Review Article

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Stress-sensing and regulatory mechanism of the endoplasmic-stress sensors Ire1 and PERK

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Abstract: Ire1 and its family protein PERK are endoplasmic reticulum (ER)-stress sensors that initiate cellular responses against ER accumulation of unfolded proteins. As reviewed in this article, many publications describe molecular mechanisms by which yeast Ire1 senses ER conditions and gets regulated. We also cover recent studies which reveal that mammalian Ire1 (IRE1α) and PERK are controlled in a similar but not exactly the same manner. ER-located molecular chaperone BiP captures these ER-stress sensors and suppresses their activity. Intriguingly, Ire1 is associated with BiP not as a chaperone substrate, but as a unique ligand. Unfolded proteins accumulated in the ER promote dissociation of the Ire1-BiP complex. Moreover, Ire1 is directly bound with unfolded proteins, leading to its cluster formation and potent activation. PERK also captures unfolded proteins and then forms self-oligomers. Meanwhile, membrane-lipid aberrancy is likely to activate these ER-stress sensors independently of ER accumulation of unfolded proteins. In addition, there exist a number of reports that touch on other factors that control activity of these ER-stress sensors. Such a multiplicity of regulatory mechanisms for these ER-stress sensors is likely to contribute to fine tuning of their activity.

Keywords: endoplasmic reticulum stress, sensor, unfolded protein

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1 Introduction

The endoplasmic reticulum (ER) is a membrane-bound cellular compartment, in which secretory and transmembrane proteins are folded. The ER also serves as the location where membrane-lipid components are biosynthesized. Kozutsumi et al. (1) reported that, in mammalian cells, stimuli that cause ER accumulation of unfolded proteins, namely ER stress, transcriptionally induce some ER-located proteins, including the HSP70-family molecular chaperone, BiP. While this phenomenon, which is today called the unfolded protein response (UPR) or ER-stress response, is observed throughout eukaryotic species, the intracellular signaling pathway for the UPR was initially uncovered through studies using budding yeast *Saccharomyces cerevisiae* as a model organism. For instance, the type-I transmembrane protein Ire1 was identified as a key factor for the UPR through genetic screenings for *S. cerevisiae* mutants that cannot evoke the UPR (2, 3). The cytosolic domain of Ire1 carries dual enzymatic activities of Ser/Thr protein kinase and endoribonuclease (4, 5). In ER-stressed *S. cerevisiae* cells, Ire1 promotes splicing of the *HAC1* mRNA, the translational product of which works as a nuclear transcription factor that is responsible for the UPR (6). It is now known that a number of genes that mainly encode proteins functioning in the ER are transcriptionally induced through this Ire1 and *HAC1*-dependent intracellular signaling pathway (7-9). While the regulated Ire1-dependent decay of mRNA (RIDD), which is described in the next paragraph, is observable in a wide variety of eukaryotic cells (10-12), *S. cerevisiae* cells do not seem to perform RIDD, since *S. cerevisiae ire1Δ* cells and *hac1Δ* cells exhibit almost the same gene expression profiles.

In metazoan cells, Ire1 splices (and thus matures) the XBP1 mRNA, which, along with *S. cerevisiae HAC1* mRNA, encodes a nuclear transcription factor that acts for the UPR (13, 14). Moreover, Ire1 degrades mRNAs encoding ER client proteins in response to ER stress (15). This phenomenon is known as RIDD, and probably results in the reduction of protein load in the ER and in the alleviation

of ER stress. According to Han et al. (16) and Upton et al. (17), the RIDD (including degradation of microRNAs), but not the splicing of the XBP1 mRNA, triggers apoptosis. Mammalian species carry two Ire1 paralogues, namely IRE1α and IRE1β, which exhibit different tissue expression and substrate specificity (18). IRE1α is thought to be expressed ubiquitously and to perform both the XBP1-mRNA splicing and RIDD. Meanwhile, IRE1β, which predominantly expressed in mucin-secreting goblet cells, is likely to be specialized for the RIDD (19, 20).

It is canonically accepted that, in addition to Ire1, vertebrate cells carry two other ER-stress sensors, namely PERK and ATF6 (21). ATF6 is a type-II transmembrane protein, which, in response to ER stress, is transported from the ER to the Golgi apparatus, and then cleaved to yield the N-terminal fragment that works as a nuclear transcription factor for the UPR together with XBP1 (22, 23). PERK is an ER-located type-I transmembrane protein that is activated as a Ser/Thr protein kinase in response to ER stress. Phosphorylation of eukaryotic initiation factor-2α by PERK causes global translational attenuation, which is thought to contribute not only to the decrease of protein load into the ER, but also to translational induction of particular proteins, including ATF4 (24, 25).

By considering the structural similarity of the luminal domains of Ire1 and PERK, we assume that they are activated in similar ways. In contrast to the review by Wu et al. (26), which describes general aspects of the cellular response to ER stress in yeast and mammalian cells, here we focus on canonical and recent insights into the molecular mechanisms by which Ire1 and PERK sense ER stress and gets regulated. Suppression of Ire1's or PERK's activity is also an intriguing issue, since both IRE1α and PERK not only act cytoprotectively, but also trigger signaling pathways toward apoptosis (27). A maladaptive UPR is also hazardous in the case of *S. cerevisiae* cells.

2 Structure of the luminal domain of Ire1 and PERK

In order to investigate the ER stress-sensing mechanism of Ire1 and PERK, an important research step is to understand the structure of their luminal domains, which should be responsible for monitoring of ER conditions. Fig. 1A represents structure of the luminal domain of *S. cerevisiae* Ire1, which is deduced from several experimental approaches. Based on 10-amino acid (AA) deletion scanning of the luminal domain of *S. cerevisiae* Ire1, Kimata et al. (28) proposed that it can be segmented into five subregions. Internal 10-AA deletions on Subregion II or IV inactivated Ire1, while Ire1 was normally activated in ER-stressed *S. cerevisiae* cells even when carrying an internal 10-AA deletion in Subregion I, III or V. Subregions I and V are likely to be loosely folded, since they are quickly digested upon *in vitro* partial deletion of a recombinant luminal-domain fragment (29). Credle et al. (30) performed X-ray crystallographic analysis of a peptide consisting of Subregions II to IV, namely the core luminal domain (cLD), which was shown to form one tightly folded module. Subregion III is a loosely folded segment sticking out from the cLD. The protein folding status described here is supported by *in silico* protein disorder prediction (31).

