

Forum Review

ERADicate ER Stress or Die Trying

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ABSTRACT

Stress within the endoplasmic reticulum (ER) induces a sophisticated network of pathways termed the unfolded protein response (UPR), which is mediated through the ER transmembrane sensors PERK, ATF6, and IRE1. The UPR coordinates the temporary downregulation of protein translation, the upregulation of ER chaperones and folding machinery, and the enhanced expression of components necessary for ER-associated degradation (ERAD) essential for decreasing ER stress by clearing terminally misfolded proteins from the ER. Repetitive but futile folding attempts not only prolong ER stress but can also result in reactive oxygen species (ROS) generation, both of which may result in cell death. Additional mechanisms for decreasing stress and the protein load in the ER have been recently revealed. They include a newly identified function of IRE1 in degradation of select secretory protein mRNAs, a “preemptive” quality control responsible for averting translocation of select secretory proteins into the ER, upregulation of forward trafficking to allow misfolded proteins with intact exit signals to exit the ER, and upregulation of autophagy. The saturation or failure of some or all of these mechanisms can result in cell death and disease, including diabetes and a number of late-onset neurologic diseases. *Antioxid. Redox Signal.* 9, 2373–2387.

INTRODUCTION

PROTEINS DESTINED for the secretory pathway are translocated from the cytosol into the endoplasmic reticulum (ER) (20, 60). Once in the ER, nascent proteins are engaged by folding machinery, which aids in achievement of the native conformation, posttranslational modifications, including disulfide bond formation and glycosylation, and multimeric protein assembly. The challenge facing the ER is formidable because as much as one third of the mammalian proteome is associated with the secretory pathway (63). This is intensified by the aberrant synthesis of as much as 30% of nascent proteins (105). To accommodate these demands, a multifaceted quality control (ERQC) monitors folding and assembly of proteins within the ER, and a coupled process termed ER-associated degradation (ERAD) degrades defective and misfolded proteins remaining in the ER (7, 23). In addition to the typical load on the ER, additional burdens can arise from a variety of causes, such as increased synthesis of secretory proteins, expression of misfolded

proteins, mutations affecting folding machinery, viral infections, disruption of calcium levels, and nutrient deprivation (43, 66). To protect against these ER stresses or perturbations in ER homeostasis, a complex signaling pathway termed the unfolded protein response (UPR) provides a multifaceted strategy to ensure the health of the ER and the associated functionality of the secretory pathway.

In higher eukaryotes, the UPR consists of three distinct pathways, modulated through three ER transmembrane sensors, IRE1, PERK, and ATF6, which are responsible for initiating the response (65). Induction of the UPR results in the general decrease in translation initiation to reduce the protein load in the ER, the increased translation of specific mRNAs (*e.g.*, ATF4), and the elegant coordination of transcriptional activation of specific genes involved in protein folding and ERAD to alleviate ER stress. Activation of different subsets of UPR genes by these transcription factors results in diverse and temporally distinct responses to ER stress. Failure of the UPR to relieve ER stress can lead to cellular dysfunction and disease.

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UPR ACTIVATION AND SIGNAL TRANSDUCTION THROUGH THE ER STRESS SENSORS IRE1, PERK, AND ATF6

IRE1

The UPR is found throughout eukaryotes from yeasts to humans and responds to both transient perturbations and to long-term ER stress (Fig. 1A and B). Accumulation of misfolded proteins in the ER results in dimerization and activation of the UPR sensor inositol-requiring transmembrane kinase and endonuclease 1 (IRE1), a type I transmembrane Ser/Thr protein kinase and site-specific endoribonuclease (RNase) (15, 84, 111). The kinase and RNase domains of IRE1 are cytoplasmic-

cally positioned, whereas the luminal N-terminal domain contains the unfolded protein sensor domain.

In the yeast *Saccharomyces cerevisiae*, Ire1p dimerization activates the endonucleolytic activity, resulting in the unconventional splicing of a 252-nucleotide intron from mRNA encoding the bZIP transcription factor Hac1p (35, 65, 111). The *HAC1* mRNA is cleaved at two specific sites by Ire1p to remove the intron (111) and the exon termini joined by the tRNA ligase Rlg1p (110). The ensuing frameshift results in the translation of the Hac1p C-terminal domain containing the transcription-activation domain and creates a potent transcriptional activator that binds to promoter UPR elements (UPREs) and upregulates transcription of >380 genes, >5% of all yeast genes (115).

