The unfolded protein response of yeast Saccharomyces cerevisiae and other organisms

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Summary: Activity, morphology and size of an organelle are not constant, but vary dependent on extracellular and intracellular conditions. The endoplasmic reticulum (ER) is the location where secretory and transmembrane proteins are folded. Dysfunction or overwork of the ER, which is mostly accompanied by ER accumulation of unfolded client proteins, leads to transcriptional induction of proteins that work in and/or for the ER. This cellular event, known as the unfolded protein response (UPR), is observed in a wide variety of eukaryotic species, and its mechanism has been mainly uncovered through studies using yeast Saccharomyces cerevisiae as a simple model organism. The intracellular signaling pathway of the UPR contains various remarkable features, which include the involvement of regulatory splicing of transcription-factor mRNAs that is performed by the ER-located transmembrane endoribonuclease Ire1 in the cytoplasm. In this article, we describe our current understanding about Ire1 and the UPR in cells of S. cerevisiae and other eukaryotic species including plants.

Key words: chaperone, endoplasmic reticulum, molecular organelle, protein folding, stress response

INTRODUCTION

For a cellular response to maintain homeostasis, it seems to be reasonable and meaningful that upon dysfunction or overwork of an organelle, its constituent proteins are transcriptionally induced, leading to functional enhancement and massive expansion of the organelle. The unfolded protein response (UPR) is likely to be the most prominent example of such a phenomenon. The UPR occurs in a wide variety of eukaryotic species, and its molecular mechanism has been mainly uncovered through frontier studies using a conventional unicellular laboratory organism, namely yeast Saccharomyces cerevisiae (S. cerevisiae). In this mini-review, we describe current knowledge about S. cerevisiae UPR and its key protein Ire1 together with roles of Ire1 in other organisms including plants.

THE UPR AND Ire1 IN S. cerevisiae CELLS

The endoplasmic reticulum (ER) is an intracellular membrane-surrounded compartment which eukaryotic cells commonly carry. In general, the ER takes a flat or tubular shape, and is expanded throughout cells. Under fluorescence-microscopic observation, ER-located fluorescent proteins show a double-ring-like distribution pattern in S. cerevisiae cells (Figure 1). The outer ring represents the cortical ER, which is proximately located near the plasma membrane. The inner ring represents the nuclear envelop, which also works as the ER.

Ribosomes are attached to the ER to form the rough ER. One of the roles of the ER is to serve as the location where secretory and transmembrane proteins are folded. The ER thus carries various molecular chaperones including “binding immunoglobulin protein” (BiP). As its name denotes, BiP was initially isolated from mammalian cells as a protein that is intracellularly associated with premature immunoglobulin heavy chain. However, BiP has subsequently been shown to be conserved among eukaryotic species including S. cerevisiae (Normington et al. 1989, Rose et al. 1989) and is now known as an essential factor for translocation and folding of ER client proteins. After being folded correctly, ER client proteins are transported to the cell surface or target organelles via vesicle transport.

Under some circumstances, collectively called ER-stress conditions, ER protein folding is impaired, which leads to accumulation and aggregation of unfolded proteins in the ER. Since proteins are often modified to carry N-linked sugar chains and cysteine disulfide bonds in the ER, impairment of these post-translational modification processes causes serious ER stress. Thus, in addition to genetic mutations that disturb protein folding in the ER, disulfide-reducing reagent dithiothreitol (DTT) and N-glycosylation inhibitor tunicamycin are conventionally used for induction of ER stress in S. cerevisiae cells.

Transcriptional induction of BiP upon ER stress was initially found through a mammalian cell study (Kozutsumi et al. 1988) and named as the unfolded protein response (UPR). Subsequent studies that addressed S. cerevisiae UPR identified an ER stress-responsive promoter element in S. cerevisiae BiP gene (Mori et al. 1992, Kohno et al. 1993). Yeast genetic methodology was then employed to find proteins responsible for the intracellular signaling of the UPR, resulting in an identification of Ire1. Cells carrying Ire1-gene knockout mutations (ire1D) exhibit only a very weak UPR and are hypersensitive to ER stress.

Figure 1 The ER in yeast cells. Yeast cells producing an ER-located version of GFP were observed under a fluorescent microscope.
stress (Cox et al. 1993, Mori et al. 1993).

