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A Review of the Mammalian Unfolded Protein Response

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Abstract

Proteins requiring post-translational modifications such as N-linked glycosylation are processed in the endoplasmic reticulum (ER). A diverse array of cellular stresses can lead to dysfunction of the ER and ultimately to an imbalance between protein-folding capacity and protein-folding load. Cells monitor protein folding by an inbuilt quality control system involving both the ER and the Golgi apparatus. Unfolded or misfolded proteins are tagged for degradation via ER associated degradation (ERAD) or sent back through the folding cycle. Continued accumulation of incorrectly folded proteins can also trigger the Unfolded Protein Response (UPR). In mammalian cells, UPR is a complex signaling program mediated by three ER transmembrane receptors: activating transcription factor 6 (ATF6), inositol requiring kinase 1 (IRE1) and double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK). UPR performs three functions, adaptation, alarm and apoptosis. During adaptation, the UPR tries to reestablish folding homeostasis by inducing the expression of chaperones that enhance protein folding. Simultaneously, global translation is attenuated to reduce the ER folding load while the degradation rate of unfolded proteins is increased. If these steps fail, the UPR induces a cellular alarm and mitochondrial mediated apoptosis program. UPR malfunctions have been associated with a wide range of disease states including tumor progression, diabetes, as well as immune and inflammatory disorders. This review describes recent advances in understanding the molecular structure of UPR in mammalian cells, its functional role in cellular stress, and its pathophysiology.

Keywords

Protein Folding; UPR; PERK; ATF6; IRE1; Apoptosis

Introduction

Protein folding is strategically important to cellular function. Secreted, membrane-bound and organelle-targeted proteins are typically processed and folded in the endoplasmic reticulum (ER) in eukaryotes (Kaufman et al., 2002; Naidoo, 2009; Ron, 2002). Intracellular perturbations caused by a variety of stressors disturb the specialized environment of the ER leading to the accumulation of unfolded proteins (Ellgaard and Helenius, 2003; Fonseca et al., 2009). Glucose deprivation, aberrant calcium regulation, viral infection and hypoxia can all alter protein folding and induce ER stress (Kaufman et al., 2002; Ron, 2002). Physiological processes such as aging can also influence protein folding (Naidoo, 2009). Normally, cells ensure that proteins are correctly folded using a combination of molecular chaperones, foldases and lectins (Naidoo, 2009). However, when proper folding can not be restored, incorrectly folded proteins are targeted to ER Associated Degradation (ERAD)

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pathways for processing (Kaufman et al., 2002). If unfolded or misfolded proteins continue to accumulate, eukaryotes induce the unfolded protein response (UPR).

In mammalian cells, UPR is a complex signaling program mediated by three ER transmembrane receptors: activating transcription factor 6 (ATF6), inositol requiring kinase 1 (IRE1) and double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK). UPR performs three functions, adaptation, alarm and apoptosis. During adaptation, the UPR tries to reestablish folding homeostasis by inducing the expression of chaperones that enhance protein folding. Simultaneously, translation is globally attenuated to reduce the ER folding load while the degradation of unfolded proteins is increased. If these steps fail, the UPR induces a cellular alarm and apoptosis program. The alarm phase involves several signal transduction events, ultimately leading to the removal of the translational block and the down-regulation of the expression and activity of pro-survival factors such as the B-cell lymphoma 2 (Bcl2) protein. After the alarm phase, cells can undergo apoptosis, although ER stress can also initiate autophagy (Bernales et al., 2006; Fujita et al., 2007; Høyer-Hansen et al., 2007; Kamimoto et al., 2006; Kouroku et al., 2007; Ogata et al., 2006; Yorimitsu et al., 2006). Thus, ER folding homeostasis strongly influences physiology (Fonseca et al., 2009). Aberrant protein folding and UPR have been implicated in a number of pathologies. For example, the onset of diabetes (Schnell, 2009) as well as myocardial ischaemia, cardiac hypertrophy, atherosclerosis and heart failure (Glembotski, 2007) have all been linked with aberrant folding or UPR signaling.

UPR reprogramming could also be a means to increase recombinant monoclonal antibody (MAb) production in mammalian cells. Therapeutic protein production using recombinant mammalian cell lines has been a critical component of the biologics industry since the mid 1980s with drugs such as tissue plasminogen activator (tPA) (Becker et al., 2008, Mohan et al., 2008). Several other current biopharmaceutical products are also secreted proteins, for example interferon- γ (IFN γ) and erythropoietin (EPO) (Ku et al., 2008). Typical mammalian production hosts for this class of product include immortalized Chinese Hamster Ovary (CHO) cells (Wurm, 2004) or other mammalian cell types, such as murine lymphoid cells (NS0, SP2/0) (Khoo et al., 2007). These cell lines are often engineered or selected for high specific protein production rates (Seth et al., 2007, Barnes and Dickson, 2006), high growth rates (Khoo et al., 2007) and reduced apoptosis (Connor et al., 2009). However, beyond the robustness of the production host, protein production may also be sensitive to intracellular processing bottlenecks affecting transcription, translation or post-translational modifications. Thus, engineering the folding subsystems of mammalian cells might also be a promising route to increase the production of secreted protein biopharmaceuticals.

The folding cycle, quality control and ER associated degradation (ERAD)

Newly synthesized polypeptide chains enter the ER through a peptide translocon in the ER membrane composed of four proteins, Sec61 α,β,γ and TRAM (Matlack et al., 1998). Upon entering the ER, these nascent chains begin to fold, often as they are being co-translationally modified (Fedorov and Baldwin, 1997). In the cytosol, protein folding is largely driven by the collapse of hydrophobic side chains which ultimately form the core of the folded protein. Another key factor, the burial of electrostatic interactions, e.g., salt bridges or hydrogen bonds in the hydrophobic core, constrains possible folding choices in a very complex free energy landscape. The forces that govern cytosolic folding are also active in the ER. However, protein folding in the ER is more complex because of post-translational modifications such as disulfide bond formation or N-linked glycosylation. Interestingly, the folding landscape in the ER can sometimes be traversed quickly; small proteins like the Semliki Forest virus capsid protein can fold in approximately 50ms (Sanchez et al., 2004).

However, for larger and more complex proteins, e.g., the human coagulation factors V and VIII, folding can take several minutes to hours to complete (Pittman et al., 1994).

