The p97 ATPase Dislocates MHC Class I Heavy Chain in US2-expressing Cells via a Ufd1-Npl4-independent Mechanism*

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The human cytomegalovirus (HCMV) protein US2 hijacks the endoplasmic reticulum (ER)-associated degradation machinery to dispose of MHC class I heavy chain (HC) at the ER. This process requires retrotranslocation of newly synthesized HC molecules from the ER membrane into the cytosol, but the mechanism underlying the dislocation reaction has been elusive. Here we establish an in vitro permeabilized cell assay that recapitulates the retrotranslocation of MHC HC in US2-expressing cells. Using this assay, we demonstrate that the dislocation process requires ATP and ubiquitin, as expected. The retrotranslocation also involves the p97 ATPase. However, the mechanism by which p97 dislocates MHC class I HC in US2 cells is distinct from that in US11 cells: the dislocation reaction in US2 cells is independent of the p97 cofactor Ufd1-Npl4. Our results suggest that different retrotranslocation mechanisms can employ distinct p97 ATPase complexes to dislocate substrates.

The endoplasmic reticulum (ER) is a major site of protein synthesis for secretory and membrane proteins. To ensure that only properly folded polypeptides and correctly assembled protein complexes enter the secretory pathway to reach their final destinations, eukaryotic cells have evolved a conserved quality control mechanism. This quality control program selectively retains polypeptides that fail to reach native conformation and export them from the ER into the cytosol for degradation by the 26 S proteasome. This process was termed ER-associated protein degradation (ERAD) or retrotranslocation (1–3). Efficient degradation of misfolded ER proteins requires concerted actions by the p97 ATPase and the ubiquitin proteasome system (4). Polypeptides emerging into the cytosol via one or more protein conducting channels are polyubiquitinated by an ER membrane-associated ubiquitin ligase (5, 6). The p97 ATPase complex, which comprises of the AAA (ATPase associated with various cellular activities) ATPase p97 and the dimeric adaptor complex Ufd1-Npl4, then use a dual recognition mode to recognize both polypeptide chain and the attached polyubiquitin conjugates, leading to the extraction of the polypeptide from the ER membrane (7, 8). p97 subsequently delivers the substrate to the proteasome for degradation with the assistance from a collection of shuttling factors that are capable of interacting with both p97 and the proteasome (9–11).

The retrotranslocation pathway can be hijacked by viruses to facilitate viral evasion of host immune defense. The murine γ-herpesvirus 68 encodes a type III membrane protein named mK3, which down-regulates newly synthesized major histocompatibility complex (MHC) class I heavy chain (HC) by co-opting the ERAD mechanism (12). Two HCMV-encoded proteins, US2 and US11, can each target MHC class I HC to the ERAD pathway for degradation. In US11- or US2-expressing cells, MHC class I HC, a type I transmembrane protein carrying a single glycan, is rapidly moved back into the cytosol. Once emerging into the cytosol, HC molecules are modified with polyubiquitin chains, whereas the N-glycan is cleaved off. The polyubiquitinated polypeptide chain is subsequently degraded by the 26 S proteasome (13, 14). The elimination of the antigen presenting MHC HC molecules from the ER membrane apparently allows viruses to escape detection by the host immune system.

Both US11 and US2 directly bind MHC class I HC to initiate the retrotranslocation process, and both pathways involve ubiquitin, the 26 S proteasome, and a translocating chain-associated membrane protein-1 (15–19). Nonetheless, these two processes each possess its own unique features, indicating that these two viral proteins may use different retrotranslocation routes to dislocate MHC class I HC from the ER membrane. For example, on the ER lumen side, although the Hsp70 family protein BiP has been implicated in both pathways (20), the ER chaperone protein-disulfide isomerase is required only for US2-mediated retrotranslocation (21). The difference between the two pathways is most drastic when the membrane components involved are compared (Fig. 1). In the ER membrane, US11 and US2 proteins engage almost completely different sets of membrane proteins to accomplish the dislocation reaction. US2 targets MHC HC to a protein complex comprised of the signal peptide peptidase and an ER-membrane bound ubiquitin ligase termed TRC8 for subsequent export into the cytoplasm (22, 23). US11 does not interact with these proteins. Instead, it targets HC to a large protein complex containing Derlin-1, VIMP, UbxD8, SEL1L, and AUP1 for retrotranslocation (24–27) (Fig. 1). On the cytosolic side, previous studies have demonstrated the involvement of the p97-Ufd1-Npl4 complex in US11-mediated retrotranslocation (7, 28). However, few studies have examined the cytosolic requirement for US2-induced retrotranslocation. In particular, it is unclear whether or not the p97 ATPase participates in this process, and if so, how it...
can cooperate with such distinct membrane retrotranslocation complexes.

