Endoplasmic reticulum chaperones participate in human cytomegalovirus US2-mediated degradation of class I major histocompatibility complex molecules

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Inhibition of cell-surface expression of major histocompatibility complex class I molecules by human cytomegalovirus (HCMV, a β-herpesvirus) promotes escape from recognition by CD8+ cytotoxic T cells. The HCMV US2 and US11 gene products induce class I downregulation during the early phase of HCMV infection by facilitating the degradation of class I heavy chains. The HCMV proteins promote the transport of the class I heavy chains across the endoplasmic reticulum (ER) membrane into the cytosol by a process referred to as ‘dislocation’, which is then followed by proteasome degradation. This process has striking similarities to the degradation of misfolded ER proteins mediated by ER quality control. Even though the major steps of the dislocation reaction have been characterized, the cellular proteins, specifically the ER chaperones involved in targeting class I for dislocation, have not been fully delineated. To elucidate the chaperones involved in HCMV-mediated class I dislocation, we utilized a chimeric class I heavy chain with an affinity tag at its carboxy terminus. Interestingly, US2 but not US11 continued to target the class I chimera for destruction, suggesting a structural limitation for US11-mediated degradation. Association studies in US2 cells and in cells that express a US2 mutant, US2–186HA, revealed that class I specifically interacts with calnexin, BiP and calreticulin. These findings demonstrate that US2-mediated class I destruction utilizes specific chaperones to facilitate class I dislocation. The data suggest a more general model in which the chaperones that mediate protein folding may also function during ER quality control to eliminate aberrant ER proteins.

INTRODUCTION

Many viruses utilize strategies to interfere with class I major histocompatibility complex (MHC) antigen presentation in order to prevent the detection and clearance of infected cells (Hewitt, 2003; Tortorella et al., 2000). The class I MHC molecule is a stable trimeric complex consisting of a glycosylated heavy chain, β2-microglobulin, and an antigenic peptide that is recognized by a cytotoxic CD8+ T lymphocyte (CTL) (Townsend & Bodmer, 1989). Human cytomegalovirus (HCMV) is proposed to avoid CTL-induced killing by limiting the cell surface presentation of antigenic peptides. HCMV encodes multiple gene products [glycoproteins (gp)US2, gpUS3, gpUS6, gpUS11, gpUS10 and pUL82] that can either associate with class I and/or modulate class I antigen presentation (Lin et al., 2007; Trgovcich et al., 2006). Most of these gene products are expressed during the immediate-early/early phase of HCMV infection, indicating a critical time for HCMV to avoid immune detection.

The HCMV type I transmembrane gpUS2 and gpUS11 prevent surface expression of class I molecules by mediating the proteasome destruction of class I heavy chains (Wiertz et al., 1996a, b). gpUS2 and gpUS11 induce the extraction of class I heavy chains from the endoplasmic reticulum (ER) into the cytosol by the AAA-ATPase p97-Npl4-Ufd1 complex (Ye et al., 2001). Upon exposure to the cytosol, class I heavy chains are deglycosylated by N-glycanase and then degraded by the proteasome. gpUS2 and gpUS11 mediate the destruction of class I heavy chains in a manner similar to how ER quality control disposes of misfolded ER proteins. A number of human diseases that are caused by the degradation of defective proteins include lung diseases (i.e. cystic fibrosis), neurological diseases (i.e. Fabri disease) and diabetes mellitus (Aridor & Hannan, 2002). Essentially, ER quality control enables cells to cast away ER polypeptides that do not reach their proper native conformation due to inherent mutations or improper glycosylation (Hiller et al., 1996; Sitia & Braakman, 2003). The specific molecular events of controlling HCMV-mediated class I degradation, and the
cellular proteins involved in this process, have not yet been fully characterized. US2- and US11-mediated class I degradation is a robust model system used to study HCMV host–pathogen interactions of proteins, and has also provided insight into the general mechanism of dislocation and degradation of aberrant ER proteins.

