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Protein Folding in the Endoplasmic Reticulum and the Unfolded Protein Response

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Abstract In all eukaryotic cells, the endoplasmic reticulum (ER) is an intracellular organelle where folding and assembly occurs for proteins destined to the extracellular space, plasma membrane, and the exo/endocytic compartments (Kaufman 1999). As a protein-folding compartment, the ER is exquisitely sensitive to alterations in homeostasis, and provides stringent quality control systems to ensure that only correctly folded proteins transit to the Golgi and unfolded or misfolded proteins are retained and ultimately degraded. A number of biochemical and physiological stimuli, such as perturbation in calcium homeostasis or redox status, elevated secretory protein synthesis, expression of misfolded proteins, sugar/glucose deprivation, altered glycosylation, and overloading of cholesterol can disrupt ER homeostasis, impose stress to the ER, and subsequently lead to accumulation of unfolded

or misfolded proteins in the ER lumen. The ER has evolved highly specific signaling pathways called the unfolded protein response (UPR) to cope with the accumulation of unfolded or misfolded proteins. Elucidation of the molecular mechanisms by which accumulation of unfolded proteins in the ER transmits a signal to the cytoplasm and nucleus has led to major new insights into the diverse cellular and physiological processes that are regulated by the UPR. This chapter summarizes how cells respond to the accumulation of unfolded proteins in the cell and the relevance of these signaling pathways to human physiology and disease.

Keywords Endoplasmic reticulum · Unfolded protein response · Translational control · ERAD · Apoptosis

Abbreviations

UPR	Unfolded protein response
ER	Endoplasmic reticulum
GRP	Glucose-regulated protein
IRE1	Inositol requiring 1
ATF6	Activating transcription factor 6
PERK	PKR-like ER kinase
XBP1	X-box binding protein 1
UPRE	Unfolded protein response element
ERSE	ER stress response element
ERAD	ER-associated protein degradion

1 Polypeptide Modification, Folding, and Assembly in the Endoplasmic Reticulum Lumen

The endoplasmic reticulum (ER) is the site of biosynthesis for sterols, lipids, and membrane and secreted proteins. Approximately one-third of all cellular protein synthesis occurs on the membrane of the rough ER. For some specialized cells that function to secrete proteins, such as plasma cells, hepatocytes, pancreatic acinar and islet cells, over 90% of the translated polypeptides are directed into the ER lumen, the entrance site into the secretory pathway (Kaufman 2004). Since the protein concentration in the ER lumen is approximately 100 mg/ml, it is essential that protein chaperones facilitate protein folding by preventing aggregation of protein folding intermediates and by correcting misfolded proteins that are caught in kinetic low-energy traps. These energy-requiring processes ensure high-fidelity protein folding in the lumen of the ER. For example, the most abundant ER chaperone BiP/GRP78 uses the energy from ATP hydrolysis to promote folding and prevent aggregation of proteins within the ER. In addition, the oxidizing environment of the ER creates a constant demand for cellular protein disulfide isomerases to catalyze and monitor disulfide bond formation in a regulated and ordered manner. Only those polypeptides that are properly folded and assembled in the ER can transit to the Golgi compartment, a process called quality control. Proteins

that are misfolded in the ER are retained and eventually translocated back through into the cytosol for degradation by the 26S proteasome in a process called ER-associated degradation (ERAD) (Tsai et al. 2002).

The recognition and modification of oligosaccharide structures in the lumen of the ER is intimately coupled to polypeptide folding (Helenius 1994). As the growing nascent chain is translocated into the lumen of the ER, a 14-oligosaccharide core, *N*-acetylglucosamine$_2$-mannose$_9$-glucose$_3$($GlcNAc_2$–Man_9Glc_3), is added to consensus asparagine residues (Asn-X-Ser/Thr; where X is any amino acid except Pro). Immediately after the addition of this core, the three terminal glucose residues are cleaved by the sequential action of glucosidases I and II to yield a $GlcNAc_2Man_9$ structure. If the polypeptide is not folded properly, a UDP-glucose:glycoprotein glucosyltransferase recognizes the unfolded nature of the glycoprotein and reglucosylates the core structure to re-establish the glucose-α(1,3)-mannose glycosidic linkage (Ritter and Helenius 2000). Monoglucosylated oligosaccharides containing this bond bind to the ER-resident protein chaperones calnexin and calreticulin. Glycoprotein interaction with calnexin and calreticulin promotes interaction with the oxidoreductase ERp57 that promotes proper disulfide bond rearrangement. This quality control process ensures that unfolded glycoproteins do not exit the ER.

The ER has evolved highly specific signaling pathways to ensure that its protein-folding capacity is not overwhelmed. Upon accumulation of unfolded proteins in the ER lumen, several adaptive pathways are activated to reduce the amount of new protein translocated into the ER lumen, to increase the retrotranslocation and degradative potential of ER-localized malfolded proteins, and to increase the protein-folding capacity of the ER.

2
Historical Perspective of the Unfolded Protein Response

In the mid-1970s, it was observed that transformation of fibroblasts with Rous sarcoma virus induces expression of a set of genes (Pouyssegur et al. 1977). The same set of genes was found to be upregulated upon glucose deprivation, hence the products of these genes were termed glucose-regulated proteins (GRPs, i.e., GRP78 and GRP94) (Lee et al. 1984, 1983). Independently, a protein was identified that binds unassembled immunoglobulin heavy chains in the ER of pre-B cells and prevents their secretion until the immunoglobulin light chains are expressed. This protein was named the immunoglobulin binding protein (BiP) (Haas and Wabl 1983; Lee 1987) and is identical to GRP78. Subsequently, it was demonstrated that overexpression of an unfolded mutant of the influenza hemagglutinin protein was sufficient to induce expression of BiP and GRP94 (Gething and Sambrook 1992), leading to the designation of this signaling pathway as the unfolded protein response (UPR). In addition, overexpression of a wild-type protein, coagulation factor VIII, was also shown to induce expression of BiP and GRP94 (Dorner et al. 1987, 1988).

