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The Recognition and Retrotranslocation of Misfolded Proteins from the Endoplasmic Reticulum

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Abstract

Secretory and membrane proteins that fail to fold in the endoplasmic reticulum (ER) are retained and may be sorted for ER-associated degradation (ERAD). During ERAD, ER-associated components such as molecular chaperones and lectins recognize folding intermediates and specific oligosaccharyl modifications on ERAD substrates. Substrates selected for ERAD are then targeted for ubiquitin- and proteasome-mediated degradation. Because the catalytic steps of the ubiquitin–proteasome system reside in the cytoplasm, soluble ERAD substrates that reside in the ER lumen must be retrotranslocated back to the cytoplasm prior to degradation. In contrast, it has been less clear how polytopic, integral membrane substrates are delivered to enzymes required for ubiquitin conjugation and to the proteasome. In this review, we discuss recent studies addressing how ERAD substrates are recognized, ubiquitinated and delivered to the proteasome and then survey current views of how soluble and integral membrane substrates may be retrotranslocated.

Keywords

degradation; ER; glycosylation; molecular chaperone; proteasome; proteolysis; transport; ubiquitin

Over one-third of newly synthesized proteins are translocated into the endoplasmic reticulum (ER), which ensures their delivery into the secretory pathway (1). Because of this high flux of proteins through the ER and because the folding of multi-domain proteins may be inefficient, the ER lumen contains a high concentration of molecular chaperones that maintain polypeptide solubility, enzymes that posttranslationally modify proteins, and factors that directly assist in the folding of newly synthesized polypeptides. Proteins that fail to acquire their native conformations, because of genetic error, cellular stress or stochastic events, may harm the cell. Therefore, components within the ER lumen also mediate quality control ‘decisions’ that result in the resolution of terminally misfolded proteins from correctly folded proteins and folding intermediates. This decision-making process is known as ER quality control (ERQC) and may result in the retention of aberrantly folded proteins in the ER (2).

To offset the potentially catastrophic consequences of misfolded protein accumulation, ER-retained species are most commonly destroyed. The primary mechanism of disposal is ER-associated degradation (ERAD) (3–9). During ERAD, misfolded proteins are delivered to the 26*S* proteasome, which resides in the cytoplasm. Therefore, the destruction of ERAD substrates requires polypeptide recognition, delivery from the ER to the cytoplasm (which has been termed retrotranslocation or dislocation) and in most cases ubiquitination, which ensures efficient delivery to the proteasome. The importance of ERAD is underscored by the fact that a growing number of ERAD substrates are linked to human diseases (10) and that the accumulation of ERAD substrates may induce the unfolded protein response (UPR), which if

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unresolved will trigger apoptosis (11). Another mechanism utilized by the cell to destroy misfolded proteins, especially those that aggregate in the secretory pathway or in the cytoplasm, is autophagy, and the reader is referred to a recent review on this topic (12). In this article, we will focus on and briefly summarize current views of how substrates are recognized during ERAD. We will then discuss the ER membrane-associated degradation machineries that facilitate ERAD substrate retrotranslocation and ubiquitination.

ERAD Substrate Recognition

Soluble substrates

It is imperative that ERAD substrates, especially those that are aggregation prone, are retained in solution to ensure efficient retrotranslocation. Thus, the simplest mechanism underlying molecular chaperone-mediated selection during ERAD is that early folding intermediates and misfolded proteins remain bound to chaperones. The chaperones would then deliver these species to secondary quality control complexes that escort selected polypeptides from the ER and to the proteasome. In fact, molecular chaperones such as BiP (an Hsp70) and its Hsp40 co-chaperones have been shown to prevent soluble substrate aggregation prior to degradation (13). Protein disulfide isomerase (PDI) and PDI homologues have also been shown to act prior to substrate retrotranslocation (14–16).

Another method of ERAD substrate retention employs ER-resident chaperone-like lectins (2, 9,17). Nearly, all secreted proteins contain a core glycosylation consensus site onto which an *N*-linked oligosaccharide (NAc-Gln_2-Man_9-Glc_3) is appended during or immediately after translocation. Soon thereafter, two of three glucose residues in the *N*-glycan are trimmed by glucosidase I and II. This species is a substrate for calnexin and calreticulin, which catalyzes polypeptide folding and prevents aggregation. Indeed, in the absence of calnexin function, BiP provides a backup mechanism to maintain substrate solubility and retain aberrant proteins in the ER (18). In what is most probably a stochastic process, the last glucose residue may be trimmed by glucosidase II, which releases the protein from the lectins. If, however, the substrate is still misfolded, it is recognized by the glucosyl transferase (GT), which preferentially recognizes unfolded or molten globule (19–21) species and reglucosylates the polypeptide. This permits lectin rebinding and prevents the misfolded protein from leaving the ER.

