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Protein Folding and Quality Control in the Endoplasmic Reticulum: Recent Lessons from Yeast and Mammalian Cell Systems

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Summary

The evolution of eukaryotes was accompanied by an increased need for intracellular communication and cellular specialization. Thus, a more complex collection of secreted and membrane proteins had to be synthesized, modified, and folded. The endoplasmic reticulum (ER) thereby became equipped with devoted enzymes and associated factors that both catalyze the production of secreted proteins and remove damaged proteins. A means to modify ER function to accommodate and destroy misfolded proteins also evolved. Not surprisingly, a growing number of human diseases are linked to various facets of ER function. Each of these topics will be discussed in this article, with an emphasis on recent reports in the literature that employed diverse models.

Introduction

Most secreted proteins in eukaryotes are initially targeted to the Endoplasmic Reticulum (ER) where they are inserted, or translocated, into the ER lumen or lipid bilayer, depending respectively on whether they are soluble or possess transmembrane domains. Because up to one-third of all proteins interact with the ER, and because some specialized cells produce large amounts of secreted proteins, the flux through this compartment can be formidable. Fortunately, the ER provides a safe-haven for protein folding; the compartment is oxidative, which facilitates disulfide bond formation, and is loaded with proteins that augment folding. These molecular chaperones facilitate protein folding and prevent the formation of off-pathway aggregates.

Most secreted proteins fold and are modified in an error-free manner, but stress, mutations, or stochastic errors during synthesis can decrease the folding yield or rate of folding. Resident chaperones might then be unable to prevent the generation of toxic unfolded species. Indeed, toxic proteins in the ER are observed in some disease states, and can initiate apoptosis [1,2]. However, if misfolded proteins transiently accumulate, or if a stress is sufficiently brief, the problems that accompany protein unfolding may be repaired. Either the ER increases its ability to handle misfolded proteins, or misfolded proteins are destroyed. The first pathway is the unfolded protein response (UPR), and the second is ER associated degradation (ERAD).

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In this review, we discuss the protein folding machineries in the ER and then turn our attention to recent highlights on the mechanisms of UPR induction and the ERAD pathway. We conclude with a few notable links between ER function and disease.

The Protein Folding Machinery in the ER

ER protein folding is a complex interplay between a polypeptide's primary structural information and linked cellular networks. Folding begins as synthesis initiates on cytosolic ribosomes, and ends when the native protein is packaged for ER exit (Figure 1). As the signal recognition particle recognizes a signal sequence, the ribosome-nascent chain complex is targeted to the cytosolic face of the “translocon”. This aligns the ribosome exit tunnel with an aqueous channel formed by the Sec61 αβγ complex [3,4] such that the elongating polypeptide passes directly into the ER lumen coincident with synthesis. For membrane proteins, transmembrane segment compaction is stimulated within the ribosome exit tunnel. This shortens nascent polypeptide length [5,6], and triggers a dynamic rearrangement that closes the lumenal end of the translocon, terminates translocation, and redirects protein movement to the cytosol [7,8]. For polytopic membrane proteins, transmembrane helix formation is coordinated with orientation across the membrane, membrane integration, and helical packing in the bilayer [9], and involves changes in transmembrane segment boundaries and topology after targeting [10,11]. Future studies will surely determine how other early folding events are facilitated.

Secreted proteins entering the ER immediately encounter a network of chaperone systems that minimize aggregation, facilitate native structure formation, and ensure oligomeric assembly [12] (Figure 1). These systems involve: i) non-covalent interactions with Hsp40 (J-proteins), Hsp70 (Grp78/BiP/Kar2) and Hsp90 (Grp94) chaperones, (ii) lectin-based chaperones, such as calnexin (CNX) and calreticulin (CRT), and (iii) protein disulfide isomerases (PDIs). The relative contribution to folding of each component is dictated by the properties and requirements of individual clients. Because nascent polypeptides are delivered into the ER in a relatively unstructured state [13], ER chaperones are organized in both space and time. For example, ERj1 is a type I membrane protein in mammals that binds the exit tunnel of 80S ribosomes and contains a lumenal J-domain that stimulates the ATPase activity of BiP, thereby facilitating client loading [14,15]. In the absence of BiP, ERj1 binds ribosomes with high affinity and allosterically inhibits translation, whereas BiP binding reduces ribosome affinity and allows translation to continue [16]. Thus, ERj1 recruits BiP to the translocon and signals the ribosome that chaperones are available on the opposite side of the membrane. Two translocon components, Sec63 (a J-protein) and its partner Sec62, may perform a similar role [17].

