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Forum Review

How Transmembrane Proteins Sense Endoplasmic Reticulum Stress

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ABSTRACT

The unfolded protein response (UPR) is an adaptive stress response in which cells recover from the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) by increasing its protein-folding capacity. The IRE1 pathway in the UPR is evolutionarily conserved from yeast to human, and two other pathways involving PERK and ATF6 have also evolved in higher eukaryotes. These three intracellular signaling pathways originate in the ER lumen, where unfolded or misfolded proteins are recognized by the three transmembrane ER stress sensors IRE1, PERK, and ATF6. This review focuses on current progress with efforts to elucidate how stress sensors recognize the accumulation of unfolded proteins. *Antioxid. Redox Signal.* 9, 2295–2303.

INTRODUCTION

Almost all secretory and membrane proteins attain their final folded or assembled conformation in the endoplasmic reticulum (ER) before making their way to their target organelles or the cell surface. Numerous ER-resident molecular chaperones and catalysts assist protein folding and assembly into correct tertiary and quaternary structures (14, 15, 26). Because the ER is the site of both protein and lipid synthesis, it has the ability to supervise the capacity of endomembrane systems to optimize membrane biogenesis (6, 10, 49, 71). When cells are exposed to environmental or physiologic stresses (such as glucose starvation, calcium depletion from the ER, strong reducing conditions, viral infection, or hypoxia), unfolded or misfolded proteins accumulate in the ER (29, 61). As a protein-folding compartment, the ER monitors the status of newly synthesized proteins and the levels of unfolded/misfolded proteins in the ER lumen (13, 26). When unfolded/misfolded proteins accumulate in the ER, cells activate a signaling pathway from the ER to the cytosol/nucleus, termed the unfolded protein response (UPR), by which they restore protein-folding capacity and adapt to new conditions (4, 29, 32, 33, 43, 56, 62). The primary signal is sensed by transmembrane proteins in the ER, whose structures resemble those of the cell-surface receptors for growth factors. In growth-factor receptors, ligands specifically bind to cell-surface receptors, resulting in a conformational change such as dimerization that activates the receptor and transmits the signaling to target molecules (60). By contrast, what the sensor proteins recognize in ER stress is not a specific ligand, but the accumulation of unfolded proteins in the ER. This article reviews recent progress toward elucidating the sensing mechanisms used by ER transmembrane sensor proteins.

TRANSMEMBRANE SENSOR PROTEINS FOR ER STRESS

The three major transmembrane sensors are IRE1 (inositol-requiring 1; ERN1, endoplasmic reticulum-to-nucleus signaling 1) (8, 41), PERK [double-stranded RNA-activated protein kinase (PKR)-like ER kinase; PEK, pancreatic eukaryotic initiation factor 2α kinase; EIF2AK3] (20, 67), and ATF6 (activating transcription factor 6) (24, 76) (Fig. 1). IRE1 and PERK are type I transmembrane proteins with protein kinase activity

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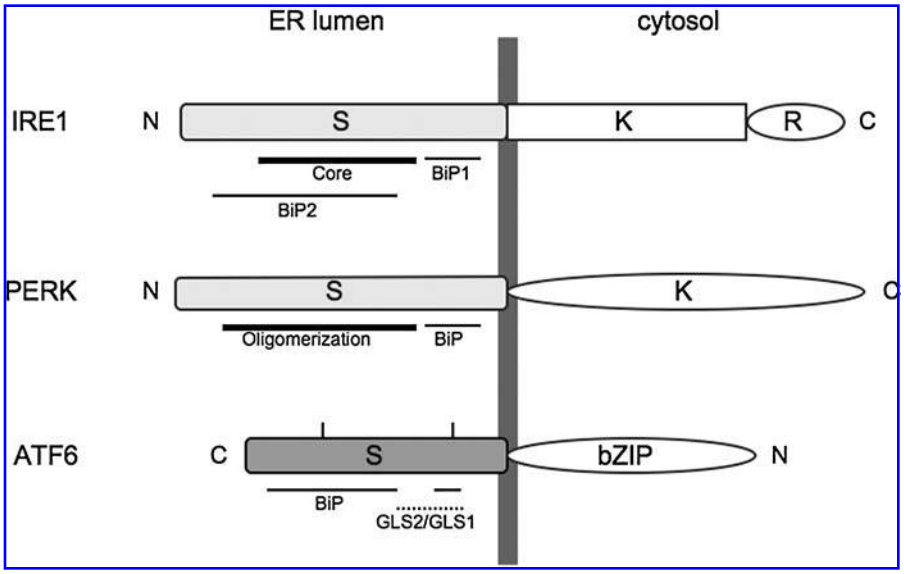