Misfolded proteins in the ER cannot cycle between calnexin/calreticulin and the GT perpetually and thus a folding ‘timer’ aborts this cycle and induces the destruction of dead-end products. The timer is an ER mannosidase that trims mannose residues from the core glycan (22–24). This reduces the efficiency of calnexin/calreticulin rebinding and diverts substrates to a second, putative lectin known as ER degradation enhancing α-mannosidase-like protein (EDEM) (25–27). Three EDEM homologues have been identified in mammals (28,29), and one homologue resides in the yeast ER (30,31). Although the protein was originally thought to simply recognize polypeptides with trimmed mannose residues, recent data suggest that EDEM may be a mannosidase and exhibits chaperone-like activity (32,33). It was also originally thought that EDEM might recognize only modestly mannose-trimmed species, but again more recent data suggest that ERAD substrates may be trimmed more extensively (34). Many of these issues, and the nature of ERAD substrate hand off between ER-resident chaperones, lectins and the retrotranslocation machinery, will clearly be aided by the development of reconstituted systems in which each event can be recapitulated.

Another intermediary that appears to act between ERAD substrate selection and retrotranslocation is Yos9, which resides in the yeast ER (35–37). Yos9 is also a lectin that appears to act as a component of a ‘gate-keeping’ complex through which ERAD substrates must pass prior to retro-translocation (38). Other components of the Yos9 complex include BiP and Hrd3 (39), which is a partner of the Hrd1 E3 ubiquitin ligase (see below). Because

Hrd3 recognizes misfolded proteins independent of Yos9, and BiP might as well, these data suggest that a multiple-step recognition mechanism may be employed prior to the final decision-making process that targets some ERAD substrates for degradation. In mammals, two homologues of Yos9, OS-9 and XTP3-B, have been noted. Similar to yeast Yos9, OS-9 and XTP3B bind to misfolded proteins and to SEL1L, a homologue of Hrd3, suggesting that these two proteins link ERAD substrates to the membrane-associated ubiquitination machinery. OS-9 also associates with Grp94, which is required for misfolded protein degradation. Therefore, Grp94 may contribute to substrate recognition and/or to the regulation of the assembly and disassembly of the OS-9–SEL1L–Hrd complex (40).

Although an *N*-glycan seems to be a critical element for the ERAD of misfolded glycoproteins (see above), it is less clear how nonglycosylated misfolded proteins are recognized and targeted for degradation. The importance of this pathway is underscored by the fact that treatment of cells with tunicamycin, an inhibitor for *N*-glycosylation, results in the accumulation of misfolded proteins in the ER and UPR induction. A recent study demonstrates that HERP, a membrane-associated cytoplasmic protein in mammals, binds to nonglycosylated BiP substrates and to the 26*S* proteasome but not to substrates whose degradation depends on calnexin function. Moreover, reduction of HERP levels inhibits the degradation of nonglycosylated BiP substrates but has no effect on calnexin substrates. These results suggest that there is some distinction in the degradation pathways for glycosylated and nonglycosylated proteins (41).

Integral membrane substrates

Far less is known about how misfolded membrane proteins are recognized and sorted to the ERAD pathway, although the cytoplasmic Hsp70/Hsp40 system and other chaperones clearly help maintain the solubility of large, cytoplasmic loops in selective ERAD substrates (42–44). We recently reported that ER-associated, cytoplasmic Hsp70–Hsp40 chaperones assist the interaction of this class of ERAD substrates with E3 ubiquitin ligases and suggested that a chaperone complex may play an active role during degradation rather than simply retaining polypeptides in solution (45). This view is consistent with other recent reports suggesting that a cytoplasmic chaperone network may lead substrates to a folding and/or degradation pathway (46,47). Interestingly, defects in cytoplasmic Hsp90 function accelerate the degradation of integral membrane proteins with misfolded cytoplasmic domains, implying that selective chaperones are ‘prodegradative’, whereas others are primarily involved in folding (48–50). In any event, integral membrane ERAD substrates with misfolded cytoplasmic domains have been proposed to utilize the ‘ERAD-C’ (cytoplasmic) pathway, and ongoing work has defined many of the requirements for the degradation of proteins in this class (Table 1 and Figure 1) (51,52).