The cLD of *S. cerevisiae* Ire1 exhibits significant primary structural similarity with the corresponding sequences of higher eukaryotic Ire1 orthologues and metazoan PERK (Fig. 1B). On the contrary, the juxtamembrane intrinsically disordered regions (JIDRs), which correspond to Subregion V of *S. cerevisiae* Ire1, seem to be evolutionarily less conserved in terms of amino acid sequences. Fungal Ire1 and metazoan PERK, but not metazoan or plant Ire1 orthologues, carry N-terminal intrinsically disordered stretches, which are called N-terminal unconserved regions (NUCRs) and correspond to Subregion I of *S. cerevisiae* Ire1. The luminal domains of Ire1 and PERK do not seem to carry any known functional motifs or to exhibit significant similarity with any other prokaryotic or eukaryotic proteins.

3 Negative regulation of Ire1 by BiP

While the UPR is considered a cellular response to cope with ER accumulation of unfolded proteins, the heat shock response, which is governed by a transcription factor protein Hsf1 in *S. cerevisiae* cells, is likely to be triggered by disturbance of protein folding in the cytosol and/or the nuclei. By analogy to an insight arguing that cytosolic and/or nuclear HSP70-family chaperones control the activity of Hsf1 (32), it is plausible that BiP regulates Ire1 and/or PERK. According to Kohno et al. (33), the UPR is attenuated in *S. cerevisiae* cells that overproduce BiP. This finding suggests that BiP negatively regulates Ire1, while it is also possible that BiP overproduction enhances protein-folding ability of the ER and alleviates ER stress. Okamura et al. (34) reported that BiP is associated with Ire1 in non-stressed *S. cerevisiae* cells, and that ER stress causes dissociation of the Ire1-BiP complex. The UPR is attenuated in *S. cerevisiae* cells carrying BiP mutants which cannot be detached from Ire1 (35). Deletion mutagenesis studies have shown that the BiP-binding site of *S. cerevisiae* Ire1

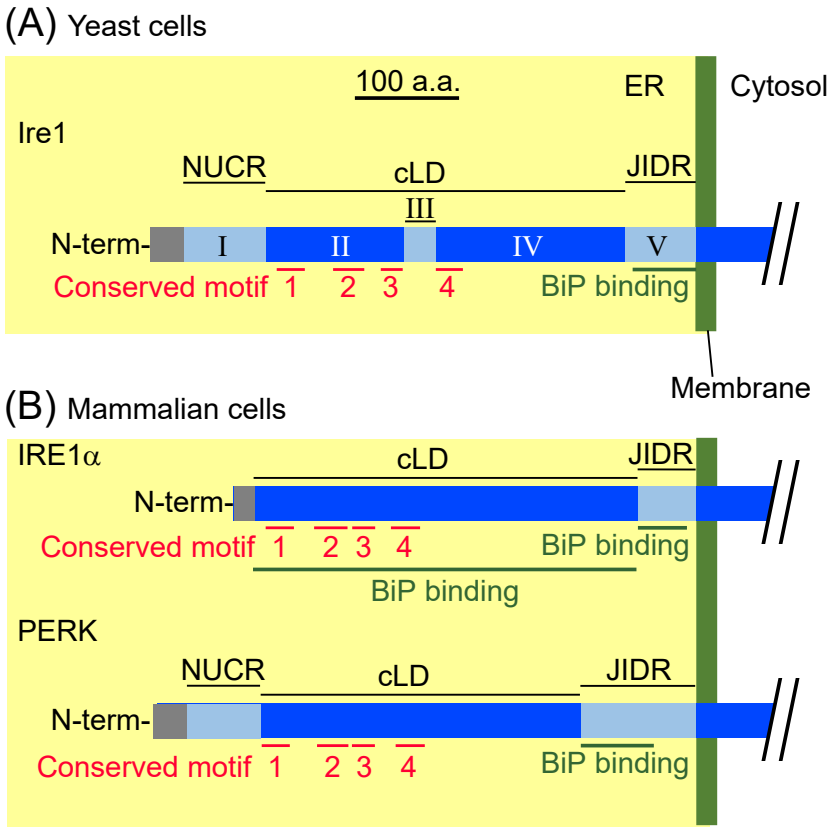

Figure 1: Structure of the luminal domains of Ire1 and PERK

According to Kimata et al. (28), the luminal domain of *S. cerevisiae* Ire1 can be segmented into Subregions I to V. Subregions II to IV compose the conserved cLD (A). Structure of the luminal domains of mammalian IRE1α and PERK is also presented (B). The highly conserved motives found on the cLDs (86) are also indicated. Amino-acid sequences of the NUCRs and the JIDRs are less conserved. The N-terminal ER-translocation signals are gray colored. The BiP-binding sites were deduced through deletion mutational analyses shown in Ma et al. (38), Kimata et al. (28) and Oikawa et al. (39).

is located in Subregion V (28, 36). Intriguingly, an *S. cerevisiae* Ire1 mutant not carrying Subregion V was not constitutive active, but was almost normally regulated by ER stress as well as wild-type Ire1, though inappropriately activated upon some specific stressing stimuli (28). This finding indicates that, as described later, BiP is not the sole determinant of Ire1 activity in *S. cerevisiae* cells.

Bertolotti et al. (37) reported physical interaction of BiP with PERK, IRE1α, and IRE1β, which dissociates upon ER stress, in mammalian cells. As well as in the case of yeast Ire1, the BiP-binding site of PERK is mapped on the JIDR (38). According to Ma et al. (38), a PERK mutant lacking the JIDR was constitutively autophosphorylated in mammalian cells, while wild-type PERK underwent autophosphorylation, which is a prerequisite of its activation, only in response to ER stress. This finding indicates that BiP is a negative regulator of PERK. However, as described later, it is now evident that, similar to *S. cerevisiae* Ire1, PERK is regulated not only by its association/dissociation with BiP. We thus speculate that, in Ma et al. (38), activity of the PERK mutant lacking the JIDR was somewhat overestimated because of its overexpression in mammalian cells.

According to Oikawa et al. (39), Carrara et al. (40) and Amin-Wetzel et al. (41), the BiP-binding sites of IRE1α are located both on the cLD and on the JIDR. This insight may imply that *S. cerevisiae* Ire1 and IRE1α are regulated by BiP in different ways. J proteins, which commonly carry the conserved J-motif sequences, are known to facilitate substrate binding of the HSP70-family chaperones. In general, J proteins are more varied than the HSP70-family chaperones, and it is widely believed the former confer specific roles to the latter. Amin-Wetzel et al. (41) demonstrated that an ER-located J protein, ERdj4, promotes association between BiP and IRE1α in mammalian cells. As described later in this article, self-

association of Ire1 is a prerequisite for its activity to evoke the UPR. Intriguingly, ERdj4 is likely to not only inhibit self-association of IRE1α, but also to contribute to the dissociation of the already existing IRE1α homo-dimers. The ERdj4 knockout mutation considerably activated IRE1α, but not PERK, in non-stressed mammalian cells.