FIG. 1. Schematic representation of three transmembrane ER stress sensors, IRE1, PERK, and ATF6. *Thin lines*, BiP-binding regions (BiP1, assigned by yeast Ire1; BiP2, a potential binding region of human IRE1α). *Bold lines*, Regions that are indispensable for the stress-sensing (Core; core stress-sensing region of yeast Ire1). *Broken line*, A region necessary for Golgi translocation (GLS, Golgi localization signal). *Vertical bars*, Conserved cysteine residues in the ER luminal region of ATF6. S, ER stress-sensing domain; K, Ser/Thr kinase domain; R, endoribonuclease domain; bZIP, transcription factor containing basic leucine zipper; N, amino terminus; C, carboxyl terminus.

(20, 41), whereas ATF6 is a type II transmembrane protein encoding a transcription factor (24, 25). IRE1, the prototype ER stress sensor, is evolutionarily conserved from yeast to human, indicating that the UPR is critical for coping with perturbation of the eukaryotic endomembrane system caused by environmental or pathophysiologic stress. *IRE1* was isolated as a gene required for inositol auxotrophy in budding yeast (50). PERK possesses a luminal domain showing limited sequence homology to its counterpart in IRE1, whereas the cytosolic domain of PERK has kinase activity that phosphorylates eukaryotic initiation factor 2α (eIF2α), resulting in attenuation of protein synthesis (20, 21). PERK thus contributes to maintaining ER homeostasis through translational control in metazoans (22, 70). The third member, ATF6, is a basic leucine zipper (bZIP) transcription factor that induces ER chaperone genes during ER stress (76, 77). The luminal region of ATF6 has no homology to those of IRE1 or PERK. Interestingly, however, the luminal regions of all three sensors associate with the ER chaperone BiP/Grp78 (BiP, immunoglobulin heavy chain–binding protein; Grp78, glucose-regulated protein), an Hsp70 family member (19, 44, 51, 57), under nonstressed conditions; BiP dissociates under ER stress, changing these sensors to their active states (1, 55).

STRESS-SENSING MECHANISM OF IRE1

Evolutionarily conserved IRE1-dependent pathway from yeast to human

Two groups independently isolated the same gene encoding a factor for signal transduction between the ER and the nucleus in the UPR (8, 41). Yeast *IRE1 (*Ire1*)* encodes a 1,115-amino acid type I transmembrane protein whose N-terminal half is located in the ER lumen and C-terminal half in the cytosol. The cytosolic domain is composed of two functional domains, a serine/threonine protein kinase and an endoribonuclease domain containing RNase L–like homology (see Fig. 1) (3, 69). This orientation of Ire1 implies that the N-terminal portion is a sensor for recognizing accumulation of unfolded proteins in the ER. Once Ire1 senses the accumulation of unfolded proteins, it forms a dimer or oligomer, *trans*-autophosphorylates (63, 81), and activates ribonuclease activity, resulting in the initiation of spliceosome-independent splicing (cytosolic splicing; see Dr. Yoshida) of the precursor form of *HAC1* mRNA encoding the bZIP transcription factor Hac1 (9, 18, 42, 68). The mature form of Hac1 enters the nucleus and binds the UPR element (UPRE) (32, 40), enhancing the transcription of downstream genes encoding ER chaperones such as BiP, the components of ER-associated protein degradation (ERAD) such as Der1, and the components of the secretory machinery, which in turn enhances protein-folding capacity in the ER (43, 56, 74).

Mammalian cells have two IRE1 paralogues, IRE1α and IRE1β (28, 72, 80). IRE1α is expressed in various tissues, and to an especially high level in placenta, liver, and pancreas (72, 83), whereas abundant IRE1β expression is restricted to the epithelial cells of digestive tissues, particularly of the colon and stomach (2). Disruption of mouse IRE1α is embryonic lethal (75, 83), whereas knockout of IRE1β causes no abnormalities compared with wild-type mice under normal conditions, but results in increased sensitivity to dextran sodium sulfate to induce inflammatory bowel disease (2). Activated IRE1α generates a mature form of *XBP1* mRNA, which encodes a bZIP transcription factor, by unconventional splicing, as does yeast Ire1 (5, 66, 78). Activated XBP1 upregulates ER chaperone genes and the genes encoding components of ERAD (35, 52, 79). Thus, the IRE1-Hac1/XBP1 pathway is evolutionarily conserved from yeast to human (Fig. 2). Although the amino acid sequence identity in the ER luminal regions of IRE1 is low between yeast and human, the N-terminal luminal region of mammalian or nematode IRE1 can substitute for yeast Ire1 in the yeast UPR, suggesting that the stress-sensing mechanism is conserved among different species (36, 37). In yeast cells having a disrupted IRE1 gene, the UPR is almost completely abolished, and they show hypersensitivity to ER stress or inositol deprivation (9). Mouse embryonic fibroblasts prepared from IRE1α knockout mice also exhibit no splicing of *XBP1* mRNA after