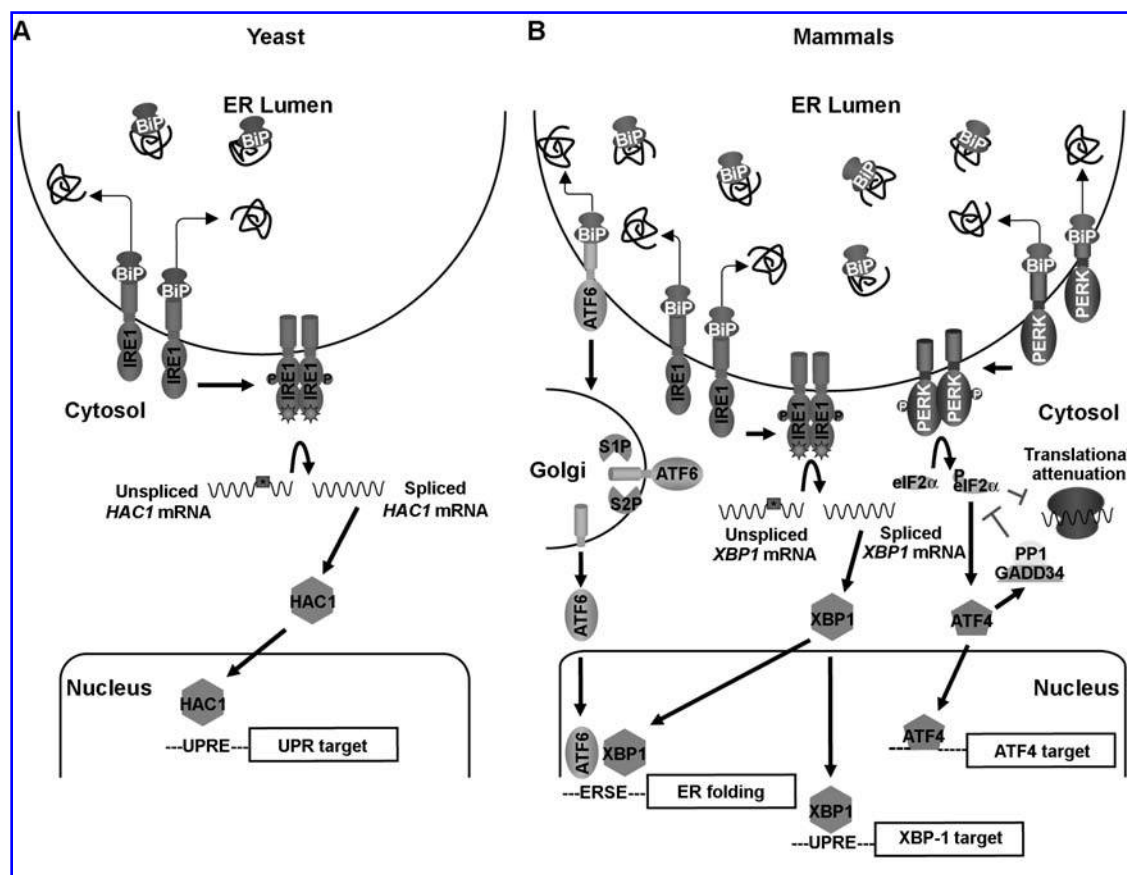


FIG. 1. The yeast and mammalian unfolded protein response (UPR). (a) In yeast, ER stress leads to dimerization and autophosphorylation of the transmembrane sensor Ire1p, presumably as a result of Kar2p/BiP dissociation. Activated Ire1p cleaves a 252-bp intron from *HAC1* mRNA, resulting in translation of a more active, spliced transcription factor Hac1p. Hac1p binds to UPR elements (UPREs) of target genes, resulting in transcriptional upregulation of >380 genes, including those involved in ER folding and ERAD. (b) In contrast, the mammalian stress response is much more complex, involving three transmembrane sensors, ATF6, IRE1, and PERK. Activation of the mammalian UPR leads to (1) transcriptional upregulation of folding machinery, (2) transcriptional upregulation of ERAD machinery, and (3) general translational attenuation. Similar to yeast, ER stress is sensed through the preferential binding of chaperones (BiP) to misfolded proteins, leading to activation of the transmembrane sensor and transmission of the stress signal across the ER membrane. Proteolytic cleavage of ATF6 in the Golgi releases the transcription factor that upregulates ER folding machinery in an attempt to lessen the load on the ER. IRE1 activation results in alternative slicing of the transcription factor XBP-1, which upregulates not only folding machinery, but also ERAD machinery to degrade misfolded proteins. PERK activation results in the phosphorylation of eIF2 α , inhibiting general translation but leading to the translation of the transcription factor ATF4, which also upregulates folding machinery. The three pathways are elegantly coordinated to allow a temporary general decrease in protein synthesis to prevent further burdening the ER, whereas transcriptional upregulation of UPR target genes results in increased folding capacity and ERAD machinery for clearing of misfolded proteins.

In higher eukaryotes, the UPR is more diverse and sophisticated in both the mechanisms of detecting ER perturbations and the broad spectrum of responses elicited. Similar to the mechanism in yeast, IRE1 activation in mammalian cells results in the endonucleolytic cleavage of a 26-nucleotide intron, causing a frame-shift in the translation of the transcription factor XBP-1, a bZIP transcription factor X-box DNA-binding protein and functional equivalent of Hac1p (10, 107, 130, 132). XBP-1, like Hac1p, is a potent transcriptional activator that increases the expression of a subset of UPR-activated genes (75).

PERK

The second UPR signal transducer is protein kinase-like ER kinase (PERK/PEK/EIF2AK3), a type-I transmembrane ER protein that is highly expressed in professional secretory cells where ER stress is anticipated (Fig. 1B). ER stress leads to activation of the cytosolic kinase domain of PERK, which rapidly and reversibly phosphorylates the translation-initiation factor eIF2 α , resulting in tight binding of phosphorylated eIF2 α to the guanine nucleotide exchange factor eIF2B, inactivating eIF2 α , and reducing initiation of general translation (109). This arm of the UPR acts to minimize the synthesis of proteins entering the ER to avoid exacerbating ER stress.

Ironically, PERK activation and the resulting eIF2 α phosphorylation responsible for diminished general translation also cause the selective increased translation of a subset of genes, including the transcription factor ATF4, an effect involving two short open reading frames (ORFs) upstream of the ATF ORF. ATF4, in conjunction with XBP-1, in turn up-regulates the expression of specific UPR-activated genes, one third of which require eIF2 α phosphorylation for their induction (22). Translation of ATF4 allows continued activation of genes, such as the ER resident Hsp70 chaperone BiP (heavy-chain binding protein, also known as GRP78) and the proapoptotic transcription factor CHOP (C/EBP homologous protein), under conditions of ER stress when general protein synthesis is inhibited (40, 104).

Although yeast lack a direct equivalent of PERK in the UPR pathway, a similar mechanism for translation inhibition exists during times of stress, such as amino acid starvation. The kinase Gcn2p phosphorylates eIF2 α , inhibiting protein synthesis and specifically enhancing translation of the ATF4 homolog Gcn4p, which activates the transcription of >500 genes (21, 50, 86). As with ATF4 regulation, GCN4 expression involves short ORFs within the mRNA. The role of Gcn4p and its activator Gcn2p has now been expanded to include the yeast UPR, as both these proteins and Hac1p are required for the upregulation of the majority of UPR target genes during ER stress (94).

ATF6

A third sensor of ER stress, which like PERK is unique to metazoans and higher eukaryotes, is activating transcription factor 6 (ATF6), a type-II membrane protein resident in the ER with a luminal sensor domain and a cytosolic bZIP transcription-factor domain (see Fig. 1B) (44, 130). During ER stress, ATF6 is transported from the ER to the Golgi, where it is proteolytically cleaved by S1P and S2P proteases (126), which are

also responsible for the cleavage of the ER membrane transcription factor SREBP (sterol response element-binding protein) (103, 121). Release of the ATF6 cytosolic fragment permits the transcription factor to localize to the nucleus and, in cooperation with the general transcription factor NF-Y, to activate transcription of target UPR genes (44, 131).

DETECTION OF MISFOLDED PROTEINS AND ER STRESS

ER stress sensors may detect misfolded proteins by either binding misfolded protein directly or alternatively through an interaction with an ER chaperone capable of binding misfolded proteins (see Fig. 1B). It has been proposed that IRE1 is maintained in an inactive state by binding of the ER chaperone BiP to the luminal unfolded protein sensor domain of IRE1 (3). Under conditions of ER stress and the presence of unfolded proteins, BiP may preferentially bind to misfolded proteins, releasing IRE1, and allowing IRE1 oligomerization and activation. PERK is also maintained in an inactive state by luminal BiP binding, as PERK is normally found bound to BiP, and this association is lost during ER stress (3). Although only sharing weak similarity in primary sequence, the luminal domains of IRE1 and PERK may function similarly in detecting ER stress. A chimeric protein generated by swapping the luminal domain of PERK with the IRE1 luminal domain maintained the ability to transmit the stress response (3). During ER stress, both activated IRE1 and PERK are found as oligomeric complexes that lack BiP (3), making a strong argument for the competition mechanism in sensing ER stress.

Recent work in yeast suggests that BiP release may not be the sole mechanism of sensing misfolded proteins and activation of IRE1 (16, 69). First, deletion of the BiP-binding region of Ire1p did not affect the ER stress-sensing and signaling ability of Ire1p (69). Second, new work by Credle and colleagues (16) identified a groove in yeast Ire1p, formed by α -helices, that is similar in structure to the peptide-binding domains of major histocompatibility complexes (MHCs). Mutation of side chains that face into the groove resulted in a reduced UPR response, suggesting that this groove may be responsible for sensing misfolded proteins in the ER lumen. The authors suggested that the stress response could be conveyed by direct binding of a misfolded protein, bringing Ire1p monomers in close proximity in the membrane and promoting oligomerization and trans-autophosphorylation. However, more recent work by Zhou *et al.* (137) suggests that the corresponding groove in human IRE1 is too narrow to accommodate an unfolded peptide, and the groove is oriented so that it faces the ER membrane instead of the ER lumen, making the groove inaccessible to misfolded proteins. Zhou *et al.* support the original proposal that BiP binding may keep IRE1 in an inactive state, and release from BiP allows IRE1 oligomerization and activation.

Residency of ATF6 in the ER may also be mediated by BiP binding, as loss of this binding correlates with trafficking of ATF6 to the Golgi (108). Accumulation of misfolded proteins in the ER lumen may cause dissociation of BiP, unmasking the two Golgi localization signals of ATF6, allowing transport of

ATF6 from the ER to the Golgi. Given the BiP binding of ATF6, it is most likely that ATF6 does not bind misfolded proteins directly, but instead is activated on BiP recruitment away from ATF6 during perturbations in ER homeostasis. In addition to masked Golgi signals, the glycosylation state of ATF6 may also play a role in its retention in the ER, preventing its transport to the Golgi and subsequent activation (53). Mutation of Asn-linked glycosylation sites in ATF6 made ATF6 a poor substrate for the ER lectin chaperones calnexin and calreticulin, leading to trafficking of ATF6 to the Golgi. A loss of ER homeostasis would likely perturb the ER glycosylation machinery, resulting in the underglycosylation of proteins, including ATF6, and this may provide an additional mechanism for sensing the protein-folding status of the ER.