An early modification on nascent proteins involves transfer of a core oligosaccharide ($GlcNAC_2Man_9Glc_3$) to Asn-X-Ser/Thr. N-linked glycosylation improves protein solubility, decreases aggregation, provides a binding site for CNX and CRT, facilitates PDI interaction, and can be a marker for ERAD [18]. Glycan attachment is catalyzed by the SST3 subunit(s) of oligosaccharyltransferase (OST) [19], a heterogeneous membrane complex that associates with the translocon [20,21]. Translocon residence helps OST scan for consensus sites and attach sugars cotranslationally. However, not all consensus sites are utilized due to premature folding, disulfide bond formation, and/or peptide release from the ribosome. Ruiz-Canada et al. demonstrated that glycosylation efficiency is also improved by the existence of two OST forms, containing either SST3A or SST3B subunits [22]. Both catalyze cotranslational glycosylation, with SST3A having less activity and more selectivity. OST containing the SST3B isoform can also glycosylate substrates after release from the transloocn, thereby recognizing sequons missed during cotranslational scanning. Glycosylation is further improved by a different OST subunit, also with two homologs,

OST3/6 [23]. Each contains a thioredoxin-like fold capable of forming mixed disulfides that improve glycosylation for a subset of clients. An important future question is how other OST subunits, whose activities remain unknown, contribute to these events.

After glycan attachment, the terminal glucose is removed by glucosidase I (Glc I), followed by removal of the second glucose by Glc II (Figure 1). This generates $GlcNAC_2Man_9Glc_1$ that forms a high affinity ligand for CNX and CRT [24-26]. The membrane protein CNX is associated with the translocon and binds substrates cotranslationally. In contrast, CRT is a soluble protein and interacts primarily with secreted proteins after ribosome release. Once bound, CNX/CRT retain substrates in the ER, prevent aggregation and degradation, and facilitate folding by recruiting a PDI, ERp57. Removal of the last glucose by Glc II generates $GlcNAC_2Man_9$, which does not bind CNX/CRT. Folded substrates can exit the ER, whereas nearly native substrates with exposed hydrophobic patches, usually near the N-linked site, are transferred to BiP or reglucosylated by UDP-Glc glycoprotein glycosyltransferase (UGT) and enter a second CNX/CRT cycle. The crystal structure of CRT complexed with a Man_3Glc_1 tetrasaccharide recently revealed the basis for specificity of $GlcNAC_2Man_9Glc_1$ over $GlcNAC_2Man_9$ whereby the terminal glucose fits into CRT via extensive hydrogen bonding [27]. Interestingly, the bound glucose is inaccessible to Glc II, which raises the question of how the substrate is released prior to glucose removal. The structural homology between CRT and CNX indicates that glycan binding is mediated by identical mechanisms, suggesting that their different specificities are due to protein-protein interactions [28] or their distinct locations in the ER.

Deglucosylation and reglucosylation are in kinetic competition with ER/Golgi mannosidases. These enzymes catalyze the slow removal of mannose, which decreases reglucosylation by UGT [18] and generates a ligand for a different set of ER lectins. Among them, EDEM, a mannosidase [29-31], and Yos9 (in yeast)[32] and OS-9 and XTP3-B (in mammals)[33,34] target substrates to an ER quality control (ERQC) compartment. The net result is a molecular clock in which glucose trimming and reattachment provide proteins a limited time, and multiple chances to fold, after which the profolding program is replaced by a prodegradative program.

Because the vectorial nature of translocation allows formation of non-native disulfide bonds, many initial disulfides are subsequently isomerized by the PDIs. The Cys-X-X-Cys motif in PDI's thioredoxin domain accepts oxidizing equivalents, primarily from Ero1 and Erv2 (but other mechanisms have recently been uncovered [35]). Regardless, oxidizing equivalents are transferred to clients via mixed disulfide intermediates. Mammalian cells contain ~20 PDIs (yeast contain 5) that vary in substrate specificity as well as oxidation, reduction, and isomerization activities [36]. While mammalian PDI is ubiquitously expressed and interacts with a range of substrates, other homologs show remarkable selectivity [37].