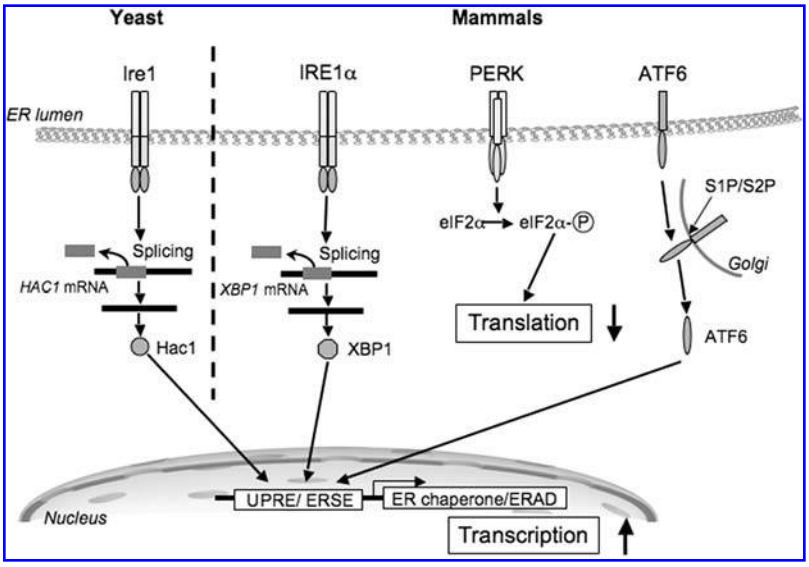

FIG. 2. Overview of the unfolded protein response in yeast and mammals. When IRE1 (yeast Ire1 and mammalian IRE1α) is activated by the accumulation of unfolded proteins, it cleaves *HAC1/XBP1* mRNA to remove an intron by spliceosome-independent unconventional splicing, which changes the translational reading frame. The mature form of Hac1/XBP1 binds specific elements (UPRE, unfolded protein response element; ERSE, ER stress-response element) and transcriptionally upregulates genes encoding ER chaperones and the components of ERAD. Activated PERK attenuates translation by phosphorylating eIF2α. ATF6 (α, β) is transported from the ER to the Golgi in response to ER stress, and the N-terminal cytosolic portion of ATF6 is released by the sequential cleavage with proteases S1P and S2P. The free ATF6 fragment activates transcription of ER chaperone genes by binding to ERSE.

ER stress stimuli (5, 34). In addition, the RNase activity of IRE1 has various effects on RNA stability. IRE1α autonomously downregulates its own expression by cleaving specific sites within IRE1α mRNA (73), and IRE1β cleaves 28S rRNA at specific sites and induces translational repression (28). In *Drosophila*, IRE1 rapidly cleaves a specific subset of mRNAs localized to the ER membrane under ER stress, which is thought to relieve the burden on the ER by selectively blocking new protein synthesis and clearing the translocation and folding machinery for the subsequent remodeling process (27).

Sensing mechanism of accumulation of unfolded proteins by IRE1

In the luminal region of yeast and human IRE1, some potential N-linked glycosylation sites and cysteine residues are found. Although N-linked glycosylation and disulfide bond formation are important for correct folding of newly synthesized proteins in the ER, neither process influences the UPR, indicating that the impairment of posttranslational modification of IRE1 is not the trigger for IRE1 activation in response to ER stress (37, 53).

Three important observations regard the sensing mechanism of the UPR: (a) overexpression of BiP attenuates induction of the UPR (12, 32); (b) yeast cells harboring a mutant BiP lacking the ER retrieval signal slowly secrete BiP into the medium; however, the UPR autonomously restores normal levels of BiP in the ER (23); and (c) an incompletely folded simian virus 5 hemagglutinin-neuraminidase mutant glycoprotein fails to bind BiP and cannot induce UPR (48). These data suggest the idea that the amount of BiP negatively regulates the activation of IRE1 (Fig. 3) (16, 62). In this model, under normal conditions, BiP binds to IRE1, as a result of which IRE1 dimer/oligomer formation is repressed. This association maintains IRE1 in an inactive, monomeric form. However, under ER stress conditions, increased amounts of unfolded or misfolded proteins in the ER compete with IRE1 for binding to BiP, resulting in dimer/oligomer formation of IRE1 (a competition model).

