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Roles of Molecular Chaperones in Endoplasmic Reticulum (ER) Quality Control and ER-Associated Degradation (ERAD)

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Secreted proteins are synthesized at the endoplasmic reticulum (ER), and a quality control mechanism in the ER is essential to maintain secretory pathway homeostasis. Newly synthesized soluble and integral membrane secreted proteins fold into their native conformations with the aid of ER molecular chaperones before they are transported to post-ER compartments. However, terminally mis-folded proteins may be retained in the ER and degraded by a process called ER-associated degradation (ERAD). Recent studies using yeast have shown that molecular chaperones both in the ER and in the cytosol play key roles during the ERAD of mis-folded proteins. One important role for chaperones during ERAD is to prevent substrate protein aggregation. Substrate selection is another important role for molecular chaperones during ERAD.

Key words: endoplasmic reticulum, ERAD, Hsp70, molecular chaperone, quality control.

Abbreviations: ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERAC, ER-associated compartment; UPR, unfolded protein response; BiP, binding protein; PDI, protein disulfide isomerase; ΔGpαF, unglycosylated mutant form of pro α-factor; CPY*, mutant form of carboxypeptidase Y; CFTR, cystic fibrosis transmembrane conductance regulator; BPTI, bovine pancreatic trypsin inhibitor.

Nearly all secreted proteins enter the endoplasmic reticulum (ER) in eukaryotic cells. Proteins destined for the secretory pathway are translocated into the ER either during or soon after their synthesis, and protein folding and maturation in the ER are essential for their subsequent transport through the secretory pathway. Mis-folded or aggregated proteins are potentially cytotoxic, and consequently cells possess quality control systems to remove these aberrant proteins. As a result, only correctly folded proteins are deployed to their final destinations (*1*). Because aberrant proteins may expose hydrophobic regions and free cysteines and have a tendency to aggregate, molecular chaperones play key roles in ER quality control because they recognize mis-folded and aggregation-prone proteins (*2*). In this mini review, we will summarize recent findings on chaperone functions in ER quality control, and will focus on studies performed in the budding yeast, *Saccharomyces cerevisiae*.

ER chaperones and ER quality control

The ER harbors three groups of molecular chaperones and folding enzymes: chaperones of the heat shock protein family, including BiP (Hsp70) and its co-chaperone partners (*e.g.* Hsp40), chaperone lectins such as calnexin and calreticulin, and thiol oxidoreductases of the protein disulfide isomerase (PDI) family (Table 1). Many of these chaperones interact with translocating nascent polypeptide chains soon after their entry into the ER lumen and facilitate protein folding, oligomerization, maturation, and post-translational modifications, which may include glycosylation and disulfide bond formation (*2*, *3*). Substrate proteins are released from ER chaperones once their native structures are attained, and correctly folded and assembled proteins are transported from the ER. However, prolonged interactions of non-native proteins with ER chaperones and folding enzymes during chaperone-mediated folding may well contribute to the retention of non-native proteins in the ER.

When mis-folded proteins accumulate in the ER, ER chaperone binding reduces the concentration of free chaperones. To cope with the resulting ER stress, cells elicit an unfolded protein response (UPR). The UPR induces the transcription of gene products that facilitate the processing of aberrant proteins and that attenuate protein translation, which reduces the amount of newly imported proteins into the ER (*4*). The sensor for mis-folded protein accumulation is Ire1p, and a severe growth defect is observed in UPR-defective *ire1* yeast mutants when mis-folded proteins are over-expressed (*5*, *6*). This growth defect can be overcome by BiP overexpression, indicating that ER chaperones protect cells from ER stress, at least in budding yeast (*5*).