Disulfide trapping, has revealed that mammalian PDIs, ERP57, ERP18, and ERp64 interact with Ero1 and exhibit distinct yet overlapping substrate specificity [38]. The PDI P5 also forms a noncovalent interaction with BiP and mixed disulfides with BiP clients. BiP binding is strengthened by P5 oxidation, suggesting that P5 recruitment facilitates the refolding of oxidized BiP substrates. By analogy, the recruitment of ERp57 to CNX/CRT likely explains its preference for facilitating glycoprotein folding, as demonstrated by siRNA knockdown experiments [39]. In yeast, PDI interacts with the EDEM homologue, suggesting that PDI chaperones this enzyme and/or that the two proteins function together during protein folding [30,40]. Structural studies have also provided new clues into PDI specificity. Yeast PDI forms a twisted “U”, formed by four thioredoxin domains, of which a and a' domains contain active CXXC motifs [41]. Substrate binding occurs at a flexible hydrophobic pocket on the b' domain, which can be partially occluded by a linker peptide [42]. The flexible nature of

this site in PDI may explain its broad substrate specificity. In contrast, the b' domain of ERp57 forms a high affinity binding site for calnexin [43], while ERp72 contains a related structure that lacks this site [44].

One might predict that ERQC prevents the export of unfolded proteins. However, recent studies suggest that ER retention and export are competing processes. In yeast, an ERAD substrate exits the ER if appended to a strong exit signal [45]. Also, misfolded variants of a mammalian prion, which are neither retained nor degraded in the ER, traffic to the Golgi and are sorted to the endo-lysosomal system [46]. This may be explained by the discovery of bipartite degradation motifs that require a locally unfolded peptide region and an adjacent mannose-trimmed glycan [47]. In the absence of glycan, misfolded substrates may lack an ERAD determinant and become subject to Golgi quality control. But, some substrates undergo mannose trimming in the Golgi and return to the ER for ERAD [48,49]. Overall, ERQC is one of several mechanisms to protect the cell against proteotoxicity. Folding, trafficking and degradation are therefore highly integrated, utilize common machinery, and generate complex signals that determine a protein's fate in multiple compartments.

The Unfolded Protein Response: An End-Run Around Folding Problems in the ER

Through UPR induction, the stresses arising from the accumulation of misfolded ER proteins can be ameliorated. The UPR is initiated by a single (in yeast) or three-pronged (in mammals) signal transduction pathway. Common to both systems is IRE1, which encodes a single-pass membrane protein that in yeast activates synthesis of the Hac1 transcription factor by splicing a translational inhibitory region within the Hac1 mRNA [50]. Once translated, Hac1 binds to promoters that contain a UPR element. A significant number of secondary UPR targets are also activated, but the identities of all contributing transcription factors are unclear [51]. The transcriptional response increases the levels of lumenal chaperones and of components required for ERAD, protein transport to other compartments, and lipid biosynthesis [52]. ER expansion might provide the most critical means to ameliorate stress [53]. The UPR appears to be triggered from select regions of the ER because Ire1 clusters upon activation [54], which may reflect self-assembly [55] and a more efficient response. Overall, the UPR reduces the concentration and toxicity associated with misfolded proteins in the ER.

The accumulation of misfolded proteins in the ER—especially those with disulfide bonds—generates oxidative stress, and some of the enzymes that mitigate oxidative stress are UPR targets. The use of a real-time reporter has confirmed, in yeast, that misfolded proteins trigger oxidative stress [56]. Moreover, disulfide-containing, misfolded proteins in the ER arrest cell growth when the UPR is disabled, an effect that arises from oxidative stress [57].

Can ER stressed yeast recover? ER stress prevents the inheritance of cortical ER by daughter cells and delays cytokinesis. However, all is not lost: once cell division is reinitiated, the selfless mother cell retains the stressed ER but passes undamaged ER on to the daughter [58].

In mammals, IRE1 is joined by two additional UPR transducers, PERK and ATF6 (Figure 2). The downstream target of Ire1 is the XBP1 transcription factor, which like Hac1 induces the synthesis of products that repair the ER. However, the more specialized demands in mammals are evident by PERK and ATF6, which transiently inhibit new protein synthesis and further increase the levels of components that ameliorate stress. At later times, these transducers can initiate an apoptotic program [1]. Indeed, there is a growing appreciation that the UPR plays a role in several disease states, as exemplified by observations in mouse

models. For example, chemical chaperones and anti-oxidants that attenuate ER stress respectively reduce complications from type 2 diabetes and improve the folding and secretion of a protein linked to hemophilia [59,60]. *Drosophila* and mammalian IRE1 also possesses a nonspecific RNAase activity, termed regulated Ire1-dependent decay (RIDD) [61]. ER-targeted mRNAs, which mostly encode secretory pathway cargo, are destroyed when the ER cannot handle more proteins. Recent data suggest that RIDD is regulated by conditions that perhaps favor XBP1 splicing over message destruction [62,63].