The folding quality of proteins in the ER is maintained by an in-built quality control (QC) system (Fig. 1) which ensures proteins are in their functional folded state before exiting the ER (Ellgaard and Helenius, 2003; Ellgaard et al., 1999). A protein is correctly folded if it has attained its native conformation after required co- or post-translational modifications. On the other hand, exposed hydrophobic regions, unpaired cysteine residues, or aggregation are all markers of an unfolded or misfolded conformation (Ellgaard et al., 1999). The best characterized QC system in the ER is the so-called *glycan-code* (Hebert et al., 2005). Most polypeptides entering the ER are modified by adding preassembled oligosaccharides to asparagine side chains appearing in ASN-X-SER/THR motifs (Helenius and Aebi, 2004). Once attached, these oligosaccharide groups can be sequentially modified by glucosidases I and II to form mono-glucosylated intermediates that are recognized by the ER lectins calnexin and calreticulin (Hammond et al., 1994) and the associated oxidoreductase ERp57 (Hebert et al., 1995; Liu et al., 1999; Oliver et al., 1999, 1997; Ware et al., 1995). Calnexin is a type I membrane protein with a β -sandwich carbohydrate binding domain and a hairpin, called the P-domain, extending away from the carbohydrate binding domain (Schrag et al., 2001). Calreticulin is the soluble paralog of calnexin with only minor structural differences in the P-domain (Ellgaard et al., 2001a,b; Schrag et al., 2001). Calnexin and calreticulin binding increases the efficiency of glycoprotein folding by protecting against aggregation (Hebert et al., 1996) and ensures that misfolded proteins are retained in the ER (Rajagopalan et al., 1994). These ER glycoprotein chaperones also promote disulfide bond formation through their interaction with ERp57; ERp57 binds the P-domain of both calnexin and calreticulin thereby promoting disulfide bond formation at specific glycoprotein locations (Frickel et al., 2002; Oliver et al., 1999, 1997). Glycoproteins are released from calnexin and calreticulin by further glucose residue cleavage by glucosidase II. Once released, these proteins can fold into their native conformation, they can be glycosylated and re-processed by the calnexin/calreticulin cycle, or they can be targeted for ER-associated degradation (ERAD) (recently reviewed in Maattanen et al., 2010). Interestingly, while calnexin and calreticulin ultimately promote folding, they do not recognize misfolded proteins. Misfolded proteins can be targeted (or re-targeted) to the calnexin/calreticulin cycle by glucosylation with UDP-glucose; the C-terminal domain of glycoprotein glucosyltransferase (GT) glucosylates near-native conformations by using its N-terminal protein sensor to locate exposed hydrophobic residues (Caramelo et al., 2004; D'Alessio et al., 2010; Sousa and Parodi, 1995; Trombetta and Parodi, 2003). In this way, GT acts as an adapter allowing attachment of calnexin/calreticulin and re-processing in the calnexin/calreticulin cycle (Parodi, 2000). Repeated glycosylation and deglycosylation cycles ensure misfolded glycoproteins spend sufficient time in the ER to correctly fold. Exit from the calnexin/calreticulin cycle can also lead to ERAD. In this case, terminal mannose residues are removed from the attached oligosaccharides by ER α -mannosidase I, which leads to interaction with membrane localized ER degradation enhancing α -mannosidase like proteins (EDEM and EDEM2) (Hosokawa et al., 2001; Olivari et al., 2005) and subsequent retro-translocation to the cytosol. The retro-translocon responsible for transporting unfolded or misfolded proteins out of the ER is uncertain (Tsai et al., 2002); several ER membrane proteins have been proposed including Sec61 components, Derlin family members and E3-ubiquitin ligases (reviewed in (Hebert et al., 2010)). Mannose removal decreases the likelihood that a unfolded protein will be processed in the calnexin/calreticulin cycle (Ellgaard et al., 1999), thus, increasing the probability of terminal mannose cleavage and retro-translocation. Once in the cytosol, these unfolded or misfolded proteins are degraded by the ubiquitin proteasome system (Hershko et al., 2000).

Hydrophobic unfolded or misfolded proteins are recognized in the ER by molecular chaperones which bind these proteins and increase the probability of correct folding (Fra et al., 1993; Helenius et al., 1997; Hellman et al., 1999). Similar to calnexin and calreticulin, hydrophobic chaperone binding increases the residence time of unfolded proteins in the ER lumen, giving these proteins a chance to fold (Fig. 2A). For example, the HSP70 family of chaperones recognize, in an ATP-dependent manner, exposed hydrophobic patches on a broad spectrum of unfolded or misfolded proteins (Kaufman et al., 2002). Repeated binding and release of HSP70 chaperones ensures that incorrectly folded proteins do not exit the ER (Kaufman et al., 2002). One critical member of the HSP70 family is BiP or GRP78. BiP consists of an N-terminal ATPase domain and a C-terminal peptide binding domain (Gething, 1999). When bound to ATP, BiP binds unfolded hydrophobic stretches with low-affinity. However, unfolded or misfolded protein binding stimulates the N-terminal ATPase activity of BiP resulting in an ADP-bound form with much higher affinity for hydrophobic motifs (Gething, 1999). Interestingly, the affinity of BiP (and other HSP70 family members) for ADP is approximately six-fold greater than for ATP. Thus, nucleotide exchange factors (NEFs) such as BiP associated protein (BAP), Sil1/Sis1p or GrpE-like proteins are required to catalyze the ADP/ATP exchange needed for the dissociation of BiP from unfolded proteins (Tyson and Stirling, 2000). In addition to its role as a folding chaperone, BiP also functions as an ER stress regulator by buffering Ca^{2+} levels (Reddy et al., 2003). BiP interacts with ER localized caspase-7 (Reddy et al., 2003) and prevents the activation of pro-apoptotic Bcl2 family members such as Bax (Fu et al., 2007; Ranganathan et al., 2006). Beyond these activities, BiP also regulates the activation of the three transmembrane ER stress transducers: PERK, ATF6 and IRE1. Normally, BiP is bound to these ER receptors, blocking their activation. However, in the presence of exposed hydrophobic residues BiP disassociates, allowing PERK, ATF6 and IRE1 activation (Fig. 2B). Overexpression of BiP leads to reduced activation of IRE1 and PERK (Bertolotti et al., 2000; Kohno et al., 1993). The PERK and ATF6 branches are thought to be activated before IRE1 (Szegezdi et al., 2006); this ordering is consistent with the signals that each branch transduces. The PERK and ATF6 pathways largely promote ER adaptation to misfolding, while IRE1 has a dual role, transmitting both survival and pro-apoptotic signals.

Double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) pathway

The PERK branch of UPR transduces both pro-survival as well as pro-apoptotic signals following the accumulation of unfolded or misfolded protein in the ER. However, its main function is to modulate translation. PERK is a type I trans-membrane protein, composed of a ER luminal stress sensor and a cytosolic protein kinase domain. Dissociation of BiP from the N-terminus of PERK initiates dimerization and autophosphorylation of the kinase domain at T981 (Kebache et al., 2004). The C-terminal kinase domain shares homology with the eukaryotic translation initiation factor 2α (eIF2 α) and the kinases, protein kinase R (PKR), heme-regulated inhibitor (HRI) kinase, and the gene control non-derepressible-2 (GCN2) kinase (Harding et al., 1999; Shi et al., 1999, 1998). The eIF2 α protein, which is composed of three subunits, is critical to translation initiation in eukaryotes, including GTP-dependent start-site recognition (Merrick, 2004). Phosphorylation of the α subunit of eIF2 α blocks the exchange of GDP bound to eIF2 α ; hence, eIF2 α remains bound to partner initiation factors (eIF2B) and translation initiation is blocked (Gebauer and Hentze, 2004). Activated PERK can phosphorylate eIF2 α at S51 (Harding et al., 1999; Raven et al., 2008), which leads to three downstream effects. First, phosphorylated eIF2 α globally attenuates translation initiation. Decreased translation reduces the influx of protein into the ER, hence diminishing the folding load. Translation attenuation is followed by increased clearance of the accumulated proteins from the ER by ERAD and expression of pro-survival genes. For example, PERK activation induces the expression of cellular inhibitor of apoptosis (cIAP)

