Minireview

**Signaling The Unfolded Protein Response from The Endoplasmic Reticulum**

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**Introduction**

Eukaryotic cells have a membranous labyrinth network called the endoplasmic reticulum (ER) that extends through the cytoplasm of the cell and is contiguous with the nuclear envelope. Proteins must be correctly folded and assembled in the ER prior to transit to intracellular organelles and the cell surface. As a processing plant for protein folding and posttranslational modification, the ER is exquisitely sensitive to alterations in homeostasis. A number of cellular stress conditions, 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 interfere with oxidative protein folding and subsequently lead to accumulation of unfolded or misfolded proteins in the ER lumen, which constitutes a fundamental threat to the cells. The ER has evolved highly specific signaling pathways to alter transcriptional and translational programs to cope with the accumulation of unfolded or misfolded proteins in the ER lumen. This adaptive response that couples the ER protein folding load with the ER protein folding capacity is termed the unfolded protein response (UPR) (1-4). Over the past ten years, significant progress has been made in understanding the mechanisms and roles of UPR signaling. Here we discuss the basic components of the UPR pathways, the physiological roles of the UPR signaling and the future direction of the field.

**Three UPR Transducers and One Master Regulator**

The basic components of the UPR pathway were first characterized in the budding yeast *Saccharomyces cerevisiae* in the early 1990s. The ER transmembrane protein
kinase/endoribonuclease IRE1p/Ern1p was found to be essential for cell survival during ER stress, and was identified as the transducer to initiate UPR signaling (5,6). Thereafter, researchers found that all eukaryotic cells have conserved the essential and unique properties of IRE1p-mediated UPR signaling identified in yeast, but also evolved additional transducers to generate a diversity of responses. In mammals, the counterpart of yeast IRE1p has two isoforms: IRE1α and IRE1β. While IRE1α is expressed in most cells and tissues, IRE1β expression is primarily restricted to the intestinal epithelial cells (7,8). IRE1 encodes an N-terminal ER stress-sensing domain in the ER lumen, an ER transmembrane domain, and a serine/threonine kinase domain and a carboxy-terminal endoribonuclease domain in the cytosol (6,9,10) (Figure 1). In addition to IRE1, higher eukaryotic cells have two additional UPR transducers: PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6). PERK contains a large ER luminal stress-sensing domain that is functionally interchangeable with the IRE1 luminal domain, and a cytosolic domain that phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α) (11-13). ATF6 is a transcription factor with an N-terminal basic leucine zipper (b-ZIP) domain in the cytosol and a C-terminal ER luminal domain to sense stress (14). Each UPR transducer is localized to the ER membrane, and is constitutively expressed in all known metazoan cells.

Upon accumulation of unfolded proteins in the ER, the UPR is activated to reduce the amount of new protein translocated into the ER lumen, to increase retrotranslocation and degradation of ER-localized proteins, and to bolster the protein-folding capacity and secretion potential of the ER. The UPR is orchestrated by transcriptional activation of
multiple genes mediated by IRE1 and ATF6, a general decrease in translation initiation and a selective translation of specific mRNAs mediated by PERK.

An essential unresolved issue for UPR signaling is how the different transducers are activated by a common stimulus, the accumulation of unfolded proteins in the ER lumen. Current studies support that an ER chaperone protein, BiP (also known as GRP78), serves as a master UPR regulator and plays essential roles in activating IRE1, PERK, and ATF6 in response to ER stress (15-17). BiP is a peptide-dependent ATPase and member of the heat shock 70 protein family that binds transiently to newly synthesized proteins translocated into the ER and more permanently to underglycosylated, misfolded or unassembled proteins. Under non-stressed conditions, BiP also binds to the luminal domains of IRE1, PERK, and ATF6 to maintain them within the ER. Upon accumulation of unfolded proteins, BiP is released from IRE1 and PERK to permit their spontaneous dimerization/oligomerization, trans-autophosphorylation, and subsequent activation (16,18). Release of ATF6 from BiP permits ATF6 transport to the Golgi compartment where it is cleaved to generate the cytosolic domain of ATF6 that translocates to the nucleus to activate transcription (17). Thus, this BiP-regulated activation provides a direct mechanism for all three UPR transducers to sense the “stress” in the ER and an autoregulatory mechanism by which the UPR is shut off upon increased expression of BiP (Figure 1).