To identify ER chaperones that complex with class I heavy chains prior to their dislocation, a class I heavy chain molecule fitted with an affinity tag at its carboxy terminus (Puig et al., 2001) was co-expressed with gpUS2 and gpUS11 continuous cell lines. gpUS11 was incapable of mediating the degradation of the chimeric class I molecules, hence, our studies focused on US2-mediated class I degradation. Association studies implicated the involvement of calnexin, calreticulin, and BiP in US2-mediated degradation of class I molecules. These results suggest for the first time that a specific chaperone complex participates in US2-mediated destruction of class I heavy chains. The data imply that the chaperones involved in protein folding/maturation may also act as ER quality control 'sensors' to target misfolded proteins for degradation.

**METHODS**

**Cell lines and antibodies.** Human U373-MG astrocytoma cells and U373 transfectants that stably express class I heavy chains–CTAP molecules (see below) were maintained in Dulbecco’s modified Eagle’s medium (Oresic et al., 2006). Rabbit polyclonal anti-US2, anti-class I heavy chain and anti-protein disulfide isomerase (PDI) were generated as described previously (Fiebiger et al., 2002; Rehm et al., 2001; Tortorella et al., 1998). The anti-gp96 serum was provided by Dr Ploegh (MIT, Cambridge, MA, USA). The monoclonal antibody W6/32 (Parham et al., 1979) was purified from hybridoma cultured supernatant (Harlow et al., 1985). The anti-calnexin monoclonal antibody (AF8) (Hochstenbach et al., 1992) was a gift from Dr Brenner (Harvard Medical School, Boston, MA, USA). Anti-calreticulin and anti-BiP antibodies were purchased from Assay Design and BD Biosciences, respectively.

**Generation of the HC–CTAP cDNA and retrovirus transduction.** Class I heavy chain chimera containing a tandem affinity purification (TAP) tag (Gavin et al., 2002; Puig et al., 2001) at its carboxy terminus (HC–CTAP) was generated. The TAP tag comprises the *Staphylococcus aureus* protein A IgG-binding domain and calmodulin-binding domain separated by the tobacco etch virus (TEV) protease cleavage site (Fig. 1) (Rigaut et al., 1999). The class I heavy chain cDNA was subcloned into the CTAP vector, a gift from Dr Katze (University of Washington, Seattle, WA, USA) and shuttled into the retroviral vector, pLgPW (Oresic et al., 2006). HC–CTAP chimeric cDNA was transduced into U373 (U373HC–CTAP), US11 (US11HC–CTAP), US2 (US2HC–CTAP) and US2–186HA (US2–186HAHC–CTAP) cells (Oresic et al., 2006). EGFP-expressing cells were sorted by the Flow Cytometry Core Facility at Mount Sinai School of Medicine.

**Immunoprecipitation.** Immunoprecipitation experiments were performed from 10^6 cells unless otherwise noted and the data are representative of at least three independent experiments. The total cell lysates represent an equivalent of 350,000 cells. Cells were treated with the proteasome inhibitor carbobenzyloxy-leucyl-leucyl-leucyl vinyl sulfone (ZL3VS) [a gift from Dr Ploegh (MIT, Cambridge, MA, USA)] as described in text. Cells were lysed in 1 ml NP-40 lysis buffer [50 mM Tris, pH 7.4, 150 mM NaCl, 20 mM MgCl₂, 0.5 % (v/v) NP-40] per 10^6 cells containing protease inhibitors (1 µM leupeptin, 1 µg aprotinin ml⁻¹ and 0.5 mM PMSF). Cell lysates were incubated with IgG-Sepharose (GE Healthcare) or the respective antibody. The immunoprecipitates were washed with wash buffer [50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5 % (v/v) NP-40]. When TEV protease cleavage was performed, the precipitates were washed in wash buffer and then TEV cleavage buffer (10 mM HEPES, 150 mM NaCl, 0.25 % NP-40, 0.5 mM EDTA, 1 mM DTT). IgG-Sepharose precipitates were resuspended in at least three times the volume of TEV cleavage buffer and 10 U TEV/10⁶ cells overnight at 4 °C. The polypeptides were resolved using SDS-PAGE and detected by a standard immunoblot. The HC–CTAP, endogenous heavy chain polypeptides and the molecular weight standards are indicated.