(Hamanaka et al., 2009). Interestingly, decreased protein translation is not universal; genes with internal ribosome entry site (IRES) sequences in the 5' untranslated regions bypass the eIF2 α translational block (Schroder and Kaufman, 2005). One of the most well-studied of these, ATF4, encodes a cAMP response element-binding transcription factor (C/EBP) (Lu et al., 2004) ATF4 that drives the expression of pro-survival functions such as amino acid transport and synthesis, redox reactions and protein secretion (Harding et al., 2003). Taken together, these effects seem to be largely pro-survival. However, ATF4 can also induce the expression of pro-apoptotic factors. For example, ATF4 induces the expression of the transcription factor C/EBP homologous protein (CHOP), which is associated with apoptotic cell-death. CHOP (also known as GADD153) is a 29 kDa protein composed of an N-terminal transcriptional activation domain and a C-terminal basic-leucine zipper (bZIP) domain that is normally present at low levels in mammalian cells (Ron and Habener, 1992). The transcriptional activator domain is positively regulated by phosphorylation at S78 and S81 by p38 MAPK family members (Maytin et al., 2001; Wang and Ron, 1996) while the bZIP domain plays a key role in the homodimerization of the protein (Matsumoto et al., 1996; Maytin et al., 2001). CHOP activity promotes apoptosis primarily by repression of Bcl2 expression and the sensitization of cells to ER-stress inducing agents (Gotoh et al., 2001; McCullough et al., 2001). For example, Matsumoto *et al.* showed that ectopic expression of CHOP in M1 myeloblastic leukemia cells reduced Bcl2 protein concentrations, while Bax levels remained constant (Matsumoto et al., 1996). They further established a link between CHOP expression and apoptosis in these cells. However, while CHOP expression is negatively correlated with Bcl2 levels, there is no CHOP binding site in the *bcl2* promoter (McCullough et al., 2001). McCullough *et al.* have suggested that the bZIP domain of CHOP could act with other bZIP transcription factors to regulate *bcl2* expression (McCullough et al., 2001). Thus, it's likely that the connection between CHOP expression and apoptosis is more complex than simple down-regulation of Bcl2 expression. Given its central role in translation attenuation, cells have evolved multiple axes to regulate PERK activity. First, the cytosolic kinase domain of PERK can be inhibited by the action of the DNAJ family member P58^{IPK}, which was initially discovered as an inhibitor of the eIF2 α protein kinase PKR (Lee et al., 1990). P58^{IPK}, whose expression is induced following ATF6 activation, binds to the cytosolic kinase domain of PERK, inhibiting its activity (van Huizen et al., 2003; Yan et al., 2002). Inhibition of PERK kinase activity relieves eIF2 α phosphorylation, thereby removing the translational block. Interestingly, P58^{IPK} expression occurs several hours after PERK activation and eIF2 α phosphorylation. Thus, P58^{IPK} induction may mark the end of UPR adaptation, and the beginning of the alarm/apoptosis phase of the response (Szegezdi et al., 2006). In addition to its direct interaction with PERK, P58^{IPK} is also involved in cotranslational protein degradation (Oyadomari et al., 2006). In this role, P58^{IPK} is thought to recruit Hsp70 to the cytosolic opening of the ER translocon in an effort to extract stalled nascent proteins. Interestingly, P58^{IPK} has also been identified as an ER-luminal co-chaperone acting in conjunction with BiP, although BiP-independent association between P58^{IPK} and a mutant vesicular stomatitis virus envelope glycoprotein (VSV-G^{ts045}) was also observed (Petrova et al., 2008). The activity of P58^{IPK} appears to be regulated; recently, Ni *et al.* reported a novel UPR-inducible cytosolic BiP isoform (GRP78va), generated by alternative splicing and IRES mediated translation, that antagonizes cytosolic P58^{IPK} in several human and mouse cell-lines (Ni et al., 2009). Second, PERK induces a negative feedback loop, through its downstream effector CHOP, involving the direct de-phosphorylation of eIF2 α . CHOP induces the expression of GADD34 which, in conjunction with protein phosphatase 1 (PP1), assembles into a phosphatase which dephosphorylates the S51 residue of eIF2 α (Novoa et al., 2001). GADD34 is a member of the GADD family of genes which are induced by DNA damage and a variety of other cellular stresses (Zhan et al., 1994). The GADD34 binding partner in this complex appears to be responsible for PP1 α recognition and targeting of the phosphatase complex to the ER. Association between GADD34 and PP1 is encoded by a C-

terminal canonical PP1 binding motif, KVRFF, while approximately 180 residues, near the N-terminus of GADD34, appear to be responsible for ER localization (Brush et al., 2003).

Activating transcription factor 6 (ATF6) pathway

ATF6 activation involves a complex series of translocation and irreversible proteolytic processing steps, ultimately leading to the up-regulation of a pro-survival transcriptional program, in the presence of unfolded or misfolded proteins. ATF6 is a 90 kDa ER transmembrane protein with two homologs: ATF6 α (Hai et al., 1989; Haze et al., 1999) and ATF6 β (Haze et al., 2001; Khanna and Campbell, 1996; Min et al., 1995). The ATF6 α homolog is thought to be primarily responsible for transcriptional regulation of pro-survival genes following ER stress, however, ATF6 β may also play a role (Haze et al., 2001; Thuerlauf et al., 2007; Yamamoto et al., 2007). Similar to IRE1 and PERK, ER stress leads to the dissociation of BiP from the N-terminus of ATF6, followed by translocation and activation. However, the mechanism of BiP interaction with ATF6 and the factors controlling ATF6 translocation to the golgi remain uncertain. N-terminal golgi localization sequences (GLS1 and GLS2) seem to be involved with BiP regulation of ATF6. BiP binding to the N-terminal GLS1 promotes the retention of ATF6 in the ER (Shen et al., 2002). On the other hand, the GLS2 domain was required to target ATF6 to the golgi body following BiP dissociation from GLS1 (Shen et al., 2002). The lectin CRT might also have a role in keeping the ATF6 in the ER (Hong et al., 2004). Unlike the previous two kinase pathways, ATF6 activation does not involve phosphorylation of a C-terminal kinase domain. Rather, after translocated to the golgi, ATF6 undergoes regulated intramembrane proteolysis (RIP); the luminal domain is first cleaved by serine protease site-1 protease (S1P) followed by metalloprotease site-2 protease (S2P) cleavage (Chen et al., 2002; Haze et al., 1999; Shen and Prywes, 2004; Ye et al., 2000). Cleavage at the juxtamembrane site allows the 50 kDa transcriptional domain of ATF6 to be translocated to the nucleus where it regulates the expression of genes with ATF/cAMP response elements (CREs) (Wang et al., 2000) and ER stress response elements (ERSE) in their promoters (Kokame et al., 2001; Yoshida et al., 1998).

Cleaved ATF6 induces a gene expression program, in conjunction with other bZIP transcription factors and required co-regulators, such as nuclear factor Y (NF-Y) (Kokame et al., 2001; Yoshida et al., 2000), that increases chaperone activity as well as the degradation of unfolded proteins (Wu et al., 2007; Yamamoto et al., 2007). For example, ATF6 upregulates BiP, protein disulfide isomerase (PDI) and ER degradation-enhancing alpha-mannosidase-like protein 1 (EDEMI) expression. Additionally, ATF6 induces the expression of the X box-binding protein 1 (XBP1) which, after processing by activated IRE1 α , induces the expression of chaperones as well as control elements such as P58^{INK} (Yoshida et al., 2001). The ATF6-induced gene expression program is also cytoprotective. For example, ATF6 induces regulator of calcineurin 1 (RCAN1) expression (Belmont et al., 2008). RCAN1 sequesters calcineurin (Belmont et al., 2008), a calcium activated protein-phosphatase B, that dephosphorylates Bcl2-antagonist of cell death (BAD) at S75 or S99 (Wang et al., 1999). This leads to sequestering of Bcl2 by Bad, which inhibits its downstream anti-apoptotic activity (Wang et al., 1999). Recently, a number of ATF6 homologs have been identified, e.g., OASIS, CREBH, LUMAN/CREB3, CREB4 and BBF2H7 that are processed in a similar way as ATF6, yet their function remains unknown (Ron and Walter, 2007). Thus, ER-stress induced ATF6 signaling may be responsible for additional undiscovered functionality.

