HHS Public Access

Author manuscript

Crit Rev Biochem Mol Biol. Author manuscript; available in PMC 2015 March 13.

Published in final edited form as:

Crit Rev Biochem Mol Biol. 2015 ; 50(1): 1–17. doi:10.3109/10409238.2014.959889.

Ubiquitin-dependent Protein Degradation at the Yeast Endoplasmic Reticulum and Nuclear Envelope

Dimitrios Zattas and **Mark Hochstrasser***

Yale University, Department of Molecular Biophysics & Biochemistry, 266 Whitney Avenue, New Haven, CT 06520-8114

Abstract

The endoplasmic reticulum (ER) is the primary organelle in eukaryotic cells where membrane and secreted proteins are inserted into or across cell membranes. Its membrane bilayer and luminal compartments provide a favorable environment for the folding and assembly of thousands of newly synthesized proteins. However, protein folding is intrinsically error-prone, and various stress conditions can further increase levels of protein misfolding and damage, particularly in the ER, which can lead to cellular dysfunction and disease. The ubiquitin-proteasome system (UPS) is responsible for the selective destruction of a vast array of protein substrates, either for protein quality control or to allow rapid changes in the levels of specific regulatory proteins. In this review, we will focus on the components and mechanisms of ER-associated protein degradation (ERAD), an important branch of the UPS. ER membranes extend from subcortical regions of the cell to the nuclear envelope, with its continuous outer and inner membranes; the nuclear envelope is a specialized subdomain of the ER. ERAD presents additional challenges to the UPS beyond those faced with soluble substrates of the cytoplasm and nucleus. These include recognition of sugar modifications that occur in the ER, retrotranslocation of proteins across the membrane bilayer, and transfer of substrates from the ER extraction machinery to the proteasome. Here we review characteristics of ERAD substrate degradation signals (degrons), mechanisms underlying substrate recognition and processing by the ERAD machinery, and ideas on the still unresolved problem of how substrate proteins are moved across and extracted from the ER membrane.

Key terms

Ubiquitination; Proteasome; ER-associated degradation; Proteolysis; Cdc48/p97

The ubiquitin-proteasome system (UPS)

Metabolic turnover of proteins is a tightly regulated process that occurs in all kingdoms of life. Intracellular protein degradation controls the levels and activities of specific enzymes and regulatory proteins, and it also serves to eliminate misfolded or otherwise aberrant and potentially toxic proteins from the cell (Varshavsky, 2012). Protein folding and assembly are

*To whom correspondence should be addressed: mark.hochstrasser@yale.edu; Phone: (203) 432-5101; Fax: (203) 432-5158.

Declaration of Interest:

None of the authors have a conflict of interest.

imperfect processes, and genetic mutations, cellular stress or exposure to toxic compounds can also disturb the native conformations of proteins. The accumulation of misfolded proteins is associated with many diseases including cystic fibrosis, Alzheimer's disease, and Parkinson's disease (Schwartz, 2009).

In eukaryotes, proteins are frequently targeted for destruction through their covalent modification by the small, extremely conserved protein ubiquitin (Hochstrasser, 1996; Pickart, 2000). One or more ubiquitin molecules can be attached to the target substrate as monomers or in the form of one or more polyubiquitin chains. Such chains involve amide (isopeptide) linkages between the C-terminus of one ubiquitin and either the α-amino group or any of the seven different Lys side chains in the next ubiquitin of the polymer (Xu, 2009). The polyubiquitinated substrates are recognized and degraded in an ATP-dependent manner by the 26S proteasome, a large multisubunit proteolytic machine (Chau, 1989; Thrower, 2000; Ravid, 2008). The set of enzymes and co-factors responsible for ubiquitin tagging of substrates and their proteasome-mediated degradation comprise the ubiquitin-proteasome system (UPS).

The UPS enables the selective and rapid turnover of thousands of different proteins. Much of the selectivity is ensured by attachment of ubiquitin to specific substrates via an elaborate enzymatic cascade. In the first step, ubiquitin is adenylated by the ubiquitin-activating enzyme, E1. The anhydride bond in the ubiquitin-adenylate intermediate is attacked by a conserved cysteine side chain in the E1, which forms a thioester linkage with the C-terminal glycine (G76) of ubiquitin. Through a transthiolation reaction, ubiquitin is then transferred to a cysteine residue on an E2 ubiquitin-conjugating enzyme. Finally, an E3 ubiquitin ligase holds the E2~ubiquitin complex in proximity to the substrate and stimulates ubiquitin transfer to the substrate, usually to a lysine side chain on the target to form an isopeptide bond. These modifications are dynamic and can be readily reversed by deubiquitinating enzymes (DUBs) (Hochstrasser, 2009; Schulman, 2011).

For the majority of organisms, there is only a single E1 for activating ubiquitin (Pickart, 2004), whereas there are many different E2 and especially E3 enzymes. For example, in budding yeast, there are one E1 (Uba1), 11 E2s and between 60 and 100 different E3 enzymes that function in ubiquitin conjugation (Pickart, 2001; Finley, 2012). As one might guess from the large number of E3 ligases, substrate recognition depends principally on these enzymes. E3s have two roles: to recognize the degrons in substrates and to stimulate ubiquitin transfer from the E2 to the target protein (Metzger, 2014). For some classes of E3s, a transthiolation of ubiquitin from the E2 to E3 occurs, and ubiquitin is only then finally transferred to a substrate (Scheffner, 1995; Wenzel, 2011).

Many critical cellular processes such as the cell cycle, signal transduction, DNA repair, and transcription are dependent on protein modification by ubiquitin. (Glotzer, 1991; Hicke, 1996; Pickart, 2001; Laney, 2003). Ubiquitination and degradation through the UPS can occur in diverse compartments within the cell. Here we will review the mechanisms governing protein degradation at the endoplasmic reticulum (ER). Since much of the basic work in this field has been performed with *Saccharomyces cerevisiae*, we will primarily

focus on studies in this model eukaryote but will also touch on related research in plants and mammals.

Protein degradation at the ER

Adaptations of the UPS in ER-associated protein degradation (ERAD)

The ER was first described ultrastructurally in 1945 by Porter and colleagues (Porter, 1945). Palade and his coworkers subsequently demonstrated that the ER serves as a site for the synthesis of secretory proteins followed by their sorting within and secretion from the cell (Redman, 1966). Over a quarter of all nascent proteins synthesized in yeast cells are thought to enter the ER, primarily through the Sec61 translocon during or shortly after their synthesis (Ghaemmaghami, 2003; Rapoport, 2007). Although the ER contains a significant population of molecular chaperones and other factors that promote the correct folding and assembly of newly synthesized proteins, many proteins in both the lumen and membrane fail to form correctly and are eventually degraded.

The earliest evidence suggesting that degradation of specific proteins was occurring at the ER and that improper regulation of this process can result in human disease was presented in the late 1980s and early 1990s (Lippincott-Schwartz, 1988; Bonifacino, 1991). Degradation of a mutated variant of the cystic fibrosis transmembrane conductance regulator (CFTR), T-cell receptor subunits, and mutant α1-antitrypsin were all found to be subject to such ER-localized degradation (Cheng, 1990; Wileman, 1991; Le, 1992). Notably, all were examples of protein quality control (PQC), wherein aberrant proteins or proteins present in stoichiometric excess over their binding partners are rapidly degraded.

Surprisingly, the cytosolic UPS machinery was found to be responsible for much of the degradation of these ER luminal and integral membrane proteins (Sommer, 1993; Ward, 1995; Jensen, 1995; Hiller, 1996; Wiertz, 1996). Substrates are ubiquitinated at the ER membrane surface and must ultimately be degraded by the 26S proteasome. Because the majority of the components of the UPS, including ubiquitin and the proteasome, reside in the cytoplasm and nucleoplasm, degradation of proteins initially localized in the ER lumen or embedded in the membrane requires their retrotranslocation across the membrane bilayer back to the cytoplasm. Although some details about the retrotranslocation process are well established, such as the general involvement of the Cdc48/p97 AAA ATPase (Ye, 2001; Jarosch, 2002; Rabinovich, 2002), many questions remain. The collective processes of protein ubiquitination at the ER, retrotranslocation to the cytoplasm, followed by proteasomal degradation have been termed ER-associated protein degradation or ERAD (McCracken, 1996).

In *S. cerevisiae*, there are two ER membrane E3 ligases, Hrd1/Der3 and Doa10, which appear to catalyze most protein ubiquitination at the ER (Figure 1; Bays, 2001; Deak, 2001; Swanson, 2001). Both enzymes are integral membrane proteins, and they belong to the RING class of E3 ligases. The RING domain, which coordinates a pair of zinc ions, directly contacts its cognate E2 and promotes ubiquitin transfer from the E2 to the substrate (Weissman, 2001). The Hrd1 and Doa10 ligases have clear orthologs in the vast majority of eukaryotes, including humans, reflecting the widespread occurrence and physiological

importance of ERAD. Additional E3s participate in ERAD in both yeast and metazoans, but their contributions appear to be more limited (Haynes, 2002; Stolz, 2013; Metzger, 2014).

The Hrd1 complex

The first E3 enzyme identified in the ERAD pathway was the Hrd1/Der3 ligase in *S. cerevisiae* (Hampton, 1996; Knop, 1996a; Bordallo, 1998). The gene encoding Hrd1, along with other *HRD* genes, was found in a genetic screen for mutants impaired for HMG-CoA reductase degradation (*hrd*) (Hampton, 1996). HMG-CoA reductase, an integral membrane protein of the ER, is the rate-limiting enzyme in the mevalonate pathway and a key control point for sterol synthesis. An independent genetic screen in yeast used the accumulation of two mutant proteins, CPY* (carboxypeptidase Y*) and PrA* (Proteinase A*), to identify mutants defective for degradation in the ER (*der*); the wild-type counterparts of these model substrates normally follow the secretory pathway to the yeast vacuole (lysosome), but when mutated and irreversibly misfolded, they are retained in the ER and rapidly degraded (Knop, 1996a; Hiller, 1996). The *DER3* gene that was identified in this screen turned out to be identical to *HRD1* (Bordallo, 1998).

Yeast Hrd1 recognizes substrates with degrons in the membrane (ERAD-M) or lumen (ERAD-L) of the ER; the latter class includes both soluble and transmembrane proteins (Vashist, 2004; Carvalho, 2006; Nakatsukasa, 2008a). The Hrd1 protein has an N-terminal transmembrane domain with six transmembrane helices; the catalytic RING domain of the enzyme is located within the soluble C-terminal portion of the protein and faces the cytosol (Gardner, 2000; Deak, 2001). The main E2 functioning with Hrd1 is Ubc7, a cytosolic enzyme recruited to the ER via interactions with Cue1, a type-1 transmembrane protein (Biederer, 1997). The Ubc1 E2 also contributes to the Hrd1 pathway, although to a much smaller degree (Friedlander, 2000; Bays, 2001). In addition to facilitating ER localization of Ubc7, Cue1 also functions as an activator of the E2, and this is necessary for efficient substrate ubiquitination (Bazirgan, 2008; Kostova, 2009). The stability of Ubc7 itself is Cue1 dependent *in vivo* as it is rapidly degraded in the absence of Cue1 (Ravid, 2007). Orthologs of the Hrd1/Ubc7 enzymatic pair are now well studied in mammalian cells as well. HRD1 and gp78 are orthologous to yeast Hrd1, and Ube2g2 and Ube2g1 correspond to the Ubc7 E2 (Kostova, 2007). There is no apparent mammalian ortholog for Cue1; however, gp78 contains a unique segment that, like a sequence-unrelated element in Cue1, serves to activate the E2 (Das, 2009; 2013).

In addition to the Hrd1 RING subunit and its associated E2 and Cue1 co-factors, the yeast Hrd1 complex includes a number of additional subunits. Earlier genetic and biochemical studies had identified most of its components, but isolation of the ligase under gentle conditions followed by mass-spectrometry analysis revealed a more precise picture of the complex (Table 1; Figure 1A; Carvalho, 2006). Hrd1 is usually found in a equimolar ratio with the Hrd3 co-factor (Gardner, 2000). The equivalent protein in mammals is SEL1L, which has been shown to be required for ER substrate retrotranslocation (Mueller, 2006). In the absence of Hrd3, Hrd1 becomes short-lived and is degraded by the proteasome. Conversely, Hrd3 overexpression impedes substrate degradation mediated by Hrd1 (Plemper, 1999), potentially due to the excess Hrd3 associating with substrates in

nonproductive complexes. Hrd3 has a large N-terminal luminal domain that interacts directly with Yos9 (Carvalho, 2006; Denic, 2006), a lectin subunit that recognizes glycosylated, misfolded luminal proteins such as CPY* (Bhamidipati, 2005; Kim, 2005; Szathmary, 2005). It is possible that luminal substrate interaction initially occurs on Yos9, which then delivers the substrate to the Hrd1 ligase through interactions with Hrd3 (Carvalho, 2006).

Interestingly, both Hrd3 and Yos9 can associate with CPY* independently of each other and regardless of the glycosylation status of this ERAD-L substrate. Yos9 has been proposed to contribute to ER retention of misfolded luminal proteins because in its absence, fusion proteins containing CPY* continue on to the Golgi and vacuole (Izawa, 2012). The luminal molecular chaperone Kar2 (called BiP or Grp78 in mammals) also associates with Yos9 and can recognize a misfolded substrate independently of the lectin (Denic, 2006). These data suggest that luminal substrates, in addition to their sugar modifications, have protein-derived recognition signals, presumably arising from their misfolded protein domains. Finally, another yeast factor that contributes to degradation of ERAD-L substrates is the Htm1 mannosidase, which is homologous to the EDEM proteins in mammals (Jakob, 2001). Htm1 specifically recognizes $Man_8GlcNAc_2$ oligosaccharides in glycoproteins, converting them to $Man_7GlcNAc_2$, an oligosaccharide signal that is recognized by Yos9, thereby targeting the attached protein to the ERAD-L pathway (Gauss, 2011).

In addition to the luminal components Yos9 and Kar2 and the transmembrane Hrd3 subunit, which are all found in the yeast Hrd1 complex, other integral membrane subunits are also crucial for ligase function. One membrane component is the Der1 protein (Knop, 1996a). Topological analysis of Der1 suggested that it bears four transmembrane helices (Hitt, 2004). Der1 is a protein of still unknown function. Der1 is required specifically for the degradation of ERAD-L but not ERAD-M substrates (Taxis, 2003; Vashist, 2004; Carvalho, 2006). The mammalian orthologs of this protein, Derlin-1, -2, and -3, have been implicated in substrate retrotranslocation across the ER membrane (Greenblatt, 2011; Lilley, 2004; Oda, 2006). Recent evidence indicates that Der1 might have a similar role. Specifically, it was shown by site-specific photocrosslinking that residues of both the luminal and membrane portions of Der1 interact directly with a misfolded luminal substrate and these interactions are required for substrate extraction from the ER (Mehnert, 2014). *S. cerevisiae* has a paralog of Der1, presumably with a similar topology, called Dfm1 (Sato, 2006; Hitt, 2004). Unlike Der1, Dfm1 has a putative SHP-box sequence that participates in interactions with the Cdc48 ATPase hexamer. Despite Dfm1 reportedly associating with both the Hrd1 and Doa10 complexes (Goder, 2008; Stolz, 2010), its role in ERAD is still unclear since it is not required for degradation of most ERAD substrates.