**RESULTS**

**Characterization of HC–CTAP in U373 cells**

MHC class I is a 43 kDa type I ER membrane glycoprotein with a luminal domain and a cytosolic tail of 33 residues. In order to identify ER proteins involved in viral-mediated
class I degradation, a TAP tag (Gavin et al., 2002; Puig et al., 2001) was fused to the carboxy terminus of the class I heavy chain (HC–CTAP) (Fig. 1a). The HC–CTAP construct was transduced into U373 (U373HC–CTAP), US2 (US2HC–CTAP) and US11 (US11HC–CTAP) cells. The addition of the TAP tag (130 aa, ~15 kDa) to class I heavy chain (~43 kDa) would result in a polypeptide with a relative molecular mass of ~58 kDa. It is unlikely that the attachment of the affinity tag to the carboxy terminus would interfere with protein complexes between class I and ER chaperones. Hence, this would allow for the identification of ER chaperones that play a role in class I destruction.

The utilization of HC–CTAP as a tool to isolate protein complexes requires that the affinity tag be functional and the HC is recognised by a specific antibody. Equal numbers of control (U373) cells and U373HC–CTAP cells were lysed in NP-40 lysis buffer and incubated with IgG-Sepharose (Fig. 1). In order to determine whether HC–CTAP could mature into a properly folded class I molecule, lysates were incubated with the anti-class I antibody W6/32, which recognizes properly folded class I molecules. HC–CTAP was also recovered with an antibody (HC10) that reacts with unfolded class I heavy chains. The W6/32 and HC10 immunoprecipitations were performed using protein A–agarose pre-bound to the respective antibody, so as to prevent the antibodies binding the protein A-binding domain of HC–CTAP. The precipitates were subjected to immunoblot analysis using anti-heavy chain serum (Fig. 1b, lanes 1–9) and rabbit serum (Fig. 1b, lanes 10–18). Properly folded endogenous class I molecules were recovered with W6/32 from both cell lines (Fig. 1b, lanes 8–9). Unfolded class I molecules were precipitated from U373HC–CTAP cells (Fig. 1b, lane 5). At steady state, the amount of unfolded class I in U373 cells was minute (Fig. 1b, lane 6). Properly folded molecules are minimally composed of class I heavy chain and β2-microglobulin. The excess class I heavy chains in U373HC–CTAP cells (Supplementary Fig. S1 available in JGV Online) depleted the β2-microglobulin levels, causing an increase in unfolded heavy chain levels.

Analysis of HC–CTAP molecules recovered using IgG-Sepharose, HC10 and W6/32 (Fig. 1b, lanes 10–12, 13–15 and 16–18, respectively) demonstrated that the chimera interacts with an immunoglobulin molecule (Fig. 1b, lane 11) and was recognized by anti-class I antibodies (Fig. 1b, lanes 14 and 17). The specificity of IgG-Sepharose for the protein A-binding domain of HC–CTAP (Supplementary Fig. S1) does not require the use of the calmodulin-binding domain to recover strongly associated proteins to HC–CTAP. An immunoprecipitation using a purified mouse IgG immunoglobulin pre-bound to protein A agarose was used to exclude the possibility that HC–CTAP molecules were recovered by binding non-specifically to the added immunoglobulins (data not shown). The data demonstrated that HC–CTAP has a functional protein A affinity tag that does not prevent the chimera from folding into a mature class I molecule.