The HSP70-family chaperones consist of the N-terminal nucleotide-binding domains (NBDs) and the C-terminal substrate-binding domains (SBDs). As a general understanding, the NBD captures and hydrolyzes ATP, resulting in control of the capture and holding of client unfolded proteins by the SBD (42). According to Carrara et al. (40), IRE1α and PERK are associated not with the SBD, but with the NBD of BiP. In other words, the luminal domain of IRE1α and PERK dissociates from BiP upon ER stress, but not as a result of competition with ER-accumulated unfolded proteins. It is likely that unfolded proteins are associated with BiP, which then undergoes an allosteric conformational change and releases IRE1α or PERK. Todd-Corlett et al. (43) proposed that, also in the case of *S. cerevisiae* cells, Ire1 is bound to the NBD, but not to the SBD of BiP.

Direct association of unfolded proteins with the cLDs of Ire1and PERK

As described above, *S. cerevisiae* Ire1 and its mutant not carrying Subregion V exhibit almost the same sensitivity to ER-stressing stimuli (28). Therefore, *S. cerevisiae* Ire1 is likely to sense ER stress independently of BiP, though the BiP/Ire1 association seems to contribute to fine tuning of the activity of *S. cerevisiae* Ire1 (28, 36).

According to its X-ray crystallographic structure, the dimerized form of the cLD of *S. cerevisiae* Ire1 forms a groove, which may be able to capture unfolded and/or stretched peptides (30). Kimata et al. (44) and Gardner and Walter (45) reported that the cLD of *S. cerevisiae* Ire1 actually associates with unfolded proteins or peptides *in vitro*. Furthermore, physical interaction between Ire1 and a model unfolded protein in *S. cerevisiae* cells was also demonstrated (45, 46). A full-length deletion of Subregion III (Fig. 1A), which is named as the ΔIII mutation, is deduced to deform the cLD, and actually impaired the association between Ire1 and unfolded proteins (44, 46). Importantly, the ΔIII mutation considerably lowers the ability of Ire1 to induce UPR upon ER accumulation of unfolded proteins in *S. cerevisiae* cells (44, 46). Therefore, at least in the case of *S. cerevisiae* Ire1, its master stress-sensing mechanism is likely to rely on its direct interaction with unfolded proteins.

On the contrary, Zhou et al. (47) proposed that, based on X-ray crystallographic analysis, the luminal domain of IRE1α also forms the groove-like structure, which, however, seems to be too narrow to capture stretched peptides. Moreover, a recombinant fragment of the luminal domain of IRE1α failed to capture model unfolded proteins in an *in vitro* analysis performed by Oikawa et al. (39). These observations argue against the hypothesis that IRE1α directly senses ER-accumulated unfolded proteins. However, Karagöz et al. (48) presented a line of evidence suggesting that the cLD of IRE1α undergoes a conformational change, which is linked to its direct association with unfolded proteins. Supporting this idea, Sundaram et al. (49) documented *in vivo* interaction between IRE1α and misfolded secretory proteins.

The luminal domain of PERK is also likely to be able to capture unfolded proteins (50). According to Wang et al. (51), the association between PERK luminal domain and unfolded proteins promotes oligomerization of PERK, which is then activated. Oikawa et al. (52) demonstrated capturing of ER client proteins by IRE1β.

4 cLD-dependent high-order oligomerization of Ire1

The X-ray crystallographic structure of the cLD of *S. cerevisiae* Ire1 also suggests that it can self-associate via two different interfaces (30), raising the possibility that it forms concatemer-like oligomers. According to Kimata et al. (44) and Aragón et al. (53), *S. cerevisiae* Ire1 clusters to exhibit a punctate distribution, which, according to its point-mutation analysis, is due to the concatemer-like self-association of the cLD. Korennykh et al. (54) reported that *S. cerevisiae* Ire1 exhibits a potent endoribonuclease activity when clustered. Furthermore, the *HAC1* mRNA is recruited to the Ire1 clusters for its efficient splicing (53, 55). According to an *in vitro* experiment described in Gardner and Walter (45), the cLD of *S. cerevisiae* Ire1 is highly oligomerized depending on the presence of a binding-substrate peptide. These observations lead to the scenario that ER-accumulated unfolded proteins are directly captured by Ire1, which is then clustered and strongly activated as an endoribonuclease.

Li et al. (56) proposed that IRE1α also clusters upon its activation in response to ER stress. Unlike the case of *S. cerevisiae* Ire1 (44), the auto-phosphorylation of IRE1α contributes to its high-order oligomerization in mammalian cells (57). Intriguingly, oligomerization status of IRE1α is likely to affect its RNA-substrate specificity, possibly explaining why ER stress causes either cytoprotective or apoptotic response depending on the level and/or duration of ER stress (13, 57). Oligomerization status of the cLD of IRE1α is also likely to depend on its

peptide binding (48). He (58) and Ishiwata-Kimata (59) proposed involvement of the actin cables and myosin-family proteins in the cluster formation of IRE1α and *S. cerevisiae* Ire1.

Although PERK is also likely to be oligomerized when activated upon ER stress (37), to our knowledge, there exist no reports that argue for formation of clusters or substantially large oligomers of PERK. The X-ray crystallographic analyses of the luminal domain of PERK suggested that it forms tetramers (50, 60). Unlike Ire1, PERK may not have to cluster, since, as mentioned above, Ire1 is thought to cluster to exert a potent endoribonuclease activity.

5 Involvement of the NUCDs in negative regulation of PERK and S. cerevisiae Ire1

In addition to Subregion V, which serves as the BiP-binding site, another intrinsically disordered segment, namely Subregion I (or the NUCD), contributes to the downregulation of *S. cerevisiae* Ire1. A mutant version of *S. cerevisiae* Ire1 carrying deletions of both Subegions I and V is constitutively self-associated, though clustered and fully activated not constitutively but in response to ER stress (31, 61). Unlike Subregion V, Subregion I does not seem to be a BiP-binding site (61). Mathuranyanon et al. (31) proposed that Subregion I is intramolecularly captured by the groove of the cLD dimer, which is then dissociated. The NUCR of PERK is also likely to act as a negative regulatory segment (31).