Initially, it was thought that Ire1 sensed the levels of misfolded proteins indirectly because Ire1 and BiP coprecipitate in cell lysates [64]. Thus, as BiP is recruited from Ire1 to accommodate increased levels of misfolded proteins, Ire1 dimerizes and is activated via transphosphorylation. Consistent with this model, BiP diffusion within the ER decreases as misfolded proteins accumuluate, and this event serves as one of the first signs that the UPR will be activated [65]. However, the yeast Ire1 crystal structure identified a peptide-binding site that resembles the site found in MHCI; mutations in this site reduce Ire1-dependent signal transduction [66]. Nevertheless, the structure of this domain in human IRE1 is collapsed, making it less obvious whether peptides fit into this site [67]. The solution to this controversy might be explained by the fact that yeast Ire1 binds unfolded proteins and BiP prevents the formation of Ire1 oligomers, which when activated primes Ire1 to initiate the UPR [68]. In mammals, BiP may provide both a threshold and dictate UPR induction kinetics [69].

ER Associated Degradation: A Quick-Fix for Misfolded Proteins in the ER

The ERAD pathway identifies and destroys individual proteins unable to pass ERQC and can be subdivided into unique steps. First, an ERAD substrate must be recognized. Molecular chaperones were first shown to play this role, but over time it has become clear that ER-resident lectins, discussed above, aid in substrate recognition. Second, the substrate must be presented to a protease, which was initially thought to reside in the ER. Over time it became clear that the cytoplasmic proteasome degrades misfolded proteins, improperly modified proteins, and orphaned subunits of multimeric complexes in the ER [70-72]. Thus, lumenal domains of membrane proteins and soluble protein substrates must be “retrotranslocated” from the ER. Third, proteasome delivery requires ubiquitination [73], and not surprisingly, enzymes required for ubiquitin conjugation and ligation are critical for ERAD. And fourth, the proteasome recognizes and destroys the retrotranslocated substrate.

Based on the many topologies of ERAD substrates, factors required for substrate recognition were proposed to reside in the ER lumen, cytoplasm, and membrane. Substrates with folding lesions in each compartment were then analyzed to reveal distinctions in the degradation requirements for ERAD-“M” (membrane), ERAD-“L” (lumenal), and ERAD-“C” (cytosolic) substrates [74] (Figure 3). Moreover, E3 ubiquitin ligases that append ubiquitin onto ERAD-L/M versus ERAD-C substrates reside in distinct multiprotein complexes [75-77]. One caveat to these designations is that a substrate—especially those with complex topologies—might expose misfolded domains in more than one compartment or might prove too hard to handle by one pathway. Indeed, some substrates in yeast do not fall cleanly within these designations [78-80], and in mammals the membrane tethering of a soluble ERAD-L substrate alters the degradation requirements [81].

As discussed above, chaperone- and lectin-containing complexes in the ER recognize ERAD substrates. Perhaps given the evolution of diverse substrates, some proteins involved in substrate selection are unique to mammals [82,83]. Although the enzymes required for glycan quality control were characterized several years ago using overexpressed proteins, the first endogenous substrate for UGT was only recently identified [84]. There is also an

appreciation that UGT and CNX/CRT are not alone in being both lectins and peptide-binding chaperones; EDEM also recognizes non-glycosylated substrates [85].

Mannose trimmed substrates are recognized by the homologous Yos9 or OS-9/XTP3-B proteins (Figure 1), which also associate with components that recognize, ferry, and ubiquitinate ERAD substrates [33,75-77,86-88]. A critical event for retrotranslocation may be disulfide bond reduction, which is catalyzed by a J-domain and thioredoxin-domain containing protein, ERdj5 [89]. The redox potential of ERdj5 positions the enzyme as the most reducing PDI. Moreover, ERdj5 binds EDEM. Recently, an ERdj5 flavoprotein partner was discovered, ERFAD [90], but it is unknown whether ERdj5 and ERFAD form an electron relay. It also remains mysterious how yeast, which lack ERdj5, reduce ERAD substrates, or whether ERdj5 is required for all mammalian substrates. Indeed, aggregated ERAD substrates can be removed by other pathways, such as autophagy [91-93].