**HC–CTAP is degraded in a gpUS2-dependent manner**

An essential characteristic for the utilization of HC–CTAP to define the mechanism of viral-mediated class I destruction is that HC–CTAP must be degraded in a gpUS2- or gpUS11-dependent manner. To that end, levels of HC–CTAP were analysed in U373HC–CTAP, US2HC–CTAP and US11HC–CTAP cells untreated or treated with the proteasome inhibitor (ZL3VS; 2.5 μM, 12 h) (Fig. 2). Total cell lysates were subjected to immunoblot analysis using rabbit serum (Fig. 2a and b, lanes 1–4) and anti-gp96 serum (Fig. 2a and b, lanes 5–8). The anti-gp96 immunoblot was used to verify equal loading of cell lysates. Similar amounts of HC–CTAP polypeptides were observed in untreated- or inhibitor-treated U373HC–CTAP cells (Fig. 2a and b, lanes 1–2), a result consistent with protein stability. A significant increase of HC–CTAP was observed in US2HC–CTAP treated with proteasome inhibitor when compared with untreated cells (Fig. 2a, lanes 3–4). The lack of a deglycosylated intermediate was due to treatment with a low concentration of proteasome inhibitor. On the other hand, equivalent levels of HC–CTAP molecules were observed in US11HC–CTAP cells treated with or without proteasome inhibitor (Fig. 2b, lanes 3–4), implying that HC–CTAP was not degraded in US11 cells. These results indicate that an extension of the class I
cytoplasmic tail prevents US11-mediated degradation, while it has no impact on US2-mediated degradation. Since gpUS2 mediates the degradation of HC–CTAP molecules, subsequent experiments concentrated on the role of chaperones in the US2-mediated degradation process.

To characterize US2-mediated degradation of HC–CTAP, we examined whether a deglycosylated HC–CTAP is generated in US2HC–CTAP cells treated with the proteasome inhibitor ZL3VS (20 μM) for up to 6 h (Fig. 3a). A diagnostic readout for dislocated heavy chains is the generation of a faster migrating deglycosylated class I intermediate (difference of ~4 kDa) upon inclusion of a proteasome inhibitor (Wiertz et al., 1996b). The total cell lysates were subjected to immunoblot analysis using anti-heavy chain serum. Increased levels of glycosylated class I heavy chains as well as the deglycosylated HC–CTAP species were observed upon proteasome inhibition for at least 2 h (Fig. 3a, lanes 3–6). The results demonstrate that the addition of a polypeptide at the carboxy terminus of the heavy chain does not interfere with US2-mediated dislocation of the class I substrate.

To determine the kinetics of HC–CTAP degradation in US2HC–CTAP cells, we performed a pulse–chase experiment. U373HC–CTAP and US2HC–CTAP cells were metabolically labelled with [35S]methionine for 15 min and chased up to 40 min (Fig. 3b). HC–CTAP was recovered with IgG–Sepharose and subjected to SDS-PAGE. As expected, HC–CTAP was stable in U373HC–CTAP cells throughout the chase period (Fig. 3b, lanes 1–4 and Fig. 3c). In contrast, the amount of HC–CTAP molecules from US2HC–CTAP cells rapidly decreased during the chase period (Fig. 3b, lanes 5–8 and Fig. 3c). In US2 cells, the half-life of HC–CTAP (~20 min) (Fig. 3c) was longer than that of endogenous heavy chain, which has been reported to be ~3–5 min (Wiertz et al., 1996b). The observation that the chimeric class I molecules were degraded with slower kinetics than endogenous heavy chains (Fiebiger et al., 2002; Story et al., 1999; Tirosh et al., 2003). Nevertheless, these results imply that chimeric HC–CTAP molecules were targeted for the proteasomal degradation in US2-expressing cells. Therefore, HC–CTAP can be used to isolate protein complexes that potentially participate in the ER-to-cytosol extraction process.