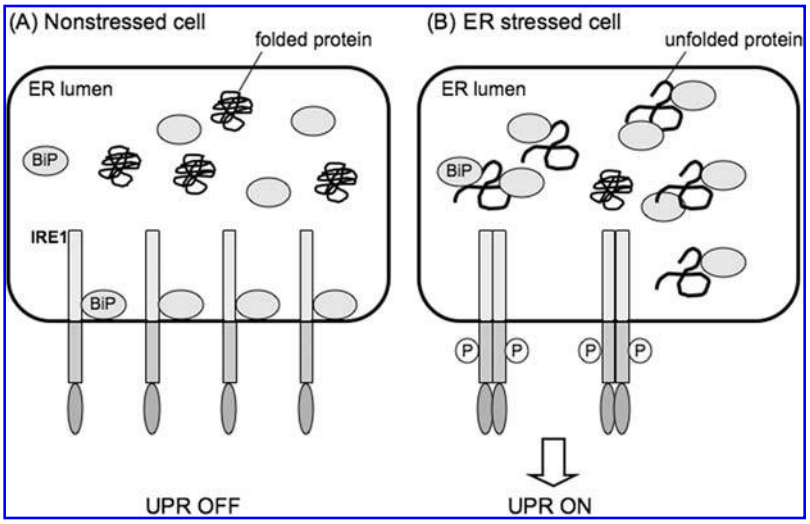

FIG. 3. Negative regulation of IRE1 activity by its interaction with the ER chaperone BiP. Under normal conditions, association with BiP keeps IRE1 in an inactive, monomeric form. In a simple competition model, under ER stress conditions, increased amounts of unfolded or misfolded proteins compete with IRE1 for binding to BiP, leading to dimerization/oligomerization of IRE1 and thereby switching on the UPR. How BiP dissociates from IRE1 is still under debate (see text).

Studies by Ron's and our groups showed that BiP binds IRE1 under normal conditions and that BiP binding declines greatly with increasing amounts of unfolded/misfolded proteins under ER stress conditions in mammals (1) and yeast (55). In mammalian cultured cells, association and dissociation of BiP and IRE1, or BiP and PERK (described later), are particularly well correlated with ER stress conditions. Genetic evidence from temperature-sensitive yeast mutants also supports the model that BiP is a negative regulator of Ire1 activation (30). When cells carrying a mutation in the substrate-binding domain of BiP were incubated at the restrictive temperature, association of BiP with Ire1 was disrupted, and the UPR pathway was activated, even in the absence of extrinsic ER stress. In contrast, cells carrying a mutation in the ATPase domain of BiP, in which BiP dissociation from Ire1 was impaired, even in the presence of ER stress, were unable to activate the UPR pathway.

Dissection of the Ire1 luminal region

Although BiP is a negative regulator of IRE1 activation, no direct evidence exists that BiP is the primary determinant of IRE1 activation. Liu *et al*. (36) reported that the yeast Ire1 luminal region could be replaced by a functional bZip dimerization motif, MafL, and proposed that ligand-independent dimerization of IRE1 is sufficient for UPR activation. This report supports the idea that BiP is the primary determinant of the UPR. To determine the precise structure–function relation of the yeast Ire1 luminal region, Kimata *et al*. (31) analyzed a series of 10-amino acids deletion scanning mutants. The results showed that the luminal region can be divided into five subregions termed I–V from the N-terminus to the transmembrane region (Fig. 4). Subregions II and IV are indispensable for activity, and the BiP-binding site was assigned to subregion V, neighboring the transmembrane domain, surprisingly, which is dispensable for activation. Unexpectedly, deletion of the BiP-binding site had no effect on ER stress inducibility: an Ire1 mutant with a deleted subregion V does not bind BiP but still responds to ER stress as well as wild-type Ire1, suggesting that BiP is not the primary determinant of the UPR but that Ire1 itself recognizes ER stress. Limited proteolysis analysis indicated that subregions II to IV are tightly folded into a domain conferring on Ire1 the ability to dimerize (53). Therefore, this domain (R112 to D454) constitutes the core stress-sensing machinery of Ire1 (31). Although an interspecies comparison of IRE1 and PERK sequences shows a low degree of homology, four conserved motifs are all included in this core stress-sensing region (29, 36). This structural and functional study of the yeast Ire1 luminal region is in good agreement with recent results obtained from crystal structure analysis of the Ire1 luminal core region (11, described later).