TIMING, COORDINATION, AND REGULATION OF THE UPR

The multiple responses of the UPR to perturbations in the ER allow the UPR to serve as a rheostat with a graduated response to cope with varying degrees of stress. Initially, ER stress results in general translational attenuation, because of eIF2 α phosphorylation, to alleviate the protein load of the ER. After PERK phosphorylation of eIF2 α , ATF6 is an efficient and rapid mediator of a transcriptional response, as production of an active transcription factor simply involves proteolytic cleavage of an existing protein. ATF6, in cooperation with the transcription factor NF-Y, binds to ER stress-response elements (ERSE) and ERSE-II cis-acting elements in the promoters of target genes (124, 132). Target genes upregulated by ATF6 include ER molecular chaperones and folding factors, to aid folding effectively (40). Continued stress results in activation of the IRE1/XBP-1 pathway and upregulation of target genes, many of which are also upregulated by ATF6. XBP-1 not only binds with NF-Y to ERSEs, but also, independent of NF-Y, to UPRES, resulting in increased expression of folding machinery and ERAD machinery, such as ER degradation-enhancing α -mannosidase-like protein (EDEM) (132, 133). ERAD machinery is functional in the absence of ER stress, but UPR activation would enhance the capacity of ERAD. The resulting response from IRE1/XBP-1 is somewhat delayed in comparison with the ATF6 response, as XBP-1 activation requires splicing and translation to produce an effective transcription factor (132, 133). However, IRE1/XBP-1 activation allows a sustained response, as XBP-1 also contains an ERSE and may transactivate itself (10, 132).

PERK activation results in general translational inhibition to alleviate protein load in the ER, whereas ATF6 and XBP-1 activation results in transcriptional upregulation of target genes responsible for protein folding and ERAD. However, PERK activation also results in the ATF4-dependent upregulation of genes involved in amino acid import, glutathione biosynthesis, and oxidative stress resistance (41). Herp (homocysteine-induced endoplasmic reticulum protein), which contains an ERSE and ERSE-II, encodes an ER membrane protein that contains an ubiquitin-like domain with unknown function and is highly induced by all three pathways of the UPR (78). CHOP/GADD153 also contains an ERSE and is activated by all three

UPR pathways (77). CHOP is responsible for induction of cell death if ER stress and the UPR are not moderated in a timely fashion (138). Therefore, the UPR must be regulated, and mechanisms to "shut off" the response are essential for cell survival.

Studies in yeast suggest some mechanisms for downregulation of the UPR. Dephosphorylation of activated Ire1p by the phosphatase Ptc2p (123) or by the dose-dependent cell-cycle regulator 2 (DCR2) (38) may serve as a regulatory mechanism of the UPR. In addition, the half-life of Hac1p is relatively short, ~ 1.5 min, which may allow fine-tuning of the UPR response (13, 67). Hac1p has a PEST degron, and degradation of Hac1p requires the presence of a functional nuclear localization signal and ubiquitin conjugating Ubc3/Cdc34p and the ubiquitin ligase SCF^{Cdc4} complex (92). A similar mechanism in mammalian cells has yet to be identified, although mechanisms of attenuating IRE1 signaling have been suggested.

Inhibition of general translation is a temporary approach to alleviate transient stress but is obviously not a long-term solution. Continued PERK activation leads to cell-cycle arrest and eventually cell death. Therefore, PERK phosphorylation of eIF2 α must be regulated. In addition, ATF4-directed expression of GADD34 targets type 1 protein phosphatase (PPI) to dephosphorylate eIF2 α , facilitating a rapid restoration of translation (136). CHOP expression also leads to the dephosphorylation of eIF2 α by directly activating GADD34 (52). PERK is directly inhibited by p58^{IPK}, which is also a target of ATF6 and XBP-1 (125). Accumulated transcripts of ATF6 and XBP-1 targets can be translated efficiently once PERK translational inhibition is removed.

Recent work suggests that the proapoptotic Bcl-2 family members BAX and BAK play an essential role in the adaptation response to ER stress through modulation of IRE1 to XBP-1 signaling (48). By using a conditional BAX-BAK double knockout (DKO) model system, researchers demonstrated that BAX-BAK DKO cells exhibited a similar phenotype to IRE1-deficient cells with diminished IRE1 signaling, XBP-1 expression, and expression of XBP-1 target genes. The cytosolic domain of IRE1 was shown to associate with BAX and BAK, and the association of IRE1 with BAX increased under ER stress conditions. The BH3 and BH1 domains of BAK, which control its proapoptotic activity through interactions with other BCL-2 protein partners, were required for the interactions with IRE1. In BAX-BAK DKO cells, more BiP associated with IRE1, preventing dimerization and autophosphorylation of IRE1. Taken together, these data suggest that BAX and BAK bind to the cytosolic domain of IRE1 and potentially stabilize its active state, thereby playing an essential role in the cell's stress response.

BAX and BAK have also been found to oligomerize to form a pore at the ER membrane, leading to Ca²⁺ efflux from the ER and UPR-induced caspase activation (139), suggesting that signaling from the ER leads to apoptosis induction. Moreover, several signals resulting from UPR activation can lead to cell death. The cytosolic domain of IRE1 binds to TRAF2, leading to JNK activation and cell death (119). TRAF2 couples IRE1 to several other proteins within the cell, such as caspase-7 and caspase-12 (129). In addition, CHOP, which is induced by all three UPR pathways, represses the expression of antiapoptotic Bcl-2, sensitizing cells to ER stress and resulting in cell death (81). Last, repetitive attempts at folding may lead to reactive

oxygen species (ROS) release and subsequently apoptosis (43), as explained later, demonstrating why upregulation of ERAD and elimination of misfolded proteins from the ER is essential for cell survival during times of stress.

ELIMINATION OF MISFOLDED PROTEINS BY ERAD

A protein that fails to achieve the correct conformation in the ER exits the folding cycle and is targeted for degradation. These misfolded or nonnative proteins are engaged by ERAD, which involves many components that recognize aberrant proteins and implement their retrotranslocation to the cytosol before degradation by the ubiquitin-proteasome system (23, 74, 80). In eliminating terminally misfolded proteins, ERAD has the capacity to reduce ER stress directly in a manner that may not be possible by simply increasing chaperone or folding-machinery levels. ERAD must have the ability to dispose of the multitude of soluble, membrane-spanning, and lipid-anchored proteins that enter the ER. The precise mechanisms for misfolded substrate recognition, engagement, and retrotranslocation from the ER remain unclear. However, a decade of research in *S. cerevisiae* contributed significantly to the current understanding of the mechanisms of ERAD. As the ERAD machinery in higher eukaryotes likely involves analogous components, our primary focus will be on recent advances from studies in yeast.

Recognition of ERAD substrates

Recognition of misfolded proteins in the ER is the first step in their removal, and a number of factors may participate. Given that models of UPR activation are based on the recruitment of the chaperone Kar2p/BiP/GRP78 from the UPR sensors to misfolded proteins, it seems inherent that this chaperone may play an early role in ERAD. BiP is considered to bind exposed hydrophobic patches of newly synthesized proteins and assist in preventing aggregation of misfolded substrates (88). BiP releases the protein, through an ATP-dependent mechanism, allowing the protein to attempt to fold (8). Protein disulfide isomerase (PDI) is another chaperone that aids in folding in the ER and is involved in the oxidation, reduction, and isomerization of disulfide bonds (25). PDI also has chaperone characteristics similar to BiP in unfolding proteins. However, the release of PDI is not ATP dependent, but rather dependent on its redox state (117).

