscher EJ. The mechanism underlying
cystic fibrosis transmembrane conductance
regulator transport from the endoplasmic reticulum to the proteasome includes
Sec61beta and a cytosolic, deglycosylated
105. de Virgilo M, Weningher H, Ivesa NE.
Ubiquitination is required for the retro-
translocation of a short-lived luminal endo-
plasmic reticulum glycoprotein to the cyto-
sol for degradation by the proteasome. J Biol
106. Clemons WM Jr, Menetret JF, Akey CW,
Rapoport TA. Structural insight into the
protein translocation channel. Curr Opin
107. Blom D, Hirsch C, Stern P, Tortorella D,
Ploegh HL. A glycosylated type I membrane
protein becomes cytosolic when peptide:
N-glycanase is compromised. EMBO J
108. Fiehiger E, Story C, Ploegh HL, Tortorella D.
Visualization of the ER-to-cytosol dis-
loction reaction of a type I membrane protein.
109. Tirosh B, Furman MH, Tortorella D,
Ploegh HL. Protein unfolding is not a pre-
 requisite for endoplasmic reticulum-to-
membrane proteins from the ER to the
cytosol is sensitive to changes in redox
111. Tsai B, Rodighiero C, Lencer WI,
Rapoport TA. Protein disulfide isomerase acts
as a redox-dependent chaperone to unfold
112. Walter J, Urban I, Volkwein C, Sommer T.
Sec61p-independent dislocation of the
tail-anchored ER membrane protein Ubc6p.


Albring J, Koopmann JO, Hammerling GJ, Momburg F. Retrotranslocation of MHC class I heavy chain from the endoplasmic reticulum to the cytosol is dependent on ATP supply to the ER lumen. Mol Immunol 2004;40:733–741.


Dalal S, Rosser MF, Cyr DM, Hanson PI. Distinct roles for the AAA ATPases NSF and Cdc48p, a cytosolic chaperone required for translocation from the ER to the cytosol: 5645–5652.


Lilley BN, Ploegh HL. Multi-protein complexes that link dislocation, ubiquitination and extraction of misfolded proteins from the endoplasmic reticulum membrane. Proc Natl Acad Sci USA 2005; in press.