Der1 association with the Hrd1 complex is mediated by another integral membrane protein, Usa1 (Carvalho, 2006). Usa1 has two transmembrane helices with large cytosolic N- and C-terminal domains. The N-terminal portion of Usa1 interacts with the cytosolic RING-bearing domain of Hrd1, whereas the C-terminal domain binds to Der1 (Horn, 2009). In this way, Usa1 provides a bridge between Der1 and Hrd1 (Figure 1A). Notably, Usa1 is crucial to both ERAD-L and ERAD-M substrate degradation (Carvalho, 2006; Horn, 2009; Carroll, 2010; Carvalho, 2010). Hence, Usa1 has functions beyond simply recruiting Der1 to Hrd1

since Der1 is only required for ERAD-L. Usa1 is required for oligomerization of the Hrd1 complex, which seems to be a necessary step for the degradation of ERAD-M substrates (Horn, 2009) and possibly luminal substrates as well (Carvalho, 2010). The mammalian ortholog of Usa1, Herp, also interacts with both HRD1 and Derlin-1 and has an equally important role in ERAD substrate degradation (Okuda-Shimizu, 2007).

A final identified membrane-anchored component of the Hrd1 complex is the Cdc48-recruiting factor, Ubx2, a double-pass transmembrane protein. Cdc48 and its co-factors Ufd1 and Npl4 also associate with the Doa10 complex at least in part through interaction with Ubx2 (Neuber, 2005; Schuberth, 2005; Carvalho, 2006). The cytosolic N-terminal part of Ubx2 has a ubiquitin-associated (UBA) domain, while the cytosolic C-terminal tail contains a ubiquitin-regulatory X (UBX) domain. UBX domains are binding modules for Cdc48, and in *S. cerevisiae* there are seven proteins that belong to the UBX family (Schuberth, 2004). Substrates that must be retrotranslocated from the ER –such as integral membrane and luminal proteins– depend on Ubx2 for their degradation. The UBA domain of Ubx2 is important for ERAD due to its interactions with ubiquitinated substrates that are retrotranslocated (Neuber, 2005). Finally, it has also been suggested that Cdc48 can associate with the ER membrane in the absence of Ubx2, possibly through interactions with Dfm1 (Schuberth, 2005; Stolz, 2010). However, the physiological importance of the Cdc48-Dfm1 interaction has not yet been verified. The function of Cdc48 in substrate retrotranslocation will be discussed in more detail in the final section of this review.

The Doa10 complex

The yeast transcription factor MATα2 was the first physiological substrate identified for the UPS (Hochstrasser, 1990; Hochstrasser, 1991). If the N-terminal 67-residue segment of MATα2 was appended to an otherwise stable protein, it caused rapid degradation of the fusion protein. The degradation signal or degron contained within this N-terminal fragment was termed *Deg1* (Chen, 1993). Multiple genetic screens were performed using different *Deg1*-reporter fusions in order to identify mutants defective for the *degradation of α2* (*doa*). From these screens, two E2 enzymes, Ubc6 and Ubc7, were found to be required for degradation of *Deg1*-fusion proteins, as was the E3 Doa10, which functions with these E2s (Table 1; Chen, 1993; Swanson, 2001; Ravid, 2006).

Doa10 is a protein of ~151 kDa that includes 14 transmembrane segments (TMs) (Kreft, 2006). Doa10 localizes throughout the ER, including both the inner and outer nuclear membranes. Inner nuclear membrane localization of Doa10 is required for the degradation of its nuclear protein substrates, such as MATα2 (Deng, 2006). In general, Doa10 mediates the ubiquitination of specific soluble proteins and membrane proteins with cytosolically disposed degrons (Ravid, 2006). These proteins are called ERAD-C substrates (Figure 1B; Vashist, 2004). The Doa10 ligase is broadly conserved among highly divergent eukaryotic species. Its mammalian ortholog, TEB4 (MARCH6), appears to have a similar localization and topology (Kreft, 2006; Hassink, 2005). Recently, plant orthologs of Doa10, named SUD1/CER9 and Bn-*CLG1A*, were identified in two different species, *Arabidopsis thaliana* and *Brassica napus*, respectively (Lü, 2012; Doblas, 2013; Lu, 2012). Of the aforementioned orthologs, TEB4 and SUD1/CER9 were tested for heterologous expression

in yeast lacking the endogenous Doa10 ligase, but no complementation was observed (Kreft, 2006; Doblas, 2013). This suggests the substantial divergence in functional aspects of the ligase during evolution. Interestingly, in the yeast genus *Kluyveromyces*, Doa10 is naturally split into two protein fragments due to the presence of an intervening sequence (IVS) in the genomic locus, resulting in two separate open-reading frames. Co-expression of the two *Kluyveromyces* Doa10 fragments, but not the individual fragments, in *S. cerevisiae* could fully complement ligase activity in the absence of endogenous Doa10 (Stuerner, 2012).

While Hrd1 and Doa10 share a common E2 enzyme, Ubc7 (and the Ubc7 co-factor Cue1), Doa10 requires an additional E2, Ubc6, for substrate ubiquitination and degradation (Figure 1B; Swanson, 2001; Ravid, 2006). Potential mechanisms for the functional interplay between the two E2s will be discussed in a later section. Ubc6 is a C-terminally tailed-anchored protein of the ER membrane (Sommer, 1993) and has two described orthologs in mammals, Ube2j1 and Ube2j2 (Kostova, 2007). Both mammalian E2s have a role in ERAD (Lenk, 2002) and were shown to interact and function with a newly identified ERAD E3, RNF185 (El Khouri, 2013). RNF185 is a paralog of another ERAD E3 ligase, RNF5/RMA1. Ube2j2, but not Ube2j1, also functions with another recently discovered mammalian ERAD E3, TMEM129 (van de Weijer, 2014; van den Boomen, 2014). However, Ube2j1/Ub2j2 do not appear to function with TEB4, which only seems to associate with the Ubc7 ortholog, Ube2g2 (Hassink, 2005). In yeast, Ubc6 is a short-lived protein and its degradation is dependent on Doa10, the ligase with which it normally works (Walter, 2001; Swanson, 2001). Ubc6 function is linked to the conserved ~130-residue TEB4-Doa10 (TD) domain of Doa10, which includes three transmembrane helices. The TD domain regulates Ubc6 metabolic stability, potentially through a direct interaction with the E2. In addition, the C-terminal transmembrane anchor of Ubc6 has been shown to contribute to the degradation of the protein (Walter, 2001; Kreft, 2011). Ubc6 degradation mediated by Doa10 appears not to be required for Doa10 activity towards other substrates, although only a limited set of substrates were tested (Kreft, 2011).

As noted above, Doa10 also interacts with the Ubx2 and $Cdc48^{Npl4\text{-}Ufd1}$ co-factors. However, Cdc48 appears to be necessary only for the degradation of ERAD-C substrates that are embedded in the ER membrane (Ravid, 2006). This suggests that the primary role for the $Cdc48^{Npl4\text{-}Ufd1}$ ATPase in the Doa10 pathway is in substrate extraction from the membrane. Doa10 also interacts directly with the Der1 homolog, Dfm1, but the data regarding the role of this protein in the degradation of Doa10 substrates have been conflicting (Kreft, 2006; Stolz, 2010).

Generally, there is minimal redundancy between Doa10 and Hrd1 in terms of ERAD substrate specificity. However, several lines of evidence suggest synergistic effects for the two ligases in protein quality control under certain cellular stress conditions. For example, yeast cells lacking both Doa10 and Hrd1 show increased sensitivity to cadmium, a heavy metal that causes oxidative stress. The doubly mutant cells also exhibit an elevated ER unfolded protein response (UPR), a homeostatic transcriptional response to increased levels of misfolded proteins in the ER (Swanson, 2001). Similarly, the *doa10*Δ *hrd1*Δ double mutant, but neither single mutant, showed strong suppression of an *npl4-1* mutation, presumably by reducing the load of polyubiquitinated ER membrane proteins that

accumulate due to the defect in $Cdc48^{Npl4\text{-}Ufd1}$ (Hitchcock, 2003). Further supporting a shared role in alleviating ER stress, both ERAD ligases and many of their co-factors are transcriptionally upregulated by the UPR (Travers, 2000; Friedlander, 2000).

Substrate Recognition Themes in ERAD

What sequence and structural features of a protein make it a substrate of the UPS in general and of the ERAD system in particular? Characterization of substrate degradation signals (degrons) still remains an area of intense investigation; however, there are some general themes regarding degron properties that have emerged (Ravid, 2008; Varshavsky, 2012). Many degrons, particularly in protein quality control substrates, are conditional in that they require exposure of a protein region(s) that is normally buried within a properly folded polypeptide or in a protein-protein interface. Such regions in soluble proteins often contain a high number of hydrophobic residues, and their recognition frequently depends on specific molecular chaperones. Conversely, in ERAD-M substrates, transmembrane regions with high hydrophilicity may be preferentially recognized (Sato, 2009). Another common theme in substrate recognition by ubiquitin E3 ligases is the importance of prior co- or post-translational modification of the target substrate. Examples include glycosylation, phosphorylation, sumoylation, N_{α}-acetylation, and hydroxylation. For a more general discussion of degron recognition by the UPS, the reader is referred to earlier reviews (Ravid, 2008; Nakatsukasa, 2008a; Varshavsky, 2012). Here we will focus on what is known about the recognition of ERAD substrates, a process that is complicated by the fact that all the enzymatic functions of the core ubiquitination machinery lie at the cytosolic face of the ER.

Degradation of ERAD-L Substrates

The first yeast proteins shown to be ERAD substrates were CPY* and PrA*, mutated forms of the vacuolar proteases carboxypeptidase Y and proteinase A, respectively (Finger, 1993). Both mutant proteins localize to the ER or an ER-related compartment and are rapidly degraded. Although originally thought to be proteasome-independent, later studies showed that CPY* is targeted for degradation by the UPS (Hiller, 1996). This implied that luminal substrates must somehow be recognized in the ER, transported across the membrane and ubiquitinated by enzymes in the cytosol or on the ER membrane. An early candidate for a factor involved in substrate recognition in the ER lumen was Der1 (Knop, 1996a). While Der1 has weak affinity for CPY*, more recent evidence suggest that it functions downstream of initial substrate recognition, which is instead thought to occur through luminal lectins and Hrd3 (Mehnert, 2014; Denic, 2006; Carvalho, 2006). CPY* is N-glycosylated at four asparagine residues, and its glycosylation was demonstrated to promote its turnover (Knop, 1996b). However, a non-glycosylated CPY* substrate is still degraded, albeit at a lower rate, by ERAD. The position of the N-glycan, rather than the number of N-glycans added to the protein, proved to be most critical. Glycosylation of a single asparagine on CPY* (Asn368) was sufficient for promoting its degradation (Kostova, 2005).

The current model for the recognition of glycosylated substrates such as CPY* is roughly as follows: During ER entry of the nascent protein, its asparagine acceptor sites are modified *en bloc* with $Glc_3Man_9GlcNAc_2$ glycan complexes by oligosaccharyltransferase. If the protein does not fold in a timely fashion, then through the action of glucosidases I and II and

mannosidase I, the glycan chain is trimmed to $Man_8GlcNAc_2$ (Helenius, 2004). As noted earlier, the Htm1 mannosidase recognizes the latter N-glycan modification and trims it to $Man_7GlcNAc_2$, revealing the terminal α1,6-linked mannose recognized by Yos9 (Quan, 2008; Clerc, 2009; Gauss, 2011). Along with the presence of unfolded protein segment(s) recognized by Kar2, Hrd3, and even Yos9, substrates are then ubiquitinated by Hrd1. The mechanism of luminal substrate recognition is conserved to a significant degree in mammalian cells. Two Yos9 orthologs have been identified in humans, OS-9 and XTP3-B/Erlectin, which are required for degradation of misfolded ERAD-L substrates such as mutant a1-antitrypsin. Like Yos9, both lectins associate with the Hrd3 ortholog, SEL1L, and can recognize nonglycosylated substrates as well (Christianson, 2008; Hosokawa, 2009). Thus, in a manner similar to their yeast counterparts, specific N-glycan signals are produced by the actions of glucosidases I and II, ERManI and EDEM mannosidases and are recognized by OS-9/XTP3-B, which ultimately promotes substrate ubiquitination and degradation (Olzmann, 2013). It has also been suggested that *O*-mannosylation of ER proteins by the Pmt1/Pmt2 complex in yeast acts as the termination signal for proteins that fail to fold (Xu, 2013). However, whether or not this contributes significantly to substrate targeting to ERAD remains to be determined.

The luminal Hsp70 chaperone Kar2 is also crucial for ERAD of CPY* in a manner that is dependent on its protein fold but not its glycosylation status (Plemper, 1997; Huyer, 2004; Denic, 2006). Interestingly, when a CPY*-chimeric protein is anchored to the ER membrane via a single transmembrane helix, it is still targeted for ERAD by the Hrd1 complex, but Kar2 and Der1 are now dispensable for its degradation (Taxis, 2003). This suggested that Kar2 and Der1 specifically recognize and facilitate the degradation of soluble misfolded proteins within the ER lumen. However, results with additional model substrates led to a modification of this generalization (Vashist, 2004; Carvalho, 2006). For example, the artificial KHN substrate is a fusion of the Kar2 signal sequence with the simian virus 5 hemagglutinin-neuraminidase ectodomain. This soluble ERAD-L substrate is *O*-mannosylated, transported to the Golgi and then retrieved to the ER before being degraded by the Hrd1 pathway (Vashist, 2001). When KHN was anchored to the ER membrane by fusing it to a TM-containing fragment of the Wsc1 protein (creating the KWW protein), the KWW substrate retained the same degradation properties as KHN, including its dependency on Der1 (Kar2 was not tested). This result suggested that it is the site of the lesion/misfolded domain that dictates the degradation pathway of an ERAD substrate (Vashist, 2004). For the membrane-anchored CPY* derivatives analyzed by Taxis et al. (2003), specific features of the chimeras or their strong overexpression might have resulted in a switch from ERAD-L to ERAD-M characteristics.

Overall, from the studies performed on yeast ERAD-L substrates, we can conclude that: i) the presence of misfolded luminal domains is a requirement for substrate recognition; ii) substrate-specific sugar modifications can influence the interactions with specific luminal cofactors of the ERAD pathway and iii) substrate recognition and retrotranslocation across the ER bilayer are likely to be performed in a sequential manner rather than through simultaneous processes. Protein retrotranslocation will be discussed in more detail in the last section of this review.