Ire1 is a type-I transmembrane protein conserved throughout eukaryotic species. As schematically represented in Figure 2, the cytosolic domain of Ire1 has activities as a Ser/Thr protein kinase and an endoribonuclease (Mori et al. 1993, Sidrauski and Walter 1997). Upon ER stress, Ire1 becomes self-associated and auto-phosphorylated to be an activator of the UPR (Shamu and Walter 1996). In other words, the kinase activity of Ire1 per se is not required for the intracellular signaling cascade for the UPR. Some kinase-dead mutants of Ire1 have activity to promote the UPR, as long as they form an appropriate structure (Papa et al. 2003, Chawla et al. 2011, Rubio et al. 2011). In contrast, the endonuclease activity of Ire1 is essential for UPR evocation because the proximal target molecules of Ire1 for the intracellular UPR signaling are mRNAs but not proteins. In addition to the auto-phosphorylation of Ire1, the kinase reaction center of Ire1 plays another essential role, which is not accompanied with phosphotransfer. According to X-ray crystal-structure analyses and in vitro biochemical assays, incorporation of ADP into the kinase reaction center is required for activation of Ire1 as an endoribonuclease (Lee et al. 2008, Korennykh et al. 2009, Rubio et al. 2011).

The spliced form of HAC1 mRNA, namely HAC1i, is translated into a basic leucine zipper (bZIP)-family transcription-factor protein (Figure 3B), which directly and positively acts at the promoter element of BiP gene thereby resulting in UPR evocation (Mori et al. 1996). In contrast, the translation product from the non-spliced form of the HAC1 mRNA (HAC1u) has only weak UPR inducing activity, and is degraded by the proteasome quickly (Figure 3B, Mori et al. 2000; Di Santo et al. 2016). Moreover, HAC1i mRNA inhibits its own translation through intramolecular hybridization as shown in Figure 3B (Rüegsegger et al. 2001). These insights explain well the reason why HAC1-mRNA splicing leads to transcriptional induction of the UPR.

BiP gene is not the sole target of HAC1i protein. According to Casagrande et al. (2000) and Friedlander et al. (2000), the UPR facilitates ER-associated protein degradation (ERAD), which is carried out through the retrieval transport and proteasomal degradation of ER-accumulated unfolded proteins (Hiller et al. 1996). Comprehensive gene-expression analyses demonstrated transcriptional induction of various genes that function in ER protein transport, folding and modification, as well as those encoding ERAD factors (Travers et al. 2000, Kimata et al. 2006). Fordyce et al. (2012) argued for a wide

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**Figure 2** Activation of Ire1. Ire1 is activated as an endoribonuclease via its self-association, auto-phosphorylation and capture of ADP.

**Figure 3** HAC1 mRNA is the target of yeast Ire1. (A) IRE1+ (wild-type) or ire1D S. cerevisiae cells were stressed by treatment with tunicamycin (2 µg/ml, 60 min) or remained unstressed. Their total RNA samples were then fractionated by electrophoresis and analyzed by Northern blotting to detect HAC1-mRNA species. (B) HAC1 mRNA is spliced by Ire1 and Rlg1 in the cytoplasm.
diversity of promoter DNA sequences to which \textit{HAC1} protein binds. Moreover, \textit{HAC1} protein causes transcriptional repression of ER client proteins that are finally transported to the cell surface (Kimata et al. 2006), possibly resulting in reduction of protein load in the ER.

Genome-wide analyses performed by Niwa et al. (2005) failed to find proximal target molecules of Ire1 other than \textit{HAC1} mRNA in \textit{S. cerevisiae} cells.

**CLUSTER FORMATION OF Ire1 IN \textit{S. cerevisiae} CELLS**

Except for metazoan PERK, which will be described later in this article, no proteins identified thus far in eukaryotic and prokaryotic organisms have significant primary-structural similarity with the luminal domain of Ire1. Therefore, it is difficult to speculate about the structure-function relationship of the luminal domain of Ire1 from its amino-acid sequence. We and others thus employed other approaches to understand the structure of the luminal domain of Ire1. Figure 4A shows a subregion segmentation of the luminal domain of \textit{S. cerevisiae} Ire1, which we propose as a result of 10-a.a. deletion scanning (Kimata et al. 2004). Internal 10-a.a. deletions in Subregions II and IV inactivated Ire1, while the UPR was normally induced in response to ER stress even when Ire1 carried a 10-a.a. deletion in Subregion I, III or V. Moreover, Subregions I and V were quickly digested in partial proteolysis of recombinant luminal domain of Ire1 (Oikawa et al. 2005). Therefore, also as speculated from the \textit{in silico} prediction of protein folding status (Mathuranyanon et al. 2015), Subregions I and V are likely to be intrinsically disordered or unfolded (Figure 4B). According to the X-ray crystal structural analysis (Credle et al. 2005), a peptide carrying Subregions II to IV forms one tightly folded module, which is named as the Core. As expected, Subregion III exists as a loosely folded segment that stretches out from the Core (Figure 4B, Credle et al. 2005).