In addition to aiding in nascent protein folding, BiP and PDI may play important mechanistic roles in the recognition and engagement of misfolded proteins by ERAD, ranging from preventing aggregation of misfolded substrates (62, 88) to participating in returning ERAD substrates to the retrotranslocation machinery (see later) (61, 96). PDI and its family members may also play a role in presenting substrates to the retrotranslocation machinery or in reducing disulfide bonds before retrotranslocation (32, 117).

The site of the lesion of the misfolded protein may influence which chaperone(s) are involved in recognition and targeting. Degradation of proteins with luminal lesions are near universally dependent on the luminal chaperone BiP, whereas degra-

dation of substrates with cytosolic mutations is dependent on the cytosolic SSA family of Hsp70 chaperones (49, 95, 114).

It is apparent that other ERAD components may play a role in misfolded substrate recognition, based on the properties of the misfolded substrate. Misfolded glycoproteins with luminal mutations may be presented by the luminal ER putative lectin Htm1p/Mnl1p (EDEM in mammals) or Yos9p or both (4, 9, 58, 68, 82, 85, 89, 113). Htm1p/Mnl1p is a mannosidase-like protein that lacks the critical catalytic residue and mannosidase activity, but has been proposed to act as a lectin that targets *N*-glycosylated proteins for ERAD (58, 85). Another luminal ER lectin with homology to the mannose-6-phosphate receptor, Yos9p, also recognizes luminally misfolded *N*-linked glycoproteins and assists in ERAD targeting (4, 9, 30, 68, 113). Interestingly, the lectin domain of Yos9p is required for degradation of substrates by ERAD but is not required for substrate binding or recognition (4). Moreover, Yos9p is capable of binding to a misfolded protein that lacks *N*-glycosylation (4). These data suggest that substrate recognition by Yos9p involves querying both the folding and glycosylation status of the substrate. Weissman and co-workers (118) propose that although carbohydrate binding is not necessary for substrate recognition, it may be necessary for interaction with downstream ERAD components. Alternatively, Yos9p may also use the sugar status of the protein to determine its folding status in a manner similar to that of calnexin and calreticulin.

In higher eukaryotes, the lectin-like chaperones calnexin (CNX) and calreticulin (CRT) aid in folding, in what is referred to as the calnexin/calreticulin cycle (46). Although CNX and CRT are homologous and have glycoprotein-binding abilities, they bind to distinct substrates. The *N*-linked glycosylation of nascent glycoproteins is initiated in the ER, and oligosaccharide chains added to Asn residues are modified before the glycoprotein exits the ER. After attachment of the initial oligosaccharide chain, Glc₃Man₉GlcNAc₂ trimming by glucosidase I and II produces a glycoprotein with the oligosaccharide Glc₁Man_{9,6}GlcNAc₂, which is bound by CNX/CRT (47, 93). Bound to CNX/CRT, the glycoprotein undergoes folding assisted by other folding machinery, such as the PDI homologue Erp57, an oxidoreductase that promotes disulfide-bond isomerization (28, 93, 98). The terminal glucose is then removed by glucosidase II, and the glycoprotein is released from the CNX/CRT cycle. Uridine diphosphate (UDP)-glucose/glycoprotein glucosyl transferase (GT) monitors the folding state of the released glycoprotein (116). If the native conformation has not been achieved, GT reglucosylates the folding intermediate, which is reengaged by CNX/CRT for another cycle (45). Once the correct conformation has been achieved, the glycoprotein is released from the CNX/CRT cycle for transport from the ER. Although CNX and CRT bind specifically to glycoproteins, binding to unglycosylated proteins has been observed as well, similar to unglycosylated protein binding by Yos9p (55, 102). Glycoproteins that fail to fold are eventually removed from the CNX/CRT cycle and targeted for ERAD. It has been proposed that mannose trimming to produce Man₆GlcNAc₂ and Man₅GlcNAc₂ prevents the misfolded glycoprotein from the re-entering the CNX/CRT cycle and commits the substrate to degradation by ERAD (26, 27, 72). Mannose trimming has also been shown to target misfolded glycoproteins for ERAD in yeast, although yeast lack the CNX/CRT cycle (140).

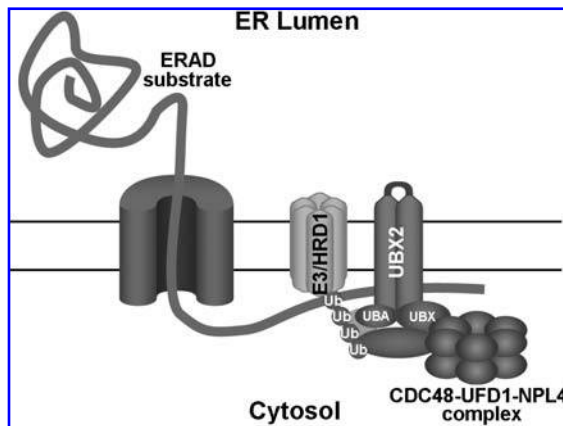


FIG. 2. Protein dislocation from the yeast ER involves Ubx2p-dependent recruitment of the Cdc48–Ufd1–Npl4p complex to the ER membrane. As misfolded substrates are retrotranslocated from the ER lumen, they are ubiquitinated by a ubiquitin ligase (E3). The ubiquitinated substrate is likely engaged by the ubiquitin-associated (UBA) domain of Ubx2p, as the substrate emerges from the ER. The Cdc48–Ufd1–Npl4p complex is recruited to the ER membrane *via* interaction with the ubiquitin regulatory X (UBX) domain of Ubx2p, which provides a bridge linking the ubiquitinated substrate and the dislocation machinery.

Retrotranslocation/dislocation, ubiquitination, and degradation

Misfolded substrates must be retrotranslocated from the ER before degradation in the cytosol. The mechanisms for retrotranslocation are widely debated. The Sec61p translocation pore through which nascent polypeptides enter the ER has long been suggested to function as both the retrotranslocation channel and the initial translocation pore (33, 97). Although a number of structural and genetic studies support this role, an equally convincing number of studies suggest otherwise. Other candidates for pore components include the multimembrane-spanning ubiquitin ligases Hrd1p, comprising six membrane-spanning

domains, and Doa10p, with 10 to 14 membrane-spanning domains (18, 31, 112). Another contender is Der1p, which has four membrane-spanning domains (51, 76, 128). Although the function of Der1p is unknown, it is required for the degradation of many but not all ERAD substrates (73). A mammalian Der1p homologue, Derlin-1, has been found in complex with VIMP (p97-interacting protein) and the cytosolic ATPase p97 (Cdc48 in yeast), and this complex interacts with retrotranslocating substrates (76, 128). These recent studies provide persuasive evidence that alternate membrane proteins may serve as components of the retrotranslocation pore, and perhaps the composition of the pore is substrate dependent.

Although soluble misfolded proteins require a pore to gain access to the cytosol, multimembrane-spanning substrates typically have domains already in the cytosol that may be degraded independent of retrotranslocation. Ste6-166p is degraded independent of Sec61p or Der1p, and instead, the proteasome may simply proteolytically remove cytosolic loops of Ste6-166p, leaving the remaining membrane and luminal fragments to be retrotranslocated independent of Sec61p or to be degraded by an alternative protease (54).