**HC–CTAP complexes with both wild-type US2 and the degradation mutant US2–186HA**

To investigate whether HC–CTAP-associated proteins from US2HC–CTAP cells are involved in the dislocation/degradation reactions, HC–CTAP was introduced into a cell line that expresses a US2 mutant (US2–186HA), which does not induce class I degradation (Fig. 4) (Oresic et al., 2006). The cytoplasmic tail of US2 was replaced with the influenza haemagglutinin epitope tag generating the US2–186HA chimera. Total cell lysates from equal numbers of U373, U373HC–CTAP, US2HC–CTAP and US2–186HAHC–CTAP cells untreated or treated with the proteasome inhibitor ZL3VS (25 μM, 5 h) were analysed by an immunoblot using HC10 antibodies (Fig. 4a). Whilst, only endogenously expressed class I heavy chains were observed from control (U373) cells (Fig. 4a, lanes 1–2), similar amounts of HC–CTAP and class I heavy chains were observed in U373HC–CTAP and US2–186HAHC–CTAP cells, independent of proteasome inhibition (Fig. 4a, lanes 3–6). Only low levels of HC–CTAP and endogenous class I heavy chains were observed in US2HC–CTAP cells and these class I levels increased upon treatment with proteasome inhibitor (Fig. 4a, lanes 7–8). Importantly, the slightly faster migrating deglycosylated HC–CTAP species, HC(−)CHO, was observed in proteasome inhibitor-treated US2HC–CTAP cells (Fig. 4a, lane 8), demonstrating that the HC–CTAP was dislocated across the ER membrane. Overall, these data show that a degradation defective gpUS2 molecule, gpUS2–186HA, is unable to induce destruction of either class I or HC–CTAP.

In order to determine whether the gpUS2-binding domain of class I was preserved in HC–CTAP molecules, we performed an association experiment using US2 and US2–186HA cells (Fig. 4b). IgG–Sepharose was incubated with
lysates from U373, U373HC–CTAP, US2HC–CTAP and US2–186HAHC–CTAP cells. Since HC–CTAP is degraded in US2 cells, an increased number of US2 cells (~4 x 10^6) was used to visualize the associated US2 polypeptides. The precipitates were analysed by an immunoblot using rabbit serum (Fig. 4b, lanes 1–8) and anti-gpUS2 serum (Fig. 4b, lanes 9–16). Total cell lysates were used as a control for the migration pattern of HC–CTAP (Fig. 4b, lanes 5–8) and US2 polypeptides (Fig. 4b, lanes 13–16). As expected, HC–CTAP molecules from U373 HC–CTAP, US2 HC–CTAP and US2–186HAHC–CTAP cells were successfully recovered with IgG–Sepharose (Fig. 4b, lanes 2–4). Notably, both gpUS2 and gpUS2–186HA co-precipitated with HC–CTAP (Fig. 4b, lanes 11–12), demonstrating that both gpUS2 and gpUS2–186HA complexed with HC–CTAP. These results confirm that the luminal domain of HC–CTAP molecules can bind to gpUS2 (Gewurz et al., 2001). More importantly, US2–186HAHC–CTAP cells serve as an ideal control cell line for protein specificity of HC–CTAP-associated proteins in US2-mediated degradation.

ER chaperones complex specifically with class I molecules in a gpUS2-dependent manner

A select set of chaperones including calnexin and calreticulin mediate the folding of class I heavy chains into a mature class I trimeric molecule (Harris et al., 1998; Pamer & Cresswell, 1998). To examine whether the chaperones that assist in class I protein folding may also be involved in their extraction from the ER, we performed association experiments in US2HC–CTAP cells (Fig. 5). To allow for the stoichiometric analysis of the chaperones that interact with HC–CTAP in US2HC–CTAP cells, association experiments were performed in a manner to recover equivalent numbers of HC–CTAP molecules from HC–CTAP-expressing cells. HC–CTAP molecules recovered from U373HC–CTAP, US2HC–CTAP and US2–186HAHC–CTAP cells using IgG–Sepharose and total cell lysates were subjected to immunoblot analysis using rabbit serum (lanes 1–8) and anti-gpUS2 serum (lanes 9–16). The glycosylated HC–CTAP, deglycosylated HC–CTAP (HC–CTAP[–CHO]), glycosylated endogenous heavy chain (HC), deglycosylated endogenous heavy chain HC[–CHO], wild-type gpUS2, gpUS2–186HA polypeptides and the molecular mass standards are indicated.