Degradation of ERAD-M Substrates

The Hrd1/Der3 E3 ligase also recognizes specific ER membrane proteins with lesions (degrons) in their transmembrane segments, the ERAD-M class of substrates (Nakatsukasa, 2008a). The first validated Hrd1 membrane substrate in *S. cerevisiae* was the Hmg2 isoform of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGR) (Hampton, 1994; Hampton, 1996). Hmg2 levels are regulated by the UPS by specific sterol and isoprenoid intermediates of the mevalonate pathway (Hampton, 1996). How can an E3 ligase recognize an integral membrane protein such as Hmg2? Extensive mutagenesis of Hmg2 unveiled a “distributed degron,” a degradation signal that was not confined to a single domain or short sequence segment (Gardner, 1999). Binding to downstream products of the mevalonate pathway influences structural features of Hmg2 such that only under high sterol levels is Hmg2 recognized as a substrate by Hrd1. It was shown that Ubc7 and Hrd1 association with Hmg2 occurs in a mevalonate pathway-dependent manner, consistent with structural changes in the substrate that change its recognition by the ERAD pathway (Gardner, 2001).

Degradation of mammalian HMGR is also regulated in a sterol-dependent manner by the UPS. There is disagreement regarding the relevant ubiquitin ligases involved. Initial studies using RNA interference (RNAi) methods implicated the mammalian Hrd1 ortholog gp78 as well as a second ERAD E3, TRC8 (Song, 2005; Jo, 2011). However, a more recent study that used gp78(−/−) mouse embryonic fibroblasts and RNAi argued against a requirement for either of these E3s in sterol-stimulated HMGR degradation (Tsai, 2012). These differences remain to be resolved. An important regulator of HMGR degradation is the protein Insig-1 (and its paralog, Insig-2). In mammals, Insig-1 binds to the sterol-sensing domain (SSD) of HMGR and is required for sterol-dependent degradation of the enzyme, probably by promoting its association with a specific E3 ligase(s) (Sever, 2003). In contrast, in yeast, binding of the ortholog of Insig-1, Nsg1, actually stabilizes Hmg2 (Theesfeld, 2013). Thus, the sterol-regulated binding of Insig to HMGR is conserved from yeast to human, but the functional consequences differ.

In yeast, different residues within the transmembrane segments of Hrd1 modulate recognition of specific ERAD-M substrates (Sato, 2009). Hrd1 has a high number of hydrophilic residues within its transmembrane regions. Hydrophilic residues in the transmembrane regions of substrates might serve as a signature for their misfolding. Thus, it was reasonable to hypothesize that these transmembrane residues in Hrd1 could interact with hydrophilic residues in substrate segments that reside within the lipid bilayer. Specific mutations of conserved hydrophilic and hydrophobic residues in the Hrd1 transmembrane domain affected different ERAD-M substrates differently. These findings suggest that the Hrd1 ligase can directly recognize intramembrane motifs in ER membrane substrates without the need of a substrate-binding intermediary (Sato, 2009).

A number of Hrd1 substrates behave as ERAD-M substrates despite having primary lesions outside of their transmembrane segments. For instance, the yeast membrane co-factor Der1 is degraded via Hrd1 in the absence of N_{α}-acetylation of its short, cytoplasmically disposed N-terminal segment. Der1 lacking this modification is still recruited to the Hrd1 complex

and appears to retain its normal topology (Zattas, 2013). Loss of acetylation might cause subtle perturbations to the membrane (or luminal) domain of Der1, which could theoretically expose a degron to the associated Hrd1 protein. A similar induced degron exposure is thought to occur in the Sec61-2 ERAD-M substrate. Degradation of this defective, temperature-sensitive protein depends at least partially on the ERAD E2s and Hrd1 (Sommer, 1993; Biederer, 1996; Plemper, 1997; Bordallo, 1998). The mutant Sec61-2 protein contains a G213D mutation at the cytosolic edge of a transmembrane helix (Nishikawa, 2001). In principle, this mutation could cause structural changes in the protein's membrane segments, creating an ERAD-M substrate (Ravid, 2006). A mutant version of the Pdr5 ATP-binding cassette (ABC) transporter, Pdr5*, is also a likely ERAD-M substrate even though its single missense mutation (C1427Y) also lies outside the intramembrane region, specifically, in a luminal loop (Plemper, 1998; Carvalho, 2006).

Finally, an unusual type of Hrd1 membrane substrate was generated by fusing the *Deg1* degradation signal to the Sec62 protein, which has two transmembrane helices connected by a short luminal linker. *Deg1*-protein fusions, including ones embedded in the ER membrane, are usually substrates for Doa10-dependent ubiquitination (Chen, 1993; Swanson, 2001; Ravid, 2006). Surprisingly, *Deg1*-Sec62 is targeted for degradation via Hrd1 in a manner that requires persistent binding of the substrate with the Sec61 translocon, and degradation correlates closely with a topological rearrangement of the Sec62 domain (Rubenstein, 2012). Human apolipoprotein B also appears to be targeted for degradation by Hrd1 while associated with the translocon. Therefore, it is likely that Hrd1 can generally target proteins aberrantly associated with the translocon, although the recognition mechanism remains to be determined. Degradation of this subclass of ERAD substrates has been termed ERAD-T (translocon-associated) (Rubenstein, 2012).

In summary, yeast Hrd1 and its mammalian counterparts are likely to be able to recognize membrane substrates through direct contacts between membrane segments of the ligase and substrate. In some cases, this requires the presence of another membrane co-factor, for example, Insig-1. Changes in the ER lipid environment appear to contribute to degron exposure or structure in ERAD-M substrates such as HMGR. In most cases, the determinants of ERAD-M degrons important for recognition have not yet been elucidated. Biophysical and structural studies will be needed in order to provide insight into these issues.

Degradation of ERAD-C Substrates

In yeast, degradation of the ERAD-C class of substrates is largely mediated by the Doa10 E3 ligase. These substrates are either soluble proteins in the cytoplasm or nucleus or are integral membrane proteins of the ER and inner nuclear membrane (INM). For all of these substrates, the degron is present in the cytoplasm or nucleoplasm (Ravid, 2006). Doa10 is localized to all regions of the ER membrane, including the INM (Swanson, 2001; Deng, 2006). Doa10 needs to localize to the INM in order to target nuclear substrates such as MATα2, whereas its INM localization is not necessary for degrading cytoplasmic substrates (Deng, 2006).

In mammals, the assignment of ERAD-L and ERAD-M substrates to orthologs of Hrd1 and ERAD-C substrates to the Doa10 ortholog TEB4 is unlikely to be as strict as in yeast. For example, it is known that the soluble nuclear protein p53 can be ubiquitinated by Hrd1 (synoviolin) in mammalian cells (Yamasaki, 2007), and the Insig-1 membrane protein discussed in the previous section appears to be targeted by Hrd1, TEB4, and another ER-associated ligase, TRC8 (Tsai, 2012; Faulkner, 2013). Even in yeast, these divisions might not be as definitive as previously thought. For instance, Ubc6 degradation is mediated by Doa10 despite the E2 having an intramembrane anchor that is important for its degradation (Walter, 2001). Additional ERAD ligases in yeast are also expected (Theesfeld, 2013).

What are the molecular characteristics of *Deg1* that allow it to be recognized by Doa10? A key determinant was found by mutagenesis to be localized to a ~20-residue segment of the *Deg1* degron (Johnson, 1998). Secondary structure prediction and helical-wheel analysis of this *Deg1* determinant suggested it forms an amphipathic helix, consistent with circular dichroism studies of the MATα2 N-terminal domain. The hydrophobic surface of the helix is the most sensitive to mutation. Under conditions where MATα2 can heterodimerize with the MATa1 transcription activator, which occurs physiologically in a/α diploid cells, MATα2 is stabilized. This is because protein-protein interactions mask the hydrophobic surface of the *Deg1* determinant and thus prevent recognition by the Doa10 pathway (Johnson, 1998). The importance of these residues in Doa10 substrate recognition was recently validated in an elegant high-throughput mutational analysis of *Deg1* substrate stability in yeast (Kim, 2013). Whether the *Deg1* sequence interacts directly with Doa10 and if so, which features of Doa10 are important for the interaction are questions that remain to be resolved.

Additional degrons recognized by Doa10 have also been identified, and these degrons appear to share at least some common features with *Deg1*. A series of artificial degrons fused to the C-terminus of a reporter protein were found to be Doa10 pathway-specific (Gilon, 1998; Ravid, 2006). Several of these, including the CL1 degron, were predicted to contain an amphipathic helix, and the hydrophobic surface of CL1 was shown to be important for degradation (Gilon, 2000). Recent studies of another degron, derived from the Ndc10 kinetochore protein, suggested the importance of two amphipathic helices interacting through their hydrophobic surfaces (Furth, 2011). Disruption of this predicted interaction and exposure of a hydrophobic helical surface triggered Doa10-dependent ubiquitination and degradation. Based on the cumulative observations described above, recognition of helix amphipathicity, or at least a hydrophobic helical surface, may be a general substrate feature that Doa10 recognizes. It is possible that Doa10 has complementary helical elements for interaction with these substrate segments, but this idea remains speculative (Swanson, 2001).

Soluble proteins carrying the CL1 degron are found in the cytoplasm (Ravid, 2006). Their localization to the ER membrane for Doa10-dependent ubiquitination requires the action of Hsp70 and Hsp40 chaperones. Specifically, the Ssa family of Hsp70s and the Ydj1 co-chaperone are directly involved in this process and are necessary for substrate ubiquitination and degradation by Doa10 (Metzger, 2008). For substrates with the Ndc10-derived degron described above, the Sis1 Hsp40, but not Ydj1, is required for E3-substrate interaction and

degradation (Alfassy, 2013; Shiber, 2013). Thus, Hsp40 co-chaperones might contribute to substrate specificity, potentially by mediating substrate binding to Doa10 or handing the substrates off to the ligase. A membrane ERAD-C substrate, Ste6*, also depends on chaperone proteins for interaction with and subsequent ubiquitination by Doa10 (Nakatsukasa, 2008b). Ste6 is a 12-transmembrane ABC transporter and the Ste6* mutant (aka Ste6-166) was identified as an ER quality control substrate (Loayza, 1998). The mutant contains a truncation at the cytoplasmic C-terminus, presumably leading to misfolding of that domain and recognition by Doa10 (Huyer, 2004; Vashist, 2004; Carvalho, 2006). Interestingly, the cytosolic Ubr1 E3 ligase also ubiquitinates Ste6* in an Hsp70-dependent manner (Stolz, 2013). Chaperone proteins may maintain solubility of misfolded substrates, preventing their aggregation, or promote E3 ligase-substrate interaction (or both).

A number of physiological membrane substrates have been identified for Doa10-dependent degradation, although their degrons are generally not yet well characterized. Human iodothyronine deiodinase (hD2), a single-pass ER membrane protein, is unstable in human cells (Steinsapir, 2000), and heterologous expression in *S. cerevisiae* showed that its degradation depended on the ERAD E2s and Doa10 (Botero, 2002; Ravid, 2006). Importantly, the human ortholog of Doa10, TEB4, also mediates proteasomal degradation of hD2 (Zavacki, 2009). Another membrane Doa10 substrate whose degradation is conserved in mammals is the Erg1 squalene monooxygenase (SM in humans) (Foresti, 2013). When the sterol biosynthetic intermediate lanosterol builds up in yeast, Erg1 becomes susceptible to Doa10-dependent degradation. In the case of human SM, it is accumulation of the end product of the pathway, cholesterol, that stimulates TEB4-mediated degradation. However, another study showed that the domain of human SM that is required for recognition by TEB4 is not conserved in *S. cerevisiae* (Zelcer, 2014). The same study also showed, surprisingly, that TEB4 contributes to degradation of HMGR; however, this effect could be indirect. Finally, Pca1, a P-type ATPase in yeast that exports cadmium from cells, contains a cadmium-sensitive N-terminal degron. In the absence of cadmium, Pca1 is rapidly degraded in a Doa10-dependent manner at the ER (Adle, 2009). Cadmium binding to the cysteine-rich N-terminal domain of Pca1 appears to mask the degron, allowing the transporter to traffic to the cell surface. This regulatory mechanism helps tune Pca1 levels to environmental cadmium levels (Adle, 2009).

In summary, a variety of degrons have been described for the Doa10 pathway. Some, such as *Deg1*, have been characterized in substantial detail, but key questions remain: i) What are the substrate interaction sites on Doa10 and are different degrons recognized by independent/non-overlapping sites on the ligase? ii) What are the exact mechanistic roles of the molecular chaperones? Do they interact directly with the ligase or is their role limited to preventing misfolded substrate domains from aggregating? iii) And finally, is recognition or ubiquitination of membrane substrates physically coupled to their retrotranslocation from the ER membrane?

Mechanisms of ERAD E2s in Ubiquitin-Chain Formation

The ERAD ubiquitination machinery generally builds polyubiquitin chains on its substrates, allowing their recognition by the 26S proteasome (Thrower, 2000). Chain assembly occurs

through formation of an amide bond between the C-terminal carboxyl group of a donor ubiquitin and an amine group on the acceptor ubiquitin. The acceptor amine can be an e-amino group from one of seven lysines in ubiquitin (K6, K11, K27, K29, K33, K48, and K63) or its free N-terminal α-amino group (Hochstrasser, 2006; Kravtsova-Ivantsiv, 2013). Whereas formation of ubiquitin chains based on K48 linkages are the most common and are usually important for substrate degradation by the proteasome (Chau, 1989), various structures for ubiquitin chains, including branched and linear chains, are now known to contribute to the UPS, as is mono-ubiquitination of multiple substrate sites (Kravtsova-Ivantsiv, 2013). Ubiquitin chain linkage is dictated by the E2 ubiquitin-conjugating enzyme(s) that is responsible for chain assembly (Wenzel, 2011). The primary ERAD E2s, Ubc6 and Ubc7, appear to assemble distinct types of ubiquitin chains, which raises questions regarding the nature of the chains formed when both enzymes function together, as is the case with most Doa10 substrates (Xu, 2009).

Both Ubc7 and its mammalian ortholog Ube2g2 form K48-linked chains *in vitro* (Li, 2007; Bazirgan, 2008). Notably, these E2s have been observed to assemble polyubiquitin chains on their active site cysteine *in vivo* and *in vitro* (Ravid, 2007; Li, 2007). In yeast cells, this E2-linked K48-linked chain helps to eliminate excess Ubc7. The preassembled ubiquitin chain on the active site of Ube2g2 formed *in vitro* could transfer as a unit to a lysine residue of a substrate (Li, 2007). Dimerization of Ube2g2 is required for chain assembly *in vitro* and ERAD activity *in vivo* (Liu, 2014).