An Ire1 mutant lacking both the N-terminal and the loosely folded juxtamembrane subregions (R92 to K462) was designated a "core" mutant (54). Surprisingly, the Ire1 core mutant self-associates under normal conditions, but this is not sufficient for activation; ER stress is still needed for full activation of Ire1 *in vivo*. Interestingly, introduction of the single amino-acid substitutions S103P or S103R in the core mutant results in constitutive activation of Ire1, suggesting that a change in the tertiary structure of the Ire1 dimer on the luminal side is required for activation (54). So what is the physiologic significance of BiP binding to Ire1? Subregion V deletion mutant cells are more sensitive to ethanol or high temperature than are wild-type cells, suggesting that Ire1 has an intrinsic ability to sense not only conventional ER stress, but also other stresses that actually cause little or no activation of Ire1. BiP may therefore function to confine or adjust Ire1 to responding to ER stress (31).

Crystal structure of IRE1

Two groups have recently determined the crystal structures of luminal region of yeast Ire1 and human IRE1. Credle *et al*. (11) succeeded in determining the core region of the yeast Ire1 luminal domain (cLD; N111 to Y449) and suggested an attractive mechanism for sensing unfolded proteins in the ER. Dimerization of cLD monomers across a large interface creates a shared central groove, reminiscent of the peptide-binding domains of major histocompatibility complexes (MHCs) (Figs. 4 and 5). Introduction of mutations into conserved amino acid side chains in Ire1 that face into the groove reduces the UPR, suggesting that the groove directly recognizes loosened and flexible regions of unfolded proteins during Ire1 activation. Credle *et al*. therefore speculated that direct binding of unfolded polypeptide in the groove might lead to formation of cLD dimers, or a higher-order quaternary structure of cLD dimer (Fig. 5A; ligand-dependent model). It is not clear whether dimer

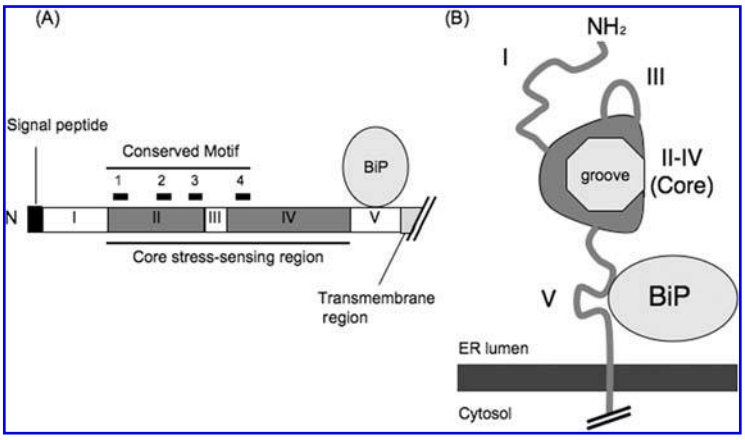

FIG. 4. Schematic representation of the N-terminal ER luminal region of yeast Ire1 (31). (**A**) The luminal region of Ire1 is divided into five subregions, I–V. *Shaded boxes,* The region essential for stress-sensing, which form a tightly folded domain termed the core stress-sensing region. BiP binds subregion V, which is dispensable for Ire1 activation. *Small black rectangles*, Conserved motifs identified by sequence alignment of Ire1 and PERK homologues (36). (**B**) A diagram of Ire1 luminal region. The structure of the core stress-sensing region of Ire1 includes a putative peptide-binding groove reported by Credle *et al*. (11).