In this report, we established an in vitro permeabilized cell assay that recapitulates important features of the dislocation reaction in intact US2-expressing cells (referred thereafter as US2 cells). Using this system, we demonstrate that the retrotranslocation of HC induced by US2 expression requires ubiquitin and the p97 ATPase. Surprisingly, the canonical adaptor complex Ufd1-Npl4 implicated in retrotranslocation of most ERAD substrates studied to date is dispensable for US2-induced retrotranslocation. We propose that adaptor switch may allow the p97 ATPase to cooperate with distinct retrotranslocation machineries in the ER membrane to serve different substrates.

MATERIALS AND METHODS

Constructs, Antibodies, Protein, and Chemicals—The pLNCX2-US2 plasmid was constructed in two steps. First, a DNA fragment comprised of the coding sequence for the signaling sequence (SS) of the proolacin gene and the FLAG tag (MDSKGSQKGSRLLLLLVSNLLLCCGVVSTPVDYKDDDK) was amplified by PCR and inserted into the BglII and NotI sites of the pLNCX2 vector (Clontech, Mountain View, CA) to make pLNCX2-SS-FLAG. The US2 coding sequence was then amplified by PCR and cloned in the NotI and SalI sites of the pLNCX2-SS-FLAG. The US2 cells were used for detection. The fluorescent blots were imaged using a LI-COR Odyssey infrared imager. Protein bands were quantified using the Odyssey 2.1. Astrocytoma or 293T cells stably expressing FLAG-US2 were generated using the pLNCX2-based retroviral system as described previously (29). 293T cell stably expressing YFP tagged T-cell receptor α chain and astrocytoma cells stably expressing US11 were used previously (28, 29).

Preparation of Cow Liver Cytosol—Fresh bovine liver tissue was cut into small pieces to remove blood vessels and connective tissue. The resulting tissue (~300 g) was thoroughly rinsed in ice-cold homogenization buffer (50 mM HEPES, pH 7.5, 80 mM KCl, 15 mM NaCl, 3 mM MgCl2, 250 mM sucrose, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF)). Homogenization buffer (~300 ml) containing extra protease inhibitors was added. The tissue was homogenized in a Polytron blender followed by further homogenization using a Potter homogenizer rotating at ~1000 rpm. The homogenate was centrifuged at 9000 × g in a Beckman JA-10 rotor for 15 min. The supernatant was filtered through eight layers of cheesecloth, re-centrifuged, and filtered through cheesecloth a second time. The supernatant was then centrifuged in a Beckman Ti45 rotor at 45,000 × g for 3 h. The cytosol supernatant was saved carefully. The protein concentration of the cytosol was 20–30 mg/ml, as measured with the use of the Micro BCA Protein Assay (Pierce).

Protein Purification and Biochemical Depletion Experiments—GST-Ube2B C88S and GST-p97 proteins were purified from Escherichia coli as previously described (30). Purified proteins were further fractionated by size exclusion chromatography on Superdex 200 and Superose 6 columns, respectively, in 50 mM Tris-HCl, pH 7.5, and 150 mM sodium chloride. 40 mg of cow liver cytosol was subjected to two rounds of depletion, each with glutathione beads containing 125 μg of GST-p97 protein. The beads were removed by centrifugation. To deplete ubiquitin from cow liver cytosol, cow liver cytosol (40 mg) was incubated with 3 mg of GST-Ubc2m in the presence of GST-Uba1 (1 μg) and an ATP regenerating system (16). To monitor the depletion efficiency, fluorescein-labeled ubiquitin (0.25 μg) was included and the reaction was incubated at 37 °C for 15 min. Ubiquitin aldehyde was added to a final concentration of 1 μM. GST-Ubc2m-ubiquitin complex was then removed by glutathione beads. The depletion efficiency was monitored by comparing the fluorescence intensity prior to and after the depletion reaction.