The UPR is conserved in all eukaryotic cells. Analysis of the UPR pathway in the budding yeast *Saccharomyces cerevisiae* identified a 22-bp *cis*-acting UPR element (UPRE) that was necessary and sufficient for ER stress induction of a reporter gene upon accumulation of unfolded proteins in the ER (Mori et al. 1992). A genetic screen was used to isolate mutants in this pathway. The first mutant identified independently by two groups was defective in a gene encoding an ER transmembrane serine/threonine protein kinase (Ire1p/Ern1p). Ire1p was characterized as a proximal UPR transducer required for transcriptional induction of UPR genes and for survival upon ER stress (Cox et al. 1993; Mori et al. 1993). Ire1p has an N-terminal lumenal domain that senses the ER stress signal and a C-terminal cytoplasmic domain that has a serine/threonine kinase activity. Subsequently, it was discovered that the C-terminus has homology with RNaseL, a nonspecific endoribonuclease that signals one arm of the interferon response in mammalian cells. Indeed, it was demonstrated that Ire1p has a site-specific endoribonuclease (RNase) activity required for activating KAR2/BiP transcription (Mori et al. 1993; Shamu and Walter 1996; Welihinda and Kaufman 1996). The presence of unfolded proteins in the ER lumen promotes dimerization and *trans*-autophosphorylation of Ire1p, activating its RNase activity to cleave at two sites within the mRNA encoding HAC1, a transcription factor that potently activates KAR2/BiP transcription. The Ire1p-dependent cleavage initiates *HAC1* mRNA splicing through an unconventional reaction (Kawahara et al. 1998; Sidrauski et al. 1996; Sidrauski and Walter 1997). The 5′ and 3′ ends of *HAC1* mRNA are subsequently ligated by transfer RNA ligase. The unconventional *HAC1*-mRNA processing reaction removes a 252-nucleotide intron, and the result is replacement of the carboxy-terminal ten amino acids in Hac1p (Hac1up) with a new 19-amino-acid segment (Hac1ip). This splicing reaction regulates UPR transcriptional activation in two ways. First, the new carboxyl terminus on Hac1ip converts Hac1p into a tenfold more potent transcriptional activator. Second, removal of the intron increases the translational efficiency of *HAC1* mRNA.

Spliced *HAC1* mRNA encodes a basic leucine zipper (b-ZIP) transcription factor that binds to a DNA sequence motif, termed the unfolded protein response element (UPRE; consensus CAGCGTG), as a dimer in the promoters of many UPR responsive genes. It was reported that Hac1p activates transcription of approximately 381 UPR target genes in yeast (Travers et al. 2000).

3 The Unfolded Protein Response Sensors in Higher Eukaryotes

All metazoan cells have conserved the essential and unique properties of the UPR in yeast, but have also evolved additional sensors and a greater number of downstream targets to generate a diversity of responses (Fig. 1). In mammals,

the counterpart of yeast Ire1p has two isoforms: IRE1α and IRE1β. While IRE1a is expressed in most cells and tissues, with high-level expression in the pancreas and placenta, IRE1β expression is primarily restricted to intestinal epithelial cells (Tirasophon et al. 1998; Wang et al. 1998). Both molecules respond to the accumulation of unfolded proteins in the ER to activate their kinase and subsequent RNase activities. In vitro cleavage reactions using yeast *HAC1*-mRNA substrate showed that the cleavage specificities of IRE1α and IRE1β are similar, indicating that they did not evolve to recognize different sets of substrates, but rather to generate temporal- and tissue-specific expression. All cells in multicellular organisms also constitutively express two additional stress sensors that respond to the accumulation of unfolded proteins in the ER lumen and coordinate either adaptive or cell-death responses: PERK and ATF6.

PERK has a carboxy-terminal protein kinase domain homologous to the double-stranded RNA-activated protein kinase PKR that phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α) (Fig. 1). However, where PKR is a soluble cytosolic protein, PERK contains a transmembrane domain and an amino-terminal domain that resides in the ER lumen and responds to the accumulation of unfolded proteins in the ER lumen (Harding et al. 1999; Liu et al. 2000; Shi et al. 1998). The luminal domain of PERK shares a low degree of homology with the IRE1 luminal domain. Surprisingly, the human PERK luminal domain can functionally substitute for the yeast Ire1p luminal domain, although the yeast genome does not have a PERK

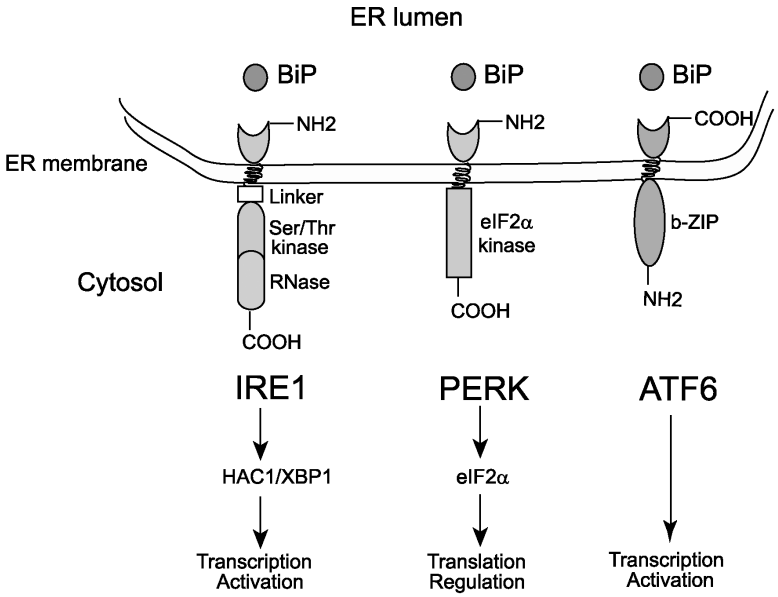

Fig. 1 Depiction of the three UPR transducers. The functional domains of IRE1, PERK, and ATF6 are shown