At this time, it is unclear how misfolded, integral membrane proteins that lack prominent soluble domains are recognized for destruction, and only a few members of this ‘ERAD-M’ class of species have been studied (5,52). It is possible that these substrates are recognized directly by E3 ubiquitin ligases; it is worth noting that most of the E3s known to be required for ERAD substrate ubiquitination contain multiple transmembrane-spanning domains (53–55), suggesting that intramembrane substrate recognition may occur. E3-encoded transmembrane domains may also constitute a component of the long-sought retrotranslocation channel (see below).

Do ERAD substrates possess a common recognition motif?

Because secretory proteins are diverse, there are potentially an infinite number of states at which proteins may misfold, depending on the positions of the mutation, environmental and cellular stress, or expression level. As noted above, the accumulation of misfolded and/or non-

native proteins in the ER can trigger the UPR, which leads to the synthesis of ER chaperones and enzymes involved in secretory protein maturation and to the synthesis of components required to divert misfolded proteins to the ERAD and other degradation pathways (11). UPR induction appears to be enhanced by both a chaperone-dependent mechanism (i.e. BiP) and an UPR-inducing sensor in the ER (56). Not surprisingly, the ERAD and UPR pathways are linked such that the simultaneous disruption of genes required for each pathway results in synthetic phenotypes (57–59). This suggests that diverse misfolded proteins may be targeted to ERAD.

Although ERAD seems to be a major mechanism to dispose misfolded secretory proteins, some misfolded proteins in the ER are degraded through different pathways. In fact, a quality control system exists in subsequent secretory organelles (60). In addition, highly accumulated misfolded proteins may aggregate and can be delivered to the autophagic pathway (61). Therefore, at this time, the existence of a common biophysical feature of ERAD substrates cannot be assumed. Notably, a recent study has suggested that ERAD efficiency does not simply correlate with a protein's thermodynamic instability or with its rate of folding (62). Moreover, there seems to be a complex interplay between ERAD, the ER-associated folding machinery, secretion efficiency and a substrate's innate biophysical properties (63).

One attribute of secreted proteins that might confound attempts to generalize mechanisms of ERAD regards the number and placement of appended oligosaccharides. Several recent reports indicate that the specific position of the *N*-glycan controls ERAD substrate selection (64,65). In mammalian cells, a C-terminal 19 amino acid cassette with one *N*-glycan is sufficient to mediate the entry of cyclooxygenase-2 into the ERAD pathway (66). In the future, it will be vital to extend these important studies to include more diverse substrates and to examine whether the embedded polypeptide sequence plays a contributing role in glycan position-dependent selection.

ERAD Substrate Retrotranslocation

Nearly, all ERAD substrates are ubiquitinated prior to their degradation, a process that requires the sequential action of an E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases, each of whose catalytic sites are located in the cytoplasm. Therefore, ER lumenal substrates must first gain access to the cytoplasm before they can be modified. It remains unknown how this occurs. Next, a critical, minimal length of the polyubiquitin chain is imperative for retrotranslocation (67), and it was shown that ubiquitinated and/or unmodified substrates can be detected in the cytosol or on the cytosolic face of the ER membrane when the proteasome is inhibited (68–72). The extraction of ubiquitinated substrates from the ER is driven by the Cdc48/p97 complex, an ATP-requiring hexameric AAA adenosine triphosphatase (ATPase) that is thought to couple ATP hydrolysis with polypeptide retrotranslocation (67,73,74). However, a selective group of soluble ERAD substrates retrotranslocate in an ubiquitin-independent manner, and these use a different energy-requiring complex, the 19*S* particle of the proteasome (see below). These substrates also accumulate in the cytosol when the proteolytic activity of the proteasome is inhibited (75–77). Together, these data imply that a protein-conducting channel facilitates the retrotranslocation of substrates to the cytoplasm (8).

Guilt by association: which protein – if any – constitutes the ‘retrotranslocon’?

The identity of the retrotranslocation channel and mechanism of retrotranslocation remain contentious, even though defined integral membrane proteins in the ER interact with ERAD substrates *en route* to their degradation and interact with components of the ubiquitination/degradation machineries (Table 1 and Figure 1). In this section, we summarize evidence that implicates the involvement of each candidate as the retrotranslocon.