**UPR Signaling Mediated by IRE1 and ATF6**

To deal with accumulation of unfolded or misfolded protein caused by ER stress, UPR is activated to alter transcriptional programs through IRE1 and ATF6 (Figure 2). In
yeast, ER stress induces IRE1 homo-dimerization and trans-autophosphorylation to activate its RNase activity to initiate removal of a 252-base intron from the mRNA encoding the transcription factor HAC1 (19). Splicing of HAC1 mRNA increases its translational efficiency and alters the carboxy-terminal sequence of HAC1 protein to generate a potent transcriptional activator. The protein encoded by spliced HAC1 mRNA binds and activates transcription from the UPR element (UPRE, minimal motif TGACGTGC/A) upstream of many UPR target genes (2,20). Analysis of the promoter regions of UPR-inducible genes in mammals, such as BiP, GRP94 and calreticulin, identified a mammalian ER stress response element (ERSE, minimal motif: CCAAT(N9)CCACG) that is necessary and sufficient for UPR gene activation (21). By using an ERSE as a probe in a yeast one-hybrid screen, two UPR-specific b-ZIP transcription factors, the X-box DNA binding protein (XBP1) and ATF6, were isolated. XBP1 was identified as a homologue of yeast HAC1 that is a substrate for mammalian IRE1 RNase activity (22-24). On activation of the UPR, IRE1 RNase cleaves XBP1 mRNA to remove a 26-nucleotide intron, generating a translational frame-shift. As the precedent of HAC1 regulation in yeast, the spliced XBP1 mRNA encodes a protein with a novel carboxy-terminus that acts as a potent transcription activator for many UPR target genes.

ATF6 is a UPR transducer that can bind ERSE elements in the promoters of UPR responsive genes (21). There are two forms of ATF6, ATF6α (90 kDa) and ATF6β (110 kD, also known as CREB-RP), both of which require the presence of the transcription factor NF-Y to bind to an ERSE (14,25,26). On activation of the UPR, ATF6 is transported to Golgi where it is cleaved by site-1 protease (S1P) and site-2 protease (S2P)
to generate a 50 kD cytosolic b-ZIP-containing fragment that migrates to the nucleus to activate transcription of UPR target genes (17,27) (Figure 2). Notably, S1P and S2P are also the processing enzymes that cleave the ER-associated transmembrane sterol-response element binding protein (SREBP) upon cholesterol deprivation. The cytosolic fragment of cleaved SREBP migrates to the nucleus to activate transcription of genes required for sterol biosynthesis (27).

ATF6 regulates a group of genes encoding ER-resident molecular chaperones and genes encoding folding enzymes, while XBP1 regulates a subset of ER resident chaperone genes that are essential for protein folding, maturation, and degradation in the ER (28,29). It was previously proposed that XBP-1 mRNA is induced by ATF6 in response to ER stress to generate more substrate XBP-1 mRNA for IRE1-mediated splicing (22,25,30). However, UPR induction of XBP1 transcripts and proteins was not altered in the cells having defective or reduced ATF6 cleavage (29,30). Induction of ATF6 mRNA upon ER stress was partially compromised in the absence of XBP-1, therefore it was proposed that ATF6 lies downstream of XBP-1 in some cases (29). These results suggest that XBP1 and ATF6 are situated largely in parallel pathways and may interact with each other upon ER stress.