homolog. This supports the idea that the fundamental mechanism for sensing the accumulation of unfolded proteins in the ER is conserved between IRE1 and PERK and between yeast and humans (Harding et al. 1999; Liu et al. 2000; Shi et al. 1998). In an evolutionary sense, it is significant that the eIF2α kinase PKR and the endoribonuclease RNaseL are both components of the interferon antiviral response that respond to the presence of double-stranded RNA produced during viral replication and prevent virus production. PERK and IRE1 share a common ancestry with PKR and RNAse L, although they have evolved to respond to accumulation of unfolded proteins in the ER. As the effector domains of PERK and PKR and of IRE1 and RNAse L share functions, it is interesting to speculate that PERK and IRE1 may also provide a protective function in infectious disease.

ATF6 is transcription factor with a b-ZIP domain in the cytosol that also contains a large ER luminal domain to sense ER stress (Haze et al. 1999). There are two isoforms of ATF6, ATF6α (90 kDa) and ATF6β (110 kDa, also known

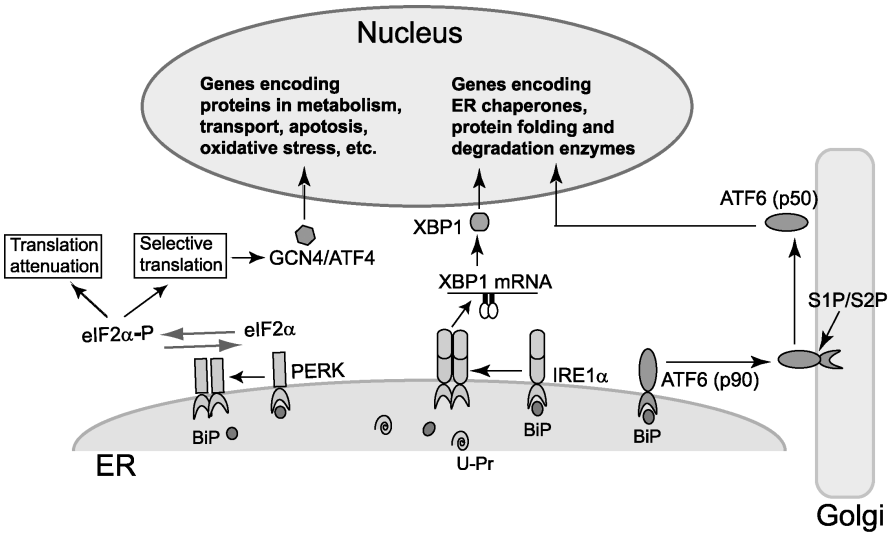

Fig. 2 Translational and transcriptional regulation upon ER stress. Upon accumulation of unfolded proteins in the ER lumen, PERK is released from BiP, thus permitting its dimerization and activation. Activated PERK phosphorylates eIF2α to reduce the frequency of the mRNA translation initiation in general. However, selective mRNAs, such as *GCN4* and *ATF4* mRNA, are preferentially translated in the presence of phosphorylated eIF2α. Upon accumulation of unfolded protein in the ER lumen, BiP release from IRE1 permits dimerization to activate its kinase and RNase activities to initiate *XBP1* mRNA splicing. Spliced *XBP1* mRNA encodes a potent transcription factor that binds to UPRE and ERSE sequences of many UPR target genes. BiP release from ATF6 permits ATF6 transport to the Golgi compartment where full-length ATF6 (90 kDa) is cleaved by S1P and S2P proteases to yield a cytosolic fragment (50 kDa) that migrates to the nucleus to activate transcription of UPR responsive genes. U-Pr unfolded protein

as CREB-RP). On activation of the UPR, ATF6α and ATF6β transit to the Golgi where they are cleaved by site-1 protease (S1P) and site-2 protease (S2P) to generate 50-kDa cytosolic b-ZIP-containing fragments that migrate to the nucleus to activate transcription of UPR target genes (Shen et al. 2002; Ye et al. 2000) (Fig. 2). Notably, ER stress-induced cleavage of ATF6 is processed by the same proteases S1P and S2P that cleave the ER-associated transmembrane sterol-response element binding protein (SREBP), which is a transcription factor required for induction of sterol biosynthetic genes (Ye et al. 2000). Regulated intramembrane proteolysis of ATF6 and SREBP is controlled at the step of trafficking of these transcription factors from the ER to the Golgi compartment. Whereas only unfolded protein accumulation in the ER promotes ATF6 transit to the Golgi for cleavage, cholesterol deprivation induces trafficking of SREBP to the Golgi.