Misfolded substrates are polyubiquitinated before degradation by the 26S proteasome. Addition of ubiquitin to misfolded substrates occurs through a sequence of enzymatic reactions. A ubiquitin-activating enzyme (E1) first activates the ubiquitin, which is transferred to a ubiquitin-conjugating enzyme (Ubc or E2). Ubiquitin is then attached to lysine residues of the substrate by a ubiquitin protein ligase (E3). Several E3 enzymes participating in ERAD have been identified in yeast. Hrd1p is a transmembrane protein with a cytosolic RING-H2 face that is stabilized by association with Hrd3p, a transmembrane protein with a large luminal domain (1, 6, 39). Hrd1p can interact with either E2 enzyme, Ubc1p or Ubc7p (1, 29). Ubc7p is a soluble enzyme that associates with the ER through interaction with Cue1p (5). Doa10p is an alternative E3 ER-associated membrane protein that relies on the E2 enzymes Ubc6p and Ubc7p (112). A third E3 involved in ERAD, Rsp5p, uses the E2 enzymes Ubc4p and Ubc5p (34, 42, 71). A number of mammalian E3s have been implicated in ERAD, including gp78 (24), the Fbs1/Fbs2 complex (134), and two E3s, membrane-

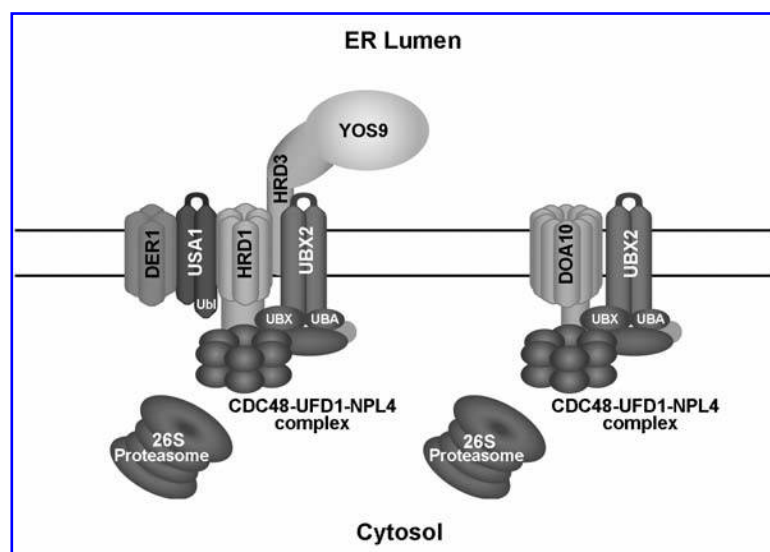


FIG. 3. Misfolded substrates are engaged by specific ERAD complexes. Degradation of lumenally misfolded proteins requires communication between the ER lumen and the cytosol. Association of yeast luminal (Yos9p), transmembrane (Hrd3p, Ubx2p, Hrd1p, Usa1p, and Der1p), and cytosolic (the Cdc48–Ufd1–Npl4p complex) ERAD components provides the linkage between ER lumen and cytosol necessary for efficient degradation of the substrates. In contrast, recognition and targeting of cytosolically misfolded substrates is accomplished by a network of transmembrane (Doa10p and Ubx2p) and cytosolic (the Cdc48–Ufd1–Npl4p complex) components.

associated RMA1 and cytosolic CHIP, that act sequentially to degrade CFTR (26, 135). Successive ubiquitin molecules are added by these E3s, and the polyubiquitinated ERAD substrate is engaged and subsequently degraded by the 26S proteasome.

Dislocation from the ER membrane and likely unfolding before degradation is aided by the Cdc48–Ufd1–Npl4p complex (59, 99, 127). Cdc48p is a cytosolic AAA ATPase protein that associates with the ER membrane during the extraction of misfolded proteins. Cdc48p participates in a number of cellular functions, and its specificity appears dictated by differing associated factors. Recently Ubx2p (Sel1p) was found to recruit Cdc48p to the ER membrane (87, 106). Ubx2p is an ER transmembrane protein that contains both a cytosolic ubiquitin-associated (UBA) domain and a cytosolic ubiquitin-regulatory X (UBX) domain, which is responsible for the interaction with Cdc48p (87, 106). Ubx2p mediates the interaction between the Cdc48–Ufd1–Npl4p complex and the ubiquitin ligases Hrd1p and Doa10p (87, 106). Moreover, Ubx2p mediates the interaction of Cdc48p with ERAD substrates (106). The UBA domain of Ubx2p may function in the dislocation of ERAD substrates from the membrane, by associating with the ubiquitinated substrate (87). The absence of Ubx2p results in delayed degradation of ERAD substrates, likely because of the loss of Cdc48p recruitment to the ER (106). Therefore, a model for dislocation of misfolded proteins has emerged in which an E3 enzyme polyubiquitinates a retrotranslocating misfolded protein, which is engaged by the UBA domain of Ubx2p (Fig. 2). Ubx2p recruits the Cdc48–Ufd1–Npl4p complex *via* the UBX domain, providing a link between misfolded proteins, the E3, and the Cdc48 complex for substrate dislocation. Loss of Ubx2p reduces membrane association of Cdc48p by ~50% (106), raising the question whether other ERAD components are also sufficient to recruit the Cdc48 complex to the ER membrane.

ERAD components associate into substrate specific complexes

Recent work has added significantly to our understanding of the mechanisms of ERAD by linking several ERAD components in pathways specific to different types of misfolded substrates (Fig. 3). Denic and colleagues (19) used FLAG-tagged Yos9p and found Yos9p in complex with Kar2p, Hrd3p, Hrd1p, Ubx2p, and Usa1p. This complex is composed of luminal, membrane-spanning, and cytosolic components, providing a mechanism for communication between the ER lumen and the cytosol. Interestingly, Der1p and Htm1p were not found, and their absence did not affect the composition of the complex. However, other key interactions were found to be important for complete complex assembly. Yos9p and Kar2p were found to associate independent of other complex members in a luminal subcomplex. Yos9p was also shown to interact specifically with the luminal domain of Hrd3p, as deletion of the cytosolic domain of Hrd3p had no effect. Hrd3p was demonstrated to bridge Yos9p and Hrd1p, which interacts with Ubx2p and brings the cytosolic Cdc48p in complex with luminal Yos9p. Both Yos9p and Hrd3p recognize misfolded substrates independently, implying that Hrd3p serves as more than a scaffold for the interaction between Yos9p and Hrd1p. Two groups suggest that the Yos9p/Hrd3p subcomplex surveys substrates before the commitment to degradation to prevent aberrant activity of

Hrd1p and the degradation of proteins that should not be condemned to ERAD (19, 30). Yos9p was demonstrated to recognize substrates independent of their glycosylation status, as previously reported, raising an intriguing question regarding the nature of the signal on misfolded proteins that is recognized by Yos9p.

Carvalho *et al.* (11) identified two independent complexes dedicated to the degradation of either lumenally or cytosolically misfolded substrates (Fig. 3). TAP-tagged Doa10p was found in complex with Ubc7p, Cue1p, Ubx2p, Cdc48p, and Npl4p. This complex is suggested specifically to recognize and target misfolded proteins with cytosolic mutations. An Hrd1p-dependent complex is suggested to target substrates with luminal mutations. TAP-tagged Hrd1p was found in complex with Der1p, Yos9p, Hrd3p, Ubx2p, Cdc48p, and Usa1p. Specific interactions were also identified as necessary for complex formation, such as the requirement of Hrd3p for the interaction between Hrd1p and Yos9p.

Usa1p is a membrane-spanning protein of unknown function that is highly upregulated by the UPR (115). Usa1p spans the membrane twice with both the N-terminal ubiquitin-like (UBL)-containing domain and the C-terminal domain on the cytosolic face of the ER. Usa1p is required to recruit Der1p to Hrd1p, as deletion of Usa1p resulted in loss of the interaction between Hrd1p and Der1p (11). In addition, Usa1p is required for the efficient degradation of misfolded luminal substrates. The authors suggest that mammalian Herp, although not similar in sequence, may be a functional homologue. Both Usa1p and Herp have a UBL domain and Herp can restore the degradation of misfolded CPY* in a *USA1* deletion (11).

Interestingly, neither Carvalho *et al.* (11) nor Denic *et al.* (19) found Sec61p in their complexes. These recent studies provided additional insight into the coordination of the components of ERAD. Another interesting approach would be to identify complexes associated with known ERAD substrates.