6 Involvement of Ire1's kinase motif in its activation as an endoribonuclease

Shamu and Walter (4) proposed that, in ER-stressed *S. cerevisiae* cells, Ire1 self-associates, which leads to its auto-phosphorylation and UPR evocation. In other words, the kinase activity of Ire1 *per se* is not absolutely required for the UPR, since kinase-functionless mutants of *S. cerevisiae* Ire1 can trigger the UPR if taking an appropriate structure (62-64). X-ray crystallographic structure of the cytosolic domain of Ire1 clearly presents the mechanism by which its auto-phosphorylation leads to its activation as an endoribonuclease (65). It should be also noted that nucleotide binding to the catalytic cleft of Ire1's kinase module is not only for the phospho-transfer reaction (54, 65). ADP (more effectively than ATP) binding affects the structure of the cytosolic domain of Ire1, which then exerts a potent ribonuclease activity. Also, a kinase inhibitor can activate a kinase-inactive mutant of IRE1α (16).

7 An overall scenario for the regulation of Ire1 in non-stressed and ER-stressed cells

How are the issues described so far in this article interconnected and how do they contribute to evocation and attenuation of the UPR?

Fig. 2A represents our current understanding of the regulation of *S. cerevisiae* Ire1 in response to ER stress. As described above, an *S. cerevisiae* Ire1 mutant carrying neither Subregion I nor V is constitutively self-associated, but clusters depending on ER stress (31, 61). We thus infer that BiP is associated with Ire1, which is then kept non-self-associated, in non-stressed *S. cerevisiae* cells. It may also be possible that BiP can forcibly dissociate self-associated Ire1 molecules. In addition, Subregion I is intramolecularly captured by the groove of the cLD dimer, which is then dissociated (31). In contrast, under ER-stress conditions, unfolded proteins accumulated in the ER are bound to BiP, which then dissociates from Ire1. Moreover, instead of the intramolecular association with Subregion I, the cLD dimer intermolecularly captures unfolded proteins and are then bundled (45), leading to the cluster formation of Ire1. It should also be noted that the self-association of Ire1 causes its auto-phosphorylation (4). According to Korennykh et al. (54), clustered and phosphorylated Ire1 molecules exhibit a potent endoribonuclease activity to splice the *HAC1* mRNA.

Under recovery phase after peak induction of the UPR, Ire1 is reported to be downregulated through its dephosphorylation (63, 64). It is also likely that BiP binding (re-association) to Ire1 contributes to the attenuation of Ire1's activity on this time point (36, 66). Since BiP is transcriptionally induced by the UPR, this phenomenon can be viewed as a negative feedback regulation.

Although being still controversial, the stress-sensing and regulatory mechanism of IRE1α is speculated to be similar to that of *S. cerevisiae* Ire1, as shown in Fig. 2B. One different point is that, in the case of IRE1α, BiP-binding sites have been mapped on both the JIDR and the cLD (39). Moreover, IRE1α does not have the NUCR.

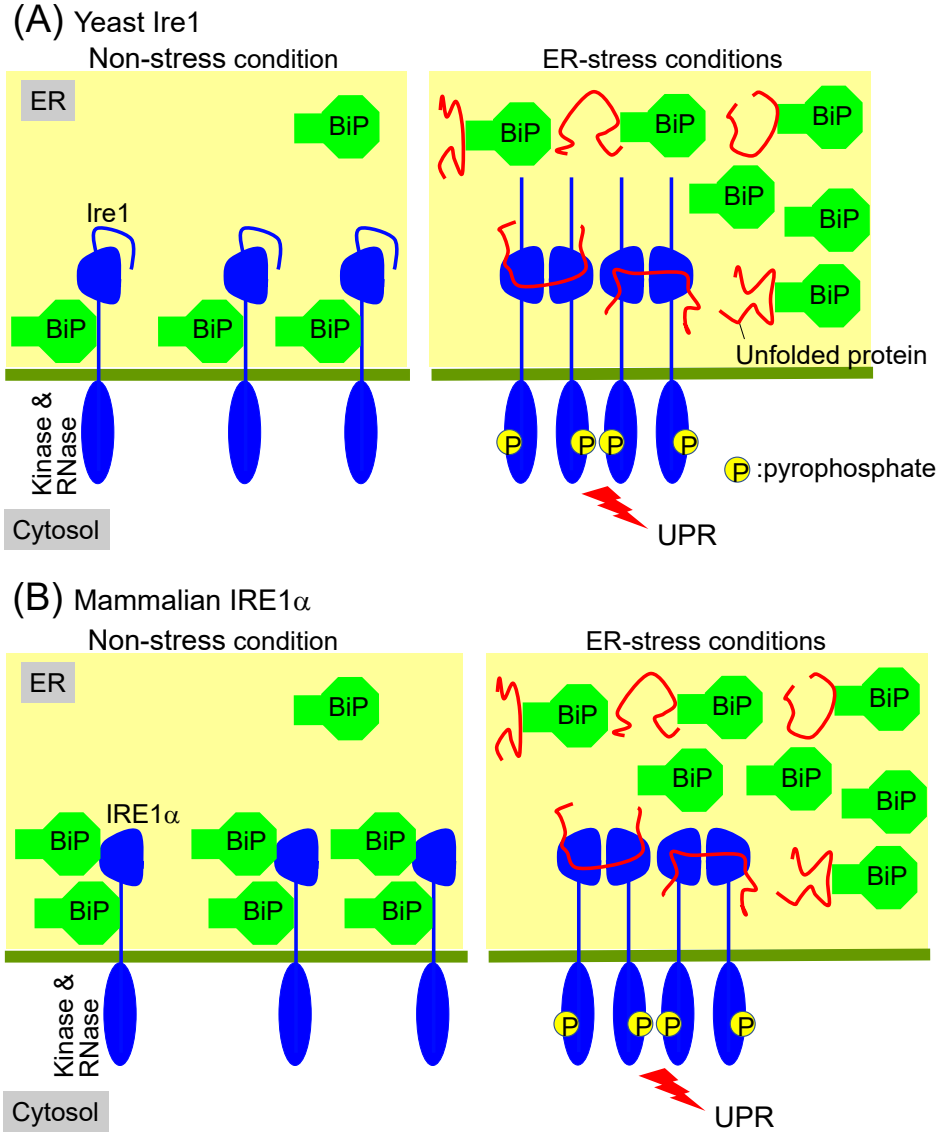

Figure 2: Current model for activation of Ire1 upon ER accumulation of unfolded proteins

8 Activation of Ire1 and PERK by membrane-lipid aberrancy and calcium depletion

Ire1, which is named for "Inositol Requiring," was initially identified as a factor that is involved in inositol prototrophy of *S. cerevisiae* cells (67). Ire1 is activated in *S. cerevisiae* cells cultured under inositol deficient conditions (68). Inositol is a membrane-lipid component, and the UPR is reported to induce genes encoding enzymes for membrane-lipid biogenesis as well as ER-located chaperone genes (7, 8, 68). Thus, at least in the case of *S. cerevisiae* cells, the UPR can be considered as a cellular response not only to cope with ER accumulation of unfolded proteins but also to maintain membrane-lipid homeostasis. Intriguingly, inositol depletion and genetic mutations that cumulatively perturb membrane-lipid integrity activated the ΔIII mutant version of Ire1 as well as wild-type Ire1 in *S. cerevisiae* cells (46). This observation strongly suggests that, in these cases, Ire1 is activated independently of unfolded proteins accumulated in the ER.