4
Activation of Unfolded Protein Response Sensors

UPR signaling is an adaptive mechanism for cells to survive accumulation of unfolded proteins in the ER lumen. The UPR reduces the amount of new protein translocated into the ER lumen, increases retrotranslocation and degradation of misfolded ER-localized proteins, and bolsters the protein-folding capacity of the ER. The UPR is orchestrated by transcriptional activation of multiple genes mediated by IRE1 and ATF6, and a general decrease in translation initiation and a selective translation of several specific mRNAs mediated by PERK.

The most proximal UPR event is the activation of the ER stress sensors by a common stimulus, the accumulation of unfolded proteins in the ER lumen. Current studies support the idea that BiP serves as a master UPR regulator that plays a central role in activating all three transducers IRE1, PERK, and ATF6 in response to ER stress (Bertolotti et al. 2000; Dorner et al. 1992; Shen et al. 2002). BiP is a member of the heat shock protein family of 70 kDa that has a peptide-dependent ATPase activity. BiP binds to exposed hydrophobic patches on unfolded proteins and requires ATP to promote release. BiP interaction with unfolded proteins is part of the cellular quality control mechanism that only permits trafficking of properly folded proteins to the Golgi compartment. Reduction in the level of free BiP leads to UPR activation, whereas overexpression of BiP inhibits UPR activation (Dorner et al. 1988, 1992; Morris et al. 1997). Under nonstressed conditions, BiP binds to IRE1, PERK, and ATF6 to prevent their signaling. As the ER is overloaded by newly synthesized unfolded proteins or is "stressed" by agents that cause protein misfolding, the pool of free BiP in the ER lumen is depleted. As the free pool of BiP drops, IRE1 and PERK are released to permit homodimerization and autophosphorylation, leading to their activation (Bertolotti et al. 2000; Liu et al. 2003). Concomitantly, release of ATF6 from BiP permits ATF6 transport to the Golgi compartment,

where it is cleaved to generate the cytosolic activated form of ATF6 (Shen et al. 2002). Thus, this BiP-regulated activation provides a direct mechanism for the three UPR transducers to sense the "stress" in the ER. However, in certain cells, different stresses or physiologic conditions can selectively activate only one or two of the ER stress sensors. For example, in B cell differentiation, the IRE1α-mediated UPR subpathway is activated and indispensable while the PERK-mediated UPR subpathway through phosphorylation of eIF2α is not required for the B cell differentiation process (Gass et al. 2002; Zhang et al. 2005). In contrast, in pancreatic β cells, glucose limitation appears to activate PERK prior to activation of IRE1 (Scheuner and Kaufman, unpublished observation). It will be important to elucidate how a general BiP repression mechanism permits the selective activation of individual components of the UPR that mediate various downstream effects.

5
The Transcriptional Response to Endoplasmic Reticulum Stress

To cope with accumulation of unfolded or misfolded protein in the ER lumen, the UPR is activated to alter transcriptional programs through IRE1 and ATF6 (Fig. 2). In mammals, the promoter regions of many UPR-inducible genes, such as BiP, GRP94, and calreticulin, contain a mammalian ER stress response element (ERSE, minimal motif: CCAAT(N_9)CCACG) that is necessary and sufficient for ER stress-induced gene transcription (Yoshida et al. 1998). Using ERSE as a probe in a yeast one-hybrid screen, researchers isolated two UPR-specific b-ZIP transcription factors, the X-box DNA binding protein 1 (XBP1) and ATF6 (Yoshida et al. 1998). XBP1 was identified as a homolog of yeast Hac1p that is a substrate for mammalian IRE1 RNase activity (Calfon et al. 2002; Shen et al. 2001; Yoshida et al. 2001). On activation of the UPR, IRE1 RNase cleaves *XBP1* mRNA to remove a 26-nucleotide intron, generating a translational frame-shift. Spliced *XBP1* mRNA encodes a protein with a novel carboxy-terminus that acts as a potent transcriptional activator for many UPR target genes. ATF6 is a UPR transducer that can bind ERSE motifs in the promoter regions of UPR responsive genes (Yoshida et al. 1998). Both ATF6α and ATF6β, in the presence of the CCAAT-binding factor (CBF; also called NF-Y), bind to the 3′ half ERSE sequence (CCACG) in the promoter regions of UPR-responsive genes to activate transcription. Whereas CBF is a factor that constitutively binds the CCAAT motif, ATF6 is the inducible factor that binds the CCACG motif (Haze et al. 1999; Li et al. 2000; Yoshida et al. 2000).

The two bZIP transcription factors of ATF/CREB family, XBP1 and ATF6, serve as key regulators of transcriptional control in response to ER stress. ATF6 regulates a group of genes encoding ER-resident molecular chaperones and folding enzymes, while XBP1 regulates a subset of ER-resident chaperone genes that are essential for protein folding, maturation, and degradation in

the ER (Lee et al. 2003; Okada et al. 2002). It was previously proposed that *XBP1* mRNA is induced by ATF6 in response to ER stress to generate more substrate *XBP1* mRNA for IRE1-mediated splicing (Lee et al. 2002; Yoshida et al. 2000, 2001). However, UPR induction of *XBP1* transcripts and proteins was not altered in the cells having defective or reduced ATF6 cleavage (Lee et al. 2003; Lee et al. 2002). Induction of *ATF6* mRNA upon ER stress was partially compromised in the absence of XBP1; therefore it was proposed that ATF6 lies downstream of XBP1 in some cases (Lee et al. 2003). These results suggest that XBP1 and ATF6 are situated largely in parallel pathways and may interact with each other upon ER stress.

