In eukaryotic cells, secreted and resident proteins of the endomembrane system fold into their native structures within the endoplasmic reticulum (ER). The ER is a network of membranous tubules and sheets whose luminal environment is crowded with molecular chaperones and protein-modification enzymes that are specialized to fold proteins. In addition, the ER contains stringent quality-control systems that selectively export correctly folded proteins and selectively extract terminally misfolded proteins for ubiquitin-dependent proteolytic degradation in the cytosol, a process known as ER-associated protein degradation (ERAD) (Vembar and Brodsky, 2008). The ER is a dynamic organelle, and its capacity to fold proteins can be adjusted in response to changes in cellular protein-folding requirements through several intracellular signaling pathways that are collectively known as the unfolded protein response (UPR) (Ron and Walter, 2007). Dysregulation of the UPR contributes to the pathology of several important human diseases, including diabetes, neurodegeneration and cancer (Kim et al., 2008). In this article and its accompanying poster, we summarize how the mammalian UPR influences cell fate by promoting either cell adaptation or apoptosis when protein folding homeostasis is perturbed.

**What is the UPR?**

The unfolded protein response (UPR) is a network of intracellular pathways that function to maintain homeostasis in the ER. The mammalian UPR is distinguished by three ER-resident transmembrane proteins – PERK, ATF6 and IRE1 – that serve as the UPR’s proximal sensors. When ER functions are perturbed, these sensors initiate several responses that activate a number of signaling pathways. These pathways can result in homeostasis or apoptosis. One crucial issue that remains unclear is how cells interpret signals sent by the UPR to make cell-fate decisions, such as whether to adapt or whether to commit to apoptosis.

**Adaptive responses of the UPR**

- **Fast negative-feedback loops**
  - ER lumen
  - unfolded protein
  - Activation of various signaling pathways
  - Signal integration and interpretation
  - Apoptosis

- **Slow negative-feedback loops**
  - ER lumen
  - unfolded protein
  - Activation of various signaling pathways
  - Signal integration and interpretation
  - Apoptosis

**Activation of the proximal UPR sensors**

IRE1 becomes active when misfolded monomers, dimers (shown here) or higher order structures, causing trans-autophosphorylation of kinase domains, which in turn activates kinase domains. PERK is thought to be activated by similar mechanisms as its kinase domain is homologous to that of IRE1.

**Putative links between the UPR and apoptotic responses**

- **Cytosol**
  - Cytochrome c release and activation of the apoptosis-inducing complex

**Speculative model for the homeostatic-apoptotic switch**

Hypothetical threshold line that modulates cell commitment into apoptosis depending on the level of ER stress. At high levels of ER stress, adaptive responses are attenuated, leading to persistent ER stress. At these high levels, the switch flips into apoptosis. This model suggests that multiple processes are involved in high-level responses, including inhibition of anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-XL, Mcl-1), activation of pro-apoptotic Bcl-2 proteins (Noxa, Bim, Bid), DNA fragmentation and JNK phosphorylation.

**Abbreviations:** ATF, activating transcription factor; ASK1, apoptosis signal-regulating kinase 1; CHOP, C/EBP homologous protein; C/EBP, CCAAT/enhancer binding protein; ER, endoplasmic reticulum; IRE1, inositol-requiring enzyme-1; JNK, c-Jun N-terminal kinase; PERK, PKR-like ER kinase; S1 and S2, polypeptide domains; S1 and S2, polypeptide domains; XBP1s, X-box binding protein 1 (spliced); XBP1u, X-box binding protein 1 (unspliced).

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models have been proposed to explain IRE1 oligomerization. In the first model, the ER-resident chaperone immunoglobulin-binding protein (BiP) functions as a master regulator by binding to IRE1 and inhibiting its oligomerization under basal conditions. In this scheme, when unfolded proteins accumulate, BiP dissociates from IRE1 to preferentially interact with them, thus allowing IRE1 to oligomerize (Bertolotti et al., 2000). The second model proposes that unfolded proteins bind directly to the luminal domain of IRE1, which induces its oligomerization (Credle et al., 2005). More work is needed to resolve the relative contributions of BiP dissociation and direct binding of unfolded proteins to IRE1 activation.