How are soluble proteins retrotranslocated? One candidate for a retrotranslocation channel is Sec61 [94-98]. Another candidate is a polytopic E3 ubiquitin ligase that forms multimers, namely Hrd1 [99]. Hrd1, which is required for the degradation of ERAD-L and ERAD-M substrates (Figure 3), crosslinks to a lumenally trapped ERAD substrate [100]. Crosslinking requires Cdc48, a AAA-ATPase that extracts substrates from the ER (see below). Consistent with channel-like activity, Hrd1 partners determine the specificity for diverse Hrd1 substrates [101], and Hrd1 may directly recognize polar and charged residues in ERAD-M substrates that are exposed when membrane segments fail to pack properly in the bilayer [102]. In mammalian cells, the exposure of a dibasic motif in opsin's transmembrane domain triggered Hrd1-dependent ERAD [103], consistent with Hrd1 acting as both a ubiquitin ligase and a chaperone for defective transmembrane segments. Hrd1 may even be a component of or constitute the retrotranslocation channel for soluble proteins. But, whether a channel is needed for membrane proteins remains unclear, nor is the case closed on whether alternate factors—such as Sec61 or the Derlins, membrane proteins that are required for the ERAD of some substrates [104-107]—form part of the channel or are substrate-specific “retrotranslocons”.

Doa10 is another integral membrane ubiquitin ligase and is required for ERAD-C in yeast [108]. In higher cells, the list of E3s is expanded, and examples of the sequential action of unique ligases have been observed [109]. Although it was previously thought that ERAD substrates possessed Lys48-derived polyubiquitin chains, recent proteomic analyses indicate that ERAD substrates contain Lys48- and Lys11-linkages [110]. Modification on residues other than Lys, particularly Ser and Thr, during ERAD has also been observed [111-113]. In any event, once a protein is polyubiquitinated, the AAA-ATPase, Cdc48 (in yeast) or p97 (in mammals) bind the substrate by virtue of its associated Npl4-Ufd1 cofactors, and Cdc48/p97-mediated ATP hydrolysis is used to facilitate membrane extraction. Proteasome adaptors then serve as intermediaries during ubiquitinated protein targeting to the proteasome [114].

Given that AAA-ATPases function as force-generating engines, it was anticipated that p97/Cdc48-dependence is dictated by the relative difficulty in retrotranslocating a substrate. In fact, genetic evidence indicates that ERAD-M substrates are most sensitive to Cdc48 depletion [115], and p97 dependence correlates with the relative stability of a polytopic membrane protein in the bilayer [116]. In contrast, some ERAD substrates are retrotranslocated in a Cdc48/p97-independent fashion and instead use an analogous AAA complex in the proteasome cap [117-120]. One substrate even retrotranslocates in a p97-dependent but Ufd1-Npl4-independent manner [121]. Regardless, p97/Cdc48 tethering to the ER is critical for maximal ERAD, and in mammals a p97 organizing protein, erasin, may

fulfill this role [122]. In yeast, Ubx2 might play this role, or it could help recruit ubiquitinated ERAD substrates to Cdc48 [123-125].

Because a significant population of proteasomes resides at the ER membrane, retrotranslocation and degradation should be coupled. In most cases this is true, but ubiquitinated, integral membrane proteins have been observed in solution after retrotranslocation from the yeast [126,127] and mammalian ER [128-130]. How these proteins are stabilized is unclear, but the characterization of cytoplasmic inclusions may help answer this question [131,132].

Before degradation, the polyubiquitin chain on ERAD substrates is removed, presumably by the same deubiquitinating enzymes (Dubs) required for most proteasome substrates [73]. Thus, it was surprising that a p97-associated Dub in mammals contributed to the retrotranslocation of a soluble and membrane substrate [133]. It was proposed that ubiquitin removal helps ERAD substrates enter the p97 hexamer, but presumably sufficient ubiquitin remains to ensure proteasome-capture. There is also continued interest in identifying proteases that aid the proteasome during ERAD. One enzyme that fills this role is the signal peptide peptidase, which is ideally positioned to clip residual transmembrane segments [134,135]. Nevertheless, the hunt for other ER proteases is ongoing.