If protein repair by ER chaperones is unsuccessful, aberrant proteins are cleared from the ER by a mechanism termed ER-associated degradation (ERAD) (*7*–*9*). During ERAD, aberrant proteins are translocated back to the cytosol and degraded by the ubiquitin-proteasome system (Fig. 1). The Sec61 translocon plays a role in the "retrotranslocation" of lumenal ERAD substrates and at least some integral membrane substrates (*10*, *11*). However, Sec61p is dispensable for the degradation of other membrane substrates (*12*, *13*), but an ER membrane protein complex containing Derlin-1 (a mammalian homolog

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Table 1. **ER chaperones and factors involved in ER quality control in *Saccharomyces cerevisiae*.**

Description	Yeast gene products
heat shock proteins	
Hsp70	BiP/Kar2p
Hsp40 (J-domain–containing proteins)	Jem1p, Scj1p, Sec63p
Lectin-like chaperones	
calnexin/calreticulin	Cne1p
EDEM	Mnl1p/Htm1p
thiol oxidoreductases	
protein disulfide isomerase	Pdi1p, Eug1p, Mpd1p, Mpd2p, Eps1p

of yeast Der1p) has been proposed to function as a retrotranslocation channel for at least one ERAD substrate: the class I major histocompatibility complex protein in virally-infected cells (*14*, *15*).

Functions of ER chaperones in ERAD–substrate selection

ER chaperones play an important role during ERAD substrate selection. For example, ER chaperone-like lectins, such as calnexin and calreticulin, recognize specific *N*-linked carbohydrate chain structures on glycoproteins. These structures are formed when glycosylated proteins cannot fold or fold slowly in the ER. Therefore, calnexin and calreticulin bind to and retain immature glycoproteins and facilitate their folding. If, however, glycoproteins cannot acquire their native conformations within an appropriate time, they are targeted for ERAD (*3*, *16*). Glycoprotein entry into the ERAD pathway has been proposed to require the trimming of a single mannose by the ER α1,2-mannosidase I, and subsequent recognition of the Man8 moiety by another putative lectin, known as EDEM, or its yeast counterpart Mnl1p/Htm1p (*17*–*19*). Terminal mannose trimming by mannosidase I is slow, and thus this event may be rate-limiting during ERAD (*16*). In any event, mannose trimming facilitates substrate transfer from calnexin to EDEM (*20*, *21*), but it is not clear how EDEM hands-off ER substrates to the translocon prior to their delivery to the cytosol.

In contrast to the lectin-like chaperones, Hsp70 chaperones such as BiP recognize hydrophobic regions of peptides that are usually buried inside native proteins, and the binding and release of these unfolded or partially denatured substrates are ATP-dependent (*22*). Based on these attributes, BiP recognizes unfolded proteins and facilitates their folding in the ER, and retains terminally mis-folded aberrant proteins in a soluble conformation. Terminally mis-folded substrates are sorted to the ERAD pathway after they are released from BiP (*1*–*3*). The action of yeast BiP (also known as Kar2p) during ERAD was defined when a mutated form of the α-factor mating pheromone precursor, pro α-factor (ΔGpαF), was examined *in vitro* (*23*, *24*). Wild type pro α-factor contains three *N*-linked oligosaccharide chains (*25*), and this protein remains soluble even when BiP is inactivated (*24*). However, BiP is required to maintain the solubility of ΔGpαF. Overall, a high affinity association of ERAD substrates for BiP may be a key determinant in substrate selection. This is consistent with the fact that all known soluble ERAD substrates require BiP for their degradation (*2*).

