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Stress-sensing mechanisms in the unfolded protein response: similarities and differences between yeast and mammals

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The unfolded protein response is an adaptive stress response that responds to the imbalance between the entry of newly synthesized unfolded proteins and the inherent folding capacity in the endoplasmic reticulum (ER). Various environmental stresses and changes in physiological conditions can result in the accumulation of unfolded proteins in the ER, which is sensed through ER transmembrane protein sensors named inositol requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6), and the sensed signals are transduced to the cytosol and the nucleus. IRE1 is a prototype ER stress sensor that is evolutionarily conserved from yeast to humans. Higher eukaryotes have evolved two other sensors, PERK and ATF6. This review focuses on the current progress in our understanding of stress-sensing mechanisms, in particular, the similarities and differences between yeast and mammals.

Keywords: BiP/clustering/ER stress sensor/IRE1/unfolded protein response.

Abbreviations: ATF6, activating transcription factor 6; ER, endoplasmic reticulum; IRE1, inositol requiring enzyme 1; PERK, PKR-like ER kinase; UPR, unfolded protein response.

In eukarytotic cells, secretory and membrane proteins are folded and assembled in the endoplasmic reticulum (ER) before being delivered to their target organelles or the cell surface via the Golgi apparatus. Newly synthesized proteins enter the ER lumen where most become glycosylated or undergo disulphide bond formation. These processes assist in correct protein folding and assembly (*1*, *2*). ER-resident molecular chaperones also promote the correct tertiary and quaternary structures by preventing aberrant protein aggregation. However, protein folding and assembly can be altered when cells are exposed to various environmental or physiological conditions, including nutrient starvation, calcium depletion from the ER, strong reducing conditions, viral infection, hypoxia or even in the course of normal development and differentiation. In these cases, normal protein folding and assembly may be interrupted, leading to the accumulation of misfolded and unfolded proteins in the ER and consequent ER stress. In response to this deleterious situation, cells activate intracellular signaling pathways leading from the ER to the cytosol/nucleus collectively termed the unfolded protein response (UPR), which functions to restore normal protein-folding capacity and allow adaptation to new conditions (*3*–*5*).

The mammalian UPR consists of at least two responses that function to enhance the protein folding capacity of the ER under conditions of stress: one suppresses the influx of newly synthesized unfolded proteins at the translational level, and the other one increases the protein folding or degradation ability within the ER at the transcriptional level. Three transmembrane ER stress sensors, inositol requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6), have been identified (*6*–*10*). These sensors play important roles in sensing abnormal conditions in the ER lumen and function to transduce signals to the cytosol/nucleus.

Three ER Stress Sensors, IRE1, PERK and ATF6

IRE1 is an intriguing prototype ER stress sensor that is evolutionarily highly conserved among almost all eukaryotes (*6*, *7*, *11*–*13*). IRE1 is a type I transmembrane protein whose N-terminal half is located in the ER lumen and C-terminal half in the cytosol (Fig. 1A). The cytosolic domain is an effector domain composed of dual enzymes, a protein kinase and an RNase. When the luminal region senses the accumulation of unfolded proteins, IRE1 dimerizes or oligomerizes and undergoes trans-autophosphorylation (*14*, *15*), resulting in the activation of its RNase activity, which in turn triggers the unconventional splicing of the *HAC1*(yeast)/*XBP1*(mammals) mRNA in the cytosolic side of ER membrane (*16*–*19*). This unconventional splicing activity leads to the production of the active transcription factor HAC1s/XBP1s ('s' refers to spliced) (Fig. 2). Activated HAC1s/XBP1s upregulates genes encoding both ER chaperones and components of the ER-associated degradation (ERAD) machinery (*20*–*22*). The second sensor protein, PERK contains a luminal domain having weak homology to its counterpart in IRE1, and a cytosolic domain possessing a