Permeabilized Cell Assay—Pulse-chase and permeabilization experiments were adapted from a previously described protocol (28, 31). US2-expressing U373-MG astrocytoma cells (32) were starved in a Met/Cys-free DMEM for 40 min and incubated in a medium containing [35S]Met/Cys for 10 min. Where indicated, the proteasome inhibitor MG132 (20 μM) was present in the starvation and pulse labeling medium. To permeabilize the plasma membrane, cells were washed with PBS supplemented with 0.9 mM CaCl2 and then incubated at 3 × 107 cells/ml in PB buffer (25 mM HEPES, pH 7.2, 115 mM potassium acetate, 5 mM sodium acetate, 2.5 mM MgCl2, 0.5 mM EGTA)
Ufd1-independent Retrotranslocation

![Diagram](image)

**FIGURE 2. A permeabilized cell assay for US2-mediated retrotranslocation.**

- **a:** experimental schemes for the permeabilized cell assay. After cells are permeabilized, they were either incubated with an ARS and ubiquitin (Ub) directly to initiate retrotranslocation (scheme A) or subjected to centrifugation to remove cytosol (scheme B). In the latter case, the membrane pellet fraction was washed with salt before the addition of CLC, Ub, and ARS. 
- **b:** US2 cells were either untreated or treated with the proteasome inhibitor MG132 (20 μM, 1 h), radiolabeled, and permeabilized. Cells were then incubated in the presence of ARS for either 0 or 60 min at 37 °C. A portion of the sample (T) was directly analyzed, whereas the other was fractionated into supernatant (S) and membrane pellet (P). The extracts were subjected to immunoprecipitation with αHC. HC + CHO, glycosylated HC; HC-CHO, deglycosylated HC. 
- **c:** membrane 115 mM salt wash. 
- **d:** membrane 385 mM salt wash.

**RESULTS**

**An In Vitro Assay for US2-mediated Retrotranslocation—**To study the mechanism of US2-mediated retrotranslocation, we set up a permeabilized cell system. A similar assay was previously established to dissect the mechanism of US11-dependent retrotranslocation of MHC class I HC (31). To this end, astrocytoma cells stably expressing US2 protein were labeled with [35S]methionine and permeabilized with a buffer containing a low concentration of the detergent digitonin. Digitonin-permeabilized cells were incubated with an ATP regenerating system to initiate retrotranslocation (Fig. 2a, scheme A). Samples taken at different time points were either analyzed directly by solubilization in a Nonidet P-40-containing extraction buffer followed by immunoprecipitation with an anti-HC antibody or first fractionated into pellet (containing the ER membrane) and supernatant (containing the cytosol) fractions before immunoprecipitation analysis. Incubation of permeabilized US2 cells with an ARS decreased the level of MHC class I HC due to retrotranslocation and proteasomal degradation (Fig. 2b, lane 1 versus 2). When US2 cells were treated with a proteasome inhibitor MG132 for 1 h prior to pulse labeling, the degradation of HC was inhibited, leading to the accumulation of a fraction of retrotranslocated MHC class I HC during the labeling process, which had the N-glycan cleaved off by a cytosolic N-glycanase (Fig. 2b, lane 5) (33). The majority of retrotranslocated HC also had the polyubiquitin chains removed, which was likely due to...
deubiquitination by cellular deubiquitinating enzymes, as
reported for US11-mediated retrotranslocation and other
ERAD substrates (11, 31, 34, 35). After incubation, we observed
that the amount of dislocated HC product was increased with
a concurrent reduction in glycosylated retrotranslocation pre-
cursor (Fig. 2b, lane 5 versus 6). Fractionation experiments
showed that the majority of deglycosylated HC was indeed
present in the cytosol fraction, whereas the glycosylated form
was mostly associated with the membrane (Fig. 2b, lane 8 versus
7), consistent with previous studies using intact US2 cells. Thus,
our in vitro assay recapitulates a key feature of the in vivo
reaction that is the release of HC into the cytosol in a deglycosylated
form upon inhibition of the proteasome.