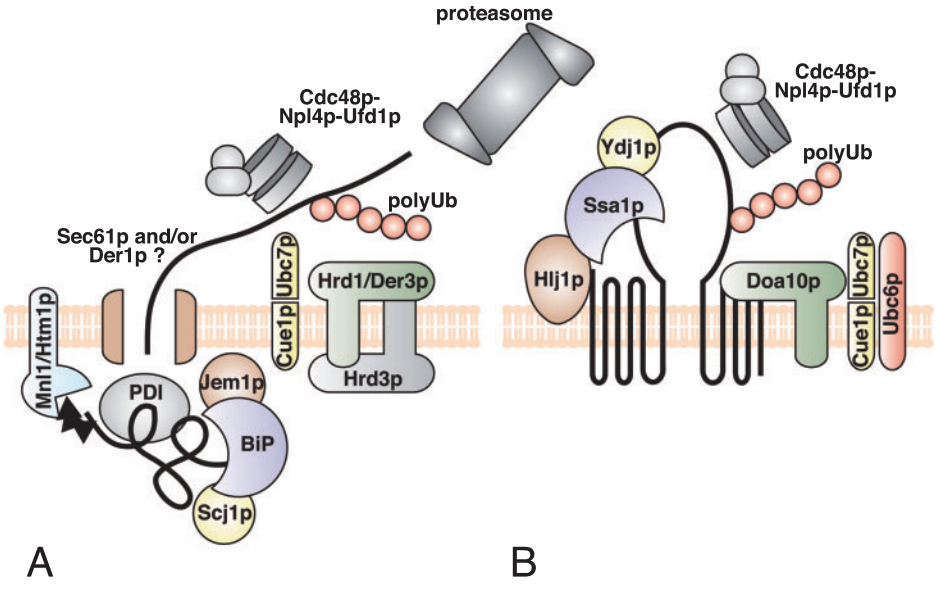

Fig. 1. **Components involved in the ERAD of soluble and membrane substrates in yeast.** A: Misfolded proteins in the ER lumen are translocated back to the cytosol and degraded by the ubiquitin-proteasome system. This process requires the functions of ER lumenal molecular chaperones, such as BiP and its J-domain–containing partner proteins, Jem1p and Scj1p. PDI and Mnl1p/Htm1p are also required for the degradation of soluble ERAD substrates. The substrates are polyubiquitinated ("polyUb") by the Hrd1p E3 ubiquitin ligase in conjunction with the E2 ubiquitin-conjugating enzyme Ubc7p. B: Degradation of several integral membrane substrates requires a cytosolic Hsp70, Ssa1p, and its J-domain–containing partner proteins, Hlj1p and Ydj1p. The Doa10p E3 ubiquitin ligase is involved in the ubiquitination of several membrane substrates in conjunction with the E2 enzymes Ubc6p and Ubc7p.

Recent studies support the hypothesis that BiP mediates ERAD substrate selection, and several unfolded proteins escape ERAD because BiP does not recognize them. One example is a mutated form of bovine pancreatic trypsin inhibitor (BPTI). When expressed in yeast, the BPTI mutant eludes BiP capture and is transported from the ER to the Golgi (*26*). Of significant interest, several ERAD substrates, including the BPTI mutant, can be modified by *O*-linked mannose (*O*-mannosylation) if they are trapped in the yeast ER (*27*, *28*). In one recent study, it was shown that *O*-mannosylation helps retain mis-folded ERAD substrates in solution independent of BiP function, and these substrates were no longer targeted for ERAD (*27*). Indeed, impaired *O*-mannosylation induces the UPR, suggesting that this modification reduces the amount of mis-folded proteins that need to be recognized by BiP (*28*).

Although BiP plays an important role during substrate selection, it does not seem to be involved in targeting of ERAD substrates to the retrotranslocon. As indicated above, EDEM appears to be involved in this process. In addition, protein disulfide isomerase (PDI) can deliver ERAD substrates to the translocon: PDI acts as a redox-dependent chaperone and unfolds cholera toxin during the retrotranslocation of the A1 chain (*29*), and appears to act downstream of BiP during the targeting of ΔGpαF to the translocon in yeast (*30*).

Functions of ER chaperones in ERAD–substrate solubilization

Another important role for ER chaperones during ERAD is to prevent the aggregation of mis-folded proteins prior to their retrotranslocation. Although substrate unfolding is not a prerequisite for retrotranslocation (*31*), ERAD substrates remain in the lumen if they aggregate. In the yeast ER, ERAD substrates such as ΔGpαF and a mutated form of carboxypeptidase Y (known as CPY*) are maintained in a soluble form by BiP and two co-chaperone partner proteins, Jem1p and Scj1p (Fig. 1A, *24*). Jem1p and Scj1p are members of the Hsp40 family of molecular chaperones, all of which are defined by the presence of an Hsp70-interacting J-domain (*32*). It was found that CPY* and ΔGpαF begin to aggregate at elevated temperatures when BiP or the co-chaperone functions are inactivated, and once aggregation occurs, their degradation is significantly impaired, even when the temperature is lowered (*24*).