ERAD GENES UPREGULATED BY THE UPR

Genomic analyses of genes transcriptionally upregulated by the UPR have been performed in the simpler IRE1-based yeast system. Interestingly, not all ERAD genes appear to be transcriptionally upregulated by the UPR. The microarray data of two groups (70, 115) found *DER1*, *HRD1*, and *UBC7* upregulated but not *YOS9* or *HTM1/MNL1*. Does this suggest that the upregulated ERAD genes (*DER1*, *HRD1*, and *UBC7*) are the critical or rate-limiting ERAD factors or, alternatively, that the ERAD genes *YOS9* and *HTM1/MNL1* are functionally upregulated by other means? The assumption is that the UPR-dependent upregulation of these ERAD components increases the ERAD capacity of the cell, although this has never been directly tested.

Transcriptional profiling of ER-stressed mammalian cells has helped identify which of the three UPR signal-transduction arms (IRE1, PERK, and ATF6) upregulates specific target genes and found that EDEM (the homologue of HTM1/MNL1) is upregulated by XBP-1 (75).

An additional issue is that overexpression of different indi-

vidual ERAD components has been seen to increase degradative rates of certain misfolded proteins. In these cases, why does the overexpression of any one component increase turnover rates, as each component cannot be the rate-limiting reaction? Alternative explanations involve that either overexpression of ERAD components may itself perturb the ER and induce the UPR, or that the individual ERAD components studied work as alternative parallel pathways, and therefore, overexpression of either one will increase turnover rates.

DECREASING ER STRESS BY REDUCING THE PROTEIN LOAD IN THE ER

In addition to the increased expression of chaperones and ERAD machinery and the PERK-dependent downregulation of general translation, other mechanisms are used by the cell to reduce the level of misfolded proteins and associated stress in the ER.

Selective degradation by IRE1 of a subset of nascent mRNAs localized to the ER

To determine whether the full metazoan IRE1 signal is transduced solely through XBP-1, researchers compared the changes in microarray expression profiles associated with the UPR in *Drosophila* S2 cells with reduced expression of either IRE1 or XBP-1 by RNAi (52). Surprisingly, activation of the UPR repressed a subset of genes in an IRE1-dependent but XBP-1-independent manner. This subset of repressed genes encoded secretory proteins but did not include genes required for ER function. These findings suggested an IRE1-dependent but XBP-1-independent process, responsible for a reduction in mRNAs of ER-targeted proteins, that would reduce the protein cargo load of the ER during stress. The selective repression was not due to decreased transcription of the genes, but rather to decreased mRNA stability. Hollien and Weissman (52) found that the mRNAs were cleaved by an endonucleolytic mechanism that was dependent on the signal sequence that targets the nascent protein for translocation into the ER. Removal of the signal sequence and mutations in hydrophobic residues that perturbed the signal-sequence function were sufficient to abolish degradation of these mRNAs. In addition, frame-shift mutations altered the degradation of the mRNAs, suggesting that correct translation of the polypeptide is required for selective degradation of the mRNA.

Because of the necessity for an intact signal sequence, it appears that the IRE1-dependent mRNA degradation occurs during cotranslational translocation into the ER. This finding suggested that IRE1 may be directly responsible for the selective degradation (52). Alternatively, activated IRE1 may recruit another ribonuclease or may be responsible for stalling the translation of the mRNA, resulting in mRNA decay. An attractive model is that translating ribosomes, associated with the ER membrane because of the nascent signal sequences, are in close vicinity to the RNase domain of activated IRE1, leading to the selective degradation of mRNA for ER-targeted proteins (Fig. 4). No evidence exists that yeast exhibit this feature, although the short half-lives of yeast mRNAs may have masked such a process.

The IRE1-dependent repression of targeted genes occurs relatively rapidly in comparison to XBP-1-dependent responses, which occur ~2 h after induction of the UPR (52). This newly identified UPR pathway of RNA degradation provides cells with an advantage when dealing with an increased ER load during ER stress. When the UPR is induced, the transcriptional activation of additional ER folding and degradation machinery can increase the load on the ER. By selectively degrading ER-targeted mRNAs for proteins that are not necessary for ER function, the cell can offset this effect. By vacating machinery required for translation and translocation into the ER, the cell is better equipped to deliver the chaperones and ERAD components whose genes have been transcriptionally upregulated by the UPR. If specific sequences for cleavage by Ire1 are contained within these targeted mRNAs, then they must be absent from the chaperone/ERAD mRNAs upregulated by the UPR.

Preemptive quality control

The previously observed variability in signal sequences may play a direct role in regulating the biosynthetic load in the ER during conditions of stress, distinct from mRNA degradation (64). Ribosomes synthesizing cargo proteins destined for the ER are delivered to the ER membrane, but during ER stress, the proteins frequently fail to be translocated. Conversely, chaperones such as BiP continue to be translocated efficiently, and this "preemptive quality control" has been attributed to variability in signal sequences, with signal sequences sensitive to this translocation failure/blockage termed "regulated." Translocation of cargo proteins with regulated signal sequences was found to require luminal factors, possibly BiP, whose availability is limited when the ER is stressed. Thus the stress-induced shortage of chaperones in the ER likely provides the mechanism for reduced entry of cargo proteins into the ER. The translocation of chaperones proceeds independent of luminal factors and thus continues during stress. Although this mechanism has outward similarities to that of IRE1-dependent degra-

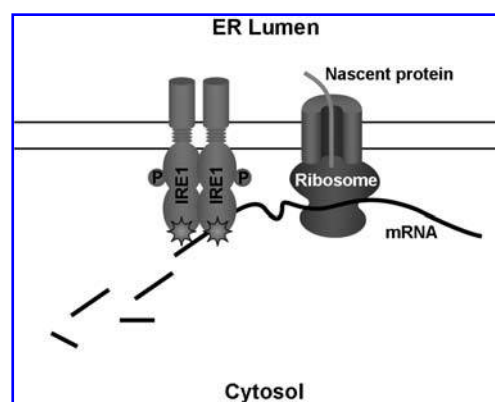


FIG. 4. Secretory protein mRNAs are selectively degraded by the ER transmembrane stress sensor IRE1 during ER stress. As a subset of nascent proteins are translocating into the ER, IRE1 mediates the endonucleolytic cleavage of the translating mRNA. This cleavage results in the degradation of the secretory protein mRNA, thereby decreasing the protein load in the ER during ER stress.

dation of mRNA, as discussed earlier, the distinction is that this mechanism acts on translated protein, not mRNA.

What is the fate of these cargo proteins rejected by the pre-emptive quality control? The dependence of the regulated signal sequences on luminal factors suggests that these proteins must initially engage the translocon, yet are unlikely to be exposed to ERAD. Instead, p58^{IPK}, a protein induced by the UPR, has been found to play a role in the extraction of nascent polypeptides before their degradation by the proteasome (91) and is a good candidate to facilitate the removal of these rejected cargo proteins in cooperation with cytosolic chaperones. The importance of the protective role of p58^{IPK} was underscored by the dramatic loss of pancreatic islet mass in p58^{IPK}^{-/-} mice when exposed to ER stress (91).

Upregulation of ER export

Other genes upregulated by the UPR in yeast include those involved in forward transport from the ER, including the ER-exit cargo receptor Erv29p (115). Is this simply a response to hasten the exit of correctly folded proteins from the ER? Contrary to a common view, some misfolded proteins are not maintained in the ER and instead traffic from the ER. Recent work in yeast demonstrated that ER exit of misfolded proteins may result from the continued presence of intact ER-exit signals within misfolded proteins, thus allowing interaction of misfolded proteins with ER-exit cargo receptors or COPII components (Fig. 5) (71). These data suggest that some misfolded proteins may still possess functional ER-exit signals, and upregulation of membrane trafficking from the ER would effectively reduce ER stress by transporting these misfolded proteins to distal compartments.