Conclusions

There has been intense interest in defining the factors that facilitate ER protein folding, but it must be appreciated that only a small fraction of all secreted proteins have been analyzed. Undoubtedly, new paradigms will emerge as additional substrates are examined. It is also clear that the UPR and ERAD have been co-opted to facilitate non-quality control activities. For example, the UPR is critical for homeostasis during unstressed conditions [136], and can initiate specific cellular responses or developmental decisions [137]; conversely, preparing the ER for a subsequent onslaught of secreted proteins—by upregulating the synthesis of ER chaperones—prevents UPR induction [138]. The ERAD pathway is also used to regulate metabolic processes [139] and can modulate cadmium detoxification in yeast [140] and proteasome levels in mammals [141]. The retrotranslocation of bacterial toxins and the mechanisms underlying cytomegalovirus pathogenesis represent other examples in which this house-keeping pathway has been usurped for defined purposes [142]. Recently, components of the ERAD machinery (e.g., Cdc48/p97) have even been shown to facilitate the extraction and proteasome-mediated degradation of ubiquitin-conjugated components in the outer mitochondrial membrane [143-145].

Perhaps misfolded proteins in other intracellular compartments also utilize this pathway.

Finally, the connection between the ER and human disease has generated interest in exploiting ERQC pathways for therapeutics. More than 100 disorders ranging from cystic fibrosis, liver disease, epilepsy, cardiac arrythmias, blindness, and Alzheimer's disease, share conserved cellular pathology, in which misfolded ER substrates are prematurely degraded or accumulate as toxic aggregates. This has prompted three strategies for intervention that are directed at either the substrate itself, specific cellular factors, or the global folding environment. The first approach arose from observations that mutant proteins retain biological function when rescued by osmolytes [146,147]. Subsequent findings revealed that protein stability was improved by specific ligands, i.e., pharmacologic chaperones [148]. Efforts to specifically correct disease-related mutants are encouraging, and the first agents that restore misfolding protein trafficking are entering the clinic. The second approach targets the ERQC machinery. Compounds that modulate Hsp70 and Hsp90 chaperones, regulatory cochaperones, and lectins are of significant interest, and

investigations to establish clinical utility are underway. Interestingly, proteasome inhibition has proven effective in treating refractory multiple myeloma because the synthetic load in malignant plasma cells sensitizes the cells to proteasome loss [149]. Proteasome stimulation by inhibiting a Dub may also improve clearance of toxic aggregates [150]. Third, the fate of ER substrates is determined by their interactions with solutes, metabolites, and macromolecules that comprise the proteostatic environment, which is in turn sensitive to stress, age and biosynthetic load [2]. Thus, manipulating proteostatic regulators, such as transcription factors, chaperone systems, or epigenetic modifiers may provide a means to treat these disorders. Similarly, upregulation of ER chaperones, such as BiP, corrects specific diseases in animal models [151,152]. The implications of these findings are profound, as proteostatic pathways are connected to pathologic processes that range from metabolic syndrome, to cancer, longevity and degenerative disease. This is clearly an exciting time to be in this field.

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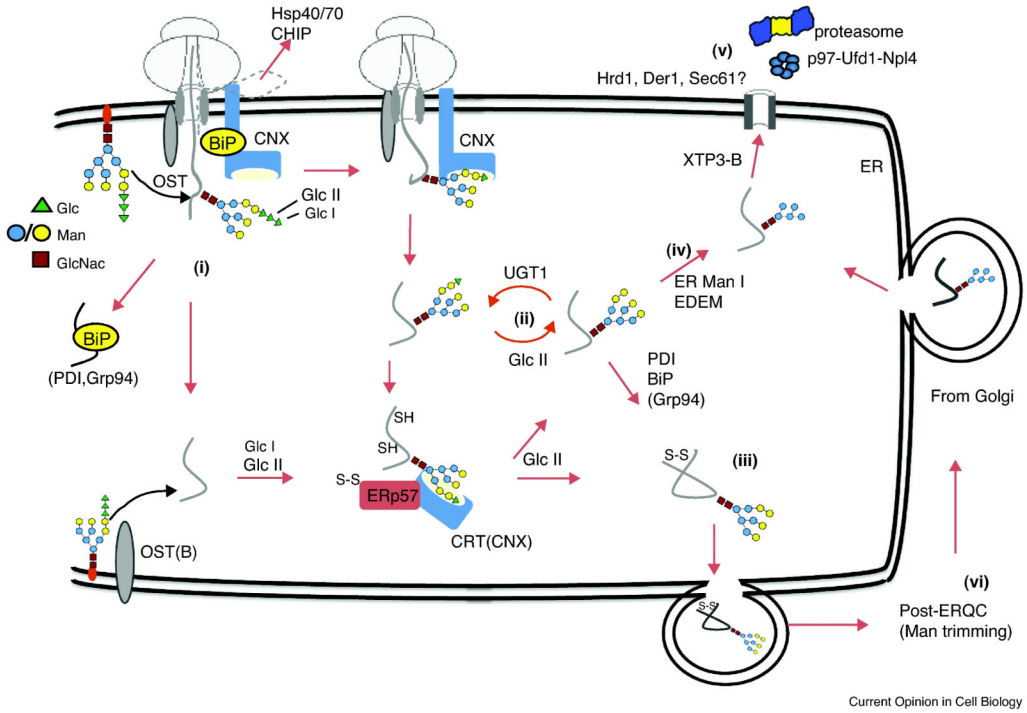