6
The Translational Response to Endoplasmic Reticulum Stress

An immediate response to the accumulation of unfolded proteins in the ER of metazoan cells, is activation of PERK to inhibit protein biosynthesis through phosphorylation of eIF2α (Kaufman 2004). When eIF2α is phosphorylated, the formation of the ternary translation initiation complex eIF2/GTP/ $tRNA_i^{Met}$ is prevented, leading to reduced efficiency of AUG initiation codon recognition and general translational attenuation to reduce the workload of the ER (Harding et al. 2000, 2001; Scheuner 2001) (Fig. 2). Murine cells deleted in PERK, or mutated at Ser51 in eIF2α to prevent phosphorylation, did not attenuate protein synthesis upon ER stress. As a consequence, these cells were not able to survive ER stress (Scheuner 2001). Whereas phosphorylation of eIF2α by PERK leads to attenuation of global mRNA translation, phosphorylated eIF2α selectively stimulates translation of a specific subset of mRNAs in response to stress (Fig. 2).

In yeast, the Gcn2p-mediated phosphorylation of eIF2α upon amino acid starvation promotes translation of *GCN4* mRNA that encodes a b-ZIP transcription factor required for induction of genes encoding amino-acid biosynthetic functions (Hinnebusch 2000). *GCN4* mRNA contains four upstream open reading frames (uORFs) in its 5′ UTR, which ordinarily inhibit the ability of the ribosome to scan through the 5′ end of the mRNA and reach the correct AUG initiation codon. Phosphorylation of eIF2α limits 60S-ribosomal-subunit joining to allow the 40S ribosomal subunit to scan through the ORFs and initiate polypeptide-chain synthesis at the authentic GCN4 ORF, thus allowing translation of *GCN4* (Kaufman 2004). This control mechanism is also utilized in mammalian cells to regulate translation in response to ER stress and amino acid starvation. For example, upon ER stress, phosphorylated eIF2α selectively promotes translation of activating transcription factor 4 (ATF4) mRNA (Harding et al. 2000; Scheuner 2001). ATF4 subsequently activates transcription of genes involved in amino acid metabolism and transport, oxidation-reduction reactions, and ER stress-induced apoptosis (Harding et al. 2003).

7
Endoplasmic Reticulum-Associated Protein Degradation

Protein folding in the oxidizing environment of the ER is an energy-requiring process (Braakman et al. 1992; Dorner et al. 1990). Under nonstressed conditions, newly synthesized proteins exist as unfolded intermediates along the protein-folding pathway. Once ER stress is imposed, for example, by depletion of energy, many folding intermediates become irreversibly trapped in low-energy states and accumulate. These unfolded proteins are retained in the ER through interactions with BiP, calnexin, and calreticulin. Eventually, unfolded or misfolded proteins in the ER lumen are retrotranslocated to the cytoplasm, where they are ubiquitinated and degraded by the proteasome (Werner et al. 1996). This process is called ER-associated degradation (ERAD) and is regulated by the UPR.

Many specific components of the ERAD pathway in yeast, such as *DER1, HRD1/DER3, HRD3*, and *UBC7*, are induced by the UPR (Travers et al. 2000). Hrd1p is an ER type I-transmembrane protein having E3 ubiquitin ligase activity (Bays et al. 2001). The interaction between the ER lumenal domains of Hrd3p and Hrd1p stabilizes the cytosolic RING-H2 motif of Hrd1p, which is required for its ubiquitin ligase activity (Gardner et al. 2000, 2001). Hrd1p prefers a misfolded protein as a ubiquitination substrate and uses only Ubc7p or Ubc1p, E2 ubiquitin-conjugating enzymes, to specifically mediate ubiquitination of ERAD substrates. Although UBC1 mRNA is unaffected by dithiothreitol treatment, induction of *UBC7* and *HRD1* upon ER stress is completely dependent on *HAC1* and *IRE1*, indicating that the UPR may upregulate components of the ERAD system in yeast (Friedlander et al. 2000). Yeast cells unable to perform ERAD are constantly susceptible to folding stress, as indicated by a constitutive activation of the UPR and a requirement for the UPR for normal growth and survival even under conditions of mild stress (Friedlander et al. 2000). For example, absence of both Ubc1p and Ubc7p or absence of Hrd1p results in marked stabilization of an ERAD substrate and induction of the UPR. Moreover, the ER-associated AAA-ATPase Cdc48p–Ufd1p–Npl4p complex that is required for ERAD functions as a cytosolic chaperone complex to extract ER degradation substrates from the ER lumen (Ye et al. 2001). Efficient dislocation of ERAD substrates ensures their subsequent proteolysis by the 26S proteasome. Although the UPR does not upregulate the expression of *CDC48*, mutations in either of *CDC48, UFD1*, or *NPL4* cause accumulation of ERAD substrates in the ER and activate the UPR (Jarosch et al. 2002). Rather than being individually dispensable, the UPR and ERAD are intimately coordinated, complementary mechanisms that prevent unfolded protein accumulation and mitigate its toxic consequences.

Regulation of ERAD by the UPR is further suggested by identification of EDEM in mammalian cells (Yoshida et al. 2003). EDEM is a type-II transmem-

brane protein localized to the ER, and its lumenal domain shows significant homology to a 1,2-mannosidase but lacks such enzymatic activity. IRE1α-deficient cells were defective in degradation of a mutant α1-antitrypsin, an ERAD substrate, and this defect was completely restored by expression of EDEM, supporting that EDEM-mediated ERAD is solely dependent on the IRE1-mediated UPR pathway. (Hosokawa et al. 2001; Molinari et al. 2003; Oda et al. 2003; Yoshida et al. 2003). On the other hand, if the overload of unfolded or misfolded proteins in the ER is not resolved, prolonged UPR activation will lead to programmed cell death.