One of the well-documented examples for lipid perturbation-induced UPR is the intracellular accumulation of saturated fatty acid (69). According to Volmer et al. (70), IRE1α and PERK directly sense membrane lipid saturation independently of their luminal domains.

Halbleib et al. (71) proposed that the transmembrane domain of Ire1 bears a unique physicochemical property that enables it to change its own behavior depending on membrane-lipid composition.

Unlike the case in *S. cerevisiae* cells (72), the ER in mammalian cells stores a large amount of calcium ions. Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), which is located on the ER membrane, transports calcium ions into the ER lumen. It is widely known that depletion of calcium ions from inside the ER (for example, by treatment of cells with the SERCA inhibitor thapsigargin) induces ER stress in mammalian cells. This is presumably because calcium ions assist protein quality control in the ER, since they are needed by calcium-binding molecular chaperones calnexin and calreticulin (73). An alternative scenario by which aberrant lipid metabolism activates IRE1α and PERK in mammalian cells was proposed by Fu et al. (74), according to which the activity of SERCA is impaired by alteration of the ER phospholipid composition.

9 Other factors that affect IRE1α and/or PERK activity

As illustrated in Chen and Brandizzi (75), a number of proteins have been reported to interact with IRE1α and modulate its activity. For example, an ER-resident protein, Bax inhibitor-1, is associated with and downregulates IRE1α (76). In contrast, according to Jwa and Chang (77), a poly(ADP-ribose) polymerase (PARP16) ADP-ribosylates and activates IRE1α and PERK depending on ER stress.

More recently, Plumb et al. (78) reported complex formation between IRE1α and the Sec61 translocon. According to Sundaram et al. (79), severe ER stress causes dissociation of this complex, which further activates IRE1α. It should also be noted that a pro-survival factor, fortilin, interacts with IRE1α, resulting in inhibition of IRE1α-induced apoptosis (80).

Covalent modifications of IRE1α that downregulate it were also reported. According to Eletto et al. (81), an ER-resident protein, disulfide isomerase PDIA6, is directly bound to IRE1α via a disulfide bridge. Nakato et al. (82) proposed that nitric oxide can S-nitrosylate and inactivate IRE1α. In contrast, S-nitrosylation of PERK is likely to activate it (82).

10 Conclusion and Perspective

As described so far in this article, the Ire1-family proteins, including PERK, is activated through BiP dissociation and direct capture of ER-accumulated unfolded proteins. The cLDs of Ire1 are highly oligomerized depending on their interaction with unfolded proteins, leading to the cluster formation of Ire1. A similar scenario seems to be applicable in the case of PERK, though PERK oligomers are smaller than those of Ire1. Moreover, a number of other factors have been reported as regulators of Ire1 and PERK. A difference between the current model from the previous model that was presented in Kimata and Kohno (83) is that the Ire1-family proteins are associated with BiP not as chaperone substrates but as specific interactors. Moreover, unlike the model described previously (39, 47), the cLD of IRE1α, as well as that of yeast Ire1, captures unfolded proteins (48).

It remains controversial whether the master sensor of unfolded proteins is the cLDs of IRE1α and PERK *per se* or the *trans*-acting factors, including BiP, for the regulation of IRE1α and PERK upon accumulation of unfolded proteins in the ER of mammalian cells. Contradictory observations have often been obtained through different experimental approaches. Although cellular expression and characterization of mutated IRE1α and PERK is a powerful methodology to explore the regulatory mechanism of these ER-stress sensors, it should be noted they can be inadequately activated when overproduced. We thus think that cellular expression of the mutant versions of IRE1α, PERK and their regulators at the endogenous levels will provide us correct insights, and that, to this end, it will be meaningful to introduce the mutations through genome-editing techniques.

Another current topic is the activation of Ire1 and PERK upon membrane-lipid aberrancy, which does not require capturing of unfolded proteins by the cLDs. It should be noted that there exist several unanswered questions regarding this issue. For instance, is BiP detached from Ire1 or PERK upon membrane-lipid disturbance stresses that activate these ER-stress sensors? We speculate that the molecular status of Ire1 activated by ER accumulation of unfolded proteins and by membrane-lipid aberrancy may be different. According to Kitai (84), membrane-lipid saturation activates IRE1α without inducing its cluster formation. Moreover, *S. cerevisiae* Ire1 seems to evoke a weak UPR in a BiP-bound and non-clustered form (66). We thus speculate that Ire1 (and probably also PERK) may be activated by membrane-lipid aberrancy without releasing BiP.

Finally, IRE1α and PERK appear to be regulated by a wider variety of mechanisms than *S. cerevisiae* Ire1, possibly reflecting the complexity of mammalian cells as the constituents of multicellular organisms. The contribution or importance of each regulatory event may differ depending

on cell types, cell lineages, stressing conditions and so on, which should be addressed in future studies. We believe that new techniques including cellular imaging of ER stress and the UPR (85) provide novel insights into dynamics and regulation of the ER-stress sensors.

Conflicts of Interest: The authors declare no conflicts of interest associated with this article.