Currently, little is known about deactivation of ATF6. Recently, XBP1u, the unspliced form of XBP1, has been implicated as a negative regulator for ATF6 (Yoshida et al., 2009). Following the induction of ER stress, two versions of XBP1 exist: XBP1u and sXBP1 (Yoshida et al., 2009). In the recovery phase following ER stress, high levels of XBP1u may

play a dual role. First, XBP1u binds sXBP1, promoting complex degradation (Tirosh et al., 2006; Yoshida et al., 2006). Second, XBP1u can bind ATF6 α rendering it more prone to proteasomal degradation (Yoshida et al., 2009). Taken together, these two steps may slow the transcription of ER chaperones and ERAD components during the recovery phase following ER stress.

Inositol-requiring kinase 1 (IRE1) pathway

IRE1 is the most evolutionarily conserved branch of UPR and its interactome has recently been reviewed by Hetz and Glimcher (Hetz and Glimcher, 2009). IRE1 initiates a program with both pro-survival and pro-apoptotic components in the presence of misfolded or unfolded proteins. IRE1 is a 100 kDa type I ER transmembrane protein with both an endoribonuclease and a serine-threonine kinase domain (Kaufman et al., 2002). IRE1 has two homologs, IRE1 α and IRE1 β ; IRE1 α is expressed in a variety of tissues (Tirasophon et al., 1998) while IRE1 β is found only in the intestinal epithelia (Tirasophon et al., 1998; Wang et al., 1998). The N-terminus of IRE1, located in the ER lumen, senses unfolded or misfolded proteins through its interaction with BiP (Cox et al., 1993; Shamu and Walter, 1996; Sidrauski and Walter, 1997). There has been some controversy surrounding the mechanism of unfolding-induced BiP dissociation and the role of possible direct interaction of unfolded proteins with IRE1. Normally BiP is bound to the N-terminus of IRE1 (Bertolotti et al., 2000; Liu et al., 2003; Okamura et al., 2000). However, in the presence of unfolding cues BiP dissociates and is sequestered by the unfolded or misfolded proteins (Kimata et al., 2003). IRE1 may also sense unfolded cues in a BiP independent manner through a N-terminal peptide binding domain (Liu et al., 2002, 2003). Interaction of this domain with unfolded or misfolded proteins has been suggested as a precursor to BiP dissociation. Current studies in yeast have suggested that BiP first dissociates, allowing IRE1 dimerization. This step is then followed by direct sensing of unfolded motifs which work to orient IRE1 into an active signaling cluster (Kimata et al., 2007). In either case, BiP dissociation allows IRE1 activation. IRE1 is activated by homo-oligomerization followed by autophosphorylation of the C-terminal kinase domain at S724 (Papa et al., 2003; Shamu and Walter, 1996; Weiss and Schlessinger, 1998; Welihinda and Kaufman, 1996). IRE1 activation enables both its kinase and endoribonuclease activities to transduce signals simultaneously through two distinct signaling axes. The endoribonuclease activity cleaves a 26-nucleotide intron from the XBP1-mRNA (Lee et al., 2002; Shen et al., 2001; Yoshida et al., 2001) which generates a 41 kDa frameshift variant (sXBP1) that acts as a potent transcription factor. sXBP1 homodimers, along with co-regulators such as nuclear factor Y (NF-Y), regulate the expression of a variety of ER chaperones and protein degradation related genes (Malhotra and Kaufman, 2007; Rao and Bredesen, 2004). sXBP1 also upregulates the expression of P58^{IPK}; as discussed previously, P58^{IPK} is a member of the DNAJ protein family that negatively regulates PERK activity, forming one of the many modes of crosstalk between the UPR branches (Yan et al., 2002).

The cytosolic domains of activated IRE1 α transduce late-phase UPR signals. Cytosolic IRE1 α dimers interact with adaptors such as tumor necrosis factor receptor-associated factor 2 (TRAF2) to drive signal-regulating kinase (ASK1) activation and then subsequently cJUN NH2-terminal kinase (JNK) and p38MAPK activation (Urano et al., 2000). IRE1 α also modulates the activation of other kinases such as extracellular signal-regulated kinases (ERKs) as well as nuclear factor κ B (NF- κ B) pathways (Hu et al., 2006; Nguyen et al., 2004). However, the role of these additional effectors in UPR is not well understood. ASK1 activity is regulated by phosphorylation/de-phosphorylation at several sites as well as by physical interaction with other proteins. The current model for ASK1 activation, at least for TNF-mediated activation, involves release of ASK1 from inhibitory proteins such as 14-3-3 (Nishitoh et al., 1998), TRAF-dependent homodimerization (Gotoh and Cooper, 1998) and

autophosphorylation at T845 (Tobiome et al., 2002). Phosphorylation of ASK1 at S83 by Akt/PKB (protein kinase B) (Kim et al., 2001) and dephosphorylation at S845 by protein phosphatase 5 (PP5) (Morita et al., 2001) both decrease ASK1 activity. More recently, the kinase PIM1 has also been shown to phosphorylate ASK1 at S83 (Gu et al., 2009). Interaction with CDC25A and 14-3-3 proteins have also been shown to decrease ASK1 activity (Zhang et al., 1999; Zou et al., 2001). ASK1 phosphorylates and activates two downstream kinases, MMK4 and MMK3 which in turn activate JNK and p38 MAP kinase, respectively. JNK is activated by dual phosphorylation at T183 and Y185 by MMK4 (Derijard et al., 1995). Once activated, JNK performs a number of functions including activation of the pro-apoptotic Bim protein (Lei and Davis, 2003; Putcha et al., 2003) and the inhibition of Bcl2 (Yamamoto et al., 1999). Activated JNK activates the proapoptotic Bcl-2 family member Bim by phosphorylation at S65 (Lei and Davis, 2003; Putcha et al., 2003). Bim is normally sequestered by motor complexes interacting with the cytoskeleton (Puthalakath et al., 1999). Following JNK-mediated phosphorylation, Bim translocates to the mitochondrial outer membrane, where it promotes cytochrome c release and caspase activation (Chen and Zhou, 2004; Puthalakath et al., 1999). Interestingly, a positive feedback loop exists between Bim and caspase 3 activation; phosphorylated Bim is a caspase 3 target, that once cleaved, is a more potent inducer of cytochrome c release (Chen and Zhou, 2004; Corazza et al., 2006). Recent ER stress studies in MCF-7 breast carcinoma-derived cells using thapsigargin (Tg) suggested that Bim expression was regulated by CHOP; following Tg treatment, a two-fold increase in Bim mRNA and a five-fold increase in Bim proteins were observed (Puthalakath et al., 2007). JNK activation also regulates the activity of anti-apoptotic protein Bcl2 (Wei et al., 2008; Yamamoto et al., 1999). Active JNK1 inhibits Bcl2 via phosphorylation at sites T69, S70 and S87 (Wei et al., 2008). In contrast, other stress induced proteins like p38 family members phosphorylate Bcl2 at S87 and T56 only (De Chiara et al., 2006). Ultimately, inhibition of Bcl2 and the activation of Bim leads to BAX/BAK dependent apoptosis. Thus, signals initiated from the cytosolic kinase domain of IRE1 α are largely pro-apoptotic.