8
Unfolded Protein Response-Induced Apoptosis

Three known pro-apoptotic pathways emanating from the ER are mediated by IRE1α, caspase-12, and PERK/CHOP, respectively (Fig. 3). Under ER stress, activated IRE1α can bind c-Jun-N-terminal inhibitory kinase (JIK) and recruit cytosolic adapter TRAF2 to the ER membrane (Urano et al. 2000; Yoneda et al. 2001). TRAF2 activates the apoptosis-signaling kinase 1 (ASK1), a mitogen-activated protein kinase kinase kinase (MAPKKK) (Nishitoh et al. 2002). Activated ASK1 leads to activation of the JNK protein kinase and mitochondria/Apaf1-dependent caspase activation (Leppa and Bohmann 1999; Nishitoh et al. 2002; Urano et al. 2000). Caspase-12 is an ER-associated proximal effector of the caspase activation cascade, and cells defective in this enzyme are partially resistant to ER stress-induced apoptosis (Nakagawa et al. 2000). ER stress induces TRAF2 release from procaspase 12, allowing it to bind activated IRE1. This also permits clustering of procaspase-12 at the ER membrane, thus leading to procaspase-12 activation (Yoneda et al. 2001). Caspase-12 can activate caspase-9, which in turn activates caspase-3 (Morishima et al. 2002). Procaspase-12 can also be activated by m-calpain in response to calcium release from the ER, although the physiological significance of this pathway is not known (Nakagawa and Yuan 2000). In addition, upon ER stress, procaspase-7 is activated and recruited to the ER membrane (Rao et al. 2001). Finally, a second death-signaling pathway activated by ER stress is mediated by transcriptional activation of genes encoding pro-apoptotic functions. Activation of the UPR transducer PERK leads to translation of the transcription factor ATF4, which subsequently activates transcription of CHOP/GADD 153, a b-ZIP transcription factor that potentates apoptosis, possibly through repressing expression of the apoptotic repressor BCL2 (Harding et al. 2000; Ma et al. 2002). In addition to its transcriptional induction by PERK/ATF4, CHOP is also regulated at the post-translational level by phosphorylation mediated by p38 MAP kinase (Wang et al. 1996; Wang and Ron 1996). CHOP forms stable heterodimers with C/EBP family members and controls expression of a set of stress-induced genes, which may be involved in apoptosis or organ regeneration. CHOP-deficient

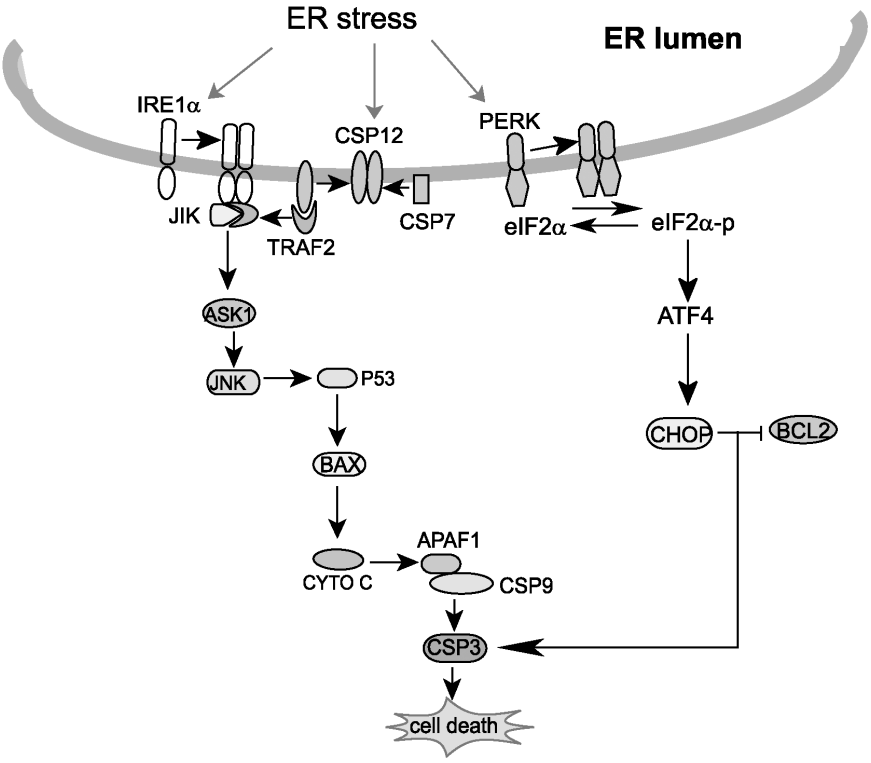

Fig. 3 Apoptosis mediated by UPR signaling. Upon ER stress, activated IRE1α can recruit JIK and TRAF2 to activate ASK1 and JNK, leading to activation of mitochondria/Apaf1-dependent caspases. Upon activation of the UPR, c-Jun-N-terminal inhibitory kinase (*JIK*) release from procaspase-12 permits clustering and activation of procaspase-12. Caspase-12 activates procaspase-9 to activate procaspase-3, the executioner of cell death. Activated PERK phosphorylates eIF2α that enhances translation of ATF4 mRNA. ATF4 induces transcription of the pro-apoptotic factor CHOP, which can inhibit expression of apoptotic suppressor BCL2. *CSP,* caspase; *CYTO C,* cytochrome C

mice have reduced apoptosis in renal epithelium in response to tunicamycin, a reagent that induces ER stress by blocking protein glycosylation (Zinszner et al. 1998).