References

[1] Kozutsumi Y., Segal M., Normington K., Gething M.J., Sambrook J., The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins, Nature, 1988, 332, 462-464; DOI: 10.1038/332462a0

[2] Cox J.S., Shamu C.E., Walter P., Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase, Cell, 1993, 73, 1197-1206

[3] Mori K., Ma W., Gething M.J., Sambrook J., A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus, Cell, 1993, 74, 743-756

[4] Shamu C.E., Walter P., Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum to the nucleus, EMBO J., 1996, 15, 3028-3039

[5] Sidrauski C., Walter P., The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response, Cell, 1997, 90, 1031-1039

[6] Cox J.S., Walter P., A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response, Cell, 1996, 87, 391-404

[7] Travers K.J., Patil C.K., Wodicka L., Lockhart D.J., Weissman J.S., Walter P., Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation, Cell, 2000, 101, 249-258

[8] Kimata Y., Ishiwata-Kimata Y., Yamada S., Kohno K., Yeast unfolded protein response pathway regulates expression of genes for anti-oxidative stress and for cell surface proteins, Genes Cells, 2006, 11, 59-69; DOI: 10.1111/j.1365-2443.2005.00921.x

[9] Fordyce P.M., Pincus D., Kimmig P., Nelson C.S., El-Samad H., Walter P. et al., Basic leucine zipper transcription factor Hac1 binds DNA in two distinct modes as revealed by microfluidic analyses, Proc. Natl. Acad. Sci. USA, 2012, 109, E3084-E3093; DOI: 10.1073/pnas.1212457109

[10] Kimmig P., Diaz M., Zheng J., Williams C.C., Lang A., Aragon T. et al., The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis, eLife, 2012, 1, e00048; DOI: 10.7554/eLife.00048

[11] Mishiba K., Nagashima Y., Suzuki E., Hayashi N., Ogata Y., Shimada Y. et al., Defects in IRE1 enhance cell death and fail to degrade mRNAs encoding secretory pathway proteins in the Arabidopsis unfolded protein response, Proc. Natl. Acad. Sci. USA, 2013, 110, 5713-5718; DOI: 10.1073/pnas.1219047110

[12] Maurel M., Chevet E., Tavernier J., Gerlo S., Getting RIDD of RNA: IRE1 in cell fate regulation, Trends Biochem. Sci., 2014, 39, 245-254; DOI: 10.1016/j.tibs.2014.02.008

[13] Yoshida H., Matsui T., Yamamoto A., Okada T., Mori K., XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell, 2001, 107, 881-891

[14] Calfon M., Zeng H., Urano F., Till J.H., Hubbard S.R., Harding H.P. et al., IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA, Nature, 2002, 415, 92-96; DOI: 10.1038/415092a

[15] Hollien J., Weissman J.S., Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response, Science, 2006, 313, 104-107; DOI: 10.1126/science.1129631

[16] Han D., Lerner A.G., Vande Walle L., Upton J.P., Xu W., Hagen A. et al., IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates, Cell, 2009, 138, 562-575; DOI: 10.1016/j.cell.2009.07.017

[17] Upton J.P., Wang L., Han D., Wang E.S., Huskey N.E., Lim L. et al., IRE1alpha cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2, Science, 2012, 338, 818-822; 10.1126/science.1226191

[18] Imagawa Y., Hosoda A., Sasaka S., Tsuru A., Kohno K., RNase domains determine the functional difference between IRE1alpha and IRE1beta, FEBS Lett., 2008, 582, 656-660; DOI: 10.1016/j.febslet.2008.01.038

[19] Nakamura D., Tsuru A., Ikegami K., Imagawa Y., Fujimoto N., Kohno K., Mammalian ER stress sensor IRE1beta specifically down-regulates the synthesis of secretory pathway proteins, FEBS Lett., 2011, 585, 133-138; DOI: 10.1016/j.febslet.2010.12.002

[20] Tsuru A., Fujimoto N., Takahashi S., Saito M., Nakamura D., Iwano M. et al., Negative feedback by IRE1beta optimizes mucin production in goblet cells, Proc. Natl. Acad. Sci. USA, 2013, 110, 2864-2869; DOI: 10.1073/pnas.1212484110

[21] Mori K., Tripartite management of unfolded proteins in the endoplasmic reticulum, Cell, 2000, 101, 451-454

[22] Yoshida H., Okada T., Haze K., Yanagi H., Yura T., Negishi M. et al., ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response, Mol. Cell. Biol., 2000, 20, 6755-6767

[23] Ye J., Rawson R.B., Komuro R., Chen X., Dave U.P., Prywes R. et al., ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs, Mol. Cell, 2000, 6, 1355-13564

[24] Harding H.P., Zhang Y., Ron D., Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature, 1999, 397, 271-274; DOI: 10.1038/16729

[25] Harding H.P., Novoa I., Zhang Y., Zeng H., Wek R., Schapira M. et al., Regulated translation initiation controls stress-induced gene expression in mammalian cells, Mol. Cell, 2000, 6, 1099-1108

[26] Wu H., Ng B.S., Thibault G., Endoplasmic reticulum stress response in yeast and humans, Biosci. Rep., 2014, 34, e00118; DOI: 10.1042/BSR20140058

[27] Hetz C., Papa F.R., The Unfolded Protein Response and Cell Fate Control, Mol. Cell, 2018, 69, 169-181; DOI: 10.1016/j.molcel.2017.06.017

[28] Kimata Y., Oikawa D., Shimizu Y., Ishiwata-Kimata Y., Kohno K., A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1, J. Cell Biol., 2004, 167, 445-456; DOI: 10.1083/jcb.200405153

[29] Oikawa D., Kimata Y., Takeuchi M., Kohno K., An essential dimer-forming subregion of the endoplasmic reticulum stress sensor Ire1, Biochem. J., 2005, 391, 135-142; DOI: 10.1042/BJ20050640

[30] Credle J.J., Finer-Moore J.S., Papa F.R., Stroud R.M., Walter P., On the mechanism of sensing unfolded protein in the endoplasmic reticulum, Proc. Natl. Acad. Sci. U S A, 2005, 102, 18773-18784; DOI: 10.1073/pnas.0509487102

[31] Mathuranyanon R., Tsukamoto T., Takeuchi A., Ishiwata-Kimata Y., Tsuchiya Y., Kohno K. et al., Tight regulation of the unfolded protein sensor Ire1 by its intramolecularly antagonizing subdomain, J. Cell Sci., 2015, 128, 1762-1772; DOI: 10.1242/jcs.164111

[32] Zheng X., Krakowiak J., Patel N., Beyzavi A., Ezike J., Khalil A.S. et al., Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation, eLife, 2016, 5; DOI: 10.7554/eLife.18638

[33] Kohno K., Normington K., Sambrook J., Gething M.J., Mori K., The promoter region of the yeast KAR2 (BiP) gene contains a regulatory domain that responds to the presence of unfolded proteins in the endoplasmic reticulum, Mol. Cell. Biol., 1993, 13, 877-890

[34] Okamura K., Kimata Y., Higashio H., Tsuru A., Kohno K., Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast, Biochem. Biophys. Res. Commun., 2000, 279, 445-450; DOI: 10.1006/bbrc.2000.3987