We next tested whether the dislocation of HC in permeabi-
lized US2 cells requires any cytosolic proteins. We introduced a
centrifugation step following the permeabilization of cells,
which removed cytosolic factors (31). The resulting membrane
pellets were washed with a buffer containing 115 mm salt before
being incubated with either buffer or cow liver cytosol (CLC)
(Fig. 2a, scheme B). Interestingly, retrotranslocation of HC
occurred even in the absence of cytosol. The addition of cytosol
did not improve the dislocation efficiency (Fig. 2c, top panel).
However, when membranes from permeabilized US2 cells were
treated with a buffer containing 385 mm salt, the dislocation
reaction became dependent on the addition of cytosol (Fig. 2c,
bottom panel). These results suggest that US2-dependent
dislocation likely involves a factor that is present both in the
cytosol and on the ER membrane. This is in contrast to the
dislocation reaction in US11 cells, which requires few cytosolic
proteins (36). Most of the factors involved in US11-induced
retrotranslocation are exclusively associated with the ER
membrane either as anchored or peripheral proteins. These
observations further consolidate the notion that the two viral
proteins induce MHC class I HC degradation via distinct mecha-
nisms. As expected, the dislocation of MHC class I HC in per-
meabilized US2 cells also required ATP as its omission com-
pletely blocked the appearance of the deglycosylated HC (Fig.
2d, lane 2 versus 4).

Ubiqitin Is Required for US2-dependent Retrotranslocation—
We tested whether retrotranslocation of MHC class I HC in
permeabilized US2 cells requires ubiquitin. We used a previ-
ously established method to deplete ubiquitin from cytosol
ubiquitin tracer (Fig. 3a). Consistent with previous results,
cytosol depleted of ubiquitin did not support US11-dependent
dislocation (Fig. 3b, lane 2 versus 4) and addition of purified
bovine ubiquitin to ubiquitin-depleted CLC restored the dislo-
cation activity, as a fraction of glycosylated HC was converted
to dislocated deglycosylated species (Fig. 3b, lane 6). When the
membranes from permeabilized US2 cells were incubated with
either mock-depleted or ubiquitin-depleted CLC, dislocation
of MHC class I HC was observed in the presence of control
CLC, but not with ubiquitin-depleted CLC (Fig. 3c, lanes 5 and
6 versus lanes 3 and 4). Like US11 cells, re-introducing ubiq-
uitin to ubiquitin-depleted cytosol restored the dislocation
activity, causing the accumulation of deglycosylated HC in the
cytosol fraction (Fig. 3c, lane 8). Together, these observations
suggest that dislocation of MHC class I HC in permeabilized
US2 cells also involves ubiquitination in agreement with previ-
ous studies in intact cells, which showed that polyubiquitinated
MHC class I HC is present in US2 cells treated with a proteasome
inhibitor (17), and that removal of potential ubiquitin conjugation
sites in MHC class I HC abolished its dislocation (18).

The Differential Effect of p97 Inhibitors on US11- and US2-
induced Dislocation—Previous studies show that polyubiquitin
acts as a recognition signal to engage a p97 ATPase complex (8),
which utilizes the energy generated by ATP hydrolysis to dislo-
cate polypeptides from the ER membrane (28). We therefore
tested whether US2-induced retrotranslocation involves p97.
Because a significant fraction of p97 is tightly associated with
the ER membrane, which is resistant to high salt treatment (28),
we used a protein termed SVIP to inhibit the p97 function. SVIP
is a small polypeptide containing a p97 interaction motif. Over-
expression of SVIP inhibits p97-dependent protein degradation
at the ER membrane (37). Addition of SVIP protein to perme-
abilized US11 cells inhibits US11-dependent retrotranslocation
(Fig. 4a). In a parallel experiment, we also treated permeabilized
US2 cells with the same dose of SVIP. However, we found that
SVIP only had a marginal effect on US2-mediated retrotranslo-
cation, leading to a small but reproducible reduction in the level of
dislocated deglycosylated HC and a corresponding increase in the
membrane-associated glycosylated HC precursors (Fig. 4b).