9 The Physiological Roles of the Unfolded Protein Response

During cell growth, differentiation, and environmental stimuli, there are different levels of protein-folding load imposed upon the ER. Cells have evolved the ability to augment their folding capacity and remodel their secretory pathway in response to developmental demands and physiological changes. Accumu-

lating evidence suggests that the UPR plays important roles in differentiation and function of specialized cells. Moreover, pathological conditions that interfere with ER homeostasis produce prolonged activation of the UPR that may contribute to the pathogenesis of many diseases.

9.1 The Unfolded Protein Response in B Cell Differentiation

On terminal differentiation of B lymphoid cells to plasma cells, the ER compartment expands approximately fivefold to accommodate the large increase in immunoglobulin (Ig) synthesis (Wiest et al. 1990). The UPR transcriptional activator XBP1 is required for plasma cell differentiation (Reimold et al. 2001). XBP1-deficient B lymphoid cells express Ig genes and undergo isotype switching, but are defective in plasma cell differentiation and do not secrete high levels of Igs. Expression of the spliced form of XBP1 efficiently restores production of secreted Igs in XBP1-deficient B cells, suggesting a physiological role for the UPR in high-rate production of secreted antibodies (Iwakoshi et al. 2003). During plasma cell differentiation, IRE1α-mediated splicing of *XBP1* mRNA was found to depend on increased translation of Ig chains (Gass et al. 2002; Iwakoshi et al. 2003; van Anken et al. 2003). These observations support the hypothesis that increased synthesis of Ig produces greater amounts of nascent, unfolded, and unassembled subunits that bind and sequester BiP, leading to UPR activation. Indeed, BiP is the most abundantly expressed UPR-dependent gene and was first identified as encoding a protein that binds Ig heavy chains in the absence of light chains (Haas and Wabl 1983). In addition, the UPR transducer ATF6 may be involved in the process of terminal differentiation of B cells by regulating secretion of Igs (Gass et al. 2002; Gunn et al. 2004).

It is possible that the UPR may signal a B cell differentiation program that occurs prior to increased antibody synthesis. Recently, our group demonstrated that mouse IRE1α is required at two distinct steps during B cell lymphopoiesis (Zhang et al. 2005). IRE1α plays essential roles in both early and late stages of B cell development. In the very early stage, IRE1α regulates transcription of the VDJ recombination-activating genes *rag1*, *rag2*, and *TdT*, which is required for initiation of VDJ rearrangement and B cell receptor formation. In the late stage of B cell differentiation, IRE1α is required to splice the *XBP1* mRNA for terminal differentiation of mature B cells into antibody-secreting plasma cells (Zhang et al. 2005).

9.2 The Unfolded Protein Response in Glucose Homeostasis and Diabetes

The metabolism of glucose is tightly controlled at the levels of synthesis and utilization through hormonal regulation. Glucose not only promotes the secretion of insulin but also stimulates insulin transcription and translation (Itoh and

Okamoto 1980; Lang 1999; Permutt 1974). UPR signaling is essential to maintain glucose homeostasis. It is noteworthy that the UPR was first characterized as transcriptional activation of a set of genes, encoding glucose-regulated proteins, in response to glucose/energy deprivation (Pouyssegur et al. 1977). We now know that pancreatic β cells uniquely require the UPR for survival during intermittent fluctuations in blood glucose (Harding et al. 2001; Scheuner 2001). Humans and mice with deletions in PERK have a profound pancreatic β cell dysfunction and develop infancy-onset diabetes (Delepine et al. 2000; Harding et al. 2001). Mice with a homozygous Ser51Ala mutation at the PERK phosphorylation site in eIF2α display a β cell loss in utero, suggesting that translational control through PERK-mediated phosphorylation of eIF2 is required to maintain β cell survival (Scheuner 2001). Pancreatic β cells are exquisitely sensitive to physiological fluctuations in blood glucose, because they lack hexokinase, an enzyme with a high affinity for glucose as a substrate. We propose that blood glucose levels influence the protein-folding status in the ER. As glucose levels decline, the energy supply decreases, so protein folding becomes less efficient and PERK is activated. The UPR regulates transcriptional induction of glucose-regulated proteins that might provide a protective function by increasing the cellular capacity for the uptake and use of glucose. Conversely, as blood glucose levels rise, eIF2α would be dephosphorylated so that translation would accelerate to increase proinsulin synthesis (Scheuner 2001). This would allow entry of new preproinsulin into the ER, and is consistent with the glucose-stimulated increase in total protein and proinsulin synthesis observed in isolated β cell preparations. Eventually, after prolonged proinsulin translation, the UPR would be turned on to inhibit further protein synthesis and prevent overload of the ER folding capacity. In this manner, a balance between glucose level and PERK-eIF2α UPR signaling is essential for the glucose-regulated periodic fluctuations in proinsulin translation, β cell function, and survival.

The UPR may also play an important role in the regulation of cellular responses to insulin. A recent study showed that ER stress serves as a central feature of peripheral insulin resistance and type 2 diabetes and that the IRE1α-XBP1 UPR pathway is critical for this process (Ozcan et al. 2004). Mice deficient in XBP1 develop insulin resistance. ER stress in obese mice leads to suppression of insulin receptor signaling through hyperactivation of c-Jun N-terminal kinase (JNK) and subsequent serine phosphorylation of insulin receptor substrate-1.