The higher-order structure of the luminal domain of Ire1 which was proposed by Credle et al. (2005) also argues that it can self-associate in two different ways, which as schematically illustrated in Figure 5A. This self-association may lead to concatemer-like high-order oligomerization. As shown in Figure 5B, Ire1 exhibits a diffuse distribution over the ER in non-stressed \textit{S. cerevisiae} cells, while ER stress drastically changes its localization pattern. The dot-like punctate distribution of Ire1, i.e. the cluster formation of Ire1, in ER-stressed cells is likely to be caused by the high-order oligomerization of the Core, since point mutations of Ire1 that are deduced to abolish the Core self-association impair the cluster formation of Ire1 (Kimata et al. 2007, Aragón et al. 2009). It is still largely obscure if factors other than Ire1 \textit{per se} promote the cluster formation of Ire1, though we previously reported a contribution of actin filaments (Ishiwata-Kimata et al. 2013a).

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**Figure 4** Structure of the luminal domain of yeast Ire1. (A) Based on our observations described in Kimata et al. (2004), we propose segmentation of the luminal domain of \textit{S. cerevisiae} Ire1 into five subregions (Subregions I to V). Subregions II and IV, but not I, III or V, are essential for Ire1’s activity to splice \textit{HAC1} mRNA. (B) Structure of the luminal domain of \textit{S. cerevisiae} Ire1 is schematically illustrated.

**Figure 5** Cluster formation of yeast Ire1. (A) The Core has an intrinsic ability to form a high-order oligomer. (B) \textit{S. cerevisiae} cells producing fluorescent protein (mNeonGreen)-tagged Ire1 were observed under a fluorescent microscope.
The point mutations of Ire1 described in the previous paragraph also markedly lowered HAC1 mRNA splicing and the UPR in ER-stressed cells (Credle et al. 2005, Kimata et al. 2007). This observation strongly suggests that Ire1 forms clusters to express its potent endoribonuclease activity. According to the X-ray crystal structure analysis, the cytosolic domain of Ire1 exhibits a potent endoribonuclease activity when clustered (Korennykh et al. 2009). Intriguingly, in vivo fluorescent-protein labeling of HAC1 mRNA demonstrated that HAC1 mRNA is actively recruited to the Ire1 clusters (Aragón et al. 2009, van Anken et al. 2014).

**UNFOLDED PROTEIN-DEPENDENT ACTIVATION MACHINERY OF Ire1**

According to Kohno et al. (1993), overexpression of BiP attenuates the UPR in *S. cerevisiae* cells, suggesting a role of BiP in suppressing Ire1’s activity. Bertolotti et al. (2000) and Okamura et al. (2000) then reported that BiP associates with Ire1 in non-stressed mammalian and *S. cerevisiae* cells and that ER stress causes dissociation of the BiP-Ire1 complex. Moreover, when cells carry mutant versions of BiP that cannot dissociate from Ire1, the UPR was evoked only weakly even in the presence of ER-stressing stimuli (Kimata et al. 2003). These observations strongly suggest that ER stress-dependent association/dissociation between BiP and Ire1 controls the UPR. Carrara et al. (2015) reported reconstitution of this reaction in vitro.

Our deletion-scanning analysis of *S. cerevisiae* Ire1 revealed that the BiP-binding site is located on Subregion V of the luminal domain of Ire1 (Kimata et al. 2004). Since an Ire1 mutant carrying a full-length deletion of Subregion V is not constitutively active but is still upregulated upon ER stress (Kimata et al. 2004), another mechanism controlling Ire1’s activity in response to ER stress should exist. According to the X-ray crystal structure analysis, dimer of the Core region of the luminal domain of Ire1 forms a groove, in which unfolded and stretched peptide may be captured (Credle et al. 2005). Indeed, recombinant proteins of the Core exhibit in vitro binding activity towards model unfolded peptides or proteins (Kimata et al. 2007, Gardner and Walter 2011). Moreover, in vivo association between Ire1 and a model unfolded protein accumulated in the ER lumen was also demonstrated (Gardner and Walter 2011, Promlek et al. 2011). Full-length deletion of Subregion III, namely the DIII mutation, of Ire1 impairs physical interaction between Ire1 and unfolded proteins both in vitro and in vivo (Kimata et al. 2007, Promlek et al. 2011). Importantly, ER accumulation of unfolded proteins evokes the UPR only weakly when cells carry the DIII mutation of Ire1 (Promlek et al. 2011). These insights strongly suggest that unfolded proteins are directly captured by the luminal domain of Ire1, leading to activation of Ire1 and evocation of the UPR. According to Gardner and Walter (2011), the physical interaction between unfolded proteins and the Core dimers bundles the latter and promotes cluster formation of Ire1.