**Fig. 4.** HC–CTAP chimera interacts with gpUS2. (a) Total cell lysates from U373, U373HC–CTAP, US2HC–CTAP and US2–186HAHC–CTAP untreated or treated with the proteasome inhibitor ZL3 VS (20 μM, 5 h) were subjected to immunoblot analysis using the monoclonal antibody anti-heavy chain HC10. (b) HC–CTAP molecules recovered from U373HC–CTAP, US2HC–CTAP and US2–186HAHC–CTAP cells using IgG–Sepharose and total cell lysates were subjected to immunoblot analysis using rabbit serum (lanes 1–8) and anti-gpUS2 serum (lanes 9–16). The glycosylated HC–CTAP, deglycosylated HC–CTAP (HC–CTAP[–CHO]), glycosylated endogenous heavy chain (HC), deglycosylated endogenous heavy chain HC[–CHO], wild-type gpUS2, gpUS2–186HA polypeptides and the molecular mass standards are indicated.
specifically with the degradation substrate in an gpUS2-dependent manner.

To investigate whether additional ER chaperones (calreticulin and BiP) also complex with class I in a gpUS2-dependent manner, HC–CTAP were recovered from U373 HC–CTAP, US2HC–CTAP and US2–186HAHC–CTAP cells using IgG–Sepharose and subjected to TEV protease digestion (Fig. 6). The released material and total cell lysates from U373HC–CTAP were subjected to immunoblot analysis using anti-calreticulin (lanes 1–4) and anti-class I heavy chain antibodies (lanes 5–8). The calnexin, HC–CTAP lacking the protein A-binding domain (HC CBD) polypeptides and the molecular mass standards are indicated.

A similar experiment was performed in which the HC–CTAP precipitates were analysed for PDI, because it is a highly expressed chaperone at millimolar levels (Noiva & Lennarz, 1992) and it is involved in dislocation (Molinari et al., 2002; Tsai et al., 2001). The anti-PDI immunoblot revealed that HC–CTAP molecules did not complex with PDI either in U373HC–CTAP or US2HC–CTAP cells. Collectively, the results imply that specific chaperones are involved in the early phases of class I dislocation.

To substantiate further the interaction of calnexin and calreticulin with HC–CTAP in US2 cells, the association of HC–CTAP with these chaperones was examined under conditions of proteasome inhibition. HC–CTAP was recovered from U373HC–CTAP and US2HC–CTAP cells untreated or treated with the proteasome inhibitor ZL3VS (2.5 μM, 12 h) with IgG–Sepharose (Fig. 7). Upon cleavage with TEV protease, samples were resolved using SDS-PAGE and subjected to immunoblot analysis using anti-calnexin (Fig. 7, lanes 1–4) and anti-calreticulin (Fig. 7, lanes 6 and 8). An increase in HC–CTAP lacking a protein A-binding domain (HC CBD) was observed in US2HC–CTAP cells treated with proteasome inhibitor (Fig. 7, compare lane 10 with 12). This increase in HC–CTAP translated into a significant recovery of both calnexin (Fig. 7, lanes 2 and 4) and calreticulin (Fig. 7, lanes 6 and 8). These results suggest that these chaperones continue to interact with ER resident HC–CTAP molecules until they are extracted out of the ER. Interestingly, in U373HC–CTAP cells, slightly more calnexin and calreticulin co-precipitated with HC–CTAP from cells treated with proteasome inhibitor (Fig. 7, compare lanes 3 and 7 with lanes 1 and 5). Inhibition of the proteasome prevents the generation of peptides, thereby blocking class I maturation and causing the
accumulation of misfolded class I molecules in the ER. Collectively, the results verify that these specific chaperones participate in early events of proteasome-mediated degradation of class I heavy chains.