Ubc7 activity is modulated by its binding to the Cue1 adaptor at the ER membrane. The presence of the soluble portion of Cue1 *in vitro* greatly enhances K48-linked polyubiquitin chain formation by Ubc7 (Bazirgan, 2008). The last 53 residues of Cue1 contain the Ubc7-Binding Region (U7BR), which is sufficient for interaction with Ubc7 and necessary for ERAD (Kostova, 2009). An analogous region, G2BR (Ube2g2-Binding Region) exists on the gp78 E3 ligase and regulates activity of Ube2g2 (Chen, 2006). NMR and X-ray crystallography data showed that G2BR interacts with the “backside” of Ube2g2, i.e., the side of the E2 opposite to its catalytic cysteine residue. This results in increased affinity of the E2 for the gp78 RING, which in return enhances discharge of the thioester-linked ubiquitin from the E2 active site, and stimulates E3-dependent polyubiquitination (Das, 2009). Structural and biochemical evidence for the Cue1 U7BR and Ubc7 suggest a similar mechanism (Metzger, 2013). While structurally divergent from G2BR, U7BR still forms interactions with the backside of Ubc7. U7BR enhances ubiquitin-loading on the E2 and increases ubiquitin transfer to ubiquitin-K48. In addition, U7BR increases E2 affinity for both the Hrd1 and Doa10 RING domains (Metzger, 2013). Structural evidence from a gp78 RING: Ube2g2: G2BR ternary complex suggested an allosteric mechanism where RING binding to the Ube2g2: G2BR complex stimulates G2BR release in order to facilitate rapid exchange of the E2 from gp78 (Das, 2013). From these data, it is clear that the backside binding of the Cue1 U7BR and gp78 G2BR to their cognate ERAD E2s is a crucial factor for efficient substrate ubiquitination.

Cue1, the founding member of the CUE domain family, also requires its CUE domain for ERAD (Bagola, 2013). This domain binds to K48-linked ubiquitin chains and is required for the formation of high-molecular weight polyubiquitin chains by Ubc7 *in vitro*. It is thought

that the CUE domain of Cue1 associates with oligo-ubiquitin conjugates to facilitate Ubc7-dependent chain elongation, thereby increasing the processivity of ubiquitin chain formation.

The other major yeast ERAD E2, Ubc6, appears to function exclusively with the Doa10 ligase (Figure 1B). For most Doa10 substrates, both Ubc6 and Ubc7 are required for maximal rates of degradation, but their relative importance can vary. The reasons for this variation are unclear but could relate to the formation of different kinds of ubiquitin chains on different substrates. Ubc6 and Ubc7 are thought to function together in the same Doa10-based complex, although to date the only evidence supporting this is a weak interaction detected between the two E2s by yeast two-hybrid analysis (Chen, 1993); the same analysis revealed dimerization of Ubc7, which is supported by biochemical studies with Ube2g2, its mammalian ortholog (Liu, 2014).

As noted earlier, Ubc6 is normally degraded in yeast cells, and this turnover requires Doa10, Ubc7, the active-site cysteine of Ubc6, and the ability to form K48-linked ubiquitin chains (Swanson, 2001; Walter, 2001; Kreft, 2011). Unexpectedly, quantitative mass-spectrometry measurements showed that Ubc6 degradation depends in part on formation of K11-linked ubiquitin chains (Xu, 2009). Moreover, GST-tagged Ubc6 autoubiquitinates itself *in vitro*, predominantly with K11-linked chains. Investigation of total polyubiquitin linkages in *S. cerevisiae* showed that Ubc6 is the main E2 contributing to K11 chain formation (~40% of linkages); loss of Doa10 leads to a similar drop in K11 ubiquitin linkages. Although yeast mutant cells that are unable to form K11-linked chains are sensitive to ER stress, the importance of K11 chains for general ERAD substrate degradation remains to be established. In mammals, the Cdc48/p97 adaptor protein, UBXD7, seems to associate with complexes containing K11-linked ubiquitin conjugates (Alexandru, 2008). By analogy, Cdc48 may preferentially recognize a subset of ERAD substrates in yeast when they are modified with K11-linked chains.

How is ubiquitin transferred onto an ERAD substrate and why do the majority of Doa10 substrates require the activities of both Ubc6 and Ubc7 for their ubiquitination? From the accumulated, albeit limited, evidence available, we can make some plausible suggestions regarding ubiquitin transfer mechanisms. In the case of Ubc7 (Ube2g2), the main E2 functioning with Hrd1, it is possible that E2 dimerization and preassembly of the polyubiquitin chain occurs prior to substrate modification. This would allow polyubiquitination of the substrate even with a single binding event on the E3 (Figure 2A). The polyubiquitin-binding CUE domain of Cue1 might enhance polyubiquitin chain formation on substrates by binding to substrates already modified with short ubiquitin chains, thereby bringing its associated polyubiquitin-charged Ubc7 into close proximity. Alternatively, by binding *in trans* to another ubiquitin-Ubc7-Cue1 complex, it might facilitate sequential ubiquitin additions on the substrate.

In the Doa10 complex, both Ubc6 and Ubc7 participate in substrate ubiquitination. Potentially, individual ubiquitin molecules might initially be ligated to the substrate in a sequential manner followed by rapid elongation of those early chains, for example, by *en bloc* polyubiquitin chain transfer. In such a two-step mechanism, one E2, possibly Ubc6,

could add a single ubiquitin or assemble a short chain on the substrate. Subsequently, the other E2, Ubc7, would extend the polyubiquitin chain by one of the mechanisms suggested above (Figure 2B). If each E2 makes a distinct type of ubiquitin chain (Ubc6 making K11-linked chains and Ubc7 K48-linked ones), it would yield a conjugate with a mixed chain linkage; this might enhance recognition by Cdc48 or its adaptors and thus stimulate ER extraction of the substrate, or it may promote proteasomal recognition.

Examples of two different E2s promoting chain initiation and elongation have been described. In mammals, the anaphase-promoting complex/cyclosome (APC/C) E3 can function with Ube2C/UbcH10 to initiate substrate ubiquitination preferentially through K11-linked chains (Jin, 2008; Williamson, 2011), while Ube2S subsequently extends with K11-linked ubiquitin chains (Williamson, 2009; Garnett, 2009; Wickliffe, 2011). Recent evidence suggests that Ube2S forms branched ubiquitin structures with multiple K11-linked chain segments, enhancing proteasomal recognition of the polyubiquitinated substrate (Meyer, 2014). The APC/C in budding yeast also uses a pair of E2s, Ubc4 and Ubc1, which preferentially catalyze mono-ubiquitination and chain extension, respectively. In this case, however, the chains are K48-linked (Rodrigo-Brenni, 2007). Overall, there are many unresolved questions about the mechanisms of ubiquitin chain formation by the yeast ERAD E2s and the potential interplay between these enzymes. It will be important to determine to what extent the nature of these chains influence ERAD retrotranslocation and proteasomal recognition.

ERAD Substrate Retrotranslocation: Search for a Path

Probably the most mysterious aspect of ERAD is how integral membrane and luminal protein substrates are moved across the ER bilayer for their destruction by the proteasome in the cytoplasm (Hampton, 2012). This relocalization appears to be an energy-dependent process, but most of the mechanistic details remain to be established. One consistent feature of retrotranslocation for both membrane and luminal substrate proteins is the requirement for the homohexameric Cdc48/p97 ATPase (Ye, 2001; Jarosch, 2002; Meyer, 2000; Hitchcock, 2001). $Cdc48^{Ufd1\text{-}Npl4}$ may help move the substrate across the membrane or dislodge it from the cytosolic face of the ER membrane (Figure 1). Proteasomal ATPases, which form a heterohexameric ring, have been implicated in the retrotranslocation process as well (Bar-Nun, 2012). A recent study suggests that Cdc48/p97 and the proteasome act sequentially on the mammalian integral membrane protein HMGR, with p97 driving the substrate out of the bilayer and the proteasome removing it from the ER surface (Morris, 2014).

ATP hydrolysis by Cdc48/p97 and binding of the polyubiquitinated substrates to Ufd1 are required for retrotranslocation, although p97 can interact with non-ubiquitinated substrates as well (Ye, 2003). Upon its recruitment to the ER by Ubx2 and possibly other co-factors, a $Cdc48^{Ufd1\text{-}Npl4}$ complex is thought to recognize the polyubiquitinated substrate exposed on the cytosolic face of the ER and drive its extraction (Neuber, 2005; Schuberth, 2005). Cdc48 has also been suggested to prevent aggregation and/or maintain solubility of misfolded nuclear proteins before they are degraded by the proteasome (Gallagher, 2014). Interestingly, in ERAD-L, Cdc48 also seems to regulate the initial recognition of luminal

substrates by Hrd1 through a mechanism that has not yet been determined (Carvalho, 2010). Inactivation of Cdc48 can generate a stalled retrotranslocation complex containing Hrd1, its membrane co-factors, the 26S proteasome, Yos9, ubiquitinated substrates, and Cdc48. This suggests that substrate recognition and retrotranslocation might be coupled, at least for some substrates (Nakatsukasa, 2013). Downstream of substrate extraction from the membrane, the Ufd2 U-box-containing enzyme can associate with the Cdc48/substrate complex and extend the substrate polyubiquitin chain, which is then recognized by the Rad23 and Dsk2 proteasomal substrate shuttle factors (Richly, 2005).

What has been most difficult to explain is the route of transfer of ERAD substrates across the ER membrane. The earliest models proposed the existence of a protein-based channel(s) at the ER membrane through which luminal proteins or integral membrane protein domains could pass (Figure 3A). The first such channel that had been hypothesized was the Sec61 translocon, whose aqueous channel was suggested to allow movement of protein chains in either direction (Plemper, 1997; Wiertz, 1996). Various mutations in the *S. cerevisiae* Sec61 protein were reported to cause defects in retrotranslocation and degradation of ERAD-L substrates, while their anterograde transport into the ER was unaffected (Plemper, 1997; Pilon, 1997; Willer, 2008). The Hrd1 ligase itself is unstable in cells lacking Hrd3; however, a *sec61* retrograde mutant can restore Hrd1 levels in this background (Plemper, 1999). This suggested that Sec61 could mediate retrotranslocation of membrane proteins as well, although this effect could be indirect.

It became clear fairly early on that certain ERAD substrates were not dependent on Sec61 for their retrotranslocation. Degradation of the yeast membrane substrates Ubc6 and Ste6* occurs normally in cells with a defective Sec61 translocon (Walter, 2001; Huyer, 2004). These are both substrates of the Doa10 pathway (Swanson, 2001). Interestingly, Hmg2-GFP, an ERAD-M substrate ubiquitinated by Hrd1, also does not require Sec61 for its removal from the ER (Garza, 2009). Elegant *in vitro* assays using yeast microsomes showed that complete retrotranslocation of intact Hmg2-GFP was Cdc48-dependent but continued in the absence of Sec61. Cdc48 is also required for retrotranslocation of Ste6* *in vitro* (Nakatsukasa, 2008b). Based on these studies, it seems likely that different substrates use distinct retrotranslocation mechanisms, potentially involving different export channels.

Another posited yeast retrotranslocation channel protein is Der1. Its mammalian ortholog, Derlin-1, was reported to associate with retrotranslocating MHC class I heavy chains in a manner that depended on the US11 human cytomegalovirus (HCMV) protein and Cdc48/p97 (Ye, 2004; Lilley, 2004). More recently, it has been suggested that Derlin-1 initially facilitates traversal of a luminal substrate across the ER membrane. In a second step, substrate extraction from the cytosolic face of the ER occurs through p97 recruitment to Derlin-1 (Greenblatt, 2011). Yeast Der1 is not believed to interact directly with Cdc48. However, site-specific crosslinking studies strongly suggest that the membrane portions of Der1 may interact with luminal substrates (Mehnert, 2014), consistent with a direct role in retrotranslocation. Ste6* degradation most likely does not depend on either yeast Derlin, Der1 or Dfm1; this would in turn exclude their involvement in Ste6* retrotranslocation (Huyer, 2004; Kreft, 2006). In short, the Derlins, like Sec61, might function as

retrotranslocation channels for specific substrates, although this remains to be directly demonstrated.

The Derlin proteins are inactive members of the rhomboid family of membrane proteases, which cleave transmembrane helices (TMs) (Lemberg, 2013; Bergbold, 2013). Interestingly, an active mammalian rhomboid intramembrane protease called RHBDL4 promotes the ERAD of membrane proteins in a manner requiring p97 (Fleig, 2012). Derlin-1 appears to share the 6-TM topology of rhomboid proteases and is predicted to be structurally similar to the *E. coli* GlpG protein, a structurally characterized rhomboid protease (Greenblatt, 2011; Wang, 2006). Yeast Der1 is thought to bear 4 TMs (Hitt, 2004), but computational modeling suggests that, as with Derlin-1, it might also have 6 TMs; this prediction has not yet been experimentally validated (Zattas, 2013). Rhomboid proteases, such as GlpG, are likely to cause deformations of the membrane bilayer in order to facilitate interaction with and hydrolysis of the intramembrane substrate cleavage site (Bondar, 2009). Derlins have been suggested to destabilize substrates through a combination of lipid bilayer thinning and TM helix unwinding, facilitating their extraction from the membrane by Cdc48/p97 (Greenblatt, 2012).

Another attractive candidate for a retrotranslocation channel is the RING domain E3 subunit of the ERAD ligase complexes. Hrd1 has 6 TMs, and Doa10 has 14. The large number of TMs in Doa10 and the high sequence conservation of a subset of them led to the original suggestion of a bifunctional ubiquitin ligase-protein exit channel in the same polypeptide (Swanson, 2001). At this point, however, more evidence for this hypothesis exists in the case of Hrd1. Crosslinking analyses *in vivo* indicated the presence of TMs in Der1, Hrd3 and Hrd1 that could all interact with a luminal (ERAD-L) substrate; however, overexpression of Hrd1 can bypass the requirement for all the integral membrane co-factors in the complex, including Hrd3 and Der1 (Plemper, 1999; Gardner, 1999; Carvalho, 2010). Carvalho et al. also showed that oligomerization of Hrd1 is required for ERAD-L. Such oligomerization is normally driven by Usa1 (Horn, 2009), but when overexpressed, Hrd1 can form oligomers in its absence (Carvalho, 2010). These data suggest a direct role for the Hrd1 transmembrane domain in ERAD substrate retrotranslocation.

However, there are data arguing against the exclusive action of a Hrd1-based retrotranslocon in the degradation of Hrd1 substrates. Hampton and colleagues created a chimera between a normally stable ER transmembrane protein, Hmg1, and the soluble C-terminal RING domain of Hrd1 (Garza, 2009). This protein autoubiquitinates itself and is rapidly degraded. Importantly, the chimera can retrotranslocate from the ER membrane in the complete absence of the Hrd1 transmembrane domain. Of course it remains possible that multiple retrotranslocons exist that function redundantly.