In contrast, BiP function is dispensable for the degradation of integral membrane ERAD substrates in yeast, including Ste6-166p, Sec61-2p, the cystic fibrosis transmembrane conductance regulator (CFTR) and unassembled, ER-retained forms of Vph1p (*13*, *24*, *33*, *34*). This is probably due to the fact that the lumenal domains in these membrane-bound ERAD substrates are properly folded (the mis-folded domain faces the cytoplasm). In opposition to this hypothesis, Wolf and colleagues showed that fusion proteins containing CPY* anchored to the ER membrane via a single transmembrane segment did not require BiP for their degradation (*35*), although it is possible that the membrane anchor might have suppressed CPY* aggregation.

Roles of cytosolic chaperones in ERAD–protein unfolding and dislocation in the cytosol

Accumulating evidence also suggests the involvement of cytosolic chaperones in ERAD. Ssa1p, a cytosolic Hsp70 in yeast, is required for the ERAD of several membrane proteins, including Ste6-166p, CFTR and unassembled Vph1p (Fig. 1B, *13*, *33*, *34*). Two Hsp40 co-chaperones, Hlj1p and Ydj1p, have been proposed to function as partner proteins of Ssa1p in the degradation of Ste6–166p and CFTR (*13*, *36*). Although one might envision that Ssa1p drives the retrotranslocation of substrates from the ER—analogous to the action of BiP during the post-translational import of secretory proteins into the ER lumen—Ssa1p does not function at this step because it is dispensable for the ERAD of lumenal proteins (*35*, *37*). Instead, Ssa1p probably maintains the solubility of mis-folded cytosolic domains on at least some integral membrane ERAD substrates, which would facilitate their delivery to and degradation by the proteasome. This may be through Ssa1p-mediated unfolding of aberrant cytosolic domains before proteasomal degradation, or because the chaperone facilitates the conjugation of polyubiquitin onto the ERAD substrate, an event that promotes proteasome-targeting (*38*).

Retrotranslocation of ERAD substrates and their delivery to the proteasome can also be catalyzed by a chaperone-like AAA ATPase, known as Cdc48p/p97, and two Cdc48p-associated cofactors, known as Ufd1p and Npl4p (*39*). The Cdc48p-Ufd1p-Npl4p complex binds first to the polypeptide backbone of the retro-translocating ERAD substrate and then to the polyubiquitin chain (*40*). Next, Cdc48p couples ATP hydrolysis to drive the completion of substrate retrotranslocation. However, at least one ERAD substrate, ΔGpαF, does not require Cdc48p for degradation. In this case, the 19S 'cap' of the proteasome, which also contains AAA ATPases, facilitates substrate retrotranslocation (*41*).

Substrate sorting in the ERAD pathway

Recent evidence indicates that the ERAD pathway is composed of multiple routes. For example, and as described above (Fig. 1), there are differences in the chaperone requirements during the degradation of lumenal and integral membrane substrates. In addition, after analyzing the degradation of a series of artificial ERAD substrates with defined topologies and locations of resident mis-folded domains, Vashist and Ng proposed that the ERAD system in yeast is composed of two surveillance mechanisms (Fig. 2, *42*). The ERAD-lumenal (ERAD-L) pathway monitors the folded state of lumenal domains, and degradation of ERAD-L substrates requires ER-to-Golgi transport, Der1p, and the Hrd1p E3 ubiquitin ligase. On the other hand, the ERAD-cytosolic (ERAD-C) pathway monitors the folded state of cytosolic domains. Degradation of the ERAD-C substrates is independent of ER-to-Golgi transport and Der1p and instead requires Doa10p, another ER-associated E3 ubiquitin ligase.