**DISCUSSION**

HCMV has evolved strategies to utilize existing cellular processes to escape immune detection (Loureiro & Ploegh, 2006). The current data extend the paradigm that HCMV co-opts host machinery to include the use of ER chaperone complexes to modulate class I antigen presentation. Following the recognition of class I molecules by HCMV gpUS2, cellular chaperones are most probably utilized to assist in the dislocation of class I heavy chains out of the ER. Even though class I is degraded in a similar manner to a misfolded protein, the unusual feature of US2-mediated class I degradation (Hegde et al., 2006). BiP is an ER chaperone involved in processing of nascent polypeptides and prevents the mixing of ER luminal contents with the cytosol. BiP can also reduce protein aggregation and is involved in the unfolded protein response (Friedlander et al., 2000; Hegde et al., 2006; Kabani et al., 2001; Nishikawa et al., 2001). During the dislocation process, the degradation substrate may be partially unfolded prior to its transport through the dislocon. At this point, BiP would interact with the misfolded protein and assist in the dislocation of the substrate. Therefore, the interaction of BiP with the degradation substrate would contribute to the efficient removal of the misfolded proteins.

Chaperones, general beneficiaries in protein folding, have a decisive role in directing misfolded proteins to the dislocon (Brodsky, 2007; McCracken & Brodsky, 2005). Eukaryotic cells have evolved to recognize efficiently structural features of polypeptides that have not achieved their native conformation and target these proteins for proteasomal destruction (Hochstrasser & Varshavsky, 1990; Johnston et al., 1998; Nishikawa et al., 2001; Whiteside et al., 1995). The ER chaperones BiP, calnexin, calreticulin, ERP57 and PDI participate in the recognition and dislocation of specific degradation substrates (Molinari et al., 2002; Nishikawa et al., 2001; Plemper et al., 1997; Romisch & Schekman, 1992). For example, calnexin is required for the degradation of pro-α factor in yeast (McCracken & Brodsky, 1996), while a mutant form of tyrosinase utilizes calreticulin and BiP for its extraction from the ER (Popescu et al., 2005). In addition, damaged glycoproteins are recognized by the ER-resident protein EDEM that interacts with mis-processed N-linked glycans and targets them for proteasomal degradation (Hosokawa et al., 2001; Molinari et al., 2003). Despite the implication of these proteins in the degradation process, selective chaperones may be assigned to the disposal of specific ER degradation substrates. Consistent with that idea, HCMV gpUS2
appears to utilize a specific set of cellular chaperones to induce destruction of class I heavy chains efficiently.

The calnexin–calreticulin-binding cycle participates in the proper folding of class I molecules (Pamer & Cresswell, 1998). It is not clear if these chaperones are recruited by gpUS2 to class I or if they are initially part of the folding apparatus and remain bound to class I in a gpUS2-dependent manner. Interestingly, calnexin and calreticulin have been proposed to act as ‘pre-protein stabilizers for the misfolded or aggregation-prone substrates that need to be discarded’ (Brodsky, 2007). Therefore, they may have a propensity to recognize misfolded proteins. On the other hand, the specific chaperones involved in the extraction of misfolded proteins may be the same proteins involved in the folding of the specific polypeptides into their mature forms. In this case, the folding chaperones will have dual function as mediators of protein folding as well as ‘sensor’ molecules that recognize misfolded proteins. Whether these misfolded proteins display structural elements that trigger their dislocation or the prolonged interaction between the chaperone and the ER protein signals its degradation is not yet clarified. Interestingly, the association of HC–CTAP with calnexin and calreticulin is most probably mediated by a structural property of class I proteins, rather than its lectin-binding specificity. An HC–CTAP molecule lacking its N-linked glycan continued to interact selectively with calnexin and calreticulin in a gpUS2-dependent manner (data not shown). In addition, gpUS2 molecules lacking an N-linked glycan continued to target class I for degradation (data not shown). Other factors such as binding affinity and possible number of N-linked glycans could also be important features in recognizing misfolded protein. To understand the molecular details of how these chaperones prepare a substrate for dislocation continues to remain an open question. Therefore, HCMV US2- and US11-mediated downregulation of class I heavy chains are ideal systems that afford a unique opportunity to delineate the dislocation reaction as well as define the interaction between HCMV and its host.