Several lines of evidence have suggested that the retrotranslocon may be Sec61, which is the major component of the translocation channel that imports polypeptides into the ER (78,79). Some ERAD substrates, including major histocompatibility complex class I (MHCI) in mammals (whose degradation is induced by a viral gene product) and a nonglycosylated yeast mating pheromone (pαF) in yeast, coprecipitate with or cross-link to Sec61 (80,81). Consistent with these data, *sec61* mutant yeast exhibit a pαF degradation defect *in vitro* and CPY* degradation defect *in vivo* (81,82). Also, the prebinding of ribosomes to ER-derived microsomes, and most likely Sec61, abolishes the retrotranslocation of a glycopeptide, which exhibits some properties in common with ERAD substrates (83). Furthermore, Sec61 depletion in reconstituted mammalian vesicles or Sec61 blockage with ribosome–nascent chain complexes prevents the export of cholera toxin and the amyloid beta-peptide from the ER to the cytosol (84,85). Less direct – but in further support of Sec61-mediated retrotranslocation – is the reported association of yeast Sec61 to the 19*S* subunit of the proteasome (86), which suggests a mechanism for the proteasome-mediated extraction of pαF from the ER (76). But, perhaps the best evidence that at least a small subset of ERAD substrates utilizes Sec61 derives from studies on the regulated degradation of apolipoprotein B (apoB) in mammals; in the lipid-deficient state, apoB translocation into the ER is halted, and the protein is then cotranslocationally degraded by the proteasome (87). It has been proposed that the regulated degradation of apoB requires an ER and Sec61-associated chaperone-like protein, p58, which may ‘shift’ Sec61 from acting as a translocon to a retrotranslocon (88).

Together, the data presented above suggest that Sec61 can directly retrotranslocate proteins or may serve as a component of the retrotranslocon, particularly those that are stalled during translation and translocation (i.e. apoB). Nevertheless, accumulating data conflict with the assumption that Sec61 is the retrotranslocon for all ERAD substrates. First, complexes containing the principle E3s utilized during ERAD (Hrd1 and Doa10) lack Sec61 (38,52,89), even though another putative retrotranslocon, known as Der1, associates with Hrd1 (see below). Second, *sec61* mutants degrade integral membrane ERAD substrates proficiently, suggesting that the channel – if it is involved in retrotranslocation – is only co-opted for the degradation of soluble substrates (50,90). Third, the realtime retrotranslocation of pαF from mammalian microsomes was unaffected by the addition of anti-Sec61 antibody, but the retrotranslocation of paF was significantly inhibited by anti-Der1 antibody. Furthermore, pαF cross-linked to this protein but not to Sec61 (77).

As noted above, the Hrd1 and Doa10 E3 ligases seem to be central components of the membrane-associated ubiquitination machineries in the yeast *Saccharomyces cerevisiae* (Table 1 and Figure 1). Hrd1 can be immunoprecipitated in complex with components in the lumen, in the ER membrane and in the cytoplasm. Each of these factors is required for the degradation of lumenal substrates or more formally substrates that harbor misfolded domains that reside in the lumen (ERAD-L) (51,52). In contrast, Doa10 mediates the turnover of ERAD-C substrates (see above). Doa10p can be immunoprecipitated in complex with a more specific group of ERAD-requiring proteins in the membrane and in the cytoplasm (52). The catalytic RING domain in both Hrd1 and Doa10 is situated on the cytoplasmic face of the ER membrane, and the proteins are estimated to span the lipid bilayer 6 times and 14 times, respectively (53–55). Based on this fact, and the fact that Hrd1 and Doa10 co-ordinate so many ERAD contributors, one view is that the enzymes function as both retrotranslocation channels and E3 ligases. Therefore, retrotranslocation and substrate modification are effectively coupled. This hypothesis has also been forwarded for gp78, a polytopic, integral membrane E3 in the mammalian ER (91).

Another protein that may constitute or be a part of the retrotranslocon is Der1, which was introduced above and was first isolated as a protein required for ‘degradation in the ER’ of two different soluble substrates (92). While Der1 is only required for the degradation of lumenal

proteins in yeast, accumulating evidence has suggested that Derlin1 – one of three homologues in the mammalian ER – is a critical component that links lumenal and cytoplasmic components during ERAD. Specifically, Derlin1 can be isolated in complex with factors in the membrane and cytoplasm and with ERAD substrates (41,93–99) (Table 2). Consistent with a central role in ERAD, Derlin1 depletion induces the UPR (97) and retards the degradation of select substrates, and – as mentioned above – Derlin1 is a central player during pαF retrotranslocation from mammalian vesicles (77). Interestingly, Derlin proteins have also recently been implicated in the liberation of polyomaviral-encoded proteins from the ER after infection (16,100) and in the retrotranslocation of cholera toxin from the ER (101). These processes require other factors required for ERQC, indicating that the ERAD pathway has been co-opted by opportunistic pathogens (102).