IRE1 α activity is regulated by several proteins, including tyrosine phosphatase 1B (PTP-1B), ASK1-interactive protein 1 (AIP1) and members of the Bcl2 protein family. PTP-1B has been implicated in a number of IRE1 α signaling events. The absence of PTP-1B reduced IRE1 α dependent JNK activation, XBP1 splicing and EDEM transcription in immortalized and primary mouse embryonic fibroblasts (Gu et al., 2004). However, no physical interaction between IRE1 α and PTP-1B was established. On the other hand, AIP1 physically interacts with both TRAF2 and IRE1 α , suggesting a model in which AIP1 facilitates IRE1 α dimerization and activation (Luo et al., 2008). The C-terminal period-like domain (PER) of AIF1 binds the N-terminal RING finger domain of TRAF2, followed by ASK1-JNK signaling (Zhang et al., 2004). Thus, based on these findings, Luo *et al.* postulated that AIF1 may be directly involved in the IRE1 α -TRAF2 complex and its activation of the ASK1-JNK signaling axis (Luo et al., 2008). This hypothesis was validated in AIP1KO mouse studies; AIP1-knockout mouse embryonic fibroblasts and vascular endothelial cells showed significant reductions in ER-stress induced ASK1-JNK activation that was rescued in AIP1 knock-in cells (Luo et al., 2008). IRE1 α has also been shown to directly interact with Bcl-2 family members Bax and Bak. Hetz *et al.* showed that Bax and Bak complex with the cytosolic domain of IRE1 α and modulate IRE1 α signaling (Hetz et al., 2006). Bax and Bak double knockout mice failed to signal through the IRE1 α UPR branch following tunicamycin-induced ER stress; however, PERK signaling markers, e.g., eIF2 α phosphorylation, responded normally (Hetz et al., 2006). This pro-activation role of Bak and Bax may be modulated by one of the few negative regulators of IRE1 α activity, Bax inhibitor 1 (BI-1). BI-1 is an anti-apoptotic protein that enhances cell survival following several intrinsic death stimuli (Xu and Reed, 1998). Bailly-Maitre *et al.* were the first to suggest that BI-1 may downregulate IRE1 α and possibly ATF6 activity (Bailly-Maitre et al.,

2006). BI-1 deficient mice displayed increased XBP1s and enhanced JNK activity in the liver and kidney, while eIF2 α phosphorylation remained normal under ER-stress conditions (Bailly-Maitre et al., 2006). Lisbona *et al.* later showed that BI-1 directly interacts with the cytosolic domain of IRE1 α , inhibiting its endoribonuclease activity (Lisbona et al., 2009). Interestingly, BI-1 interacts with several members of the Bcl2 protein family e.g., Bcl2 and Bcl-XL, even though it has no homology (Xu and Reed, 1998). Members of the HSP family of proteins have also been shown to regulate IRE1 α . For example, HSP90 interacts with the cytosolic domain of IRE1 α , potentially protecting it from degradation by the proteasome (Marcu et al., 2002). HSP72 interaction with the cytosolic IRE1 α domain has also recently been shown to enhance IRE1 α endoribonuclease activity (Gupta et al., 2010). Taken together, these modes of IRE1 α regulation with the exception of B1-1, largely promote or enhance IRE1 α signaling.

ER stress-induced autophagy and apoptosis

Ultimately, if UPR fails to restore ER homeostasis, cells initiate terminal programs such as autophagy or apoptosis. Several recent studies indicate that ER stress can trigger autophagy (Bernales et al., 2006; Fujita et al., 2007; Høyer-Hansen et al., 2007; Kamimoto et al., 2006; Kouroku et al., 2007; Ogata et al., 2006; Wei et al., 2008; Yorimitsu et al., 2006). Autophagy is an evolutionarily conserved cellular pathway, in which a cell recycles its macromolecules and organelles (Levine and Klionsky, 2004). Autophagy is initiated by the formation of an autophagosome, composed of part of the cytosol or cellular organelles, encased in a double membrane (Codogno and Meijer, 2005; Kroemer and Jaattela, 2005; Levine and Klionsky, 2004). Autophagosomes then bind endolysosomal vesicles, leading to the creation of the autolysosome. The autolysosome is then degraded, completing the autophagy cycle. The key regulatory proteins involved in the nucleation and formation of the autophagosomal membrane are class III phosphatidylinositol 3-kinase (PI3K), p150 myristylated protein kinase and Beclin-1 (Levine and Klionsky, 2004). On the other hand, the ER-stress sensors PERK, IRE1 and cytosolic Ca²⁺ all act as effectors initiating autophagy in ER stressed cells (Fujita et al., 2007; Høyer-Hansen et al., 2007; Kouroku et al., 2007; Ogata et al., 2006). JNK1 mediated phosphorylation of Bcl2 at T69, S70 and S87 may also be important; phosphorylation at these sites leads to the dissociation of Bcl2 from Beclin-1, and the activation of autophagy (Wei et al., 2008). Association of Beclin-1 with PI3K and other proteins promotes the localization of other autophagy related proteins to the preautophagosomal membrane (Levine and Kroemer, 2008). Autophagy can be inhibited in eukaryotic cells by several factors, including the mammalian target of rapamycin complex 1 (mTORC1) protein complex (Meijer and Codogno, 2006; Noda and Ohsumi, 1998).

The autophagy program is a cellular reboot mechanism while apoptosis is a terminal death program. Apoptosis is characterized by nuclear and cytoplasmic condensation, blebbing of the plasma membrane and DNA fragmentation (Kerr et al., 1972). The dying cell eventually disintegrates into membrane-enclosed apoptotic bodies which are quickly destroyed by phagocytes or neighboring cells. A common biomarker of apoptosis is the activation of aspartate-specific proteases, collectively known as caspases (Alnemri et al., 1996). Caspases rapidly dismantle cell cycle, cytoskeletal and organelle proteins by proteolytic cleavage. There are two pathways that result in caspase activation in response to apoptotic signals; the death-receptor and the stress mediated pathways. The death-receptor pathway is marked by ligand-mediated activation of death receptors on the plasma membrane. The alternative pathway for caspase activation is mediated by cellular stress e.g., ER stress. Caspases are activated from their zymogens (procaspases), in response to various death cues. First, the initiator caspases, caspase-8 and caspase-9, are activated in response to death cues (Muzio et al., 1998). This is followed by the activation of executioner caspases, such as caspase-3, caspase-6 and caspase-7. Activated executioner caspases proteolytically process several

substrates, facilitating cell death. They also activate initiator caspases, forming a positive feedback loop.

Activation of both the PERK and IRE1 pathways modulate stress-induced apoptosis through their regulation of Bcl2 expression and activity. Overall, stress induced apoptosis can occur through both mitochondrial-dependent and independent pathways. Stress signals cause oligomerization of pro-apoptotic proteins, such as Bax and Bak. These proteins are normally sequestered at the mitochondrial outer membrane by the survival protein Bcl2, under non-apoptotic conditions (Wei et al., 2001). Once Bax and Bak oligomerize, they insert into the mitochondrial membrane and breach membrane integrity (Nechushtan et al., 1999). This results in a net efflux of cytochrome-c from the mitochondria to the cytosol and the initiation of the well-studied Apaf-1 mediated caspase-9 activation pathway. Recently, crosstalk between the ER and mitochondria during apoptosis, which might initiate mitochondrial apoptotic events, has also been explored (Demaurex and Distelhorst, 2003). This crosstalk may be facilitated via the release of Ca^{2+} ions from the ER into the cytosol, in response to ER stress. Mitochondrial uptake of Ca^{2+} initiates membrane fission and caspase activation via an uncertain mechanism. However, a few key drivers of ER Ca^{2+} release in response to stress signals have been identified (Nicotera and Orrenius, 1998). Fas-mediated caspase-8 activation leads to cleavage of the membrane-bound ER protein, Bap31. Although Bap31 is membrane-bound, its caspase-recognition sequence is cytosolic, thus it is accessible by caspase-8. The truncated product, Bap20, is believed to promote the release of ER Ca^{2+} (Demaurex and Distelhorst, 2003; Distelhorst and Shore, 2004; Thomenius and Distelhorst, 2003). Interestingly, Bcl2 is also localized to the ER-membrane, but its role is uncertain (Adams, 2003). Once in the mitochondria, Ca^{2+} modulates the permeability transition (PT) pore (PTP) thereby increasing the efflux of cytochrome-c (Bernardi, 1999; Decaudin et al., 1997). Stress induced mitochondrial-independent apoptotic pathways are not well understood. Currently, caspase 12 has been suggested as a possible ER-stress apoptotic mediator in mice (Nakagawa et al., 2000; Szegezdi et al., 2006; Yoneda et al., 2001). However, caspase 12 is not expressed in human cells. Moreover, there is considerable debate about its role in stress-induced apoptotic cell-death (Saleh et al., 2006).