To further examine the role of p97 in US2-mediated dislo-
ocation, we incubated permeabilized US2 cells with a p97 mutant
lacking the second ATPase domain (ΔD2). This mutant was
shown to block retrotranslocation of MHC class I HC in US11 cells (7) (Fig. 4c).
Interestingly, addition of this mutant to permeabilized US2 cells had no significant impact on HC dislocation under the same condition (Fig. 4d). Together, these results suggest that US2-induced retrotranslocation either does not require p97 or employs a p97 complex that is different from the one used in US11 cells, so it cannot be efficiently inhibited by these inhibitory proteins.

Interaction of p97 with a Retrotranslocation Intermediate in US2 Cells—To gain more insights on the role of p97 in US2-mediated retrotranslocation, we examined the potential interaction of p97 with MHC class I HC undergoing retrotranslocation in US2 cells. Because the retrotranslocation and degradation of MHC class I HC in US2 cells occurs rapidly ($t_{1/2}$ < 5 min), the interaction between p97 and the substrate HC, if existing, must be transient in nature. To capture this transient complex, we treated cells with a proteasome inhibitor for 1 h before labeling cells with $[^{35}S]$methionine. Cell extracts were prepared using either the mild detergent DeoxyBigCHAP or a stronger detergent (Nonidet P-40). p97 was immunoprecipitated from cell extract (Fig. 5, bottom panel) and the associated proteins were subjected to a second round of immunoprecipitation with an anti-MHC HC antibody (Fig. 5, upper panels). The result showed that a fraction of MHC class I HC molecules associated with p97 only when DeoxyBigCHAP was used to prepare cell extract, consistent with our hypothesis that the p97-MHC class I HC interaction is unstable. Interestingly, unlike US11 cells in which p97 associated with both the glycosylated retrotranslocation precursor and the dislocated deglycosylated MHC molecules (38), p97 preferentially associated with deglycosylated MHC class I HC in US2 cells (Fig. 5). This observation indicates that p97 is still involved in US2-mediated retrotranslocation, but it appears to operate by a mechanism distinct from that used in US11 cells.
We then tested whether the retrotranslocation of MHC class I HC in US2 cells requires the Ufd1-Npl4 cofactor complex. Ufd1 and Npl4 are present in cells solely as a dimeric complex (39) and depletion of one subunit always causes the simultaneous loss of the other, as reported in several studies (40, 41). The Ufd1-Npl4 complex is mostly in the cytosol, but a fraction of the complex is also associated with the ER membrane. However, the interaction of the Ufd1-Npl4 complex with the membrane can be disrupted by salt (350 mM) (Fig. 6a). Because our in vitro assay includes a washing step with a buffer containing ∼400 mM salt, the only source of Ufd1-Npl4 in the in vitro retrotranslocation reactions is the cytosol. We therefore depleted the Ufd1-Npl4 complex from the cytosol using recombinant GST-p97. Consistent with previous studies, this approach efficiently removed the cofactor complex from the cytosol, as confirmed by immunoblotting with an anti-Ufd1 antibody (Fig. 6b). We first tested the effect of Ufd1 depletion on US11-mediated dislocation. When US11 cells were permeabilized and washed with salt, retrotranslocation of HC was partially inhibited, as indicated by the reduced level of deglycosylated HC during the chase period (Fig. 6c, lanes 5 and 6 versus 2 and 3). This was at least in part due to the removal of the Ufd1 complex from the membrane as depletion of Ufd1 from CLC further impaired HC retrotranslocation (Fig. 6c, lanes 8 and 9). In contrast, when US2-induced dislocation assay was performed using either the cofactor-depleted cytosol or a mock depleted cytosol, we observed no difference in their ability to support the retrotranslocation of HC (Fig. 6a). Thus, we conclude that unlike US11 cells, US2-mediated retrotranslocation likely does not require Ufd1-Npl4.