**UPR Signaling Mediated by PERK**

The most immediate response to ER stress is transient attenuation of mRNA translation by increased phosphorylation of eIF2α (31). When eIF2α is phosphorylated, the formation of the ternary translation initiation complex eIF2/GTP/Met-tRNAi^{Met} is prevented, leading to attenuation of translation in general. The ER-resident kinase PERK...
is activated in response to ER stress to phosphorylate eIF2α on Ser51, thereby attenuating protein synthesis to reduce the workload of the ER (32-34) (Figure 3). 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 (32). However, it was surprising that the increased ER stress-induced apoptosis in these cells was due to defective transcriptional activation of UPR genes required for adaptation. 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 (Figure 3). In yeast, the 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 (35). GCN4 mRNA contains multiple upstream open reading frames (uORFs) in its 5’ UTR. These uORFs, which ordinarily prevent translation of the authentic GCN4 ORF, are bypassed only when eIF2α is phosphorylated, thus allowing translation of the GCN4 ORF (31). This control mechanism is also utilized in mammalian cells to regulate gene expression 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 (34). ATF4 subsequently activates transcription of genes involved in amino acid metabolism and transport, oxidation-reduction reactions, and ER stress-induced apoptosis (37,38).
ER Stress-Associated Protein Degradation and Programmed Cell Death

Protein folding in the oxidizing environment of the ER is an energy-requiring process (38,39). Under non-stressed conditions, newly synthesized proteins exist as unfolded intermediates along the protein-folding pathway. Once ER stress is imposed, such as by depletion of energy, many such folding intermediates become irreversibly trapped in low-energy states and accumulate. Unfolded or misfolded proteins in the ER lumen are retrotranslocated through the translocon to the cytoplasm, where they are usually ubiquitinated and degraded by the proteasome (40). This process is called ER-associated degradation (ERAD), and is regulated by the UPR. Proteasomal degradation of ER-associated misfolded proteins is required to protect from UPR activation. Proteasomal inhibition is sufficient to activate the UPR, which can, in turn, induce transcription of genes encoding several components of ERAD, such as, DER1, HRD1/DER3, HRD3, and UBC7 in yeast (41). The IRE1-XBP1 UPR pathway seems to be critical in regulating ERAD. In mammalian cells, induction of the gene encoding the ER degradation-enhancing a 1,2-mannosidase-like protein (EDEM), an important ERAD component that is essential for degradation of glycoproteins misfolded in the ER, depends solely on IRE1-XBP1 UPR signaling (42-45). Cells deleted in IRE1 pathway are defective in ERAD (45).

On the other hand, if the overload of unfolded or misfolded proteins in the ER is not resolved, prolonged activation of the UPR would lead to programmed cell death. Three known pro-apoptotic pathways emanating from the ER are mediated by IRE1, caspase-12, and PERK/CHOP, respectively, although the mechanisms are unclear (1,36). Under the ER stress, activated IRE1 can bind c-Jun-N-terminal inhibitory kinase (JIK)
and recruit cytosolic adapter TRAF2, that signals through apoptosis-signaling kinase 1 (ASK1) and the JNK protein kinase to activate mitochondria/Apaf1-dependent apoptosis (46-48). 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 (49). Under ER stress, activated caspase-12 activates caspase-9, which in turn activates caspase-3, leading to apoptosis (49,50). CHOP is a b-ZIP transcription factor that induces expression of genes favoring apoptosis in response to ER stress (36). Prolonged UPR activation leads to expression of transcription factor ATF4 through the PERK-eIF2α pathway. ATF4 then induces expression of CHOP, which subsequently activates caspase-3 through unknown intermediates (34,51).

The Physiological Roles of the UPR

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. Accumulating evidence suggests that the UPR plays important roles in these processes. An excellent example is plasma cell differentiation. On terminal differentiation of B lymphoid cells to plasma cells, the ER compartment expands approximately five-fold to accommodate the large increase in immunoglobulin synthesis (52). Interestingly, the UPR transcriptional activator XBP1 is required for plasma cell differentiation (53). XBP1-deficient B lymphoid cells express immunoglobulin genes and undergo isotype switching, but are defective in plasma cell differentiation and do not secrete high levels of immunoglobulins. Expression of the
spliced form of XBP1 efficiently restores production of secreted immunoglobulins in XBP1-deficient B cells, suggesting a physiological role for the UPR in high-rate production of secreted antibodies (54). During plasma cell differentiation, IRE1-mediated splicing of XBP1 mRNA was found to depend on increased translation of immunoglobulin chains (54-56). These observations support the hypothesis that increased synthesis of immunoglobulins 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 immunoglobulin heavy chains in the absence of light chains (57). However, the current studies do not exclude the possibility that the UPR may signal a B cell differentiation program that occurs prior to increased antibody synthesis. Although cleavage of ATF6 and splicing of XBP1 is observed during B cell differentiation, the activation and roles of IRE1α and PERK in differentiating B cells have yet to be directly investigated (55).