Upregulation of autophagy to remove “excess” ER after UPR activation

Ultrastructural EM examination of the yeast ER after UPR activation found the ER to increase in volume at least fivefold (2), the effect of which is to reduce the concentration of unfolded proteins. After prolonged UPR activation, some cells also displayed autophagosome-like structures containing ER-derived membrane stacks. Further analysis found that some autophagy genes are upregulated by ER stress and that cell survival is dependent on a functional autophagy pathway in the face of acute ER stress (2). The authors speculate that such disposal of the ER might be to control ER size and hence ER homeostasis, or alternatively, that potentially toxic aggregates are segregated into ER subdomains that are subsequently removed by autophagy.

OXIDATIVE FOLDING AND STRESS IN THE ER

Disulfide bond formation in the ER is an essential process involving protein disulfide isomerase (PDI) catalyzing the oxidation of thiols within cargo proteins to form disulfide bonds. PDI can be reoxidized by ERO1, a FAD-binding protein in the ER, from which the electrons are terminally transferred to mo-

lecular oxygen (118). Both PDI and ERO1 are under UPR control (115), so that an increased demand for synthesis of cargo proteins, many of which contain disulfide bonds, is matched by an increased capacity in ER oxidative-folding capacity.

During protein folding, misfolding, or oxidative stress, proteins within the ER may form nonnative disulfides. These must be isomerized or reduced to allow the correct disulfides the opportunity to form, or if an ERAD substrate, probably reduced to decrease steric hindrance during the dislocation process. Glutathione, the main cellular redox buffer, likely plays a role in resolving these nonnative disulfides in the ER. The reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio in the ER is favorable for disulfide bond formation, which leads to the original proposal that GSSG directly provided the oxidizing equivalents for disulfide bond formation. However, the *in vitro* finding that ERO1-PDI mediated the catalysis of disulfide bonds within RNaseA in the absence of either GSH or GSSG (118) strongly suggests that GSSG is not providing the oxidative equivalents, although it does not eliminate GSSG as an alternative mechanism to oxidize PDI. Instead, several lines of evidence support the involvement of GSH in the reduction or isomerization or both of nonnative disulfides within the ER, which form even under nonstressed conditions. A decrease in total glutathione levels correlated with an increase in nonnative S-S bonds (12, 83) while the proportion of PDI in the oxidized form increased, decreasing the capacity of PDI to isomerize nonnative S-S bonds. Therefore, GSH may directly reduce nonnative disulfides or alternatively act indirectly to maintain a portion of the PDI and related oxoreductase family members in a reduced state to catalyze disulfide bond reduction or isomerization.

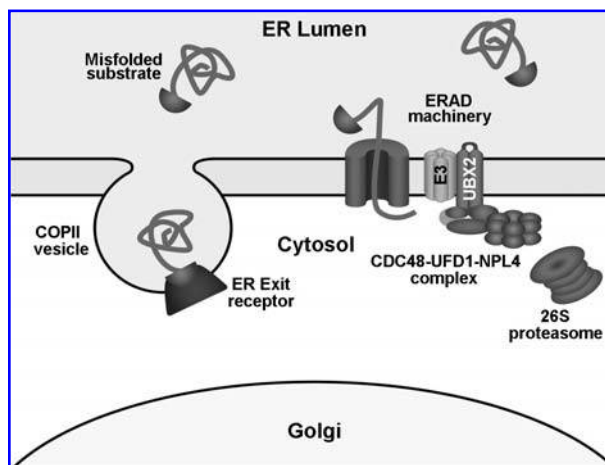


FIG. 5. Upregulation of trafficking machinery by the UPR serves as an additional mechanism to reducing misfolded protein load in the ER. Misfolded proteins are targeted for degradation by ERAD. However, some misfolded proteins maintain the ability to interact with ER exit machinery and incorporate into COPII vesicles exiting the ER. In addition to upregulating folding and ERAD machinery, induction of the UPR upregulates genes involved in forward trafficking (such as the yeast ER exit receptor Erv29p). Upregulation of forward-trafficking machinery may provide an additional mechanism for decreasing the protein load in the ER during ER stress by allowing misfolded proteins with intact ER exit signals to traffic from the ER.

The terminal electron acceptor from ERO1 is molecular oxygen, resulting in the production of stoichiometric amounts of hydrogen peroxide, a membrane-permeable reactive oxygen species (ROS) (37). This places professional secretory cells under oxidative stress during the high-level expression of correctly folded disulfide bond-containing proteins such as insulin or IgG. One mechanism by which mammalian cells have adapted to this oxidative stress involves PERK triggering the general stress response, which increases amino acid metabolism, which in turn can lead to enhanced glutathione production (41).

High-level expression of the misfolded protein CPY* in the yeast ER results in the production of ROS (Fig. 6), a rapid increase in the GSSG/GSH ratio and cell death that is suppressed by the addition of GSH (43). The cytotoxicity does not result from ROS derived from nascent disulfide bond formation within CPY*, as similar expression levels of correctly folded CPY did not generate lethal levels of oxidative stress. Instead, the rapid oxidation of GSH may result from GSH acting directly or indirectly to reduce improper disulfide bonds within misfolded CPY*. Such reductions would result in the consumption of GSH and would return the thiols of CPY* to the reduced form, allowing them to interact again with Ero1p and Pdi1p to be re-oxidized. This futile cycle of disulfide formation and reduction would continue with each cycle, generating ROS and consuming GSH, the principal cellular anti-ROS buffer. The importance of cysteine residues and disulfide-bond oxidation in this process is illustrated by the loss of cytotoxicity when the cysteines of CPY* were replaced with alanines (43). Increased Ero1p expression results in both enhanced ROS levels (43) and a decrease in GSH (17). Therefore, the five- to eightfold up-regulation of Ero1p and Pdi1p by the activated UPR (115) would greatly exacerbate the oxidative stress in the ER by further increasing the rate of ROS production and GSH expenditure to reduce nonnative disulfides. The significance of prolonged UPR activation is demonstrated by the observation that inactivation of the UPR prevented these cells from dying (43), indicating that the UPR, in acting to maintain the functionality of the secretory pathway, does not always act in the best interests of the cell.

Cytotoxic levels of oxidative stress, derived from futile thiol oxidation/reduction cycles of nonnative disulfides, would likely be found in mammalian cells expressing very large quantities of a single misfolded protein containing many disulfide bonds. An analogous situation to CPY* would likely occur with high-UPR activation and saturation of the ERAD machinery. Sev-

eral examples include insulin-secreting pancreatic β cells (101), arginine vasopressin (AVP)-secreting neurons (56), and proteolipid protein (PLP)-secreting oligodendrocytes (36). In all these cases, large quantities of mutant proteins with disulfide bonds are retained in the ER and result in the death of specific cell types. From this perspective, an important function of ERAD is to destroy misfolded proteins so as to avoid inadvertent futile cycles of oxidation/reduction that could result in cytotoxic levels of ER-derived oxidative stress.

ER STRESS, APOPTOSIS, CELL DEATH, AND DISEASE

ER stress and UPR activation have been observed in many human diseases including cancer, diabetes, and a multitude of late onset neurodegenerative diseases, including Alzheimer, Huntington, Parkinson, and familial amyotrophic lateral sclerosis (ALS). It remains a central question as to whether the observed ER stress is the primary cause of the disease or instead a downstream consequence of a different cellular dysfunction.