PERK is also a type-I transmembrane protein that has a cytosolic kinase domain and an N-terminal luminal domain that is homologous to that of IRE1. As a consequence, it has been postulated that PERK is activated by similar mechanisms to those involved in IRE1 activation (Bertolotti et al., 2000; Liu et al., 2000).

ATF6 is an ER-resident type-II transmembrane protein that exists as an oxidized monomer, dimer, and/or oligomer and that associates with BiP under basal conditions. When unfolded proteins accumulate, ATF6 dissociates from BiP and conserved intra- and intermolecular disulfide bonds in the luminal domain of ATF6 are reduced, creating ATF6 monomers (Nadanaka et al., 2006; Shen et al., 2005). Reduced monomeric ATF6 translocates to the Golgi and becomes a substrate for the Site-1 and Site-2 proteases, which liberate the N-terminal cytosolic fragment of ATF6 [ATF6(N), a basic leucine zipper (bZIP) transcription factor] via regulated intramembrane proteolysis (Haze et al., 1999). More work is needed to elucidate the mechanisms governing ATF6 disulfide bond reduction, BiP dissociation and regulation of ATF6 translocation to the Golgi.

Adaptive responses of the UPR

When the proximal UPR sensors become activated, they initiate a response to restore protein-folding homeostasis in the ER. This adaptive response involves several outputs and can be conceptualized as two negative-feedback loops acting on two different time scales: a fast negative-feedback loop that decreases the influx of proteins into the ER, and a slow negative-feedback loop that requires de novo mRNA and protein synthesis to increase the folding capacity of the ER (Trusina et al., 2008).

The kinase activity of activated PERK phosphorylates eukaryotic translation initiation factor 2α (eIF2α), which impedes subsequent rounds of translation initiation (Harding et al., 1999). In addition, IRE1 is responsible for the rapid degradation of several ER-localized mRNAs (Hollien and Weissman, 2006). Transient translation attenuation and mRNA decay constitute the fast negative-feedback loops because they rapidly reduce the protein load on the ER. This provides the ER folding machinery an extended opportunity to fold existing unfolded proteins and the ERAD machinery an extended period of time to degrade them.

IRE1 also catalyzes the non-conventional splicing of XBP1u mRNA into XBP1s mRNA, which encodes the bZIP transcription factor X-box-binding protein 1 (XBPs1) (Calfon et al., 2002; Yoshida et al., 2001). The slower phase of the adaptive response is controlled by XBPs1 together with ATF6(N). ATF6(N) and XBPs1 increase transcription rates of genes encoding ER-resident chaperones, protein-modification enzymes, ERAD components and lipid biosynthetic enzymes to augment the size and folding and degradation activities of the ER (Yamamoto et al., 2007). Translation of the activating transcription factor-4 (ATF4) also increases when eIF2α is phosphorylated by PERK, causing increased transcription of many genes that promote survival under many types of cellular stress (Harding et al., 2003). Additional transcription factors might also contribute to the transcriptional UPR program in certain cell types; for example, CREBH (cyclic AMP response element-binding protein H) appears to be involved in hepatocytes (Zhang et al., 2006). Together, these negative-feedback loops reduce the concentration of unfolded proteins in the ER to maintain cellular homeostasis in the face of changing metabolic and protein-folding requirements. As the concentration of unfolded proteins decreases, the UPR shuts off, although the molecular details of UPR attenuation remain unclear.

ER stress

The term ‘ER stress’ is often used to describe a condition in which ER homeostasis is lost because of an overload on the ER’s protein-folding capacity. In practice, however, ER stress is often used operationally to describe any condition in which cells have activated the UPR. This operational definition has evolved because it is difficult to directly measure the ER unfolded proteins that are thought to be the activating signals of the UPR. However, solely monitoring UPR signaling does not necessarily provide information about the functional state of protein folding in the ER. Therefore, it is important to consider additional physiological end-points, such as ER distention, or changes in the secretion, glycosylation or oxidation of ER proteins (Merkamer et al., 2008). In experimental settings, ER stress is generally induced by treating cells with toxic chemicals that severely impair ER protein folding or trafficking. Under these non-physiological conditions, the adaptive mechanisms of the UPR are insufficient to maintain homeostasis in the ER and cells ultimately die, typically through apoptosis.