The pathophysiology of ER-stress and aberrant UPR

ER stress plays an important role in a spectrum of diseases ranging from neurodegeneration, cardiac diseases, cancer, diabetes to muscle degeneration (Table 1). Understanding ER-stress mechanisms in the context of these diseases presents a unique opportunity for drug discovery. Current therapeutic efforts have largely focused on amplifying the adaptive, pro-survival components of the UPR signal, for example, by inducing chaperone expression, which works to restore ER homeostasis. However, in the context of cancer, the opposite outcome is sought; in this case a number of strategies have focused on inhibiting proteasome function (Table 2).

Diabetes

In the context of diseases such as diabetes, pancreatic β -cells depend on efficient UPR signaling to meet the demands for constantly varying levels of insulin synthesis. Type 1 diabetes is marked by excessive loss of pancreatic β -cells, while type 2 diabetes is marked by pancreatic β -cell dysfunction. The large biosynthetic load placed on the ER because of insulin production in response to food uptake (glucose) can overwhelm the folding capacity of the ER, resulting in ER stress. This leads to subsequent PERK activation and reduction of protein synthesis. In PERK $-/-$ cells, protein synthesis is unresponsive to the stress and leads to accumulation of unfolded proteins (e.g. proinsulin) and ultimately cell death. It has been shown that PERK deficient mice are more prone to diabetes and progressive hyperglycemia (Harding et al., 2001). In type II diabetes, ER stress leads to JNK-mediated phosphorylation

of insulin receptor substrate 1 (IRS1) at S307 (Ozcan et al., 2004). IRS1 is a substrate of Insulin receptor. Phosphorylation of IRS1 therefore inhibits insulin action. Nitric oxide (NO), is also a key player in β -cell death in type-1 diabetes and vascular complications in type-2 diabetes. NO depletes ER Ca^{2+} leading to ER stress and ultimately apoptosis. Pancreatic β -cells have shown that NO induced apoptosis is CHOP dependent (Oyadomari et al., 2001). Thus, ER stress is a critical feature of both type-I and type-II diabetes at the molecular, cellular and organismal level.

Role of UPR in hypoxia and cancers

The ER not only acts as the center for maturation of proteins, but also as a critical node for oxygen sensing and signaling. In rapidly growing tumors, cells face stressors like hypoxia and nutrient deprivation both of which can lead to ER stress and ultimately UPR. For example, oxygen deprivation has recently been shown to be an initiator of UPR (Koumenis and Wouters, 2006). Interestingly, the connection between hypoxia and UPR is through hypoxia inducible factor (HIF) independent pathways. Hypoxia leads to PERK activation and the transient phosphorylation of eIF2 α (Koritzinsky et al., 2006; Koumenis et al., 2002; Ma and Hendershot, 2003). This effect occurs on a time scale of minutes in the case of anoxia, and is slower for moderate ($\text{pO}_2 \leq 1\%$) hypoxic conditions (Koritzinsky et al., 2006). Because of their ability to transduce pro-apoptotic signals, the modulation of PERK and IRE1 activity has been explored as a potential anti-cancer strategy. Versipelostatin, a repressor of BiP expression, has been shown to produce anti-tumor activity in MKN-74 xenograft mouse models (Park et al., 2004). Enhanced apoptosis has also been observed in BiP-deficient fibrosarcoma cells, and XBP1- and PERK-deficient mouse fibroblasts (Bi et al., 2005; Jamora et al., 1996; Romero-Ramirez et al., 2004).

Another related anti-cancer strategy is the induction of ER-stress using proteasome or protein trafficking inhibitors. Bortezomib, a selective inhibitor of the 26S proteasome, is used for the treatment of relapsed multiple myeloma, a plasma cell neoplasia (Neubert et al., 2008). Anti-tumor activity was also observed with Bortezomib in preclinical pancreatic cancer studies using the L3.6pl cell-line (Nawrocki et al., 2005). Addition of Bortezomib to splenic and bone marrow plasma cells led to a 40-fold increase in CHOP expression, and a subsequent decrease in Bcl2 levels (Neubert et al., 2008). HIV type 1 (HIV-1) protease inhibitors (PI) like Nelfavir and Atazanavir are also currently being studied for the treatment of malignant gliomas (Pyrko et al., 2007). Studies in glioblastoma cells suggested that Nelfavir or Atazanavir led to inhibition of proteasome activity and subsequently CHOP-induced cell death (Pyrko et al., 2007). Thus, although no mechanistic link between proteasome inhibitors and UPR has been established, anecdotal evidence, such as CHOP induction, suggests these inhibitors are at least partially inducing ER-stress and subsequent UPR-mediated cell death. Recently, studies targeting intracellular trafficking of proteins have also been explored as an anti-cancer strategy. Brefeldin A treated chronic lymphocytic leukemia (CLL) cells showed induction of ER stress, caused by inhibition of protein trafficking, ultimately resulting in Golgi collapse and cell death (Carew et al., 2006). Inhibition of protein trafficking from the ER can also lead to ER swelling and p53-independent apoptosis (Carew et al., 2006).

Neurodegenerative disorders

Several neurodegenerative diseases like Parkinson's disease, Alzheimer's disease; have been implicated to have accumulation of misfolded proteins leading to altered neuronal connectivity, neuronal death and and dysfunctional ERAD machinery (Soto, 2003; Bence et al., 2001; Katayama et al., 1999) (Table 1). The implications of UPR in neurodegenerative diseases is yet to be determined (Lindholm et al., 2006). Alzheimer's disease (AD) is characterized by progressive decline of cognitive functions, marked by loss of neurons from

different brain regions and extracellular senile plaques formed by aggregates of amyloid b-peptide (Ab) and neurofibrillary tangles (NFT) of protein tau (Selkoe, 2001). Mutations in Ab precursor protein (APP) and Presenillins (PS1 and PS2) are associated with early onset of AD (Selkoe, 2001; Katayama et al., 1999). PS1 and PS2 mediate the cleavage of APP and Notch (Wilquet and Strooper, 2004). Mutant PS1 studies have been implicated in increased Ab production and ER Stress mediated apoptosis (Verkhatsky, 2005; Mattson, 2000; LaFarla, 2002). Mutant PS1 was shown to bind to IRE1, thereby suppressing the activation of a key branch of UPR and reduced transcriptional regulation of BiP (Katayama et al., 2004). On the contrary, increased levels of BiP and PERK was seen in AD human brain specimens (Hoozemans et al., 2005). Whether UPR is initiated for neuro-protection or for neuronal death, is still to be determined.