[35] Kimata Y., Kimata Y.I., Shimizu Y., Abe H., Farcasanu I.C., Takeuchi M. et al., Genetic evidence for a role of BiP/Kar2 that regulates Ire1 in response to accumulation of unfolded proteins, Mol. Biol. Cell, 2003, 14, 2559-2569; DOI: 10.1091/mbc.E02-11-0708

[36] Pincus D., Chevalier M.W., Aragon T., van Anken E., Vidal S.E., El-Samad H. et al., BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response, PLoS Biol., 2010, 8, e1000415; DOI: 10.1371/journal.pbio.1000415

[37] Bertolotti A., Zhang Y., Hendershot L.M., Harding H.P., Ron D., Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response, Nat. Cell Biol., 2000, 2, 326-332; DOI: 10.1038/35014014

[38] Ma K., Vattem K.M., Wek R.C., Dimerization and release of molecular chaperone inhibition facilitate activation of eukaryotic initiation factor-2 kinase in response to endoplasmic reticulum stress, J. Biol. Chem., 2002, 277, 18728-18735; DOI: 10.1074/jbc.M200903200

[39] Oikawa D., Kimata Y., Kohno K., Iwawaki T., Activation of mammalian IRE1alpha upon ER stress depends on dissociation of BiP rather than on direct interaction with unfolded proteins, Exp. Cell Res., 2009, 315, 2496-2504; DOI: 10.1016/j.yexcr.2009.06.009

[40] Carrara M., Prischi F., Nowak P.R., Kopp M.C., Ali M.M., Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling, eLife, 2015, 4; DOI: 10.7554/eLife.03522

[41] Amin-Wetzel N., Saunders R.A., Kamphuis M.J., Rato C., Preissler S., Harding H.P. et al., A J-Protein Co-chaperone Recruits BiP to monomerize IRE1 and repress the unfolded protein response, Cell, 2017, 171, 1625-1637; DOI: 10.1016/j.cell.2017.10.040

[42] Mayer M.P., Hsp70 chaperone dynamics and molecular mechanism, Trends Biochem. Sci., 2013, 38, 507-514; DOI: 10.1016/j.tibs.2013.08.001

[43] Todd-Corlett A., Jones E., Seghers C., Gething M.J., Lobe IB of the ATPase domain of Kar2p/BiP interacts with Ire1p to negatively regulate the unfolded protein response in Saccharomyces cerevisiae, J. Mol. Biol., 2007, 367, 770-787; DOI: 10.1016/j.jmb.2007.01.009

[44] Kimata Y., Ishiwata-Kimata Y., Ito T., Hirata A., Suzuki T., Oikawa D. et al., Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins, J. Cell Biol., 2007, 179, 75-86; DOI: 10.1083/jcb.200704166

[45] Gardner B.M., Walter P., Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response, Science, 2011, 333, 1891-1894; DOI: 10.1126/science.1209126

[46] Promlek T., Ishiwata-Kimata Y., Shido M., Sakuramoto M., Kohno K., Kimata Y., Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways, Mol. Biol. Cell, 2011, 22, 3520-3532; DOI: 10.1091/mbc.E11-04-0295

[47] Zhou J., Liu C.Y., Back S.H., Clark R.L., Peisach D., Xu Z. et al., The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response, Proc. Natl. Acad. Sci. USA, 2006, 103, 14343-14348; DOI: 10.1073/pnas.0606480103

[48] Karagoz G.E., Acosta-Alvear D., Nguyen H.T., Lee C.P., Chu F., Walter P., An unfolded protein-induced conformational switch activates mammalian IRE1, eLife, 2017, 6; DOI: 10.7554/eLife.30700

[49] Sundaram A., Appathurai S., Plumb R., Mariappan M., Dynamic changes in complexes of IRE1alpha, PERK, and ATF6alpha during endoplasmic reticulum stress, Mol. Biol. Cell, 2018, 29, 1376-1388; DOI: 10.1091/mbc.E17-10-0594

[50] Wang P., Li J., Sha B., The ER stress sensor PERK luminal domain functions as a molecular chaperone to interact with misfolded proteins, Acta Crystallogr. D Struct. Biol., 2016, 72, 1290-1297; DOI: 10.1107/S2059798316018064

[51] Wang P., Li J., Tao J., Sha B., The luminal domain of the ER stress sensor protein PERK binds misfolded proteins and thereby triggers PERK oligomerization, J. Biol. Chem., 2018, 293, 4110-4121; DOI: 10.1074/jbc.RA117.001294

[52] Oikawa D., Kitamura A., Kinjo M., Iwawaki T., Direct association of unfolded proteins with mammalian ER stress sensor, IRE1beta, PLoS One, 2012, 7, e51290; DOI: 10.1371/journal.pone.0051290

[53] Aragon T., van Anken E., Pincus D., Serafimova I.M., Korennykh A.V., Rubio C.A. et al., Messenger RNA targeting to endoplasmic reticulum stress signalling sites, Nature, 2009, 457, 736-740; DOI: 10.1038/nature07641

[54] Korennykh A.V., Egea P.F., Korostelev A.A., Finer-Moore J., Zhang C., Shokat K.M. et al., The unfolded protein response signals through high-order assembly of Ire1, Nature, 2009, 457, 687-693; DOI: 10.1038/nature07661

[55] van Anken E., Pincus D., Coyle S., Aragon T., Osman C., Lari F. et al., Specificity in endoplasmic reticulum-stress signaling in yeast entails a step-wise engagement of HAC1 mRNA to clusters of the stress sensor Ire1, eLife, 2014, 3, e05031; DOI: 10.7554/eLife.05031