Another scenario, which will surely muddy future attempts to define the retrotranslocon, is that the channel forms only transiently and/or is a composite of several candidates. In other intracellular organelles, such as the peroxisome, the transient pore model has been postulated, which could explain the fact that peroxisomes import folded, even oligomeric, proteins (103). Yet another scenario is that retrotranslocation requires the formation of a lipid bi-cell in the ER membrane (104). This model has been invoked to explain data indicating that protein complexes and large folded domains and *N*-glycans on ERAD substrates can be retrotranslocated (105–107). It is possible that a proteinaceous channel would be unable to expand to accommodate these large cargo molecules.

Could there be as-yet undiscovered or other candidate proteins that constitute the retrotranslocon? One factor proposed to fulfill this role is Ssh1, a Sec61 homologue in yeast (108). However, other data suggest that the yeast genetic background may dictate the Ssh1 dependence on ERAD (50) and thus it remains unclear how this protein contributes to ERQC.

Substrate Delivery to the Proteasome

After retrotranslocation, a series of ubiquitin-binding proteins escort modified substrates from the ER membrane to the proteasome (109,110). One of these factors is the AAA ATPase, Cdc48 (p97 in mammal), that in conjugation with two cofactors (Ufd1 and Npl4) contributes to ERAD after substrate ubiquitination but prior to degradation (67,73,74,111,112). Biochemical studies have shown that Cdc48/p97 extracts ubiquitinated substrates to the cytosol (45,73,113) either by actively pulling a polypeptide through the retrotranslocon and/or lipid membrane or by segregating a polypeptide that has already been liberated from the ER membrane. Cdc48/p97 might then deliver substrates to the proteasome by virtue of its interaction with ubiquitin-binding and ubiquitin-like domain-containing molecules such as Ufd2 (which has been termed an E4), Ufd3 (a protein of unknown function), Otu1 (a deubiquitinating enzyme) and Rad23/Dsk2 (109,114–118).

Accumulating evidence indicates that Cdc48/p97 is not required for the destruction of all ERAD substrates. For example, the Cdc48/p97 requirement during the extraction of a membrane protein might depend on the overall hydrophobicity of the substrate. Membrane proteins possessing multiple transmembrane-spanning segments might have a heightened requirement for the enzyme, whereas substrates containing fewer transmembrane segments or transmembrane segments with charged residues appear to be less dependent on Cdc48/p97 function (119). Second, Cdc48/p97 activity is dispensable for the retrotranslocation of pαF from yeast or mammalian microsomes (76,77). Third, the retrotranslocation of cholera toxin from the ER is p97 independent (120). For pαF and cholera toxin, the lack of Cdc48/p97-dependent export may arise because the substrates are not polyubiquitinated. Instead, the 19*S* particle of the proteasome drives the retrotranslocation of these substrates. Of note, the base of the 19*S* particle, like Cdc48/p97, harbors a ring of AAA ATPases (109), and some

recent studies have attempted to define which subunit(s) of the base are directly involved in catalyzing retrotranslocation (67,121).

How are Integral Membrane Substrates Delivered to the Proteasome?

By definition, integral membrane substrates expose one or more regions to the cytoplasm, which could be ubiquitinated before retrotranslocation. The subsequent degradation of these substrates could start from either end of the polypeptide after dislocation through the retrotranslocon or through direct membrane extraction (Figure 2). Alternatively, degradation could start from an internal site on a cytoplasmically exposed loop after an endoproteolytic clip by the proteasome. In these models, it is assumed that degradation and retrotranslocation are tightly coupled and occur at the ER membrane. On the other hand, some integral membrane ERAD substrates, such as MHCI (70) and cystic fibrosis transmembrane conductance regulator (CFTR) (122), have been observed to reside in the cytoplasm when proteasome function is compromised. These data suggested that membrane-spanning segments might be solubilized from the lipid bilayer of the ER prior to proteasome-mediated degradation. By employing a reconstituted system, we were able to demonstrate directly that Ste6p*, another 12 transmembrane-spanning ERAD substrate, is released into the cytosol in a Cdc48/p97- and ATP-dependent manner (45). The electrophoretic profiles of these species suggest that the transmembrane domain of Ste6p* became solvent exposed. Therefore, a future research challenge is to determine how transmembrane segments are maintained in solution.

Conclusions and Perspectives

As described in this review, many open questions remain on the mechanistic details by which different ERAD substrates are degraded. As is often the case in ‘new’ fields, early attempts to generalize accumulated phenomenology have invariably been met with exceptions to the rule. Indeed, we are continually humbled by the growing number of factors that impact ERAD efficiency and diverse mechanisms employed during ERAD. Thus, it is clear that the degradation pathways of new and noncanonical ERAD substrates must be defined.