The seemingly contradictory data on retrograde ERAD substrate movement across the ER membrane might be reconciled if a distinct mechanism of protein extraction is utilized in at least some types of ERAD. Different integral membrane proteins in the ERAD ligase complexes might all be able to deform or destabilize the lipid bilayer, perhaps in the way proposed for rhomboid proteases. Membrane bending, thinning, or lipid phase separation could all in principle lower the energy barrier for a hydrophilic substrate (or substrate

domain) to move from the lumen through the hydrophobic membrane or for a hydrophobic TM helix to move from the bilayer into the cytoplasm (Figure 3B). This hypothesis could explain the *in vivo* luminal substrate crosslinks observed with various Hrd1 complex factors (Mehnert, 2014; Carvalho, 2010). As the luminal substrate moves across the membrane in a HRD complex-dependent manner, but not necessarily through a protein channel, substrate interactions can occur with many of the complex's co-factors. Similar membrane deformation mechanisms might operate in the Doa10 complex. Local membrane destabilization has been proposed as a mechanism for other membrane protein translocation systems, for example, the twin-arginine translocation (Tat) system in bacteria (Patel, 2014). Others have proposed ERAD membrane substrate movement from the ER through lipid droplet formation (Ploegh, 2007), although evidence against this idea has also been presented (Olzmann, 2011). Clearly, much remains to be learned in this area.

Conclusions and Future Perspectives

Since the early days of ERAD studies, we have obtained a significant level of understanding of many if not most of the components of the pathways responsible for ER substrate recognition, ubiquitination, retrotranslocation and delivery to the proteasome. However, there are still many outstanding questions regarding the mechanisms of each of the individual steps of ERAD. What constitutes the fundamental substrate degradation signals recognized by the different ERAD pathways? Can these degrons be predicted? How do the ERAD E3 ligases organize their co-factors, E2s, and potentially protein exit factors to orchestrate substrate recognition, ubiquitination, and retrotranslocation? How do the ERAD E2s assemble polyubiquitin chains, especially in cases where different E2s prefer to generate different chain linkages? How does ATP hydrolysis by Cdc48 (or the proteasome) contribute to substrate extraction from the ER to the cytoplasm? Must substrates be fully unfolded to pass back to the cytoplasm from the lumen of the ER? What are the exact mechanistic contributions of molecular chaperones and "holdases" in ERAD? And finally, what biophysical and biochemical mechanisms mediate substrate movement through the ER membrane bilayer? Obtaining satisfying answers to these questions will require extensive new cell biological, biochemical, and biophysical analyses and will no doubt be driven by new ideas and approaches. The next 5–10 years should be an exciting time for the ERAD field.

Acknowledgments

We would like to thank Christopher Hickey, Robert Tomko Jr. and Jason Berk for their useful comments on the manuscript.

This work was funded by National Institutes of Health Grant GM046904 to M.H. and, in part, National Institutes of Health Training Grant T32GM7223 to D.Z.

References

Adle DJ, Wei W, Smith N, Bies JJ, Lee J. Cadmium-mediated rescue from ER-associated degradation induces expression of its exporter. Proc Natl Acad Sci USA. 2009; 106 (25):10189–94. [PubMed: 19515821]

Alexandru G, Graumann J, Smith GT, Kolawa NJ, Fang R, Deshaies RJ. UBXD7 binds multiple ubiquitin ligases and implicates p97 in HIF1alpha turnover. Cell. 2008; 134 (5):804–16. [PubMed: 18775313]

Alfassy OS, Cohen I, Reiss Y, Tirosh B, Ravid T. Placing a disrupted degradation motif at the C terminus of proteasome substrates attenuates degradation without impairing ubiquitylation. J Biol Chem. 2013; 288 (18):12645–53. [PubMed: 23519465]

Bagola K, von Delbrück M, Dittmar G, Scheffner M, Ziv I, Glickman MH, Ciechanover A, Sommer T. Ubiquitin binding by a CUE domain regulates ubiquitin chain formation by ERAD E3 ligases. Mol Cell. 2013; 50 (4):528–39. [PubMed: 23665229]

Bar-Nun S, Glickman MH. Proteasomal AAA-ATPases: structure and function. Biochim Biophys Acta. 2012; 1823 (1):67–82. [PubMed: 21820014]

Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY. Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. Nat Cell Biol. 2001; 3 (1):24–9. [PubMed: 11146622]

Bazirgan OA, Hampton RY. Cue1p is an activator of Ubc7p E2 activity in vitro and in vivo. J Biol Chem. 2008; 283 (19):12797–810. [PubMed: 18321851]

Bergbold N, Lemberg MK. Emerging role of rhomboid family proteins in mammalian biology and disease. Biochim Biophys Acta. 2013; 1828 (12):2840–8. [PubMed: 23562403]

Bhamidipati A, Denic V, Quan EM, Weissman JS. Exploration of the topological requirements of ERAD identifies Yos9p as a lectin sensor of misfolded glycoproteins in the ER lumen. Mol Cell. 2005; 19 (6):741–51. [PubMed: 16168370]

Biederer T, Volkwein C, Sommer T. Degradation of subunits of the Sec61p complex, an integral component of the ER membrane, by the ubiquitin-proteasome pathway. EMBO J. 1996; 15 (9): 2069–76. [PubMed: 8641272]

Biederer T, Volkwein C, Sommer T. Role of Cue1p in ubiquitination and degradation at the ER surface. Science. 1997; 278 (5344):1806–9. [PubMed: 9388185]

Bondar AN, del Val C, White SH. Rhomboid protease dynamics and lipid interactions. Structure. 2009; 17 (3):395–405. [PubMed: 19278654]

Bonifacino JS, Lippincott-Schwartz J. Degradation of proteins within the endoplasmic reticulum. Curr Opin Cell Biol. 1991; 3 (4):592–600. [PubMed: 1772654]

Bordallo J, Plemper RK, Finger A, Wolf DH. Der3p/Hrd1p is required for endoplasmic reticulum-associated degradation of misfolded lumenal and integral membrane proteins. Mol Biol Cell. 1998; 9 (1):209–22. [PubMed: 9437001]

Botero D, Gereben B, Goncalves C, De Jesus LA, Harney JW, Bianco AC. Ubc6p and ubc7p are required for normal and substrate-induced endoplasmic reticulum-associated degradation of the human selenoprotein type 2 iodothyronine monodeiodinase. Mol Endocrinol. 2002; 16 (9):1999–2007. [PubMed: 12198238]

Carroll SM, Hampton RY. Usa1p is required for optimal function and regulation of the Hrd1p endoplasmic reticulum-associated degradation ubiquitin ligase. J Biol Chem. 2010; 285 (8):5146–56. [PubMed: 19940128]

Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. Cell. 2006; 126 (2):361–73. [PubMed: 16873066]

Carvalho P, Stanley AM, Rapoport TA. Retrotranslocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p. Cell. 2010; 143 (4):579–91. [PubMed: 21074049]

Chau V, Tobias JW, Bachmair A, Marriott D, Ecker DJ, Gonda DK, Varshavsky A. A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science. 1989; 243 (4898): 1576–83. [PubMed: 2538923]

Chen B, Mariano J, Tsai YC, Chan AH, Cohen M, Weissman AM. The activity of a human endoplasmic reticulum-associated degradation E3, gp78, requires its Cue domain, RING finger, and an E2-binding site. Proc Natl Acad Sci USA. 2006; 103 (2):341–6. [PubMed: 16407162]

Chen P, Johnson P, Sommer T, Jentsch S, Hochstrasser M. Multiple ubiquitin-conjugating enzymes participate in the in vivo degradation of the yeast MAT alpha 2 repressor. Cell. 1993; 74 (2):357–69. [PubMed: 8393731]

Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, Smith AE. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell. 1990; 63 (4):827–34. [PubMed: 1699669]

Christianson JC, Shaler TA, Tyler RE, Kopito RR. OS-9 and GRP94 deliver mutant alpha1-antitrypsin to the Hrd1-SEL1L ubiquitin ligase complex for ERAD. Nat Cell Biol. 2008; 10 (3):272–82. [PubMed: 18264092]

Clerc S, Hirsch C, Oggier DM, Deprez P, Jakob C, Sommer T, Aebi M. Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. J Cell Biol. 2009; 184 (1):159–72. [PubMed: 19124653]

Das R, Liang YH, Mariano J, Li J, Huang T, King A, Tarasov SG, Weissman AM, Ji X, Byrd RA. Allosteric regulation of E2:E3 interactions promote a processive ubiquitination machine. EMBO J. 2013; 32 (18):2504–16. [PubMed: 23942235]

Das R, Mariano J, Tsai YC, Kalathur RC, Kostova Z, Li J, Tarasov SG, McFeeters RL, Altieri AS, Ji X, Byrd RA, Weissman AM. Allosteric activation of E2-RING finger-mediated ubiquitylation by a structurally defined specific E2-binding region of gp78. Mol Cell. 2009; 34 (6):674–85. [PubMed: 19560420]

Deak PM, Wolf DH. Membrane topology and function of Der3/Hrd1p as a ubiquitin-protein ligase (E3) involved in endoplasmic reticulum degradation. J Biol Chem. 2001; 276 (14):10663–9. [PubMed: 11139575]

Deng M, Hochstrasser M. Spatially regulated ubiquitin ligation by an ER/nuclear membrane ligase. Nature. 2006; 443 (7113):827–31. [PubMed: 17051211]

Denic V, Quan EM, Weissman JS. A luminal surveillance complex that selects misfolded glycoproteins for ER-associated degradation. Cell. 2006; 126 (2):349–59. [PubMed: 16873065]

Doblas VG, Amorim-Silva V, Posé D, Rosado A, Esteban A, Arró M, Azevedo H, Bombarely A, Borsani O, Valpuesta V, Ferrer A, Tavares RM, Botella MA. The SUD1 gene encodes a putative E3 ubiquitin ligase and is a positive regulator of 3-hydroxy-3-methylglutaryl coenzyme a reductase activity in Arabidopsis. Plant Cell. 2013; 25 (2):728–43. [PubMed: 23404890]

El Khouri E, Le Pavec G, Toledano MB, Delaunay-Moisan A. RNF185 is a novel E3 ligase of endoplasmic reticulum-associated degradation (ERAD) that targets cystic fibrosis transmembrane conductance regulator (CFTR). J Biol Chem. 2013; 288 (43):31177–91. [PubMed: 24019521]

Faulkner RA, Nguyen AD, Jo Y, DeBose-Boyd RA. Lipid-regulated degradation of HMG-CoA reductase and Insig-1 through distinct mechanisms in insect cells. J Lipid Res. 2013; 54 (4):1011–22. [PubMed: 23403031]

Finger A, Knop M, Wolf DH. Analysis of two mutated vacuolar proteins reveals a degradation pathway in the endoplasmic reticulum or a related compartment of yeast. Eur J Biochem. 1993; 218 (2):565–74. [PubMed: 8269947]

Finley D, Ulrich HD, Sommer T, Kaiser P. The ubiquitin-proteasome system of Saccharomyces cerevisiae. Genetics. 2012; 192 (2):319–60. [PubMed: 23028185]

Fleig L, Bergbold AN, Sahasrabudhe P, Geiger B, Kaltak L, Lemberg MK. Ubiquitin-dependent intramembrane rhomboid protease promotes ERAD of membrane proteins. Mol Cell. 2012; 47 (4): 558–69. [PubMed: 22795130]

Foresti O, Ruggiano A, Hannibal-Bach HK, Ejsing CS, Carvalho P. Sterol homeostasis requires regulated degradation of squalene monooxygenase by the ubiquitin ligase Doa10/Teb4. Elife. 2013; 2:e00953. [PubMed: 23898401]

Friedlander R, Jarosch E, Urban J, Volkwein C, Sommer T. A regulatory link between ER-associated protein degradation and the unfolded-protein response. Nat Cell Biol. 2000; 2 (7):379–84. [PubMed: 10878801]

Furth N, Gertman O, Shiber A, Alfassy OS, Cohen I, Rosenberg MM, Doron NK, Friedler A, Ravid T. Exposure of bipartite hydrophobic signal triggers nuclear quality control of Ndc10 at the endoplasmic reticulum/nuclear envelope. Mol Biol Cell. 2011; 22 (24):4726–39. [PubMed: 21998200]

Gallagher PS, Clowes Candadai SV, Gardner RG. The requirement for Cdc48/p97 in nuclear protein quality control degradation depends on the substrate and correlates with substrate insolubility. J Cell Sci. 2014; 127:1980–91. [PubMed: 24569878]

Gardner RG, Hampton RY. A ‘distributed degron’ allows regulated entry into the ER degradation pathway. EMBO J. 1999; 18 (21):5994–6004. [PubMed: 10545111]

Gardner RG, Shearer AG, Hampton RY. In vivo action of the HRD ubiquitin ligase complex: mechanisms of endoplasmic reticulum quality control and sterol regulation. Mol Cell Biol. 2001; 21 (13):4267–91.

Gardner RG, Swarbrick GM, Bays NW, Cronin SR, Wilhovsky S, Seelig L, Kim C, Hampton RY. Endoplasmic reticulum degradation requires lumen to cytosol signaling. Transmembrane control of Hrd1p by Hrd3p. J Cell Biol. 2000; 151 (1):69–82. [PubMed: 11018054]

Garnett MJ, Mansfeld J, Godwin C, Matsusaka T, Wu J, Russell P, Pines J, Venkitaraman AR. UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. Nat Cell Biol. 2009; 11 (11):1363–9. [PubMed: 19820702]

Garza RM, Sato BK, Hampton RY. In vitro analysis of Hrd1p-mediated retrotranslocation of its multispanning membrane substrate 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. J Biol Chem. 2009; 284 (22):14710–22. [PubMed: 19324879]

Gauss R, Kanehara K, Carvalho P, Ng DT, Aebi M. A complex of Pdi1p and the mannosidase Htm1p initiates clearance of unfolded glycoproteins from the endoplasmic reticulum. Mol Cell. 2011; 42 (6):782–93. [PubMed: 21700223]

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O’Shea EK, Weissman JS. Global analysis of protein expression in yeast. Nature. 2003; 425 (6959):737–41. [PubMed: 14562106]

Gilon T, Chomsky O, Kulka RG. Degradation signals for ubiquitin system proteolysis in Saccharomyces cerevisiae. EMBO J. 1998; 17 (10):2759–66. [PubMed: 9582269]

Gilon T, Chomsky O, Kulka RG. Degradation signals recognized by the Ubc6p-Ubc7p ubiquitin-conjugating enzyme pair. Mol Cell Biol. 2000; 20 (19):7214–9. [PubMed: 10982838]

Glotzer M, Murray AW, Kirschner MW. Cyclin is degraded by the ubiquitin pathway. Nature. 1991; 349 (6305):132–8. [PubMed: 1846030]

Goder V, Carvalho P, Rapoport TA. The ER-associated degradation component Der1p and its homolog Dfm1p are contained in complexes with distinct cofactors of the ATPase Cdc48p. FEBS Lett. 2008; 582 (11):1575–80. [PubMed: 18407841]

Greenblatt EJ, Olzmann JA, Kopito RR. Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant a-1 antitrypsin from the endoplasmic reticulum. Nat Struct Mol Biol. 2011; 18 (10):1147–52. [PubMed: 21909096]