We finally confirmed our findings using intact 293T cells stably expressing US2. We knocked down both p97 and Ufd1 using a siRNA approach. The depletion of these proteins from cells could be verified by immunoblotting (Fig. 7a). The depletion of either p97 or Ufd1 significantly impaired the ERAD pathway, as indicated by the increased stability of the classical ERAD substrate TCRα (Fig. 7a, lanes 1–3). Interestingly, in US2-expressing 293T cells, depletion of p97 stabilized endogenous MHC class I HC, whereas knockdown of Ufd1 had no effect on the degradation of MHC class I HC (Fig. 7a, lanes 4–6, and b). These results confirm that US2-induced retrotranslocation proceeds via a p97-dependent but Ufd1-independent mechanism.

**DISCUSSION**

The HCMV protein US2 hijacks the ERAD pathway to dispose of newly synthesized MHC class I HC. It has been shown previously that in US2-expressing cells, MHC class I HC molecules undergo polyubiquitination and are dislocated into the cytosol prior to proteasomal degradation (13). Removal of ubiquitination sites on MHC class I HC inhibits its retrotranslocation and degradation (18). To dissect the mechanism of US2-induced retrotranslocation of MHC class I HC, we establish an in vitro permeabilized cell assay that recapitulates key features of this reaction. In our in vitro assay, ubiquitin depletion completely abolishes US2-induced retrotranslocation, consistent with a previous report that US2-mediated retrotranslocation requires ubiquitination. In addition, the dislocation reaction requires ATP.

We show that in addition to ubiquitin, US2-mediated retrotranslocation also involves the p97 ATPase. At first glance, this appears to resemble US11-induced retrotranslocation. However, a thorough comparison of the two reactions indicates that the two HCMV proteins use distinct retrotranslocation complexes to down-regulate MHC class I HC. For ubiquitination, the US2 protein uses the TRC8 ubiquitin ligase to conjugate polyubiquitin chains on one or more lysine residues in HC and removal of ubiquitin acceptors in HC inhibits its retrotranslocation (23). By contrast, TRC8 is not involved in US11-induced retrotranslocation and lysine residues in HC molecules are dispensable for retrotranslocation (18, 23). Because MHC class I HC is polyubiquitinated in US11 cells and blocking this modification by either depletion of ubiquitin from the cytosol or inactivation of the ubiquitin-activating enzyme abolishes US11-
US2-mediated retrotranslocation is independent of Ufd1. a, 293T cells stably expressing TCRα-YFP or US2 were transfected with either control siRNA or siRNA targeting p97 or Ufd1. Whole cell extracts were analyzed by immunoblotting with antibodies against the indicated proteins. The number indicates the relative intensity of the Ufd1 band. Note that the GFP antibody also detects a nonspecific protein in US2 cell extract, which migrates at the same position as TCRα-YFP. b, shown are normalized HC levels in US2 cells transfected with the indicated siRNAs from three independent experiments. Error bars represent S.E. (n = 3).

dependent retrotranslocation (15, 16, 31), we assumed that ubiquitin chains may be conjugated to one or more non-lysine residues in HC, as demonstrated recently (42). For the dislocation reaction, although both reactions appear to use the p97 ATPase, the mode of p97 action in these reactions is different. In US11-expressing cells, p97 interacts with both glycosylated retrotranslocation precursor as well as deglycosylated retro-translation product (38). In US2 cells, p97 primarily interacts with the deglycosylated species. This distinction suggests that US2 and US11 may employ different p97 complexes to dislocate MHC class I HC, a notion further supported by our finding that US2-mediated retrotranslocation does not require the cofactor complex Ufd1-Npl4. This model would explain why several p97 inhibitory proteins efficiently inhibit the dislocation of HC in permeabilized US11 cells but not in US2 cells. Perhaps, different p97 complexes may have differential accessibility to these inhibitory proteins.