Figure 1. Early events during protein folding and quality control in the mammalian ER

(1) Nascent polypeptides enter the ER through the Sec61 translocon complex, which contains ~20 components (some of which are shown). Next, a core oligosaccharide, $GlcNAC_2Man_9Glc_3$, is cotranslationally transferred from a dolichol lipid precursor (orange oval) to an NXS/T (X≠P) consensus site by OST. Terminal glucoses (green triangles) are trimmed by glucosidase I and II (Glc I and Glc II) to a $GlcNAC_2Man_9Glc_1$ structure that binds the membrane protein, calnexin (CNX). Non-glycosylated substrates either bind BiP and undergo non-lectin mediated folding, and/or are posttranslationally glycosylated by the OST(B) isoform and thereby enter the CNX binding cycle. If translocation is aborted, substrates are delivered to cytosolic chaperones (e.g., Hsp70) and may be degraded. (2) Upon release from CNX, N-linked glycans are further deglucosylated by GlcII, and the substrate either folds spontaneously, is transferred to BiP-, PDI-, and/or Grp94-containing chaperone complexes, or is recognized as misfolded by UGT1 and reglucosylated. Reglucosylation enables rebinding to CNX and its lumenal homolog calreticulin (CRT), which stimulate isomerization and disulfide bond formation via the associated ERp57 PDI homolog. (3) When folded, the substrate is packaged into COPII-coated vesicles for transport to the Golgi. (4) During this process, certain terminal mannose residues (yellow circles) may be subjected to removal by ER Mannosidase I (ManI) and several EDEM homologs. Mannose trimming reduces the affinity for UGT1 and ultimately generates a $GlcNAC_2Man_5$ structure that binds additional lectins, including XTP3-B and OS-9 (not pictured), which bring the substrate to the retrotranslocation machinery (5) for retro-translocation to the cytosol, ubiquitination by E3 ligases, and degradation by the 26S proteasome. (6) In times of ER stress or excess protein load, misfolded substrates containing $GlcNAC_2Man_9$ glycans can also be delivered to the Golgi and sorted for lysosomal degradation, or modified by Golgi ManII and returned to the ER for degradation via ERAD.