Greenblatt EJ, Olzmann JA, Kopito RR. Making the cut: intramembrane cleavage by a rhomboid protease promotes ERAD. Nat Struct Mol Biol. 2012; 19 (10):979–81. [PubMed: 23037595]

Hampton RY, Gardner 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. 1996; 7 (12):2029–44. [PubMed: 8970163]

Hampton RY, Rine J. Regulated degradation of HMG-CoA reductase, an integral membrane protein of the endoplasmic reticulum, in yeast. J Cell Biol. 1994; 125 (2):299–312. [PubMed: 8163547]

Hampton RY, Sommer T. Finding the will and the way of ERAD substrate retrotranslocation. Curr Opin Cell Biol. 2012; 24 (4):460–6. [PubMed: 22854296]

Hassink G, Kikkert M, van Voorden S, Lee SJ, Spaapen R, van Laar T, Coleman CS, Bartee E, Früh K, Chau V, Wiertz E. TEB4 is a C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum. Biochem J. 2005; 388:647–55. [PubMed: 15673284]

Haynes CM, Caldwell S, Cooper AA. An HRD/DER-independent ER quality control mechanism involves Rsp5p-dependent ubiquitination and ER-Golgi transport. J Cell Biol. 2002; 158 (1):91–101. [PubMed: 12105183]

Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. Annu Rev Biochem. 2004; 73:1019–49. [PubMed: 15189166]

Hicke L, Riezman H. Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. Cell. 1996; 84 (2):277–87. [PubMed: 8565073]

Hiller MM, Finger A, Schweiger M, Wolf DH. ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. Science. 1996; 273 (5282):1725–8. [PubMed: 8781238]

Hitchcock AL, Auld K, Gygi SP, Silver PA. A subset of membrane-associated proteins is ubiquitinated in response to mutations in the endoplasmic reticulum degradation machinery. Proc Natl Acad Sci USA. 2003; 100 (22):12735–40. [PubMed: 14557538]

Hitchcock AL, Krebber H, Frietze S, Lin A, Latterich M, Silver PA. The conserved npl4 protein complex mediates proteasome-dependent membrane-bound transcription factor activation. Mol Biol Cell. 2001; 12 (10):3226–41. [PubMed: 11598205]

Hitt R, Wolf DH. Der1p, a protein required for degradation of malfolded soluble proteins of the endoplasmic reticulum: topology and Der1-like proteins. FEMS Yeast Res. 2004; 4 (7):721–9. [PubMed: 15093775]

Hochstrasser M. Ubiquitin-dependent protein degradation. Annual Review of Genetics. 1996; 30:405–39.

Hochstrasser M. Lingering mysteries of ubiquitin-chain assembly. Cell. 2006; 124 (1):27–34. [PubMed: 16413479]

Hochstrasser M. Origin and function of ubiquitin-like proteins. Nature. 2009; 458 (7237):422–9. [PubMed: 19325621]

Hochstrasser M, Ellison MJ, Chau V, Varshavsky A. The short-lived MAT alpha 2 transcriptional regulator is ubiquitinated in vivo. Proc Natl Acad Sci USA. 1991; 88 (11):4606–10. [PubMed: 1647011]

Hochstrasser M, Varshavsky A. In vivo degradation of a transcriptional regulator: the yeast alpha 2 repressor. Cell. 1990; 61 (4):697–708. [PubMed: 2111732]

Horn SC, Hanna J, Hirsch C, Volkwein C, Schütz A, Heinemann U, Sommer T, Jarosch E. Usa1 functions as a scaffold of the HRD-ubiquitin ligase. Mol Cell. 2009; 36 (5):782–93. [PubMed: 20005842]

Hosokawa N, Kamiya Y, Kamiya D, Kato K, Nagata K. Human OS-9, a lectin required for glycoprotein endoplasmic reticulum-associated degradation, recognizes mannose-trimmed N-glycans. J Biol Chem. 2009; 284 (25):17061–8. [PubMed: 19346256]

Huyer G, Piluek WF, Fansler Z, Kreft SG, Hochstrasser M, Brodsky JL, Michaelis S. Distinct machinery is required in Saccharomyces cerevisiae for the endoplasmic reticulum-associated degradation of a multispanning membrane protein and a soluble luminal protein. J Biol Chem. 2004; 279 (37):38369–78. [PubMed: 15252059]

Izawa T, Nagai H, Endo T, Nishikawa S. Yos9p and Hrd1p mediate ER retention of misfolded proteins for ER-associated degradation. Mol Biol Cell. 2012; 23 (7):1283–93. [PubMed: 22298424]

Jakob CA, Bodmer D, Spirig U, Battig P, Marcil A, Dignard D, Bergeron JJ, Thomas DY, Aebi M. Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep. 2001; 2 (5):423–30. [PubMed: 11375935]

Jarosch E, Taxis C, Volkwein C, Bordallo J, Finley D, Wolf DH, Sommer T. Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. Nat Cell Biol. 2002; 4 (2):134–9. [PubMed: 11813000]

Jensen TJ, Loo MA, Pind S, Williams DB, Goldberg AL, Riordan JR. Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. Cell. 1995; 83 (1):129–35. [PubMed: 7553864]

Jin L, Williamson A, Banerjee S, Philipp I, Rape M. Mechanism of ubiquitin-chain formation by the human anaphase-promoting complex. Cell. 2008; 133 (4):653–65. [PubMed: 18485873]

Jo Y, Lee PC, Sguigna PV, DeBose-Boyd RA. Sterol-induced degradation of HMG CoA reductase depends on interplay of two Insigs and two ubiquitin ligases, gp78 and Trc8. Proc Natl Acad Sci USA. 2011; 108 (51):20503–8. [PubMed: 22143767]

Johnson PR, Swanson R, Rakhilina L, Hochstrasser M. Degradation signal masking by heterodimerization of MATalpha2 and MATa1 blocks their mutual destruction by the ubiquitin-proteasome pathway. Cell. 1998; 94 (2):217–27. [PubMed: 9695950]

Kim I, Miller CR, Young DL, Fields S. High-throughput analysis of in vivo protein stability. Mol Cell Proteomics. 2013; 12 (11):3370–8. [PubMed: 23897579]

Kim W, Spear ED, Ng DT. Yos9p detects and targets misfolded glycoproteins for ER- associated degradation. Mol Cell. 2005; 19 (6):753–64. [PubMed: 16168371]

Knop M, Finger A, Braun T, Hellmuth K, Wolf DH. Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast. EMBO J. 1996a; 15 (4):753–63. [PubMed: 8631297]

Knop M, Hauser N, Wolf DH. N-Glycosylation affects endoplasmic reticulum degradation of a mutated derivative of carboxypeptidase yscY in yeast. Yeast. 1996b; 12 (12):1229–38. [PubMed: 8905927]

Kostova Z, Mariano J, Scholz S, Koenig C, Weissman AM. A Ubc7p-binding domain in Cue1p activates ER-associated protein degradation. J Cell Sci. 2009; 122:1374–81. [PubMed: 19366730]

Kostova Z, Tsai YC, Weissman AM. Ubiquitin ligases, critical mediators of endoplasmic reticulum-associated degradation. Semin Cell Dev Biol. 2007; 18 (6):770–9. [PubMed: 17950636]

Kostova Z, Wolf DH. Importance of carbohydrate positioning in the recognition of mutated CPY for ER-associated degradation. J Cell Sci. 2005; 118:1485–92. [PubMed: 15769847]

Kravtsova-Ivantsiv Y, Sommer T, Ciechanover A. The lysine48-based polyubiquitin chain proteasomal signal: not a single child anymore. Angew Chem Int Ed Engl. 2013; 52(1):192–8. [PubMed: 23124625]

Kreft SG, Hochstrasser M. An unusual transmembrane helix in the endoplasmic reticulum ubiquitin ligase Doa10 modulates degradation of its cognate E2 enzyme. J Biol Chem. 2011; 286 (23): 20163–74. [PubMed: 21467040]

Kreft SG, Wang L, Hochstrasser M. Membrane topology of the yeast endoplasmic reticulum-localized ubiquitin ligase Doa10 and comparison with its human ortholog TEB4 (MARCH-VI). J Biol Chem. 2006; 281 (8):4646–53. [PubMed: 16373356]

Laney JD, Hochstrasser M. Ubiquitin-dependent degradation of the yeast Mat(alpha)2 repressor enables a switch in developmental state. Genes Dev. 2003; 17 (18):2259–70. [PubMed: 12952895]

Le A, Ferrell GA, Dishon DS, Le QQ, Sifers RN. Soluble aggregates of the human PiZ alpha 1-antitrypsin variant are degraded within the endoplasmic reticulum by a mechanism sensitive to inhibitors of protein synthesis. J Biol Chem. 1992; 267 (2):1072–80. [PubMed: 1530934]

Lemberg MK. Sampling the membrane: function of rhomboid-family proteins. Trends Cell Biol. 2013; 23 (5):210–7. [PubMed: 23369641]

Lenk U, Yu H, Walter J, Gelman MS, Hartmann E, Kopito RR, Sommer T. A role for mammalian Ubc6 homologues in ER-associated protein degradation. J Cell Sci. 2002; 115:3007–14. [PubMed: 12082160]

Li W, Tu D, Brunger AT, Ye Y. A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. Nature. 2007; 446 (7133):333–7. [PubMed: 17310145]

Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. Nature. 2004; 429 (6994):834–40. [PubMed: 15215855]

Lippincott-Schwartz J, Bonifacino JS, Yuan LC, Klausner RD. Degradation from the endoplasmic reticulum: disposing of newly synthesized proteins. Cell. 1988; 54 (2):209–20. [PubMed: 3292055]

Liu W, Shang Y, Zeng Y, Liu C, Li Y, Zhai L, Wang P, Lou J, Xu P, Ye Y, Li W. Dimeric Ube2g2 simultaneously engages donor and acceptor ubiquitins to form Lys48-linked ubiquitin chains. EMBO J. 2014; 33 (1):46–61. [PubMed: 24366945]

Loayza D, Tam A, Schmidt WK, Michaelis S. Ste6p mutants defective in exit from the endoplasmic reticulum (ER) reveal aspects of an ER quality control pathway in Saccharomyces cerevisiae. Mol Biol Cell. 1998; 9 (10):2767–84. [PubMed: 9763443]

Lu YH, Arnaud D, Belcram H, Falentin C, Rouault P, Piel N, Lucas MO, Just J, Renard M, Delourme R, Chalhoub B. A dominant point mutation in a RINGv E3 ubiquitin ligase homoeologous gene leads to cleistogamy in Brassica napus. Plant Cell. 2012; 24 (12):4875–91. [PubMed: 23277363]

Lü S, Zhao H, Des Marais DL, Parsons EP, Wen X, Xu X, Bangarusamy DK, Wang G, Rowland O, Juenger T, Bressan RA, Jenks MA. Arabidopsis ECERIFERUM9 involvement in cuticle formation and maintenance of plant water status. Plant Physiol. 2012; 159 (3):930–44. [PubMed: 22635115]

McCracken AA, Brodsky JL. Assembly of ER-associated protein degradation in vitro: dependence on cytosol, calnexin, and ATP. J Cell Biol. 1996; 132 (3):291–8. [PubMed: 8636208]

Mehnert M, Sommer T, Jarosch E. Der1 promotes movement of misfolded proteins through the endoplasmic reticulum membrane. Nat Cell Biol. 2014; 16 (1):77–86. [PubMed: 24292014]

Metzger MB, Liang YH, Das R, Mariano J, Li S, Li J, Kostova Z, Byrd RA, Ji X, Weissman AM. A structurally unique E2-binding domain activates ubiquitination by the ERAD E2, Ubc7p, through multiple mechanisms. Mol Cell. 2013; 50 (4):516–27. [PubMed: 23665230]

Metzger MB, Maurer MJ, Dancy BM, Michaelis S. Degradation of a cytosolic protein requires endoplasmic reticulum-associated degradation machinery. J Biol Chem. 2008; 283 (47):32302–16. [PubMed: 18812321]

Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination. Biochimica et Biophysica Acta. 2014; 1843 (1):47–60. [PubMed: 23747565]

Meyer HH, Shorter JG, Seemann J, Pappin D, Warren G. A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. EMBO J. 2000; 19 (10):2181–92. [PubMed: 10811609]

Meyer HJ, Rape M. Enhanced protein degradation by branched ubiquitin chains. Cell. 2014; 157 (4): 910–21. [PubMed: 24813613]

Morris LL, Hartman IZ, Jun DJ, Seemann J, DeBose-Boyd RA. Sequential Actions of the AAA-ATPase Valosin-containing Protein (VCP)/p97 and the Proteasome 19 S Regulatory Particle in Sterol-accelerated, Endoplasmic Reticulum (ER)-associated Degradation of 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase. J Biol Chem. 2014; 289 (27):19053–19066. [PubMed: 24860107]

Mueller B, Lilley BN, Ploegh HL. SEL1L, the homologue of yeast Hrd3p, is involved in protein dislocation from the mammalian ER. J Cell Biol. 2006; 175 (2):261–70. [PubMed: 17043138]

Nakatsukasa K, Brodsky JL. The recognition and retrotranslocation of misfolded proteins from the endoplasmic reticulum. Traffic. 2008a; 9 (6):861–70. [PubMed: 18315532]

Nakatsukasa K, Brodsky JL, Kamura T. A stalled retrotranslocation complex reveals physical linkage between substrate recognition and proteasomal degradation during ER-associated degradation. Mol Biol Cell. 2013; 24 (11):1765–75. [PubMed: 23536702]

Nakatsukasa K, Huyer G, Michaelis S, Brodksy JL. Dissecting the ER-associated degradation of a misfolded polytopic membrane protein. Cell. 2008b; 132 (1):101–12. [PubMed: 18191224]

Neuber O, Jarosch E, Volkwein C, Walter J, Sommer T. Ubx2 links the Cdc48 complex to ER-associated protein degradation. Nat Cell Biol. 2005; 7 (10):993–8. [PubMed: 16179953]

Nishikawa SI, Fewell SW, Kato Y, Brodsky JL, Endo T. Molecular chaperones in the yeast endoplasmic reticulum maintain the solubility of proteins for retrotranslocation and degradation. J Cell Biol. 2001; 153 (5):1061–70. [PubMed: 11381090]

Oda Y, Okada T, Yoshida H, Kaufman RJ, Nagata K, Mori K. Derlin-2 and Derlin-3 are regulated by the mammalian unfolded protein response and are required for ER-associated degradation. J Cell Biol. 2006; 172 (3):383–93. [PubMed: 16449189]

Okuda-Shimizu Y, Hendershot LM. Characterization of an ERAD pathway for nonglycosylated BiP substrates, which require Herp. Mol Cell. 2007; 28 (4):544–54. [PubMed: 18042451]

Olzmann JA, Kopito RR. Lipid droplet formation is dispensable for endoplasmic reticulum-associated degradation. J Biol Chem. 2011; 286 (32):27872–4. [PubMed: 21693705]