Nevertheless, specific, general questions in the field that remain to be answered include the following: Are substrates actively released from the ERQC machinery, or is the final decision to degrade an ERAD substrate simply a stochastic event? What is the nature of the retrotranslocon, or in the end, will there be a variety of means by which the retro-translocation reaction can be engineered? Are there other specialized ERAD machineries, and how is ERAD regulated, as evidenced by the examination of apoB degradation (88) or cells under stress (123–125)? How are substrates, such as 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, converted from functional enzymes in the ER membrane to ERAD substrates (6)? Because proteasome substrates must be deubiquitinated, how is ERAD substrate deubiquitination and degradation coupled, and are specific deubiquitinating enzymes required for ERAD? What is the rate-limiting step during ERAD, or does this depend on the nature of the substrate? And finally, how is ERAD efficiency controlled during development, disease and stress? Needless to say, answers to each of these questions will keep many of us busy for some time.

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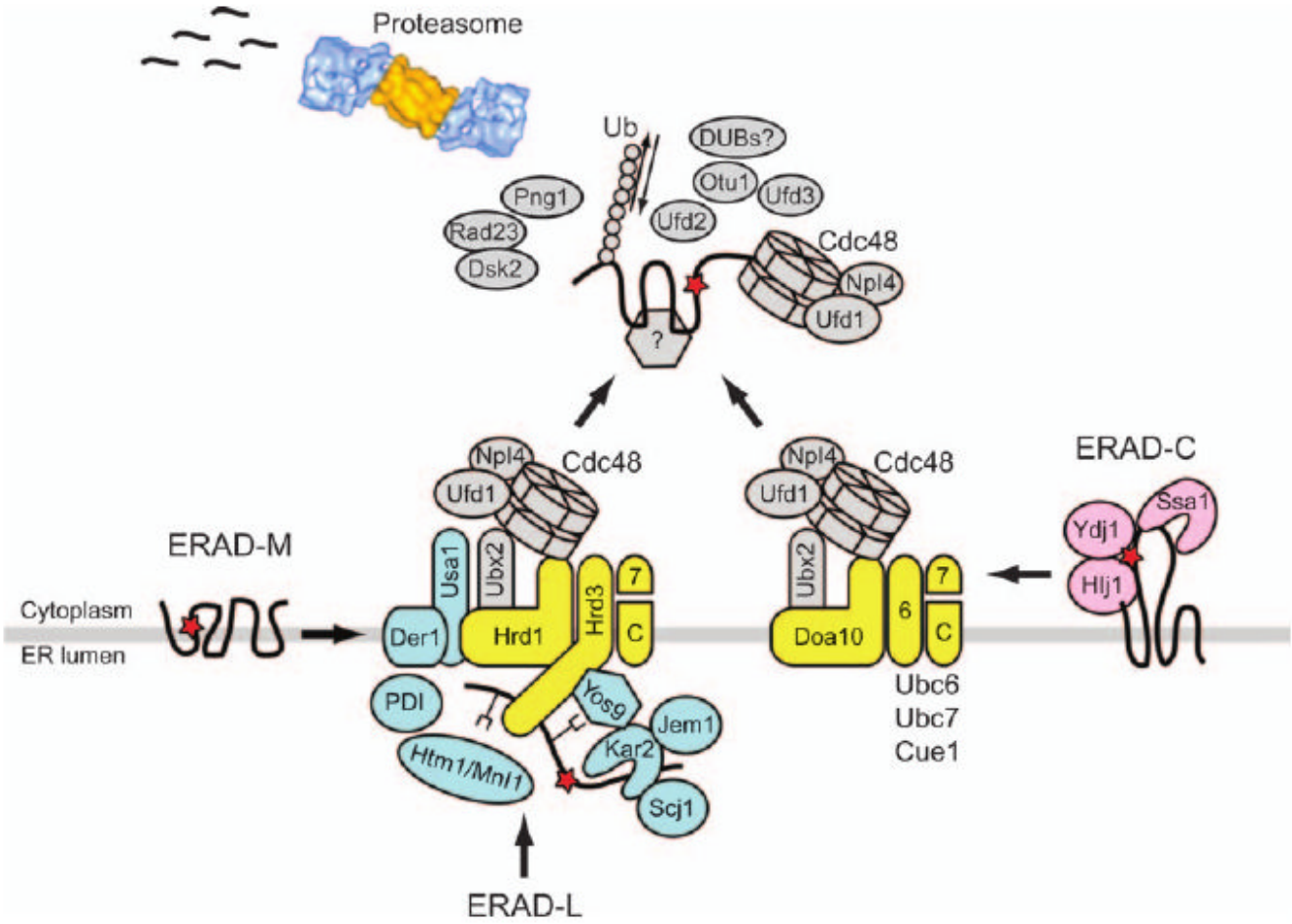