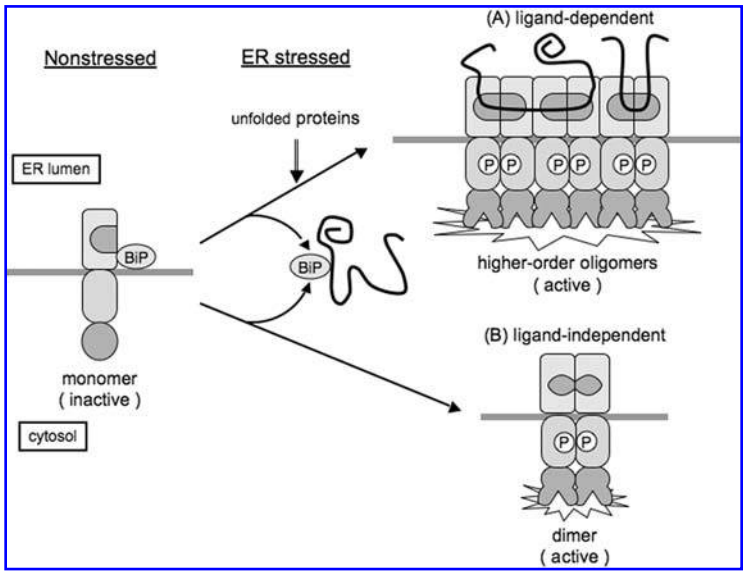

FIG. 5. A model for the activation of Ire1. Dissociation of BiP from Ire1 is prerequisite, although the precise mechanisms remain unknown. (**A**) Ligand-dependent model. Dimerization between the core stress-sensing region of monomers creates a shared central groove reminiscent of the peptide-binding domains of MHCs (11). Binding of unfolded polypeptides to the groove of Ire1 leads to higher-order quaternary structure and full activation of UPR (11). (**B**) Ligand-independent model. Dissociation of BiP from IRE1 by accumulation of unfolded proteins leads to dimerization of Ire1, resulting in *trans*-autophosphorylation and full activation of IRE1 dimer (84).

formation is enough to cause partial activation of Ire1, but mutation studies in two interfaces located on opposite faces of the cLD indicate that higher-order structure is required for full activation.

Conversely, the crystal structure of the human IRE1α luminal domain (S24-V390), recently reported by Zhou *et al.* (84), did not support this model. The monomer of the luminal domain of hIRE1α comprises a unique fold of a triangular assembly of β-sheet clusters. Dimerization creates an MHC1-like groove at the interface as occurs in yeast Ire1; however, the groove is too narrow to allow peptide binding, and the purified luminal domain (LD) spontaneously forms high-affinity dimers *in vitro*. Mutations disrupting the interface of dimerization produce hIRE1α molecules that fail either to dimerize or to activate the UPR under ER stress. In addition, the LD dimer is oriented in the ER membrane in a manner that would make it difficult for peptide-induced oligomers to form. Zhou *et al.* thus speculated that peptide binding is neither probable nor necessary for hIRE1α homodimer formation and proposed that BiP-associating IRE1 maintains an inactive monomeric state. Releasing BiP from IRE1 by accumulation of unfolded proteins in the ER would cause IRE1 to dimerize through hydrogen bonding and extensive hydrophobic interactions at the dimer interface. Dimerization-induced intermolecular autophosphorylation would then lead to activation of the RNase activity (see Fig. 5B).

The two crystallographic models obtained from analyses of yeast and human IRE1α seem incompatible, although the function of the luminal domain of both IRE1s is evolutionarily conserved. However, because both models require the release of BiP from IRE1, BiP is clearly a negative regulator of IRE1 activation (see Fig. 5). The main difference between the two models is whether a subsequent step, the direct binding of unfolded polypeptides to IRE1, is needed. The second model (Fig. 5B; ligand-independent model) shows that the release of BiP from IRE1 is necessary and sufficient for activation of IRE1, whereas the first model (Fig. 5A; ligand-dependent model) requires not only the release of BiP but also the ensuing direct binding of unfolded polypeptides to the groove of the IRE1 dimer, which triggers formation of higher-order quaternary structure by the cLD dimer. One possible explanation of this discrepancy is that the sensing mechanism is slightly different between yeast and human, as described in the two models. However, when the human IRE1 luminal domain replaces the corresponding domain of yeast Ire1, functionality in yeast is retained *in vivo*, suggesting that it is unlikely that the activation of yeast and human IRE1s is regulated in a different manner. The determined crystal structures are only tightly folded globular domains in the luminal region; the structure of the juxta-transmembrane region, which is loosely folded and assigned to the BiP-binding site in yeast, has not yet been determined. According to the ligand-independent model (Fig. 5B), deletion of the BiP-binding region should yield an Ire1 constitutive-active phenotype. However, yeast Ire1 deletion mutants that cannot bind BiP display unaltered ER stress inducibility compared with wild-type Ire1. This result goes against the ligand-independent model, but does not exclude its validity because deletion of subregion V might cause a steric conformational change in yeast Ire1. Furthermore Liu *et al.* (38) reported that the BiP-binding region in hIRE1 was assigned to the luminal globular domain, which differs from its location in yeast Ire1 (see Fig. 1) (38). To demonstrate the validity of the ligand-dependent model, it will be necessary to ascertain by further experimentation whether the cLD of Ire1 can directly bind unfolded polypeptides.