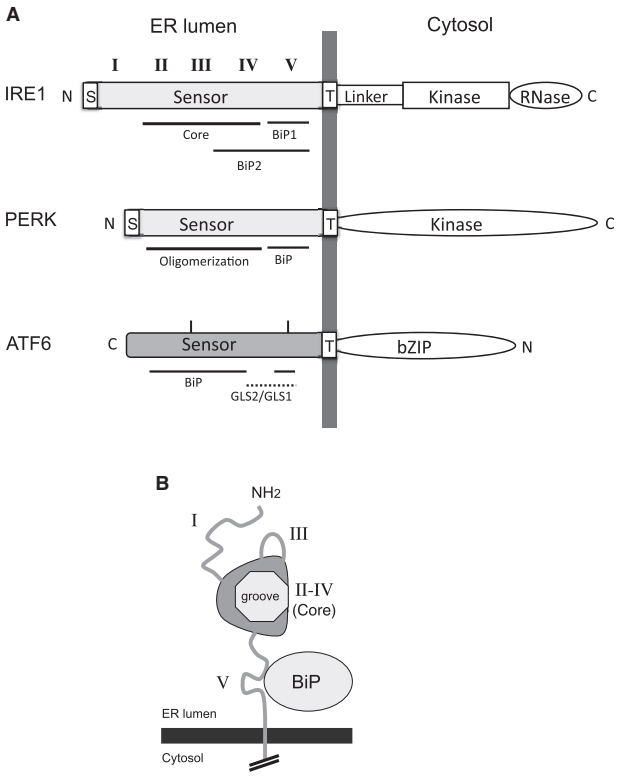

Fig. 1 Schematic representation of three transmembrane ER stress sensors, IRE1, PERK and ATF6 (*52*). (A) Thin lines indicate BiP binding regions (BiP1, assigned by yeast Ire1; BiP2, a potential binding region of human IRE1α). Bold lines represent regions that are indispensable for sensing stress (core). The broken line represents a region necessary for Golgi translocation (GLS: Golgi localization signal). Vertical bars indicate conserved cysteine residues in the ER luminal region of ATF6. S, signal sequence; T, transmembrane region; bZIP, transcription factor containing basic leucine zipper; N, amino terminus; C, carboxyl terminus; I–V, yeast Ire1 subregion. (B) A diagram of yeast Ire1 luminal region. The structure of the Core includes a putative peptide-binding groove.

kinase activity that phosphorylates eukaryotic initiation factor 2α (eIF2α), resulting in the attenuation of protein synthesis (Fig. 1A) (*8*, *9*). PERK thus contributes to maintaining ER homeostasis in metazoans by attenuating protein translation. The third sensor protein, ATF6, is a type II transmembrane protein containing an N-terminal region that protrudes into the cytosolic surface and which is composed of a basic leucine zipper (bZIP) transcription factor region (Fig. 1A) (*10*). Upon ER stress, ATF6 is transported from the ER to the Golgi body via COPII vesicles and is sequentially cleaved by the proteases S1P (site-1 protease) and S2P (site-2 protease) resident in the Golgi (*23*). The cleaved cytosolic domains are released from the membrane and are transported to the nucleus to transcriptionally induce ER chaperones and ERAD-related genes (*24–26*). The luminal region of ATF6 has no homology to those of IRE1 or PERK. In yeast, the IRE1 pathway is the sole signalling pathway mediating the UPR, while metazoans have acquired two more sophisticated pathways, ATF6 and PERK, to adapt to various environmental and pathophysiological conditions.