Olzmann JA, Kopito RR, Christianson JC. The mammalian endoplasmic reticulum-associated degradation system. Cold Spring Harb Perspect Biol. 2013; 5 (9):a013185. [PubMed: 23232094]

Patel R, Smith SM, Robinson C. Protein transport by the bacterial Tat pathway. Biochim Biophys Acta. 2014; 1843 (8):1620–1628. [PubMed: 24583120]

Pickart CM. Ubiquitin in chains. Trends Biochem Sci. 2000; 25 (11):544–8. [PubMed: 11084366]

Pickart CM. Mechanisms underlying ubiquitination. Annu Rev Biochem. 2001; 70:503–33. [PubMed: 11395416]

Pickart CM, Eddins MJ. Ubiquitin: structures, functions, mechanisms. Biochim Biophys Acta. 2004; 1659 (1–3):55–72. [PubMed: 15571809]

Pilon M, Schekman R, Römisch K. Sec61p mediates export of a misfolded secretory protein from the endoplasmic reticulum to the cytosol for degradation. EMBO J. 1997; 16 (15):4540–8. [PubMed: 9303298]

Plemper RK, Böhmler S, Bordallo J, Sommer T, Wolf DH. Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. Nature. 1997; 388 (6645):891–5. [PubMed: 9278052]

Plemper RK, Bordallo J, Deak PM, Taxis C, Hitt R, Wolf DH. Genetic interactions of Hrd3p and Der3p/Hrd1p with Sec61p suggest a retro-translocation complex mediating protein transport for ER degradation. J Cell Sci. 1999; 112:4123–34. [PubMed: 10547371]

Plemper RK, Egner R, Kuchler K, Wolf DH. Endoplasmic reticulum degradation of a mutated ATP-binding cassette transporter Pdr5 proceeds in a concerted action of Sec61 and the proteasome. J Biol Chem. 1998; 273 (49):32848–56. [PubMed: 9830032]

Ploegh HL. A lipid-based model for the creation of an escape hatch from theendoplasmic reticulum. Nature. 2007; 448 (7152):435–8. [PubMed: 17653186]

Porter KR, Claude A, Fullam EF. A study of tissue culture cells by electron microscopy: methods and preliminary observations. J Exp Med. 1945; 81 (3):233–46. [PubMed: 19871454]

Quan EM, Kamiya Y, Kamiya D, Denic V, Weibezahn J, Kato K, Weissman JS. Defining the glycan destruction signal for endoplasmic reticulum-associated degradation. Mol Cell. 2008; 32 (6):870–7. [PubMed: 19111666]

Rabinovich E, Kerem A, Fröhlich KU, Diamant N, Bar-Nun S. AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. Mol Cell Biol. 2002; 22 (2):626–34. [PubMed: 11756557]

Ravid T, Hochstrasser M. Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. Nat Cell Biol. 2007; 9 (4):422–7. [PubMed: 17310239]

Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin-proteasome system. Nat Rev Mol Cell Biol. 2008; 9 (9):679–90. [PubMed: 18698327]

Ravid T, Kreft SG, Hochstrasser M. Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. EMBO J. 2006; 25 (3):533–43. [PubMed: 16437165]

Rapoport TA. Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. Nature. 2007; 450 (7170):663–9. [PubMed: 18046402]

Redman CM, Siekevitz P, Palade GE. Synthesis and transfer of amylase in pigeon pancreatic microsomes. J Biol Chem. 1966; 241 (5):1150–8. [PubMed: 5933873]

Richly H, Rape M, Braun S, Rumpf S, Hoege C, Jentsch S. A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting. Cell. 2005; 120 (1):73–84. [PubMed: 15652483]

Rodrigo-Brenni MC, Morgan DO. Sequential E2s drive polyubiquitin chain assembly on APC targets. Cell. 2007; 130 (1):127–39. [PubMed: 17632060]

Rubenstein EM, Kreft SG, Greenblatt W, Swanson R, Hochstrasser M. Aberrant substrate engagement of the ER translocon triggers degradation by the Hrd1 ubiquitin ligase. J Cell Biol. 2012; 197 (6): 761–73. [PubMed: 22689655]

Sato BK, Hampton RY. Yeast Derlin Dfm1 interacts with Cdc48 and functions in ER homeostasis. Yeast. 2006; 23 (14–15):1053–64. [PubMed: 17083136]

Sato BK, Schulz D, Do PH, Hampton RY. Misfolded membrane proteins are specifically recognized by the transmembrane domain of the Hrd1p ubiquitin ligase. Mol Cell. 2009; 34 (2):212–22. [PubMed: 19394298]

Scheffner M, Nuber U, Huibregtse JM. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature. 1995; 373:81–83. [PubMed: 7800044]

Schuberth C, Buchberger A. Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. Nat Cell Biol. 2005; 7 (10):999–1006. [PubMed: 16179952]

Schuberth C, Richly H, Rumpf S, Buchberger A. Shp1 and Ubx2 are adaptors of Cdc48 involved in ubiquitin-dependent protein degradation. EMBO Rep. 2004; 5 (8):818–24. [PubMed: 15258615]

Schulman BA. Twists and turns in ubiquitin-like protein conjugation cascades. Protein Sci. 2011; 20 (12):1941–54. [PubMed: 22012881]

Schwartz AL, Ciechanover A. Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. Annu Rev Pharmacol Toxicol. 2009; 49:73–96. [PubMed: 18834306]

Sever N, Yang T, Brown MS, Goldstein JL, DeBose-Boyd RA. Accelerated degradation of HMG CoA reductase mediated by binding of insig-1 to its sterol-sensing domain. Mol Cell. 2003; 11 (1):25–33. [PubMed: 12535518]

Shiber A, Breuer W, Brandeis M, Ravid T. Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. Mol Biol Cell. 2013; 24 (13):2076–87. [PubMed: 23637465]

Sommer T, Jentsch S. A protein translocation defect linked to ubiquitin conjugation at the endoplasmic reticulum. Nature. 1993; 365 (6442):176–9. [PubMed: 8396728]

Song BL, Sever N, DeBose-Boyd RA. Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. Mol Cell. 2005; 19 (6):829–40. [PubMed: 16168377]

Steinsapir J, Bianco AC, Buettner C, Harney J, Larsen PR. Substrate-induced down-regulation of human type 2 deiodinase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. Endocrinology. 2000; 141 (3):1127–35. [PubMed: 10698189]

Stolz A, Besser S, Hottmann H, Wolf DH. Previously unknown role for the ubiquitin ligase Ubr1 in endoplasmic reticulum-associated protein degradation. Proc Natl Acad Sci USA. 2013; 110 (38): 15271–6. [PubMed: 23988329]

Stolz A, Schweizer RS, Schäfer A, Wolf DH. Dfm1 forms distinct complexes with Cdc48 and the ER ubiquitin ligases and is required for ERAD. Traffic. 2010; 11 (10):1363–9. [PubMed: 20579315]

Stuerner E, Kuraku S, Hochstrasser M, Kreft SG. Split-Doa10: a naturally split polytopic eukaryotic membrane protein generated by fission of a nuclear gene. PLoS One. 2012; 7 (10):e45194. [PubMed: 23071509]

Swanson R, Locher M, Hochstrasser M. A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha2 repressor degradation. Genes Dev. 2001; 15 (20):2660–74. [PubMed: 11641273]

Szathmary R, Bielmann R, Nita-Lazar M, Burda P, Jakob CA. Yos9 protein is essential for degradation of misfolded glycoproteins and may function as lectin in ERAD. Mol Cell. 2005; 19 (6):765–75. [PubMed: 16168372]

Taxis C, Hitt R, Park SH, Deak PM, Kostova Z, Wolf DH. Use of modular substrates demonstrates mechanistic diversity and reveals differences in chaperone requirement of ERAD. J Biol Chem. 2003; 278 (38):35903–13. [PubMed: 12847107]

Theesfeld CL, Hampton RY. Insulin-induced gene protein (INSIG)-dependent sterol regulation of Hmg2 endoplasmic reticulum-associated degradation (ERAD) in yeast. J Biol Chem. 2013; 288 (12):8519–30. [PubMed: 23306196]

Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. Recognition of the polyubiquitin proteolytic signal. EMBO J. 2000; 19 (1):94–102. [PubMed: 10619848]

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell. 2000; 101 (3):249–58. [PubMed: 10847680]

Tsai YC, Leichner GS, Pearce MM, Wilson GL, Wojcikiewicz RJ, Roitelman J, Weissman AM. Differential regulation of HMG-CoA reductase and Insig-1 by enzymes of the ubiquitin-proteasome system. Mol Biol Cell. 2012; 23 (23):4484–94. [PubMed: 23087214]

van de Weijer ML, Bassik MC, Luteijn RD, Voorburg CM, Lohuis MA, Kremmer E, Hoeben RC, LeProust EM, Chen S, Hoelen H, Ressing ME, Patena W, Weissman JS, McManus MT, Wiertz EJ, Lebbink RJ. A high-coverage shRNA screen identifies TMEM129 as an E3 ligase involved in ER-associated protein degradation. Nat Commun. 2014; 5:3832. [PubMed: 24807418]

van den Boomen DJ, Timms RT, Grice GL, Stagg HR, Skødt K, Dougan G, Nathan JA, Lehner PJ. TMEM129 is a Derlin-1 associated ERAD E3 ligase essential for virus-induced degradation of MHC-I. Proc Natl Acad Sci USA. 2014; 111:11425–30. [PubMed: 25030448]

Varshavsky A. The ubiquitin system, an immense realm. Annu Rev Biochem. 2012; 81:167–76. [PubMed: 22663079]

Vashist S, Kim W, Belden WJ, Spear ED, Barlowe C, Ng DT. Distinct retrieval and retention mechanisms are required for the quality control of endoplasmic reticulum protein folding. J Cell Biol. 2001; 155 (3):355–68. [PubMed: 11673477]

Vashist S, Ng DT. Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. J Cell Biol. 2004; 165 (1):41–52. [PubMed: 15078901]

Walter J, Urban J, Volkwein C, Sommer T. Sec61p-independent degradation of the tail- anchored ER membrane protein Ubc6p. EMBO J. 2001; 20 (12):3124–31. [PubMed: 11406589]

Wang Y, Zhang Y, Ha Y. Crystal structure of a rhomboid family intramembrane protease. Nature. 2006; 444 (7116):179–80. [PubMed: 17051161]

Ward CL, Omura S, Kopito RR. Degradation of CFTR by the ubiquitin-proteasome pathway. Cell. 1995; 83 (1):121–7. [PubMed: 7553863]

Weissman AM. Themes and variations on ubiquitylation. Nat Rev Mol Cell Biol. 2001; 2 (3):169–78. [PubMed: 11265246]

Wenzel DM, Lissounov A, Brzovic PS, Klevit RE. UBCH7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. Nature. 2011; 474 (7349):105–8. [PubMed: 21532592]

Wenzel DM, Stoll KE, Klevit RE. E2s: structurally economical and functionally replete. Biochem J. 2011; 433 (1):31–42. [PubMed: 21158740]

Wickliffe KE, Lorenz S, Wemmer DE, Kuriyan J, Rape M. The mechanism of linkage-specific ubiquitin chain elongation by a single-subunit E2. Cell. 2011; 144 (5):769–81. [PubMed: 21376237]

Wiertz EJ, Jones TR, Sun L, Bogyo M, Geuze HJ, Ploegh HL. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell. 1996; 84 (5):769–79. [PubMed: 8625414]

Wileman T, Kane LP, Carson GR, Terhorst C. Depletion of cellular calcium accelerates protein degradation in the endoplasmic reticulum. J Biol Chem. 1991; 266 (7):4500–7. [PubMed: 1825655]

Willer M, Forte GM, Stirling CJ. Sec61p is required for ERAD-L: genetic dissection of the translocation and ERAD-L functions of Sec61P using novel derivatives of CPY. J Biol Chem. 2008; 283 (49):33883–8. [PubMed: 18819915]

Williamson A, Banerjee S, Zhu X, Philipp I, Iavarone AT, Rape M. Regulation of ubiquitin chain initiation to control the timing of substrate degradation. Mol Cell. 2011; 42 (6):744–57. [PubMed: 21700221]

Williamson A, Wickliffe KE, Mellone BG, Song L, Karpen GH, Rape M. Identification of a physiological E2 module for the human anaphase-promoting complex. Proc Natl Acad Sci USA. 2009; 106 (43):18213–8. [PubMed: 19822757]

Xu C, Wang S, Thibault G, Ng DT. Futile protein folding cycles in the ER are terminated by the unfolded protein O-mannosylation pathway. Science. 2013; 340 (6135):978–81. [PubMed: 23704572]

Xu P, Duong DM, Seyfried NT, Cheng D, Xie Y, Robert J, Rush J, Hochstrasser M, Finley D, Peng J. Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. Cell. 2009; 137 (1):133–45. [PubMed: 19345192]

Yamasaki S, Yagishita N, Nishioka K, Nakajima T. The roles of synoviolin in crosstalk between endoplasmic reticulum stress-induced apoptosis and p53 pathway. Cell Cycle. 2007; 6 (11): 1319–23. [PubMed: 17582219]

Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature. 2001; 414 (6864):652–6. [PubMed: 11740563]

Ye Y, Meyer HH, Rapoport TA. Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. J Cell Biol. 2003; 162 (1):71–84. [PubMed: 12847084]

Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature. 2004; 429 (6994):841–7. [PubMed: 15215856]

Zattas D, Adle DJ, Rubenstein EM, Hochstrasser M. N-terminal acetylation of the yeast Derlin Der1 is essential for Hrd1 ubiquitin-ligase activity toward luminal ER substrates. Mol Biol Cell. 2013; 24 (7):890–900. [PubMed: 23363603]

Zavacki AM, Arrojo E, Drigo R, Freitas BC, Chung M, Harney JW, Egri P, Wittmann G, Fekete C, Gereben B, Bianco AC. The E3 ubiquitin ligase TEB4 mediates degradation of type 2 iodothyronine deiodinase. Mol Cell Biol. 2009; 29(19):5339–47. [PubMed: 19651899]

Zelcer N, Sharpe LJ, Loregger A, Kristiana I, Cook EC, Phan L, Stevenson J, Brown AJ. The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and the cholesterol synthesis pathway. Mol Cell Biol. 2014; 34 (7):1262–70. [PubMed: 24449766]