The use of different p97 complexes by the two HCMV protein-induced retrotranslocation processes might be due to the different mechanisms by which these viral proteins recognize and target HC molecules for retrotranslocation. US11 uses its transmembrane domain to recognize both MHC class I HC and Derlin-1. The latter is a multispanning membrane protein implicated in retrotranslocation of some misfolded ER proteins (24, 25, 43). By serving as an adaptor that links MHC class I HC to Derlin-1, US11 targets HC to a retrotranslocation complex comprising Derlin-1, one or more membrane-bound ubiquitin ligases, and the AAA ATPase p97 (38, 44). In contrast, Derlin family members are dispensable for US2-mediated retrotranslocation. Instead, US2 uses its ER luminal domain to interact with HC and its cytosolic tail to bind the signal peptide peptidase (22). Signal peptide peptidase is in complex with the TRC8 E3 ubiquitin ligase, which appears to mediate the transfer of MHC class I HC across the ER membrane in US2 cells (23). Whether or not TRC8 also mediates retrotranslocation of misfolded ER proteins is currently unknown. Nonetheless, it becomes apparent that distinct retrotranslocation complexes exist in the ER membrane in both yeast and higher eukaryotes, which serve different cohorts of ERAD substrates. Because almost all retrotranslocation mechanisms studied to date use the p97 ATPase for substrate dislocation, the difference in the membrane machinery underscores the importance of tailoring p97 complex by affiliating it with different co-factors, which would allow the same enzyme to cooperate with different membrane-bound retrotranslocation complexes. In agreement with this idea, a recent study shows that the p97 adaptor UbxD8, a Ubx-domain-containing protein is only involved in retrotranslocation of HC molecules in US11 cells but not in US2 cells (27).