STRESS-SENSING MECHANISM BY PERK

PERK is a type I transmembrane protein whose luminal region resembles the stress-sensing luminal region of IRE1, whereas its cytosolic region contains an eIF2α kinase domain (20, 67). When ER stress is sensed by the luminal region, PERK dimerizes or oligomerizes and autophosphorylates, and becomes activated (1, 39). Activated PERK phosphorylates eIF2α, thereby inhibiting protein synthesis at the initiation step, as expected in view of the sequence similarity between the cytoso-

lic domain of PERK and PKR (20, 21, 59). During ER stress, the attenuation of protein synthesis by PERK is very important for cell survival: $PERK^{-/-}$ cells are highly sensitive to ER stress conditions (21). By impeding translation, PERK can thus transiently block the influx of newly synthesized protein under ER stress as part of a physiologic process that balances rates of translation on the cytosolic side with rates of protein folding on the luminal side (21, 22).

Although the luminal regions of PERK and IRE1 show only limited sequence homology, the predicted secondary structure for PERK indicates that its folding status is similar to that of the IRE1α luminal region (11, 84). The luminal region of PERK is associated with BiP under nonstressed conditions, and BiP is released from PERK, as it is from IRE1, by ER stress (1). Association and dissociation of BiP and IRE1 correlate well with the absence and presence of ER stress, respectively; BiP is thus considered to be a negative regulator of PERK as well as of IRE1. Deletion analysis of the ER luminal region of PERK showed that the BiP-binding region is in close proximity to the transmembrane region, as it is in yeast Ire1 (39) (see Fig.1). The N-terminal domain lacking the BiP-binding region is required both for dimer/oligomer formation and for activation of PERK. These results suggest that the mechanism of activation for PERK is quite similar to that for IRE1. Indeed, the ER luminal region of PERK can replace the yeast Ire1 luminal region to signal UPR (36), and the luminal region of IRE1β can replace that of mammalian PERK (1). However, a PERK mutant lacking the BiP-binding region was constitutively active, suggesting that BiP directly controls PERK activation. This result is not consistent with the behavior of yeast Ire1 (39).

STRESS-SENSING MECHANISM BY ATF6

ATF6 is an ER membrane–bound transcription factor that undergoes proteolytic cleavage in Golgi apparatus during ER stress (82). Its N-terminal cytosolic region contains DNA binding and transcriptional activation domains, whereas its C-terminal luminal region comprises the ER stress-sensing domain (see Fig. 1) (7). On ER stress, ATF6 is transported from the ER to the Golgi, where it is cleaved sequentially by a site 1 protease (S1P) and a site 2 protease (S2P); the liberated N-terminal cytosolic domain is then transferred into the nucleus (7, 24, 25) (see Fig. 2). This domain is a bZIP transcription factor that binds ER stress-response elements (ERSE-I and II) and upregulates genes encoding ER chaperones and folding catalysts (76, 77). As activation of ATF6 is required for its transport from the ER to the Golgi apparatus, the ER stress-sensing mechanism for ATF6 seems quite different from that observed for IRE1 and PERK (45).

However, their common partner molecule, BiP, also participates in activation of ATF6. Shen *et al.* (64) found that BiP is associated with the luminal region of ATF6, but that it dissociates from ATF6 after treatment with various ER stressors. Therefore, although the luminal region of ATF6 lacks sequence homology with the corresponding regions of IRE1 and PERK, BiP seems to be a key binding partner for all three sensors of ER stress. Under normal conditions, both ATF6 and BiP are components of a large complex (>600 kDa), but glycerol-gradient sedimentation revealed that under ER stress, this complex shifted to a lower molecular mass. The luminal region of ATF6 has two Golgi localization signals, GLS1 and GLS 2 (64). Because the BiP-binding sites overlap with or are close to the two GLSs, BiP binding could cause a conformational change of the GLSs, such that they are no longer recognized by the export machinery. When the BiP-binding sites were deleted, but GLS2 retained, the resulting ATF6 mutant was constitutively processed to the mature form, even under nonstressed conditions. Conversely, overexpression of BiP retarded the transport of wild-type ATF6 from the ER to the Golgi. These results suggest that BiP keeps ATF6 in the ER by inhibiting its GLSs and that dissociation of BiP allows ATF6 to be transported to the Golgi (64).

However, the same group recently proposed that the active regulatory mechanism is required for the dissociation of BiP from the BiP–ATF6 complex (65). Contradictory to the competition model, which is based on dynamic binding of BiP to ATF6, they reported that BiP bound stably to ATF6, yielding a complex that was resistant to ATP-induced BiP dissociation *in vitro*. They speculated that ER stress dissociates BiP from ATF6 by actively restarting the BiP ATPase cycle.