Putative links between the UPR and apoptotic responses

Cells experiencing irreparable ER stress commit to apoptosis when the outer mitochondrial membrane (OMM) is permeabilized and cytochrome c is released to activate executioner caspases. This intrinsic (mitochondrial) apoptotic pathway, which is typically triggered in response to intracellular stresses including DNA damage and viral infections, is regulated by the Bcl-2 protein family (Youle and Strasser, 2008). The Bcl-2 family can be divided into three groups: multi-domain proapoptotic proteins (e.g. Bax, Bak), anti-apoptotic proteins (e.g. Bcl-2, Bcl-XL) and proapoptotic BH3-only proteins (e.g. Bid, Bad, Bim, Noxa, Puma) (Brunelle and Letai, 2009). In response to ER stress, the proapoptotic BH3-only proteins are transcriptionally or post-translationally activated to stimulate proapoptotic Bax and Bak either directly or indirectly through antagonizing anti-apoptotic members. Once activated, Bax and/or Bak form homomultimers in the OMM to initiate mitochondrial permeabilization (Wei et al., 2001). At this time, it is unclear whether and how UPR signaling components communicate with the Bcl-2 family members or other apoptotic signaling molecules to initiate apoptosis. In the following section, we summarize some of the more compelling data supporting such a link.

Of the 11 members of the BH3-only family, Puma, Noxa, Bid and Bim have been described to mediate apoptosis triggered by ER stress (Li et al., 2006; Puthalakath et al., 2007; Upton et al., 2008). However, it remains possible that other BH3-only proteins serve important roles, with the relative contribution(s) of each BH3-only member varying in different tissues. Recently, the transcription factor C/EBP homologous protein (CHOP) was found to increase the rate of Bim transcription during ER stress, marking an important connection between a UPR signaling component and a BH3-only protein (Puthalakath et al., 2007). CHOP mRNA levels increase sharply during ER stress, an effect that is mediated primarily through the upstream transcription factor ATF4. In addition to regulating Bim expression, CHOP has been reported to antagonize the expression of anti-apoptotic Bcl-2. Although CHOP is
clearly an important mediator between the UPR and the apoptotic machinery, CHOP 

ultimately depend on how Bcl-2 proteins interpret the mix of survival and apoptotic signals transmitted by the UPR: such interpretation results in cell survival under conditions of remedial stress and cell death when homeostasis cannot be restored following catastrophic ER protein misfolding. To make this decision, cells might incorporate a time factor in which sustained UPR signaling (as could occur during chronic ER stress) increases the likelihood of apoptosis. In support of such a model, the mRNA and protein half-lives of proapoptotic CHOP were found to be short lived compared with pro-survival UPR outputs such as the ER chaperone BiP (Rutkowski et al., 2006). Sustained PERK activity (which is primarily responsible for CHOP upregulation) might thus be necessary to build CHOP levels to a required threshold to stimulate Bcl-2 proteins to commit to apoptosis. In addition, sustained PERK activity should result in protracted translation attenuation, which should be incompatible with survival. Similarly, sustained mRNA degradation mediated by IRE1 might deplete ER cargo and protein folding activities (Han et al., 2009). In support of this notion, overexpression of PERK or IRE1, which leads to their spontaneous oligomerization and activation, is typically sufficient to cause apoptosis. This is reminiscent of apoptosis that occurs during the sustained activation of other protein kinases such as JNK (Ventura et al., 2006).

In addition, the severity of ER stress might alter the relative activation levels of certain UPR output pathways to influence cell-fate decisions. For example, IRE1 has at least three established outputs: XBP1 mRNA splicing, non-specific mRNA cleavage and JNK activation (Han et al., 2009; Hollièn et al., 2009; Hollièn and Weissman, 2006; Urano et al., 2000). It is possible that different degrees of protein misfolding differentially affect which of these IRE1 outputs are realized by differentially altering its oligomerization state.

**Perspectives**

Over the past 20 years since the UPR was first described, many of its molecular components have been identified and characterized. To move forward, it will be necessary to investigate how these individual components function as a signaling network to direct cell-fate decisions. To this end, we will need to develop quantitative tools to study various UPR components dynamically in individual living cells as they experience ER stress. In addition, it will be important to challenge cells with physiologically relevant stressors to understand how the UPR contributes to cellular physiology and pathogenesis of protein-misfolding diseases. It is likely that the elucidation of key components of the UPR’s homeostatic-apoptotic signaling network will reveal pharmacological targets for drug discovery and potential therapeutics for ER-stress-related diseases.

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