How might the UPR contribute to disease? One possible avenue is that, by attempting to maintain the function of the secretory pathway, the UPR causes cytotoxic consequences, such as the oxidative stress discussed earlier. Another possibility is that, by acting to assist in cell survival, the UPR might protect cells that endanger the organism, such as those in solid tumors. Hypoxic conditions are commonly experienced by solid tumors and are also known to induce ER stress strongly, likely because of the lowered oxidative folding capacity in the ER when ERO1 lacks sufficient oxygen to act as electron acceptors. The central protective role of XBP-1 in hypoxic conditions is indicated by the robust induction of genes regulated by XBP-1, whereas the loss of XBP-1 sensitizes cells to hypoxia (100). The UPR is strongly implicated in assisting the cells within these tumors to survive this stress, as Xbp1-deficient cells develop into smaller tumors when transplanted into SCID mice (100). Modulating the protective role of the UPR and XBP-1 in these situations could potentially be a good therapeutic target for preventing tumor development.

Prolonged ER stress can trigger apoptosis *via* the UPR to eliminate damaged cells. The manner in which the UPR switches from a protective to apoptotic role is complex, and many questions remain. In essence, the protective PERK path-

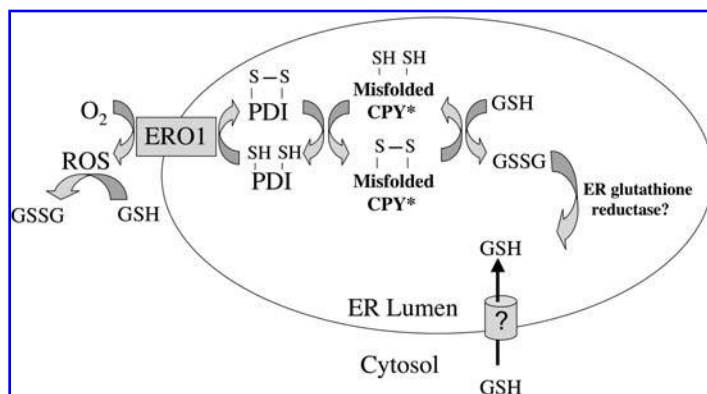


FIG. 6. ER stress-induced oxidative stress. High levels of expression of misfolded CPY* saturate ERAD in yeast. CPY* continues to interact nonproductively with PDI and forms nonnative disulfide bonds in the process. These are broken by glutathione, either directly (as shown) or indirectly *via* PDI (not shown), returning CPY* to the reduced state to allow oxidized PDI to remake the nonnative disulfide bonds. Each turn of this cycle produces ROS *via* ERO1 and depletes the cellular level of the protective GSH.

way is downregulated, whereas the expression of the transcription factor CHOP/GADD153 is upregulated. CHOP is considered proapoptotic, as Chop^{-/-} mice display partial resistance to ER stress-induced apoptosis (138). The delay in programmed cell death in the Chop^{-/-} mice indicates that CHOP is not an essential transducer of the ER stress-induced apoptotic signal, or alternatively, that other apoptotic pathways are subsequently triggered in these severely stressed cells. CHOP induces expression of a number of proteins including the ER oxidase ERO1 α (79). This elevated ERO1 α expression is thought to cause hyperoxidizing conditions in the ER that would likely further perturb protein folding, as well as produce additional ROS. It is unclear as to whether the CHOP-mediated upregulation of ERO1 α is a deliberate pro-cell death response to increase both ER and oxidative stress, or alternatively, the elevated levels of ERO1 α are unintended cytotoxic consequences of the cell overproviding components essential to the ER and the secretory pathway *via* the UPR.

CHOP induction also downregulates the antiapoptotic BCL2. Overexpression of BCL2 or a deficiency of Bax and Bak confers resistance against lethal levels of ER stress, demonstrating involvement of the Bcl2 family members (22, 122). Although it remains to be determined how ER stress regulates these proteins, it seems likely that Ca²⁺ efflux from the ER is involved in transducing the stress signal from the ER to the mitochondria and ultimately resulting in caspase activation.

The prevention of ER stress-induced apoptosis has been proposed as a therapeutic strategy, but it is likely to be effective only if the underlying cellular defect is a loss of ER homeostasis. A case in point is that the Parkinson's disease-associated A53T allele of α -synuclein causes cell death by inhibiting membrane trafficking from the ER, which in turn causes ER stress (14). It is unknown whether ER stress-induced apoptosis causes cell death in this situation, but if so, preventing this form of apoptosis would not restore essential membrane transport from the ER. Therefore, the cellular dysfunction and disease symptoms would likely continue because of the underlying defect, even when apoptosis was blocked.

CONCLUSIONS

The UPR and ERAD machinery acts to maintain the functionality of the ER and secretory pathway by providing sufficient folding, export, and degradative capacity. Unfortunately, it seems that ERAD and the UPR are both friend and foe, functioning in both preventing toxicity and evoking cellular responses contributing to disease pathology. The upregulation of the ER oxidative-folding machinery is likely capable of causing both ROS generation and GSH depletion, leading to potentially lethal levels of oxidative stress. ERAD and the UPR have therapeutic implications in a wide range of diseases, and the capacity to downregulate or inhibit the ERO1-PDI reaction pathway may well be a beneficial therapeutic approach. However, multiple challenges exist in targeting the UPR therapeutically. One difficulty will be to achieve tissue-specific manipulation of the UPR, so that the treatment does not induce UPR-dependent dysfunction in other tissues. Another challenge will be to understand the basis of the diseases that display ER stress so as to predict which will benefit from modulating the UPR.

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ABBREVIATIONS

ATF4, activation of transcription factor 4; ATF6, activation of transcription factor 6; BAK, Bcl-2 homologous antagonist/killer; BAX, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; BiP, heavy chain-binding protein; bp, base pair; Cdc48p, cell-division cycle protein 48; CHOP, C/EBP homologous protein; CNX, calnexin; CRT, calreticulin; Cue1p, coupling of ubiquitin conjugation to ER-degradation protein 1; Der1p, degradation in the endoplasmic reticulum protein 1; DKO, double knockout; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin protein ligase; EDEM, ER degradation-enhancing α -mannosidase-like protein; eIF2 α , eukaryotic translation-initiation factor 2; ER, endoplasmic reticulum; ERAD, ER-associated degradation; Ero1p, ER oxidase protein 1; ERQC, ER quality control; ERSE, ER stress-response elements; GADD34, growth arrest and DNA damage-inducible protein 34; Gcn2p, general control nonderepressible protein 2; GSH, reduced glutathione; GSSG, oxidized glutathione; GT, UDP-glucose:glycoprotein glucosyl transferase; Hac1p, homologous to Atf/Creb1 protein 1; Herp, homocysteine-induced ER protein; Hrd1p, HMG-CoA reductase degradation protein 1; Hrd3p, HMG-CoA reductase degradation protein 3; Htm1p, homologous to mannosidase protein 1; IRE1, inositol-requiring transmembrane kinase and endonuclease 1; JNK, c-Jun amino-terminal kinase; MHC, major histocompatibility complex; PDI, protein disulfide isomerase; PERK, protein kinase-like ER kinase; PPI, type 1 protein phosphatase; Ptc2p, phosphatase two C protein 2; Rlg1p, RNA ligase protein 1; RNase, endoribonuclease; ROS, reactive oxygen species; Rsp5p, reverses SPT-phenotype protein 5; S1P, site-1 protease; S2P, site-2 protease; Sec61p, SECretory protein 61; SREBP, sterol response element-binding protein; SSA, stress-seventy subfamily A; TRAF2, TNF receptor-associated factor 2; UBA, ubiquitin associated; Ubc, ubiquitin conjugating; Ubl, ubiquitin-like; UBX, ubiquitin regulatory X; Ubx2p, ubiquitin regulatory X protein 2; Ufd1p, ubiquitin fusion degradation protein 1; UPR, unfolded protein response; UPRE, UPR element; Usa1p, U1-Snp1-associating protein 1; VIMP, VCP-interacting membrane protein; XBP-1, X-box-binding protein 1; Yos9p, yeast OS-9 homologue.

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