[56] Li H., Korennykh A.V., Behrman S.L., Walter P., Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic

clustering, Proc. Natl. Acad. Sci. USA, 2010, 107, 16113-16118; DOI: 10.1073/pnas.1010580107
[57] Ghosh R., Wang L., Wang E.S., Perera B.G., Igbaria A., Morita S. et al., Allosteric inhibition of the IRE1alpha RNase preserves cell viability and function during endoplasmic reticulum stress, Cell, 2014, 158, 534-548; DOI: 10.1016/j.cell.2014.07.002
[58] He Y., Beatty A., Han X., Ji Y., Ma X., Adelstein R.S. et al., Nonmuscle myosin IIB links cytoskeleton to IRE1alpha signaling during ER stress, Dev. Cell, 2012, 23, 1141-1152; DOI: 10.1016/j.devcel.2012.11.006
[59] Ishiwata-Kimata Y., Yamamoto Y.H., Takizawa K., Kohno K., Kimata Y., F-actin and a type-II myosin are required for efficient clustering of the ER stress sensor Ire1, Cell Struct. Funct., 2013, 38, 135-143
[60] Carrara M., Prischi F., Nowak P.R., Ali M.M., Crystal structures reveal transient PERK luminal domain tetramerization in endoplasmic reticulum stress signaling, EMBO J., 2015, 34, 1589-1600; DOI: 10.15252/embj.201489183
[61] Oikawa D., Kimata Y., Kohno K., Self-association and BiP dissociation are not sufficient for activation of the ER stress sensor Ire1, J. Cell Sci., 2007, 120, 1681-1688; DOI: 10.1242/jcs.002808
[62] Papa F.R., Zhang C., Shokat K., Walter P., Bypassing a kinase activity with an ATP-competitive drug, Science, 2003, 302, 1533-1537; DOI: 10.1126/science.1090031
[63] Chawla A., Chakrabarti S., Ghosh G., Niwa M., Attenuation of yeast UPR is essential for survival and is mediated by IRE1 kinase, J. Cell Biol., 2011, 193, 41-50; DOI: 10.1083/jcb.201008071
[64] Rubio C., Pincus D., Korennykh A., Schuck S., El-Samad H., Walter P., Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity, J. Cell Biol., 2011, 193, 171-184; DOI: 10.1083/jcb.201007077
[65] Lee K.P., Dey M., Neculai D., Cao C., Dever T.E., Sicheri F., Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing, Cell, 2008, 132, 89-100; DOI: 10.1016/j.cell.2007.10.057
[66] Ishiwata-Kimata Y., Promlek T., Kohno K., Kimata Y., BiP-bound and nonclustered mode of Ire1 evokes a weak but sustained unfolded protein response, Genes Cells, 2013, 18, 288-301; DOI: 10.1111/gtc.12035
[67] Nikawa J., Yamashita S., IRE1 encodes a putative protein kinase containing a membrane-spanning domain and is required for inositol phototrophy in Saccharomyces cerevisiae, Mol. Microbiol., 1992, 6, 1441-1446
[68] Cox J.S., Chapman R.E., Walter P., The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane, Mol. Biol. Cell, 1997, 8, 1805-1814
[69] Pineau L., Ferreira T., Lipid-induced ER stress in yeast and beta cells: parallel trails to a common fate, FEMS Yeast Res., 2010, 10, 1035-1045; DOI: 10.1111/j.1567-1364.2010.00674.x
[70] Volmer R., van der Ploeg K., Ron D., Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains, Proc. Natl. Acad. Sci. USA, 2013, 110, 4628-4633; DOI: 10.1073/pnas.1217611110
[71] Halbleib K., Pesek K., Covino R., Hofbauer H.F., Wunnicke D., Hanelt I. et al., Activation of the Unfolded Protein Response by Lipid Bilayer Stress, Mol. Cell, 2017, 67, 673-684; DOI: 10.1016/j.molcel.2017.06.012
[72] Strayle J., Pozzan T., Rudolph H.K., Steady-state free Ca(2+) in the yeast endoplasmic reticulum reaches only 10 microM and is mainly controlled by the secretory pathway pump pmr1, EMBO J., 1999, 18, 4733-4743
[73] Helenius A., Aebi M., Roles of N-linked glycans in the endoplasmic reticulum, Annu. Rev. Biochem., 2004, 73, 1019-1049
[74] Fu S., Yang L., Li P., Hofmann O., Dicker L., Hide W. et al., Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity, Nature, 2011, 473, 528-531; DOI: 10.1038/nature09968
[75] Chen Y., Brandizzi F., IRE1: ER stress sensor and cell fate executor, Trends Cell Biol., 2013, 23, 547-555; DOI: 10.1016/j.tcb.2013.06.005
[76] Lisbona F., Rojas-Rivera D., Thielen P., Zamorano S., Todd D., Martinon F. et al., BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1alpha, Mol. Cell, 2009, 33, 679-691; DOI: 10.1016/j.molcel.2009.02.017
[77] Jwa M., Chang P., PARP16 is a tail-anchored endoplasmic reticulum protein required for the PERK- and IRE1alpha-mediated unfolded protein response, Nat. Cell Biol., 2012, 14, 1223-1230; DOI: 10.1038/ncb2593
[78] Plumb R., Zhang Z.R., Appathurai S., Mariappan M., A functional link between the co-translational protein translocation pathway and the UPR, eLife, 2015, 4; DOI: 10.7554/eLife.07426
[79] Sundaram A., Plumb R., Appathurai S., Mariappan M., The Sec61 translocon limits IRE1alpha signaling during the unfolded protein response, eLife, 2017, 6; DOI: 10.7554/eLife.27187
[80] Pinkaew D., Chattopadhyay A., King M.D., Chunhacha P., Liu Z., Stevenson H.L. et al., Fortilin binds IRE1alpha and prevents ER stress from signaling apoptotic cell death, Nat. Commun., 2017, 8, 18; DOI: 10.1038/s41467-017-00029-1
[81] Eletto D., Eletto D., Dersh D., Gidalevitz T., Argon Y., Protein disulfide isomerase A6 controls the decay of IRE1alpha signaling via disulfide-dependent association, Mol. Cell, 2014, 53, 562-576; DOI: 10.1016/j.molcel.2014.01.004
[82] Nakato R., Ohkubo Y., Konishi A., Shibata M., Kaneko Y., Iwawaki T. et al., Regulation of the unfolded protein response via S-nitrosylation of sensors of endoplasmic reticulum stress, Sci. Rep., 2015, 5, 14812; DOI: 10.1038/srep14812
[83] Kimata K., Kohno K., Endoplasmic reticulum stress-sensing mechanisms in yeast and mammalian cells, Curr. Opin. Cell Biol., 2011, 23, 135-142; DOI: 10.1016/j.ceb.2010.10.008
[84] Kitai Y., Ariyama H., Kono N., Oikawa D., Iwawaki T., Arai H., Membrane lipid saturation activates IRE1alpha without inducing clustering, Genes Cells, 2013, 18, 798-809; DOI: 10.1111/gtc.12074
[85] Lajoie P., Fazio E.N., Snapp E.L., Approaches to imaging unfolded secretory protein stress in living cells, Endoplasmic Reticulum Stress Dis, 2014, 1, 27-39
[86] Liu C.Y., Schroder M., Kaufman R.J., Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum, J. Biol. Chem., 2000, 275, 24881-24885; DOI: 10.1074/jbc.M004454200