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REFERENCES

1. Tsai, B., Ye, Y., and Rapoport, T. A. (2002) Nat. Rev. Mol. Cell Biol. 3, 246–255
2. Meusser, B., Hirsch, C., Jarosch, E., and Sommer, T. (2005) Nat. Cell Biol. 7, 766–772
3. Vembhar, S. S., and Brodsky, J. L. (2008) Nat. Rev. Mol. Cell Biol. 9, 944–957
4. Bays, N. W., and Hampton, R. Y. (2002) Cell 109, 71–84
5. Ye, Y. (2005) Essays Biochem. 41, 99–112
6. Hirsch, C., Gauss, R., Horn, S. C., Neuber, O., and Sommer, T. (2009) Nature 458, 453–460
7. Ye, Y., Meyer, H. H., and Rapoport, T. A. (2003) J. Cell Biol. 162, 71–84
8. Flierman, D., Ye, Y., Dai, M., Chau, V., and Rapoport, T. A. (2003) J. Biol. Chem. 278, 34774–34782
9. Richly, H., Rape, M., Braun, S., Rumpf, S., Hoege, C., and Jentsch, S. (2005) Cell 120, 73–84
10. Kim, I., Ahn, J., Liu, C., Tanabe, K., Apodaca, J., Suzuki, T., and Rao, H. (2006) J. Cell Biol. 172, 211–219
11. Wang, Q., Li, L., and Ye, Y. (2006) J. Biol. Chem. 281, 963–971
12. Wang, X., Ye, Y., Lencer, W., and Hansen, T. H. (2006) J. Biol. Chem. 281,
13. Wiertz, E. J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T. R., Rapoport, T. A., and Ploegh, H. L. (1996) Nature 384, 432–438
14. Wiertz, E. J., Jones, T. R., Sun, L., Bogyo, M., Geuze, H. J., and Ploegh, H. L. (1996) Cell 84, 769–779
15. Kikkert, M., Hassink, G., Barel, M., Hirsch, C., van der Wal, F. J., and Wiertz, E. (2001) Biochem. J. 358, 369–377
16. Wiertz, E. J., Eclipse, H. L., and Ploegh, H. L. (1996) Cell 84, 769–779
17. Kikkert, M., Hassink, G., Barel, M., Hirsch, H. J., and Wiertz, E. J. (2001) Biochem. J. 358, 369–377
18. Shamu, C. E., Flierman, D., Ploegh, H. L., Rapoport, T. A., and Chau, V. (2001) Mol. Biol. Cell 12, 2546–2555
19. Shamu, C. E., Story, C. M., Rapoport, T. A., and Ploegh, H. L. (1999) J. Cell Biol. 147, 45–58
20. Jones, T. R., Hanson, L. K., Sun, L., Slater, J. S., Stenberg, R. M., and Campbell, A. E. (1995) J. Virol. 69, 4830–4841
21. Blom, D., Hirsch, C., Stern, P., Tortorella, D., and Ploegh, H. L. (2004) EMBO J. 23, 650–658
22. Furman, M. H., Loureiro, J., Ploegh, H. L., and Tortorella, D. (2003) J. Biol. Chem. 278, 34804–34811
23. Stagg, H. R., Thomas, M., van den Boomen, D., Wiertz, E. J., Drabkin, H. A., Gemmill, R. M., and Lehner, P. J. (2009) J. Cell Biol. 186, 685–692
24. Ye, Y., Shibata, Y., Kikkert, M., van Voorden, S., Wiertz, E., and Rapoport, T. A. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 14132–14138
25. Bruderer, R. M., Brasseur, C., and Meyer, H. H. (2004) J. Biol. Chem. 279, 49609–49616
26. Mueller, B., Liley, B. N., and Ploegh, H. L. (2004) Nature 429, 841–847
27. Stagg, H. R., Thomas, M., van den Boomen, D., Wiertz, E. J., Drabkin, H. A., Gemmill, R. M., and Lehner, P. J. (2009) J. Cell Biol. 186, 685–692
28. Ye, Y., Shibata, Y., Kikkert, M., van Voorden, S., Wiertz, E., and Rapoport, T. A. (2004) Nature 429, 841–847
29. Liley, B. N., and Ploegh, H. L. (2004) Nature 429, 834–840
30. Mueller, B., Liley, B. N., and Ploegh, H. L. (2004) J. Cell Biol. 175, 261–270
31. Mueller, B., Liley, B. N., and Ploegh, H. L. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 12325–12330
32. Ye, Y., Meyer, H. H., and Rapoport, T. A. (2001) Nature 414, 652–656
33. Wang, Q., Li, L., and Ye, Y. (2008) J. Biol. Chem. 283, 7445–7454
34. Li, W., Tu, D., Li, L., Wollert, T., Ghirlando, R., Brunger, A. T., and Ye, Y. (2009) Proc. Natl. Acad. Sci. U.S.A. 106, 3722–3727
35. Shamu, C. E., Story, C. M., Rapoport, T. A., and Ploegh, H. L. (1999) J. Cell Biol. 147, 45–58
36. Jones, T. R., Hanson, L. K., Sun, L., Slater, J. S., Stenberg, R. M., and Campbell, A. E. (1995) J. Virol. 69, 4830–4841
37. Blom, D., Hirsch, C., Stern, P., Tortorella, D., and Ploegh, H. L. (2004) EMBO J. 23, 650–658
38. Ernst, R., Mueller, B., Ploegh, H. L., and Schlieker, C. (2009) Mol. Cell 36, 28–38
39. Sowa, M. E., Bennett, E. J., Gygi, S. P., and Harper, J. W. (2009) Cell 138, 389–403
40. Flierman, D., Coleman, C. S., Pickart, C. M., Rapoport, T. A., and Chau, V. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 11589–11594
41. Ballar, P., Zhong, Y., Nagahama, M., Tagaya, M., Shen, Y., and Fang, S. (2007) J. Biol. Chem. 282, 33908–33914
42. Ye, Y., Shibata, Y., Kikkert, M., van Voorden, S., Wiertz, E., and Rapoport, T. A. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 14132–14138
43. Bruderer, R. M., Brasseur, C., and Meyer, H. H. (2004) J. Biol. Chem. 279, 49609–49616
44. Hetzer, M., Meyer, H. H., Walther, T. C., Bilbao-Cortes, D., Warren, G., and Mattaj, I. W. (2001) Nat. Cell Biol. 3, 1086–1091
45. Ramadan, K., Bruderer, R., Spiga, F. M., Popp, O., Baur, T., Gotta, M., and Meyer, H. H. (2007) Nature 450, 1258–1262
46. Wang, X., Herr, R. A., Chua, W. J., Lybarger, L., Wiertz, E. J., and Hansen, T. H. (2007) J. Cell Biol. 177, 613–624
47. Liley, B. N., and Ploegh, H. L. (2005) Mol. Biol. Cell 14, 3690–3698
48. Liley, B. N., and Ploegh, H. L. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 14296–14301