BiP is a Common Negative Regulator or Adjuster

Immunoglobulin heavy chain binding protein (BiP, GRP78) is an Hsp70 family protein located in the ER lumen (*27*, *28*). BiP is the most abundant chaperone in the ER lumen and plays a central role in maintaining protein quality control in the ER. Interestingly, in the unstressed condition, BiP is associated with all three ER stress sensors, but in response to ER stress is rapidly released (*29–31*). The kinetics of association and dissociation between BiP and IRE1 or PERK correlate well with the kinetics of activation and repression (*29*, *30*). Binding of BiP to ATF6 does not repress the formation of ATF6 dimers/oligomers, as is observed for IRE1, but rather prevents its transport from the ER to the Golgi. Binding of BiP to the luminal region of ATF6 sterically hinders the Golgi transport signal located therein, and thus blocks transport to the Golgi (Fig. 1A) (*31*). These results suggest that BiP bound to the luminal region of ER stress sensors maintains them in an inactive state until conditions of stress are encountered (*29*, *30*). BiP also functions to limit the response of Ire1 to ER stress and prevents activation by other stresses (*32*).

Stress-sensing Mechanism by IRE1

The N-terminal luminal region of yeast Ire1 has been precisely analysed by deletion scanning mutagenesis, resulting in the identification of five subregions termed I–V from the N-terminus to the transmembrane region (Fig. 1A, B) (*32*). Regions II–IV form a tightly folded domain that is responsible for dimer/oligomer formation (*33*). This folded domain is referred to as Core that is indispensable for stress-sensing (described Core hereafter). Surprisingly a BiP binding region locates outside of Core and mutants of Ire1 lacking a BiP binding region can respond to ER stress as well as wild-type Ire1, indicating that dissociation of BiP from Ire1 is not sufficient for the activation of Ire1 (*32*, *34*). Therefore, Core contains a domain responsible for the recognition of ER stress. Analysis of the crystal structure of yeast Ire1 Core suggests a reason for this (*35*). When the Core domain dimerizes, a major histocompatibility complexes-like groove is formed, suggesting that peptides from unfolded proteins might directly bind into this groove. Analysis of the crystal structure also showed that the dimer forms higher order oligomers that could be distinguished by interfaces I and II, as shown in Fig. 2A. Point mutations that disrupt either these interfaces or that interfere with the peptide-binding groove of Core-oligomer result in a loss in sensing activity, suggesting that this higher order oligomer formation is required for the recognition of unfolded proteins (*35*).

This higher-ordered oligomer of Ire1 was observed in yeast *in vivo* and the relocalization of Ire1 during ER stress has been demonstrated (*36*). In the absence of ER stress, Ire1 is located in the ER membrane, but this pattern rapidly changes to a punctate pattern (hereafter called cluster) under conditions of ER stress.