How are ERAD substrates sorted to each pathway? Vashist and Ng analyzed the degradation of ERAD substrates with mis-folded domains on both sides of the membrane and found that these proteins showed only the characteristics of ERAD-C substrates (*42*). Although the mechanism of substrate recognition and sorting is not

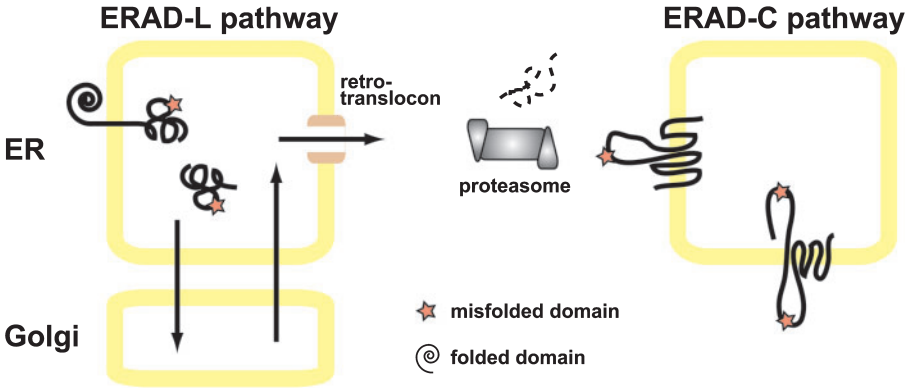

Fig. 2. **Two distinct ERAD pathways function according to the location of the mis-folded domain.** The ERAD-L system monitors the folded state of lumenal domains, and degradation of ERAD-L substrates appears to require ER-to-Golgi transport. The ERAD-C pathway monitors the folded state of cytosolic domains. ERAD substrates with mis-folded domains on both sides of the membrane are degraded by the ERAD-C pathway.

well understood, this result strongly suggests that the checkpoint for ERAD-C pathway sorting is upstream of the ERAD-L pathway. One way to interpret these data is to propose the existence of distinct ER sub-compartments into which different ERAD substrates are sequentially sorted. Indeed, some ERAD-C substrates are sorted to quality control subcompartment of the ER, a compartment that has been called the ER associated compartment (ERAC), before degradation (*43*). However, mutation of cytosolic chaperones, such as Ssa1p, did not affect substrate sorting to the ERAC, suggesting that chaperones are not involved in this step (*13*). Because the sorting of only a subset of ERAD substrates has been analyzed, further studies using other defined substrates will be necessary to understand substrate sorting during ERAD.

One interesting feature of the ERAD-L pathway is the apparent requirement for ER-to-Golgi transport. Stabilization of ERAD-L substrates was observed when *sec* mutants defective in the ER-to-Golgi transport were incubated at the non-permissive temperature or in strains mutated for the gene encoding Erv29p, a cargo receptor involved in packaging a subset of soluble proteins into COPII vesicles (*44–46*). These results suggest that substrate selection for the ERAD-L pathway may involve sorting at ER exit sites. Evidence supporting this notion comes from the observation that inhibition of ER-to-Golgi transport induced relocalization of BiP and cargo proteins to discrete regions within the ER. This region has been referred to as the BiP body (*47*).

Concluding remarks

Binding of molecular chaperones to mis-folded proteins is the key step in ER quality control. During ERAD, molecular chaperones not only assist degradation by preventing substrate aggregation but also function in substrate recognition and probably sorting. However, many questions still remain. For example, how do chaperones transfer substrates from the folding to the degradation pathway? What other molecules and steps are there between chaperone release and retrotranslocation? How do cytosolic chaperones facilitate integral membrane protein degradation? And finally, are there any consensus features in aberrant secreted proteins that define their sorting to the ERAD-L or ERAD-C pathways? We propose that the identification and analysis of new factors that facilitate ERAD and the development of new tools and assays in which the ERAD pathway can be deciphered will help answer these questions.

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