9.3 The Unfolded Protein Response in Organelle Expansion

When the protein-folding load exceeds the capacity of the ER to fold proteins, the UPR maintains ER homeostasis by inhibiting protein synthesis and enhancing transcription of resident ER proteins that facilitate protein mat-

uration, secretion, and degradation. The UPR is required for ER expansion that occurs upon differentiation of highly specialized secretory cells (Kaufman 2002). During differentiation of certain secretory cells, such as those in the pancreas or liver, membrane expansion is accompanied by a dramatic increase in protein secretion and UPR activation. Recent evidence support that one role of UPR activation is to expand the quantity of the ER in order to promote more productive protein folding and secretion. In mature B cells, ectopic expression of XBP1 induced a wide spectrum of secretory pathway genes and physically expanded the ER (Shaffer et al. 2004). Overexpression of spliced XBP1 increased cell size, lysosome content, mitochondrial mass and function, ribosome number, and total protein synthesis. Thus, XBP1 coordinates diverse changes in cellular structure and function resulting in the characteristic phenotype of professional secretory cells. Furthermore, another study showed that spliced XBP1 could induce membrane biosynthesis and ER proliferation in a cell type different from B lymphocytes (Sriburi et al. 2004). Overexpression of spliced XBP1 in NIH-3T3 cells was sufficient to induce synthesis of phosphatidylcholine, the primary phospholipid of the ER membrane. Cells overexpressing spliced XBP1 exhibit elevated levels of membrane phospholipids, increased surface area and volume of rough ER, and enhanced activity of the cytidine diphosphocholine pathway of phosphatidylcholine biosynthesis.

9.4 The Unfolded Protein Response in Neurological Diseases

Neurological disease caused by expansion of polyglutamine repeats and neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, are associated with accumulation of abnormal protein and dysfunction of the ER. Analysis of the polyglutamine repeat associated with the spinocerebrocellular atrophy protein (SCA3) in Machado-Joseph disease suggests that cytoplasmic accumulation of the SCA3 aggregate can inhibit proteasome function, thereby interfering with ERAD to induce the UPR and elicit caspase-12 activation (Nishitoh et al. 2002). Alzheimer's disease is a progressive neurodegenerative disorder that is characterized clinically by progressive loss of memory and cognitive impairment, and pathologically by the extracellular deposition of senile plaques. Mutations of genes that encode amyloid precursor protein, presenilin-1 (PS1) and presenilin-2 (PS2) were found to cause familial Alzheimer's disease (Goate et al. 1991; Levy-Lahad et al. 1995; Rogaev et al. 1995; Sherrington et al. 1995). Interestingly, it was observed that PS1 bound directly to IRE1α on the ER membrane and that the autophosphorylation of IRE1α in response to ER stress was diminished in cells expressing mutant PS1 compared with cells expressing wild-type PS1 (Katayama et al. 1999, 2001). Mutant PS1 was also found to suppress the activation of the other two UPR transducers, ATF6 and PERK, so the global ER response to stress seems to be reduced by mutant PS1. Indeed,

cells expressing mutant PS1 show increased vulnerability to ER stress (Guo et al. 1999; Katayama et al. 1999, 2001). The mechanisms by which mutant PS1 affects the ER stress response are attributed to the inhibited activation of the ER stress transducers IRE1, PERK, and ATF6. However, in sporadic Alzheimer's disease, the spliced isoform of PS2 induces expression of high-mobility group A1a protein (HMGA1a), and also downregulates the UPR signaling pathway in a manner similar to that of PS1 mutant in familial Alzheimer disease (Sato et al. 2001). It was suggested that caspase-4, the human homolog of murine caspase-12, plays critical roles in ER stress-induced neuronal cell death in Alzheimer disease (Katayama et al. 2004).

In addition, autosomal recessive juvenile parkinsonism (AR-JP) results from defects in the Parkin gene (Kitada et al. 1998), encoding a ubiquitin protein ligase (E3) that functions with ubiquitin-conjugating enzyme UbcH7 or UbcH8 to tag proteins for degradation. Overexpression of Parkin suppresses cell death associated with ER stress (Imai et al. 2000). PAEL-R is a putative transmembrane receptor protein that is detected in an insoluble form in the brains of AR-JP patients (Imai et al. 2001). Inherited Parkinson's disease is associated with the accumulation of PAEL-R in the ER of dopaminergic neurons. The accumulation of PAEL-R results from defective Parkin that does not maintain the proteasome-degrading activity necessary to maintain ER function (Imai et al. 2002).

Finally, translational inhibition through UPR activation was observed in cerebral ischemia. PERK is the only eIF2α kinase that is known to be activated after cerebral ischemia (Kumar et al. 2001) and *XBP1* mRNA splicing was detected after transient cerebral ischemia (Paschen et al. 2003). Together these findings indicate that the etiology of many neurological diseases is significantly related to impaired ER homeostasis and activation of the UPR.

10 Perspectives

Over the past 10 years, tremendous progress has been made in identifying the components that regulate the UPR upon accumulation of unfolded protein in the ER lumen. However, little is known regarding the physiological roles of the different UPR pathways in maintaining cell homeostasis and in disease pathogenesis. Additional studies are required to elucidate the mechanisms by which selective UPR transducers are activated under different physiological conditions. Research efforts to understand the upstream events in the UPR pathway promise to expand our knowledge of UPR regulation and its physiological functions. It is known that a variety of environmental insults and genetic defects result in accumulation of unfolded or misfolded proteins in the ER that contribute to the pathogenesis of different disease states. As new animal models with defects in different sig-

naling components of the UPR are generated, we will gain a more precise knowledge of how these pathways cause or are a consequence of different pathological conditions. Elucidating which components of the UPR that are beneficial versus those that are detrimental under different conditions of stress represents a major avenue for future research. As we gain a greater understanding of UPR signaling and the physiological roles of the UPR in health and disease, it should be possible to design novel therapeutic strategies to activate or inhibit UPR signaling in order to intervene in diseases associated with abnormal accumulation of unfolded or misfolded proteins in the ER.

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