Figure 1. A current working model of the ERAD pathway in yeast

ERAD-L substrates are recognized by Hsp70/Hsp40 chaperones (Kar2, Jem1 and Scj1), PDI, putative lectins (Yos9 and Htm1/Mnl1) and the lumenal domain of Hrd3. These substrates are then retrotranslocated to the cytoplasm. In contrast, ERAD-M substrates may be directly recognized by the Hrd1 E3 ligase. ERAD-C substrates are recognized by cytoplasmic Hsp70/Hsp40 chaperones (Ssa1, Ydj1 and Hlj1) and by the Doa10 E3 ligase. The ubiquitinated substrates are delivered to the proteasome core for degradation by a series of escort factors, including the Cdc48 complex and the 19*S* proteasome cap. Steps following substrate ubiquitination are not yet clear for ERAD substrates. However, the polyubiquitin chain may be further remodeled by Ufd2 and by deubiquitinating enzymes (Rpn1, and/or DUBs such as Otu1). *N*-glycans may be cleaved by Png1, and some substrates are escorted to the proteasome by Rad23 and Dsk2. Membrane-associated E2–E3 enzymes, ERAD-L-specific components and retrotranslocation components are colored in yellow, blue and gray, respectively. The red star depicts a mutation that leads to protein misfolding, and the proteasome image was adopted from Voges et al. (126).