Immune and Inflammatory disorders

Unfolded or misfolded protein accumulation and UPR-induction is observed in autoimmune diseases like rheumatoid arthritis, inflammatory bowel diseases and multiple sclerosis. UPR and the inflammatory response are connected through several modalities including increased reactive oxygen species (ROS), increased calcium release from the ER, activation of JNK and the activation of NF- κ B (Zhang and Kaufman, 2008). NF- κ B plays a central role in inflammation (Rius et al., 2008). In unstressed cells, NF- κ B is inactive, sequestered by the inhibitor of NF- κ B (I κ B). UPR induction leads to phosphorylation and subsequent degradation of I κ B and the expression of inflammatory genes (Rius et al., 2008; Tak and Firestein, 2001). UPR also plays a key role in autoimmune diseases. CREBH, an ATF6 homolog, translocates to the golgi and undergoes cleavage by S1p and S2p proteases in the presence of ER stress. Proteolytic processing releases an N-terminal fragment that migrates to the nucleus and induces C-reactive protein and serum amyloid P-component expression (Zhang et al., 2006). Both of these genes are associated with the activation of the acute inflammatory response (Zhang et al., 2006). CREBH can also induce pro-inflammatory cytokines such as IL-6 and IL-1 β (Zhang et al., 2006).

Summary and Conclusions

Protein folding is strategically important to cellular function. Perturbations to the unique folding environment of the endoplasmic reticulum (ER) lead to the accumulation of unfolded or misfolded proteins. Higher eukaryotes have evolved a complex three-pronged response to correct aberrant folding, known as the unfolded protein response. The unfolded protein response acts to restore ER homeostasis. However, following prolonged or severe ER stress, cells initiate an alarm phase ultimately leading to apoptotic cell death. Malfunctions in the folding state of critical proteins have been linked with cancer, diabetes and other diseases. Thus, the ability of UPR to regulate cell fate, has made it a primary area of study for pathophysiology and a potential therapeutic axis for the treatment of a diverse array of diseases.

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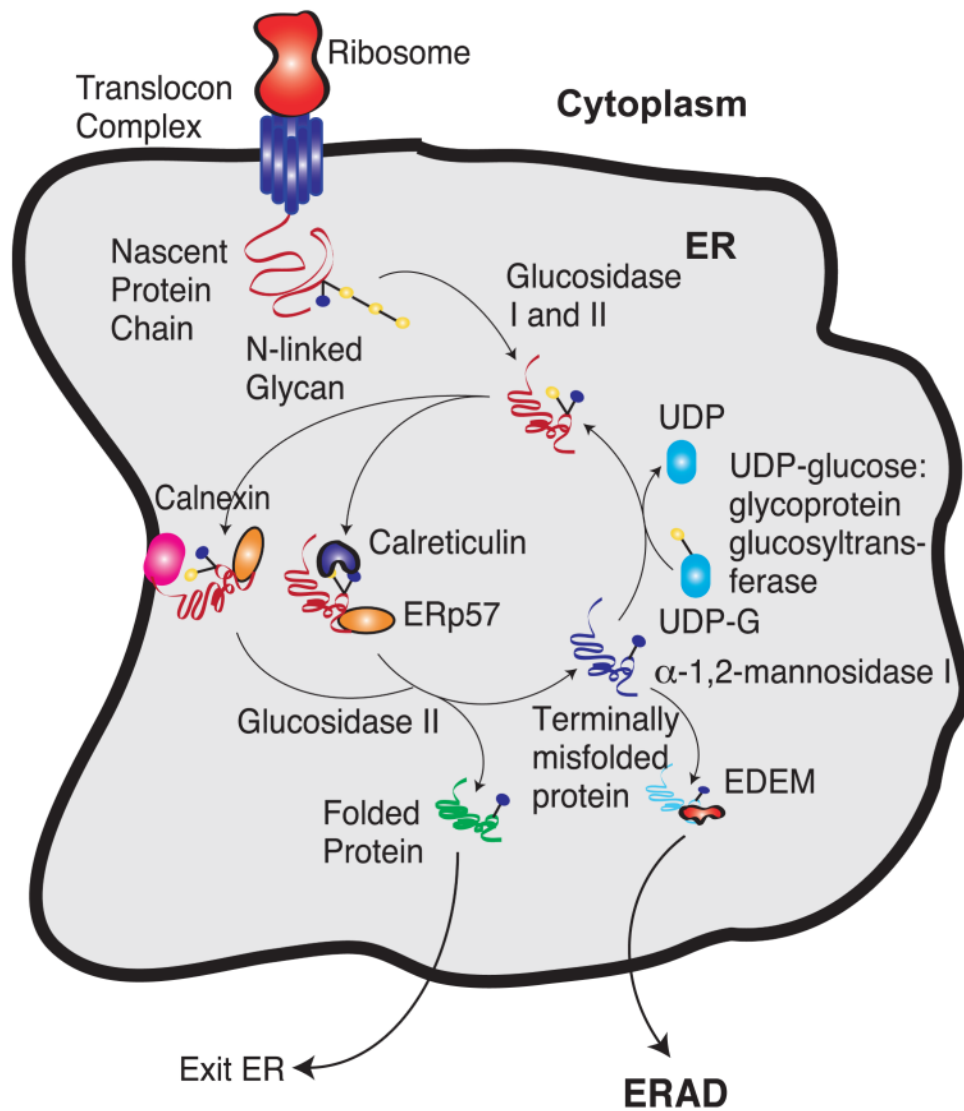
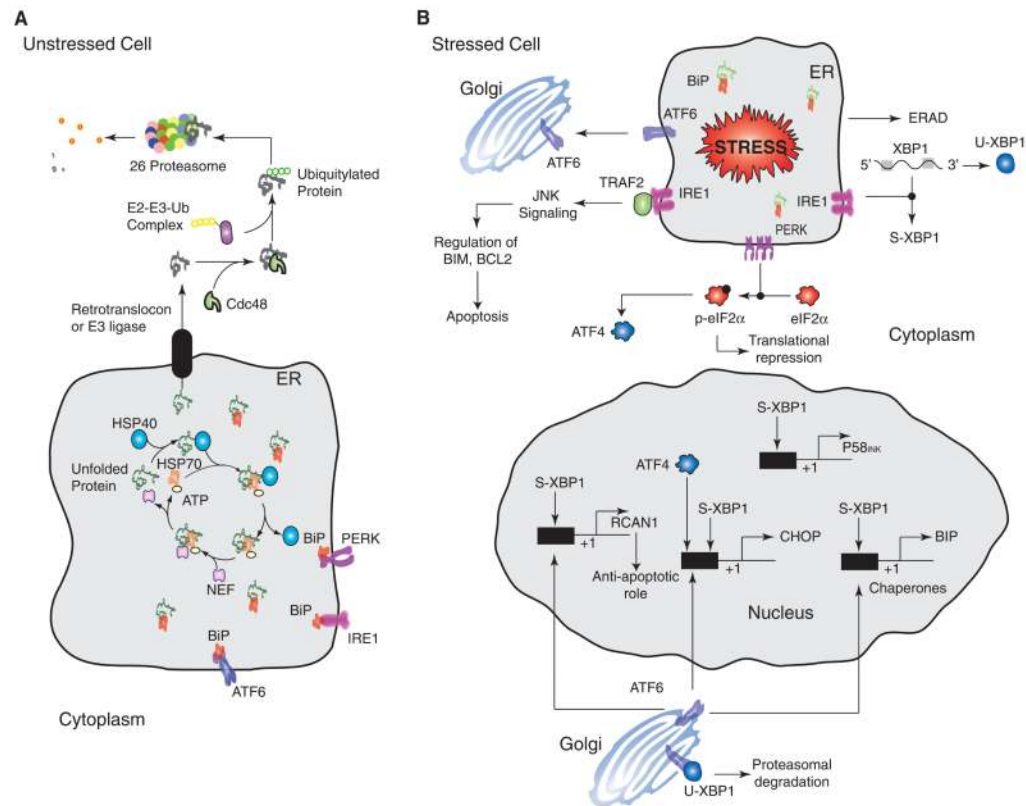