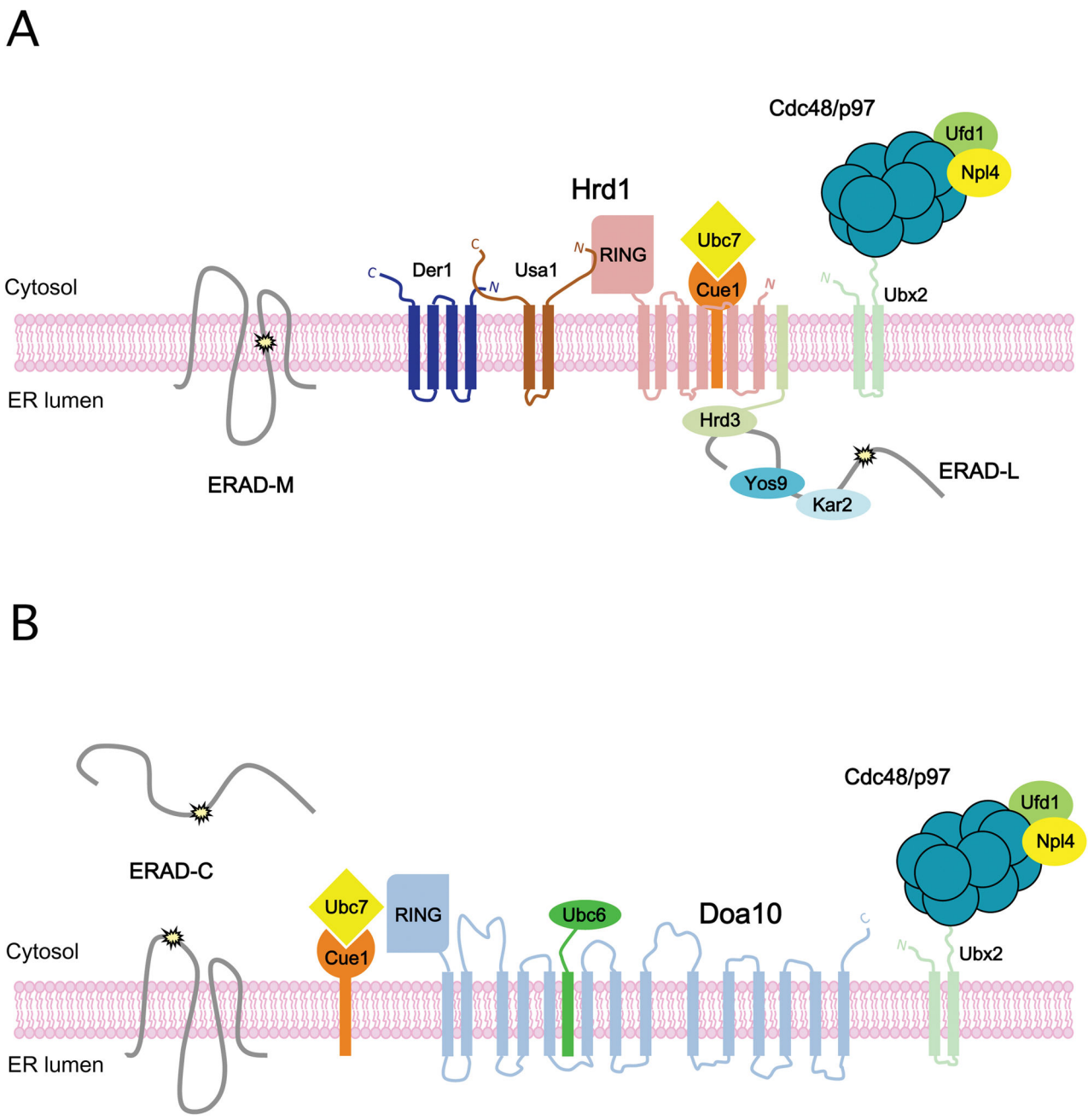

Figure 1.

Two principal ubiquitin ligase complexes in *S. cerevisiae* function in ERAD. A) The HRD1 complex. The Hrd1 E3 ligase functions mainly with the Ubc7 E2, which is recruited to the ER and activated by Cue1. Hrd3 is also a stoichiometric component of the complex, and its luminal domain mediates substrate recognition. A misfolded luminal substrate (ERAD-L) is initially recognized by the Yos9 lectin, the Kar2 Hsp70 protein and/or Hrd3. Ubiquitination and retrotranslocation of ERAD-L substrates -but not misfolded membrane substrates (ERAD-M)- require the presence of Der1. Der1 is brought to the HRD complex via Usa1. Usa1 is also required for oligomerization of the HRD complex and ERAD. The Cdc48

complex is recruited to the ER via the Ubx2 adaptor protein. Ubiquitinated substrates are extracted from the ER though the action of Cdc48 and its co-factors Ufd1 and Npl4. B) The DOA10 complex. Doa10 has 14 transmembrane segments. This ligase primarily targets for ubiquitination misfolded soluble cytosolic proteins or membrane proteins with misfolded/damaged domains in the cytoplasmic regions. These are called ERAD-C substrates. Ubiquitination of most ERAD-C substrates requires two E2s, Ubc6 and Ubc7. Ubc6 is C-terminally anchored to the ER and itself is a substrate of Doa10. The Cdc48 complex is required for degradation of membrane ERAD-C substrates but not soluble ones.

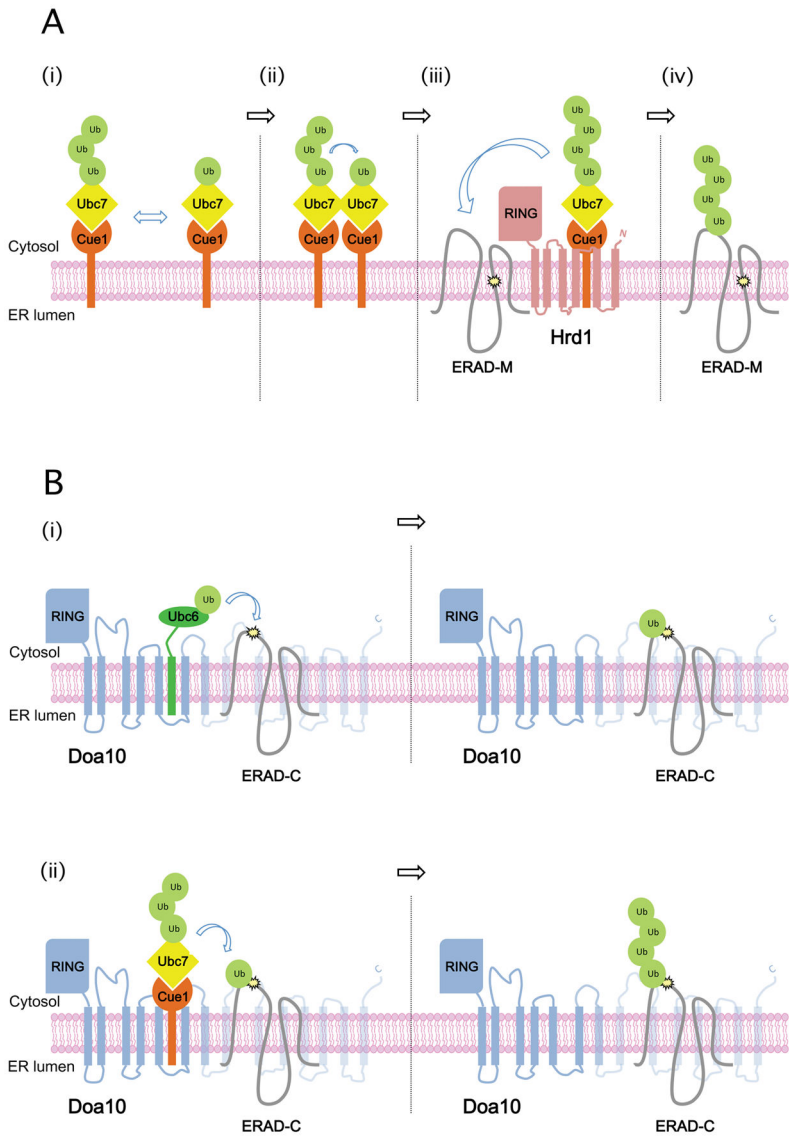

Figure 2.

Hypothetical mechanisms of ubiquitin transfer by the yeast ERAD E2s. A) (i) Ubc7 can form polyubiquitin chains on its catalytic cysteine residue. This might require transfer of ubiquitin from a second copy of Ubc7. (ii) Based on studies of its mammalian ortholog, Ube2g2, Ubc7 might also dimerize in order to form polyubiquitin chains. (iii, iv) Transfer of ubiquitin onto the substrate can be performed sequentially or *en bloc* as depicted here. Cue1 is necessary for Ubc7 activity. B) In the case of Doa10, two E2s are required for proper substrate ubiquitination. A possible explanation for why both Ubc6 and Ubc7 are required for ubiquitination is that they might work in a stepwise manner. Ubc6 prefers to make K11-linked chains. (i) It is likely that in the first step, Ubc6 acts as the “priming” E2 where it can monoubiquitinate (shown here) or form short chains on the substrate. (ii) In the second step, Ubc7 can extend those chains to a sufficient length and promote substrate degradation.

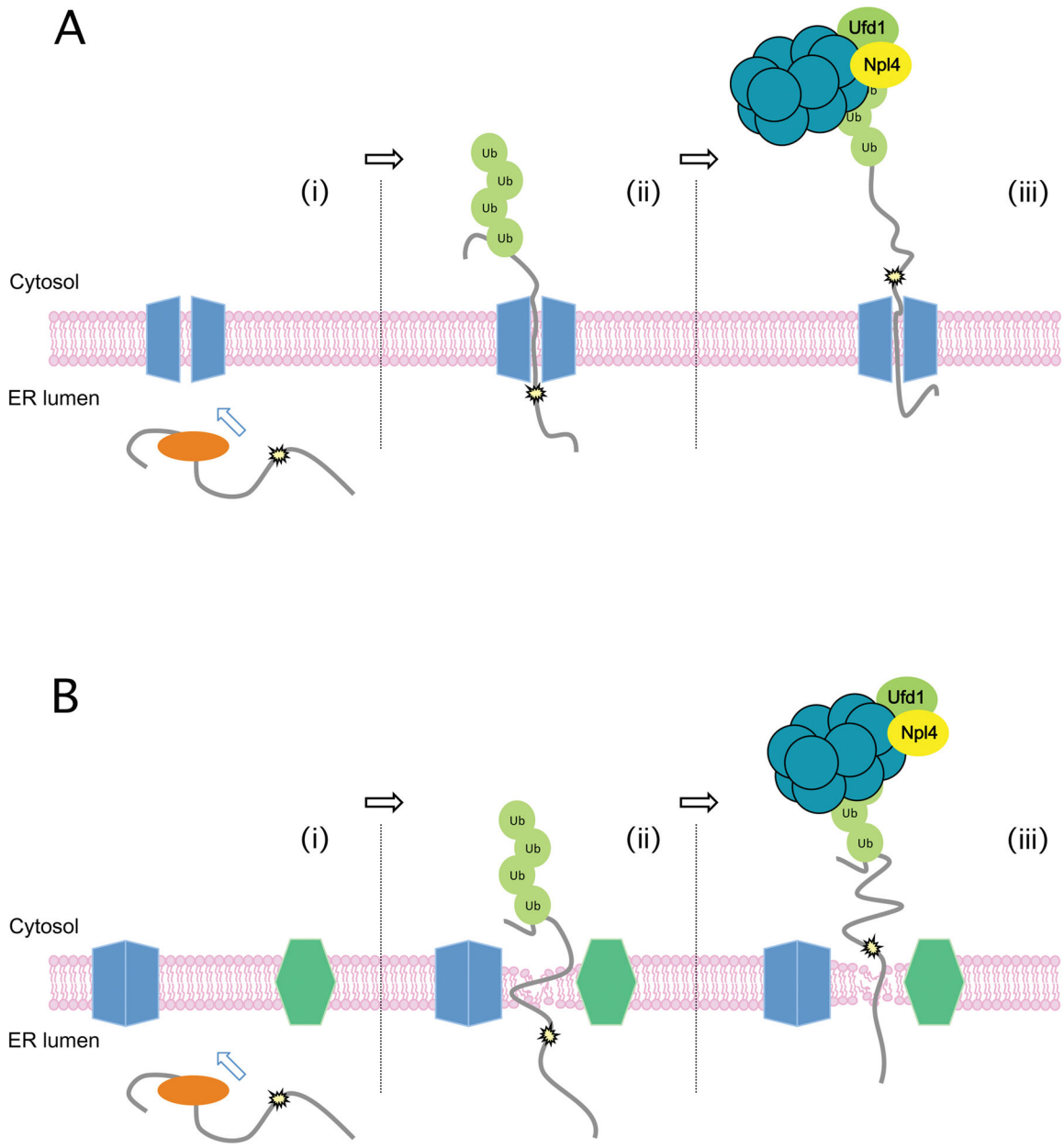

Figure 3.

Models of substrate retrotranslocation in ERAD. A) (i) At first, a hypothetical ERAD-L substrate is recognized in the ER lumen (co-factor is shown in orange). Eventually, membrane co-factors will recognize the substrate and initiate the retrotranslocation process. One model is that there are one or more ERAD co-factors that can independently act as a retrotranslocation channel (shown in blue). (ii) Substrate passes through the protein channel and its ubiquitination by the E3 in the cytosol occurs simultaneously with this movement. (iii) The Cdc48/Ufd1/Npl4 complex then interacts with the polyubiquitinated substrate and promotes its full extraction from the ER. The proteasome could participate at a latter stage or in a parallel pathway with Cdc48 B) (i) In the second model, substrate is transferred

across the membrane without the assistance of a dedicated protein channel. Instead, (ii) one or multiple co-factors (shown in blue and green) might affect the lipid dynamics of the ER membrane bilayer by causing a thinning of the membrane or increasing lipid disorder, for example. This would lower the free energy requirement for a polypeptide to move across the membrane. The substrate can still interact with ERAD co-factors as it is moving across. (iii) In the final step, the ubiquitinated substrate is extracted by Cdc48 and transported to the proteasome.

Table 1

Components of the yeast ERAD machinery and their conserved orthologs in mammals

ERAD protein	Mammalian Ortholog(s)	Characterized function(s)
Hrd1 Complex		
Hrd1	HRD1 and gp78	E3 ligase at the ER membrane, participates in retrotranslocation
Hrd3	SEL1L	Required for Hrd1 stability and ERAD-L substrate recognition
Usa1	Herp	Scaffold protein for HRD complex oligomerization and Der1 recruitment
Der1	Derlin-1, -2, and -3	Required for ERAD-L, participates in retrotranslocation
Yos9	OS9 and XTP3-B	Luminal lectin, required for ERAD-L substrate recognition
Kar2	BiP	Luminal Hsp70 protein, required for ERAD- L substrate recognition
Doa10 Complex		
Doa10	TEB4 (MARCH VI)	E3 ligase at the ER and IN membranes
Ubc6	Ube2j1 and Ube2j2	Transmembrane E2 conjugating enzyme
Shared co-factors		
Ubc7	Ube2g1 and Ube2g2	Soluble E2 conjugating enzyme
Cue1	not present	Recruits and stimulates Ubc7 activity
Cdc48	p97/VCP	AAA ATPase, promotes substrate extraction from the ER and IN membranes
Ubx2	UBXD8	Recruits Cdc48 to the ER and IN membranes
Ufd1	UFD1	Cdc48 co-factor, required for ERAD
Npl4	NPL4	Cdc48 co-factor, required for ERAD