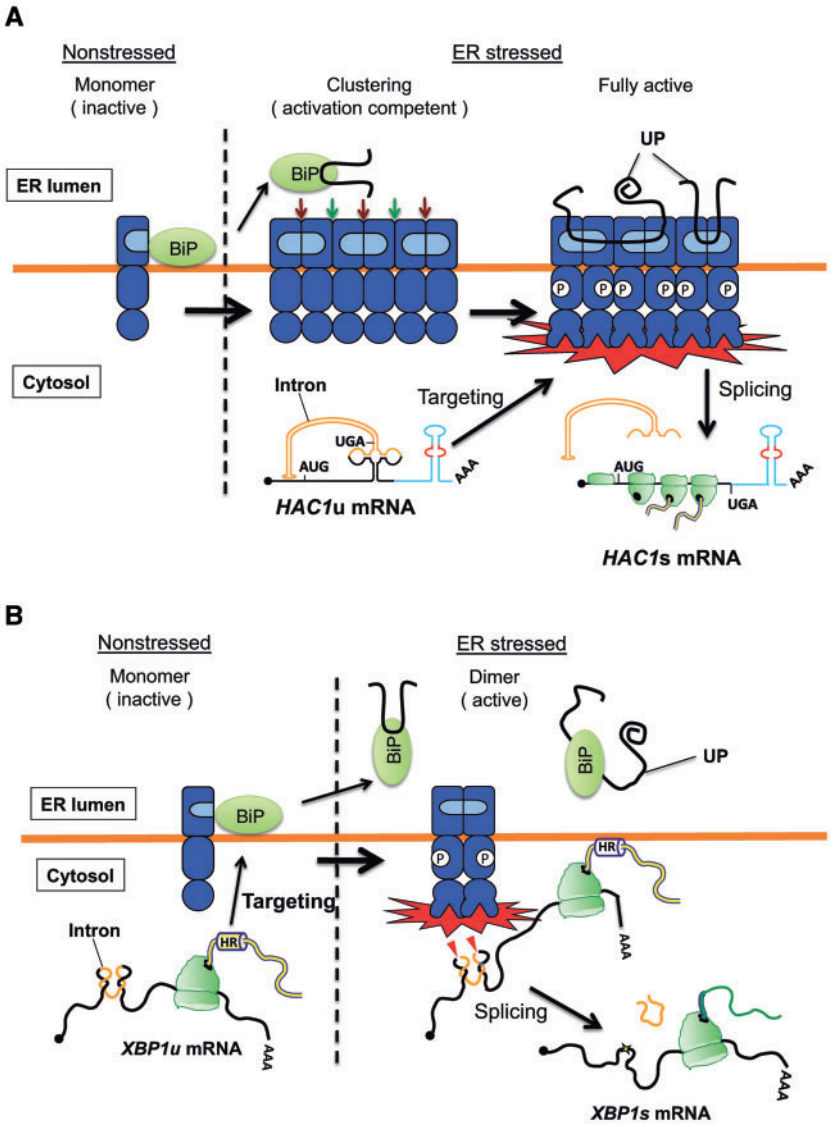

Fig. 2 Current model of IRE1 activation in yeast and mammals. (A) Ligand-dependent two-step activation model in yeast. See the text for the details of the activation step of Ire1. Ire1 clusters recruit untranslated *HAC1u* mRNA, whose translation is arrested by pairing of its 5′-UTR with the sequence of the intron (indicated by brown colour) through a bipartite targeting sequence (red) located in the 3′-UTR. Finally, this association of *HAC1u* mRNA with the Ire1 cluster initiates an unconventional splicing process that forms spliced *HAC1s* mRNA. Arrows depict Interface I (brown) and II (green). (B) Ligand-independent model in mammals. See the text for the details of the dimer formation of IRE1α. Unspliced *XBP1u* mRNA is constitutively recruited to ER membrane by tethering of the hydrophobic region (HR) of the partially translated XBP1u protein to the ER membrane, leading to facilitated splicing of *XBP1u* mRNA by IRE1α.

Cluster formation occurs in cells harbouring a kinase-deficient Ire1 mutant or those lacking *HAC1* mRNA. This suggests that the observed clustering is not a result of a cytosolic event but seems to be a pre-requisite for full Ire1 activation. Furthermore, this change in Ire1 distribution was blocked when mutations that disrupt interface I or II are introduced into Ire1. Thus, clustering of Ire1 under conditions of ER stress is believed to correspond to the formation of higher order complexes observed *in vitro* and as indicated by analysis of the crystal structure. Direct interaction of Core with unfolded proteins was demonstrated by inhibition of *in vitro* aggregation assay (*36*). From the above results, a model involving two-step sensor activation is proposed (Fig. 2A). First, upon ER stress, BiP dissociates from Ire1 due to its interaction with accumulated unfolded proteins, and/or by other mechanisms, resulting in formation of Ire1 clusters. Second, binding of unfolded proteins to the grooves causes a conformational change in the luminal domain. This conformation change leads to the reorientation of the cytosolic domain, resulting in the activation of the RNase. Ire1 mutant harbouring only Core domain spontaneously forms large clusters. However, clustering *per se* does not fully activate Ire1. Full activation of UPR still requires ER stress, indicating that clustering is not sufficient for full activation of Ire1 in yeast. This result is consistent with the two-step activation model.