Fig. 1. The calnexin/calreticulin protein folding cycle. Yellow circles denote glucose groups and while blue circles denote mannose groups. After entering the ER lumen, glucosyltransferase adds a glucose residue to the nascent protein chain. The monoglucosylated glycoprotein then interacts with calnexin/calreticulin. These chaperones interact with the thiol-disulphide oxidoreductase ERp57. Cleavage of the last glucose residue by glucosidase II leads to the release of the chaperones. At this time, the protein could have either folded and left the ER or it could have attained an incorrect state. The incorrectly folded proteins are then the substrates of UDP glucose:glycoprotein glucosyltransferase, which puts a glucose residue back to the incorrectly folded protein. This enables the protein to spend some more time in folding in the ER. If the protein fails to fold in a repeated number of cycles, the mannose residue is removed by α -1,2-mannosidase I. This enables the protein to be recognized by ER-degradation-enhancing 1,2-mannosidase-like protein (EDEM). This targets the unfolded proteins for ER-associated degradation (ERAD).

**Fig. 2.**

An array of cellular stressors can perturb the folding environment in the endoplasmic reticulum (ER) leading to unfolded or misfolded protein. In response to the folding imbalance, cells initiate the cytoprotective unfolded protein response (UPR). **A:** The problem of unfolded or misfolded proteins in the ER is addressed by increasing the folding capacity through the up-regulation of the expression of chaperone proteins, attenuating translation by regulating eIF2 α , and promoting the degradation of misfolded proteins through ER-associated degradation (ERAD). If UPR is unable to restore the folding balance, ER stress will eventually lead to apoptotic cell-death. **B:** The three signal transduction pathways mediating the unfolded protein response in higher eukaryotes. First, the PKR-like ER kinase (PERK) pathway is initiated after BiP dissociation from PERK. While PERK transduces both pro- and anti-apoptotic signals, its main function is translation attenuation through the phosphorylation of eIF2 α . Next, the activating transcription factor 6 (ATF6) pathway is activated following BiP dissociation. ATF6 induces the expression of chaperones e.g., BiP as well as apoptosis effectors such as CHOP. Lastly, the inositol-requiring kinase 1 (IRE1) pathway is activated following BiP dissociation from IRE1. Activated IRE1 has both an endoribonuclease and a serine-threonine kinase activity that drive can pro-apoptotic signals.

Table 1

Relevance of ER stress in human disease states

| Disease state | Role of ER-Stress and UPR | Key proteins | Reference |
|---|---|---|---|
| Alzheimer's disease (AD) | AD-induced Presenilin 1 regulates BiP. | Presenilin 1, PERK, eIF2 α , BiP | Katayama et al. (1999); Milhavel et al. (2002); Niwa et al. (1999); Terro et al. (2002); Unterberger et al. (2006) |
| Parkinson's disease (PD) | Suppressed ER-stress- induced apoptosis and aggregation of α -synuclein. | Parkin, α -synuclein | Dawson and Dawson (2003); Imai et al. (2000); Petrucelli et al. (2002); Ryu et al. (2002); Takahashi et al. (2003) |
| Amyotrophic lateral sclerosis (ALS) | Altered ERAD machinery and activation of ASK1. | Cu/Zn-superoxide dismutase (SOD), ASK1 and Derlin-1 | Nishitoh et al. (2008) |
| Bipolar Disorder | Current medications target induction of UPR. | XBP1 | Cichon et al. (2004); Kakiuchi et al. (2005, 2003) |
| Stroke | CHOP and UPR mediates neuronal response in Ischemia. | PERK-eIF2 α , ASK1, CHOP | Kumar et al. (2001); Paschen et al. (1998); Tajiri et al. (2004); Tanaka et al. (2000) |
| Heart Disease | Degeneration of cardiac myocytes, transaortic constriction. Myocardial infarction induces UPR. | IRE1, PERK- eIF2 α , ASK1 | Glembotski (2008); Okada et al. (2004); Pan et al. (2004); Shintani-Ishida et al. (2006); Thuerlauf et al. (2006); Vitadello et al. (2003) |
| Atherosclerosis | Regulates inflammatory genes in vascular cells. | IRE1, JNK, TRAF2, XBP1 | Gargalovic et al. (2006); Harding et al. (2001); Lee et al. (2008); Zhang et al. (2001) |
| Type 1 diabetes | Regulators in normal conditions and triggers β cell dysfunction and apoptosis in ER Stress. | PERK-eIF2 α , JNK and Calcium | Araki et al. (2003); Fonseca et al. (2005); Ishihara et al. (2004); Oyadomari et al. (2002, 2001); Scheuner et al. (2001); Yamada et al. (2006) |
| Type 2 diabetes | ER stress is a key aspect of type 2 diabetes. | XBP1, JNK | Ozcan et al. (2004) |
| Cancer | Cytoprotective branches of UPR vital to survival and progression of tumors. | BiP, XBP1, ATF6, PERK | Bi et al. (2005); Fujimoto et al. (2003); Jamora et al. (1996); Romero-Ramirez et al. (2004); Shuda et al. (2003) |
| Autoimmune disease | Development of plasma cells and dendritic cells. | XBP1, HLA-B27 | Blass et al. (2001); Corrigan et al. (2001); Todd et al. (2008) |
| Glomerulonephritis, acute kidney injury | Upregulation of BiP, CHOP and down- regulation of Bcl-2 in primary glomerular diseases. | BiP, CHOP, Bcl2 | Blass et al. (2001); Corrigan et al. (2001); Todd et al. (2008) Bando et al. (2004); Markan et al. (2009) |

Table 2

Therapeutic interventions targeting ER stress.

| Compound | Pathway | Function | Reference |
|----------------------------------|--|--|---|
| Valporate (VPA), lithium | ER chaperones (BiP, GRP94, Calreticulin) | Remove ER Stress; ER chaperone induction in Neuro-protection in Bipolar Disorders. | Bown et al. (2002); Hiroi et al. (2005); Shao et al. (2006) |
| BiP inducer X (BLX) | ER chaperones (BiP) | Remove ER Stress; Small-molecule inducer of BiP in Cerebral ischemia; stroke resulting in reduced cell death. | Kudo et al. (2008) |
| Salubrinal | eIF2 α , Bcl2 | Phosphatase inhibitor; PERK- eIF2 α pathway agonist in Parkinson's disease; anti-stroke; regulator of Bcl2. | Araki et al. (2003); Boyce et al. (2005); Harding et al. (2001); Kessel (2006); Kumar et al. (2001); Reijonen et al. (2008); Scheuner et al. (2001) |
| p38 MAPK antagonists | p38 MAPK (CHOP activity) | Apoptosis up; p38 dependent CHOP phosphorylation regulator in stroke, diabetes. | Milhavel et al. (2002); Paschen et al. (1998); Tajiri et al. (2004); Wang and Ron (1996); Yoshida (2007) |
| Versipelostatin (VST) | BiP | Apoptosis up; Alters BiP production in glucose-deprived solid tumors. | Park et al. (2004) |
| Bortezomib, Nelfavir, Atazanavir | Proteasome, XBP1 | Apoptosis up; Inhibition of HIV-1 proteasome activity. CHOP mediated cell death. | Gills et al. (2007); Lee et al. (2003); Neubert et al. (2008); Pyrko et al. (2007) |
| Brefeldin A | CHOP, BiP | Apoptosis up; Inhibition of protein trafficking, ER swelling. | Carew et al. (2006) |