UPR signaling is also essential to maintain glucose homeostasis. In this respect, it is interesting to note that the UPR was first characterized as transcriptional activation of a set of genes, encoding glucose-regulated proteins (GRPs), in response to glucose/energy deprivation (58). We now know that pancreatic β-cells uniquely require the UPR for survival during intermittent fluctuations in blood glucose (32,59). Humans and mice with deletions in PERK have a profound pancreatic β-cell dysfunction and develop infancy-onset diabetes (59,60). In addition, mice with a mutation at the eIF2α phosphorylation site display a severe β-cell dysfunction (59). A model that blood glucose levels influence the protein-folding status in the ER of the β-cell has been proposed: as glucose levels
decline, the energy supply decreases, so protein folding becomes inefficient in the ER and therefore activates the PERK-eIF2α sub-pathway of the UPR to attenuate protein translation. Conversely, as blood glucose levels rise, eIF2α would be dephosphorylated so that translation would accelerate to increase proinsulin synthesis (32). In addition, continual elevation in blood glucose, such as that which occurs during insulin resistance, would prolong proinsulin translation to overload the ER folding capacity and thereby activate the UPR. In this manner, a balance between glucose level and PERK-eIF2α UPR signaling is essential for regulated translation of insulin, β-cell function and survival.

**Concluding Remarks**

Significant progress has been made in identifying components of the UPR pathways and in understanding physiological roles of the UPR. However, our knowledge of the UPR pathway is still incomplete. There remains much to do in identifying new substrates for the known UPR transducers and new transducers for unknown UPR pathways. Furthermore, it will be extremely important to elucidate the sensing mechanisms for the UPR transducers under different physiological conditions. Studies of this most upstream event in the UPR promise to expand our understanding of the UPR signaling pathway itself and its physiological functions. It is known that a variety of environmental insults and genetic diseases 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 signaling components of the UPR are generated, a more precise knowledge of the extent to which these pathways cause or arise as a consequence of different pathological conditions will be gained. Elucidating
which components of the UPR are beneficial versus those that are detrimental under different conditions of stress represents a major avenue of research for the future. As we gain a greater understanding of the mechanisms and physiological roles of the UPR, it should be possible to design novel therapies for the diseases associated with abnormal accumulation of unfolded or misfolded proteins by activating or inhibiting the UPR signaling as desired.

**The abbreviations used are:** UPR, unfolded protein response; ER, endoplasmic reticulum; 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.

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References

Figure Legends

Figure 1. **Depiction of three UPR transducers and one master regulator.** The domain structures of IRE1, PERK and ATF6, and their associations with BiP are shown. Ser/Thr kinase, serine/threonine kinase; eIF2α, eukaryotic translation-initiation factor 2α; bZIP, basic leucine zipper.

Figure 2. **UPR signaling mediated by IRE1 and ATF6.** 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 or ERSE sequence of many UPR target genes. Paradoxically, BiP release from ATF6 permits ATF6 transport to the Golgi compartment where full length ATF6 (90KD) is cleaved by S1P and S2P proteases to yield a cytosolic fragment (50KD) that migrates to the nucleus to activate transcription of UPR responsive genes. U-Pr, unfolded protein.

Figure 3. **UPR signaling mediated by PERK.** On accumulation of unfolded protein 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 or ATF4 mRNA, can be preferentially translated by the phosphorylated eIF2α. U-Pr, unfolded protein.
Figure 1
Figure 2

Nucleus

UPRE/ERSE

ATF6 (p50)

IRE1

ER

Golgi

S1P/S2P

XBP1 mRNA

XBP1

UPR gene

BiP

BiP

U-Pr

ATF6 (p90)

IRE1
Figure 3