However, analysis of the crystal structure of the human IRE1α (hIRE1α) luminal domain does not support the above model (*37*). The groove formed by dimerization is too narrow to allow peptide binding and the purified luminal domain spontaneously forms

high-affinity dimers *in vitro*. Mutations disrupting the dimerization interface produce hIRE1α molecules that fail either to dimerize or to activate the UPR under conditions of ER stress. The authors thus speculate that peptide binding is unlikely and is unnecessary for hIRE1α homodimer generation. The dissociation of BiP from the hIRE1α due to the accumulation of unfolded proteins in the ER would cause hIRE1α to dimerize through hydrogen bonding and extensive hydrophobic interactions at the dimer interface (*38*, *39*). Dimerization-induced intermolecular autophosphorylation would then lead to activation of its RNase activity (Fig. 2B). In yeast Ire1, the BiP-binding region is clearly separated by the Core region, whereas deletion mutation analysis of hIRE1α indicates that these two domains partially overlap (*40*). Furthermore, fragments from the luminal region of hIRE1α did not exhibit anti-aggregation activity *in vitro*. These data are consistent with results obtained from analysis of the crystal structure and suggest that activation of mammalian IRE1α depends only on the dissociation of BiP from hIRE1α, and not on the interaction of unfolded proteins or other components with hIRE1α.

The data obtained from yeast and human IRE1 are somewhat inconsistent. Although the luminal domain of hIRE1α shows very limited homology to yeast Ire1, the luminal region is functionally interchangeable when chimeric human/yeast IRE1 is expressed in yeast cells.

The Role of Ire1 Clustering and the Recruitment of mRNA to the ER

Three recent papers highlight the physiological role of IRE1 clustering in response to ER stress (*41–43*). Analysis of the crystal structure of the cytosolic effector domain of yeast Ire1 indicates that clustering of the ER luminal sensor region triggers a change in the orientation of the cytosolic domain (*42*). This change results in the formation of an oligomeric helical rod structure, similar to a DNA double helix. Each effector domain in this helix is positioned so as to be subject to trans-phosphorylation by the kinase domain of the juxtaposed Ire1 molecule. This autophosphorylation results in a prominent enhancement in RNase activity compared to the Ire1 dimer. The clustering of Ire1 RNase domains in the structure may form a platform upon which the specific cleavage of the dual stem loop of *HAC1u* mRNA (unspliced form of *HAC1* mRNA) can take place. Under normal conditions, *HAC1u* mRNA is translationally arrested by a base-pairing interaction between the 5′-untranslated region and the intron of *HAC1u* mRNA (Fig. 2A) (*44*). Intriguingly, translation-arrested *HAC1u* mRNA is efficiently recruited to the Ire1 cluster in response to ER stress, and its intron is effectively cleaved off (Fig. 2A) (*41*). The targeting signal responsible for this cleavage event was identified as a bipartite sequence located in the 3′-untranslated region of the *HAC1u* mRNA. This bipartite signal was found to be sufficient for targeting other mRNAs to Ire1 clusters, as long as translation was repressed. Thus, the yeast Ire1 cluster formed under conditions of ER stress is a type of signalling centre mediating the UPR.

By contrast, mammalian *XBP1u* mRNA is translationally active and constitutively produced (*18*, *19*). Although XBP1u is a very unstable protein, it possesses the ability to form a hetrodimer with transcriptionally active XBP1s or ATF6, resulting in the sequestration of XBP1s or ATF6 from the nucleus (*45*, *46*). Thus, XBP1u functions as a negative feedback regulator of XBP1s and ATF6, shutting off the target genes in UPR during the recovery phase following ER stress. Biochemical analyses showed that most of the *XBP1u* mRNA is localized to the ER membrane, where it is spliced by IRE1α, although its product is not a secretory protein (*43*). In contrast, the *XBP1s* mRNA is localized in the cytosol. Association of *XBP1u* mRNA to the membrane depends on the co-translation of XBP1u protein, which contains a conserved hydrophobic region located at the C-terminus (Fig. 2B). *XBP1u* mRNA is tethered to the ER membrane as part of a complex consisting of the mRNA, the ribosome and the hydrophobic region of the partially translated XBP1u protein, which associates with the membrane (Fig. 2B). This mechanism of tethering mRNA to the ER membrane is reported in both yeast *Hac1* mRNA and mammalian *XBP1* mRNA (*41*, *43*), and apparently function to enhance cytoplasmic splicing efficiency and facilitate a more rapid response to ER stress. This mechanism represents a new paradigm for mRNA distribution, maturation and regulation of expression. However, there are a number of differences between the yeast and mammalian systems, including targeting signals (mRNA or protein), IRE1 dependence, translation condition and timing of recruitment (*47*). Further analysis will be needed to eludicate the similarities and differences between yeast and mammals, how they develop or evolve an appropriate response to ER stress.