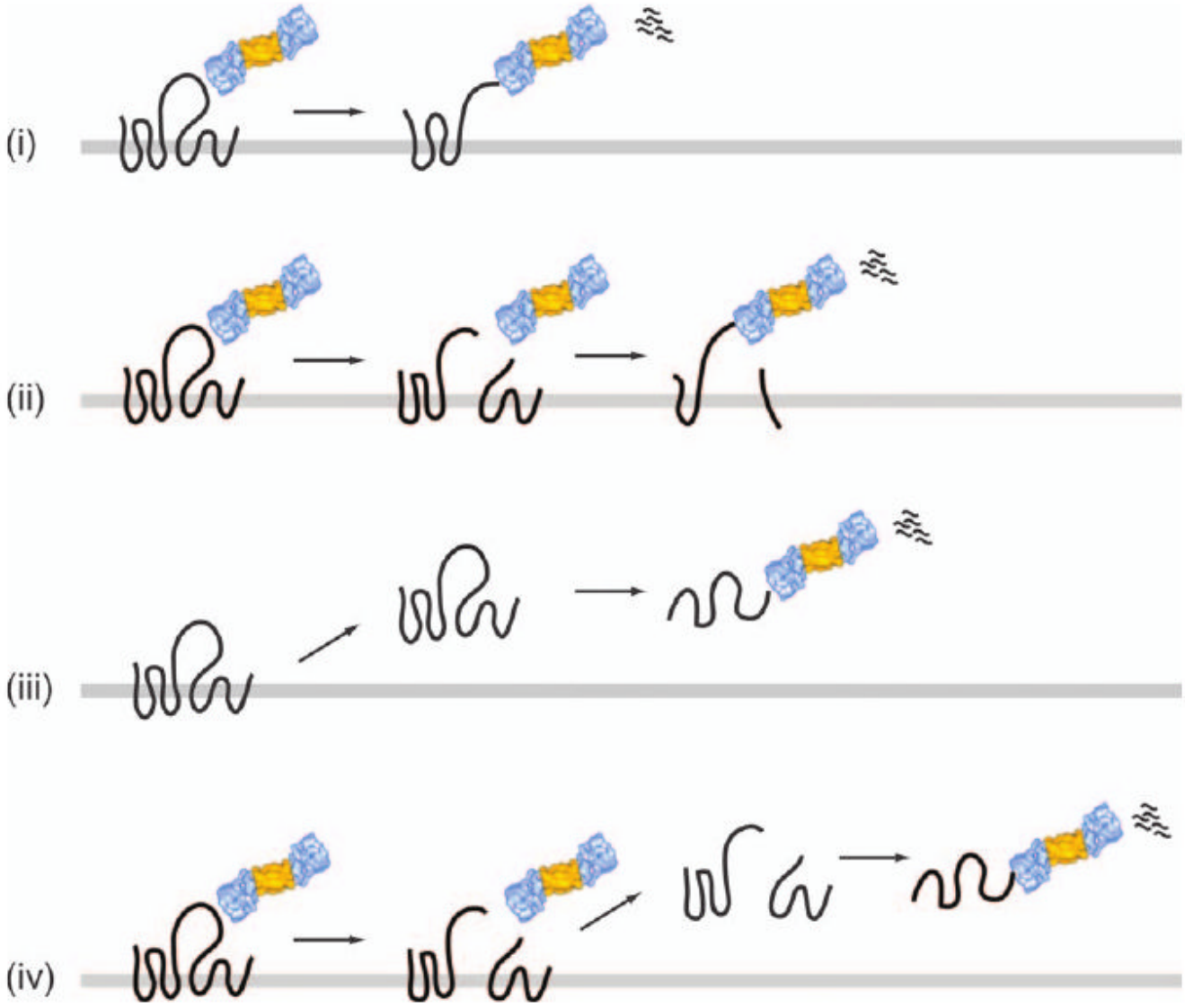

Figure 2. Models for membrane substrate delivery to the proteasome

Polytopic membrane proteins may be processively dislocated and degraded from either the N- or the C-terminus by the proteasome (i), as was proposed for the FtsH proteolytic system in bacteria (127). Alternatively, polytopic membrane proteins may be extracted to the cytoplasm and then degraded by the proteasome (iii). In this case, the solubility of hydrophobic transmembrane segments may be maintained by the Cdc48 complex, the 19*S* cap of the proteasome and/or other components including chaperones and proteasome-escorting factors. Because the proteasome has an endoproteolytic activity (128), cytoplasmic loop(s) of substrates may first be 'clipped' by the proteasome and then dislocated or extracted (ii or iv). Not shown in these models is the potential role of a retrotranslocon in substrate degradation. The proteasome image was adopted from Voges et al. (126).

Table 1

Selective components required for ERAD in yeast[a]

	Cytosol- and membrance-associated		Merribrane-associated		ER lumen- and membrane-associated	
ERAD-C	Degradation	26*S* proteasome	E3	Doa10		
	Retrotranslocation	Cdc48–Ufd1–Npl4–Ubx2	E2	Ubc6 and Ubc7–Cue1		
	Chaperones	Ssa1, Ydj1 and HIj1				
ERAD-M	Degradation	26*S* proteasome	E3	Hrd1–Hrd3		
	Retrotranslocation	Cdc48–Ufd1–Npl4–Ubx2	E2	Ubc7–Cue1		
ERAD-L	Degradation	26*S* proteasome	E3	Hrd1–Hrd3	Putative lectins	Yos9 and Htm1/Mnl1
	Retrotranslocation	Cdc48–Ufd1–Npl4–Ubx2	E2	Ubc7–Cue1	Chaperones	BiP (Kar2), Scj1, Jem1 and PDI
			Putative retrotranslocon components	Der1, Sec61, Ssh1 and Usa1		
Other components	Polyubiquitination	Ufd2				
	Deubiquitination	Rpn11 and Otu1				
	Escort	Rad23–Dsk2				
	Deglycosylation	Png1				

[a]Components involved in the ERAD-C, -M or -L pathways as listed by their locations. Note that the functions of Ufd2 and Rad23-Dsk2 have not been well characterized for multiple ERAD substrates, and the direct involvement of Rpn11, Otu1 and Png1 during ERAD is still being elucidated. Ubx2 and Cue1 are membrane proteins that recruit the Cdc48 complex and Ubc7 to the ER membrane, respectively. Hrd3 is a membrane protein that stabilizes Hrd1 and recognizes lumenal substrates through its lumenal domain. Usa1 is also a membrane protein that appears to help link the Hrd1 E3 ligase complex and Der1 and is a specific component of the ERAD-L pathway (52).

Table 2

Selective components required for ERAD in mammals[a]

Mammals	***Saccharomyces cerevisiae***
Cytosol- and membrane-associated	
p97-UFD1-NPL4	Cdc48–Ufd1–Npl4
Carboxyl terminus of Hsc70-interacting protein (CHIP)	
FBX2	
Parkin	
ATX3	
Rad23	Rad23
N-glycanase	Png1
Membrane-associated	
gp78	
HRD1–SEL1L	Hrd1–Hrd3
RMA1	
TEB4	Doa10
Ubc6e	Ubc6
Ubc7	Ubc7
HERP	Usa1
VCP-interacting membrane protein (VIMP)	
Derlin-1, -2 and -3	Der1
Sec61	Sec61
ER lumen- and membrane-associated	
OS-9 and XTP3-B	Yos9
EDEM-1, -2 and -3	Htm1/Mnl1
BiP	BiP (Kar2)
PDI	PDI

[a]Selective conserved components are listed by their locations. Proteins whose names are underlined can be coimmunoprecipitated with Derlin-1 (see text). Note that the functions of many of the components in mammals have not been fully delineated.