Another aspect of the regulation of ATF6 activation was reported recently by Nadanaka *et al.* (46, 47). In the ER lumen, ATF6 normally exists in monomer, dimer, and oligomer forms through intra- and intermolecular disulfide bridges between two conserved cysteine residues in its luminal region (see Fig. 1). Under ER stress induced by both dithiothreitol and tunicamycin, these disulfide bonds are reduced, and only monomeric ATF6 reaches the Golgi, where it is effectively cleaved by S1P and S2P. Although ER stress-induced reduction of ATF6 may be important for the activation process, it is not, by itself, sufficient for ATF6 activation. This mechanism ensures the strictness of regulation to protect mislocation of ATF6 to the Golgi under normal conditions; that is, ATF6 can react only in response to ER stress.

LIGAND-DEPENDENT OR -INDEPENDENT ACTIVATION OF SENSORS?

IRE1, PERK, and ATF6 all bind BiP under nonstressed conditions, and BiP dissociation from these sensor proteins is necessary for their full activation (1, 55, 64). The classic model in which accumulation of unfolded proteins competitively deprives BiP of IRE1 is very simple and acceptable (see Fig. 3; a simple competition model). However, one of the key remaining problems is the large difference in molecular numbers between BiP and Ire1. BiP is the most abundant chaperone in the yeast ER, with an estimated number of 337,000 molecules per cell, whereas Ire1 is present at only ~260 molecules per cell (17). To deprive BiP of Ire1, therefore, huge amounts of unfolded proteins are needed in the ER lumen. The cellular response to overexpression of BiP in yeast was analyzed (58). If BiP concentration in the cell were to double, computer simulation predicted that the UPR would be drastically downregulated and that the response would occur quite slowly, according to a simple competition model. However, in *in vivo* experiments, more than twofold overexpression of BiP only slightly attenuated the UPR (12, 32, 55, 58). Thus, a simple competition model

cannot theoretically explain the induction mechanism of the UPR. Furthermore, a yeast Ire1 core mutant, which is defective in BiP-binding subregion V, still senses increasing amounts of unfolded or misfolded proteins and can transduce the signal to the cytosol/nucleus as efficiently as wild-type Ire1 (31, 53, 54), indicating that BiP is not the primary determinant of UPR, but that Ire1 recognizes, directly or indirectly, the ER stress condition. From this standpoint, the recent report that Ire1 dimerization can form a peptide-binding groove is of considerable interest (see Figs. 4 and 5) (11). Unfolded polypeptides are strong candidates for fitting into this groove. Confirmation of this model will require identification of complexes between Ire1 dimers and unfolded proteins.

CONCLUDING REMARKS

Three major adaptive pathways exist for eukaryotic cell survival in response to ER stress. The three transmembrane proteins, IRE1, PERK, and ATF6, sense the accumulation of unfolded or misfolded proteins in the ER. Interestingly, the ER chaperone BiP binds the luminal regions of all three sensors under normal conditions, and dissociation of BiP from these sensors is needed for the first activation step under ER stress. Thus, a simple competition model has been broadly accepted. However, recent data from structural analysis of yeast Ire1 raise the possibility that the stress sensor itself can recognize unfolded proteins, although the precise details of this process are currently enigmatic. After dissociation of BiP from the sensor, dimerization/oligomerization occurs in IRE1 and PERK, a step that is necessary but not sufficient for activation in the case of yeast Ire1 (54). Direct interaction between unfolded proteins and the sensor, some conformational change, or the involvement of other components is also likely to be needed for this activation step. However, the ligand-binding model remains debatable because the crystal structure of human IRE1 supports the ligand-independent model. It will be important to clarify whether unfolded polypeptides can directly bind to the luminal domain.

Conversely, the ER stress-induced monomeric form of ATF6, which is freed from BiP, is transported from the ER to the Golgi *via* the CopII vesicle and cleaved by S1P/S2P (45). The liberated N-terminal domain of ATF6 is translocated to the nucleus, where it activates the transcription of ER chaperone genes. Even if ATF6 is mislocated to the Golgi under normal conditions, disulfide-bonded ATF6 will return to the ER because nonreduced ATF6 is resistant to cleavage by S1P (47). Activation of ER stress sensors is therefore regulated by several steps, suggesting that regulation *via* these three different pathways is critical in enabling specific cells or tissues to respond to different levels or types of ER stress. Further studies will be needed before we have a complete picture of how ER stress sensors recognize the accumulation of unfolded and misfolded proteins.

ABBREVIATIONS

ATF6, activating transcription factor; BiP, immunoglobulin heavy chain–binding protein; bZIP, basic leucine zipper; cLD, core luminal domain; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; IRE1, inositol-requiring kinase 1; PERK, PKR-like ER kinase; PKR, double-stranded RNA-activated protein kinase; UPR, unfolded protein response.

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