Mechanisms of PERK and ATF6 Activation

The mechanism of PERK activation seems to be quite similar to that of yeast Ire1. Although the luminal regions of PERK and Ire1 show little sequence homology, the predicted secondary structure for PERK indicates that its folding status is similar to that of the IRE1α luminal region (*35*, *37*). Deletion analysis of the ER luminal region of PERK showed that its BiP binding region is located in close proximity to the transmembrane region and the domain required for dimerization/oligomerization is separated from the BiP binding region, similar to yeast Ire1 (Fig. 1A) (*48*). The N-terminal domain, which lacks the BiP binding region, is sufficient both for dimer/oligomer formation and for activation of PERK. In addition, the luminal region of IRE1 can replace that of mammalian PERK (*29*). However, a PERK mutant lacking the BiP binding region is constitutively active, suggesting that BiP directly controls PERK activation (*48*). This is different than the behaviour of yeast Ire1.

ATF6 is negatively regulated by BiP, binding of which interferes with the signal responsible for transport of ATF6 to the Golgi, as has been described. However, the existence of a different system of regulation has also been reported (*49–51*). In the ER lumen, ATF6 normally exists in monomer, dimer and oligomer forms as a result of intra- and intermolecular disulphide bridges between two conserved cysteine residues in its luminal region (Fig. 1A). Under conditions of ER stress induced by either dithiothreitol or tunicamycin, these disulphide bonds are reduced, and only monomeric ATF6 reaches the Golgi, where it is effectively cleaved by S1P and S2P. This mechanism may serve to make the regulation of ATF6 more stringent, preventing the mislocation of ATF6 to the Golgi under normal conditions and ensuring that it acts only in response to ER stress.

Concluding Remarks

Recently, our understanding of the stress-sensing mechanisms mediated by IRE1 has been greatly advanced by studies in yeast and mammals. Data obtained from yeast studies strongly support the idea that dissociation of Ire1 from BiP allows it to form clustered structures. These clusters may directly bind to unfolded proteins, which in turn triggers the formation of a helical configuration of the C-terminal effector domains of Ire1. This conformation change provides a platform for the recognition and the cleavage of specific sites located in target *HAC1u* mRNA. In contrast, mammalian IRE1α may not directly interact with unfolded proteins, but rather undergoes spontaneous dimerization once freed from BiP, and this may be sufficient for its activation. Yeast *HAC1u* mRNA is recruited to the Ire1 clusters in response to ER stress by means of a conserved RNA motif localized in 3′-UTR, whereas mammalian *XBP1u* mRNA is recruited to the ER membrane by a co-translation complex, independent of IRE1α. ER membrane localization of the unspliced *HAC1u* mRNA in yeast and *XBP1u* mRNA in mammals contributes to the efficient splicing by Ire1 in yeast and by IRE1α in mammals, respectively. The mechanism of membrane localization of *HAC1u* mRNA is quite different from that of *XBP1u* mRNA. The above data suggest that yeast and mammals have developed a different system to reach the same goal. Further studies are necessary for understanding some of the aforementioned controversial experimental data, but we believe that such studies will also lead us to exploit novel fields of study. Because studies of the unfolded protein response have proven to be a fertile area of research that consistently yields new paradigms.

Conflict of interest
None declared.

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