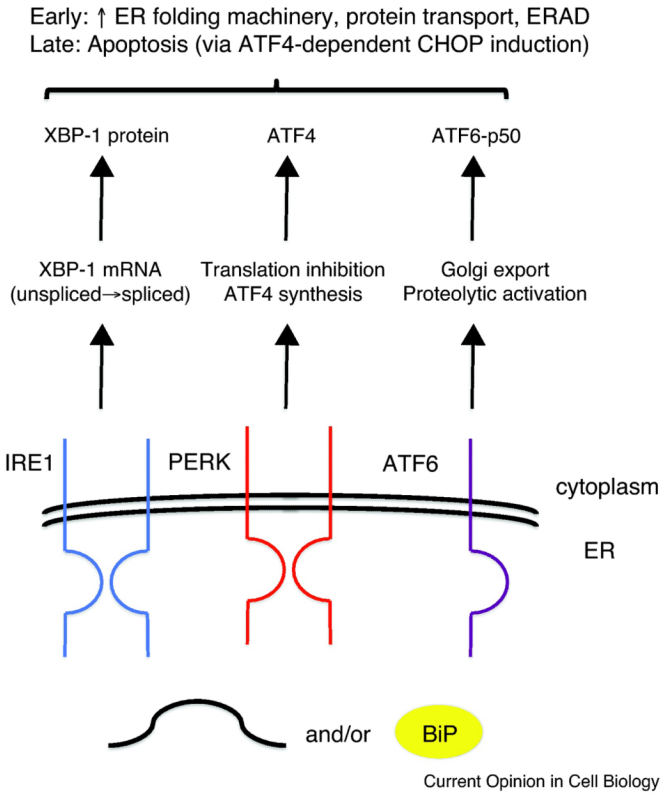

Figure 2. The mammalian UPR is triggered by a multi-pronged signal transduction pathway

The accumulation of misfolded proteins in the ER and/or liberation of BiP from IRE1, PERK, and ATF6, activate a series of down-stream events. IRE1 induces the splicing of the XBP-1 message, which is then translated into an active transcription factor. There are actually two isoforms of IRE1 (α and β) but for simplicity only a single, generic protein is depicted. PERK phosphorylates eIF2α, which inhibits general protein synthesis but the ATF4 transcription factor can still be translated because of the presence of a short, upstream ORF that is skipped when eIF2α is phosphorylated. In contrast, ATF6, when activated, is released from the ER and migrates to the Golgi where it is clipped, thus releasing an active transcription factor, ATF6-p50. The immediate effects of UPR induction help the ER cope with an increase in the concentration of misfolded proteins. However, prolonged UPR induction triggers apoptosis through the ATF4-dependent transcription of the CHOP/Gadd153 transcription factor. CHOP both increases the expression of pro-apoptotic genes and inhibits the expression of Bcl2, an antiapoptotic protein.

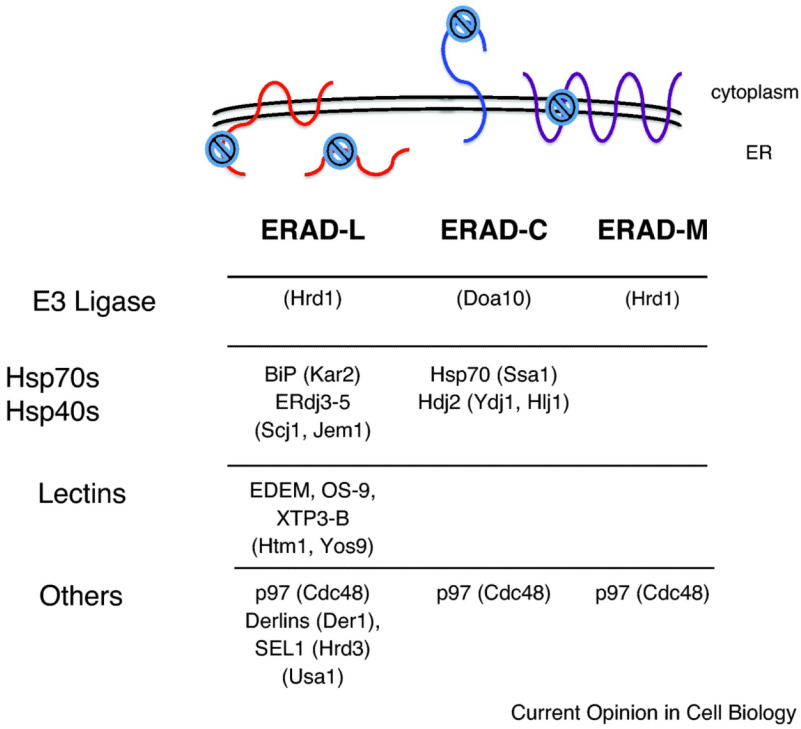

Figure 3. ERAD substrates can be classified into those with misfolded protein lesions either in the ER lumen (ERAD-L), the cytoplasm (ERAD-C), or the membrane (ERAD-M)

The site of the misfolding lesion is depicted by a . Select yeast factors (in parentheses) required for the degradation of each class are listed, and mammalian homologs are indicated in cases in which these have also been shown to function during ERAD, although not necessarily in a class-specific manner. Note: Many mammalian E3s have been identified as contributing to the ERAD of select factors (e.g., CHIP, HRD1, RMA1, gp78, TEB4, and Parkin), but for simplicity these are not shown. In addition, there is evidence that Hsp70 and its constitutively expressed isoform, Hsc70, may play unique roles during protein folding and ERAD, respectively [153].