Taken together, we thus propose the following scenario. Under non-stress conditions, Ire1 prefers to be non-self-associated, since BiP is associated with Ire1 to inhibit its self-association (Figure 6). In contrast, under ER-stress conditions, unfolded proteins are predominantly associated with BiP, which then cannot associate with Ire1. Moreover, unfolded proteins are directly captured by the Core dimer, which is then bundled for cluster formation and activation of Ire1 (Figure 6).

It should also be noted that, in a manner different from that of Subregion V, Subregion I also contributes to repression of Ire1’s activity (Oikawa et al. 2007). According to our observations presented in Mathuranyanon et al. (2015), Subregion I is intramolecularly captured by the groove of the Core dimer, causing its dissociation, in unstressed cells.

**DEACTIVATION OF Ire1 AFTER PEAK INDUCTION OF THE UPR**

Unregulated UPR, which can be caused, for example, by constitutive expression of HAC1 protein, retards growth of *S. cerevisiae* cells, probably because the UPR drastically alters the transcriptome (Travers et al. 2000, Kimata et al. 2006). This insight explains the reason why Ire1’s activity is tightly regulated by multiple regulatory mechanisms. Moreover, under conditions with modest ER stress, HAC1 mRNA splicing is quickly attenuated after peak induction of the UPR (Pincus et al. 2006).
According to Chawla et al. (2011) and Rubio et al. (2011), HAC1 mRNA inappropriately continued to be highly spliced when cells carried cytosolic-domain point mutants of Ire1 that can be activated as an endoribonuclease without being auto-phosphorylated. Moreover, these Ire1-mutant cells quickly lost their viability during long-term stress imposition. These observations strongly suggest an involvement of dephosphorylation of Ire1 in the post-activation turn-off of the UPR, which contributes to cell viability. This insight may explain the reason why Ire1 has kinase activity in addition to the endoribonuclease activity and executes autophosphorylation, which is not absolutely required for evocation of the UPR.

The association of BiP with Ire1 also contributes to deactivation of Ire1 after peak induction of the UPR. According to Pincus et al. (2010) and Ishiwata-Kimata et al. (2013b), Ire1 mutants that lack Subregion V, which serves as the BiP-binding site, continued to be highly activated even after prolonged ER-stress imposition. Because BiP is transcriptionally induced through the UPR, deactivation of Ire1 by BiP can be considered as a negative-feedback regulatory mechanism. Nevertheless, we also think that, upon continuous ER stress, Ire1 maintains weak activity as a BiP-bound and homo-dimerized form (Ishiwata-Kimata et al. 2013b).

CONDITIONS IN WHICH THE UPR FUNCTIONS IN S. cerevisiae CELLS

For laboratory studies of the UPR, S. cerevisiae cells are potently ER-stressed by exposure to DTT or tunicamycin or by artificial expression of model unfolded proteins. A reasonable question therefore is to under what conditions other than those artefactual laboratorial situations Ire1 works in S. cerevisiae cells.

S. cerevisiae is an industrial microorganism, for example, used for ethanol fermentation. Miyagawa et al. (2014) reported that culturing cells under high-ethanol conditions causes protein denaturation and aggregation, which activate Ire1. Moreover, acetic acid, a byproduct of bioethanol production from lignocellulosic biomass, is reported to be an ER stressor (Kawazoe et al. 2017). The environmental pollutant cadmium also activates Ire1 through impairment of protein folding in S. cerevisiae ER (Le et al. 2016). Under these stress conditions, the UPR contributes considerably to cellular survival.

Inositol starvation is also known as a stress stimulus that activates Ire1 and induces the UPR in S. cerevisiae cells (Cox et al. 1997). Interestingly, the DIII mutant version of Ire1, which cannot sense unfolded proteins accumulated in the ER, was normally activated by some stress stimuli, such as inositol starvation, which are likely to predominantly damage membrane-lipid homeostasis (Promlek et al. 2011). We thus propose that membrane-lipid aberrancy activates Ire1 through a yet-uncovered mechanism, which is different from that for ER-accumulated unfolded proteins. According to Halbleib et al. (2017), the transmembrane domain of Ire1 contributes to sensing of membrane-lipid aberrancy. It should be also noted that the UPR transcriptionally induces a number of genes including those encoding lipid-metabolizing enzymes (Travers et al. 2000). By considering also the role of the ER as a location where membrane-lipid components are biosynthesized, we now think that the UPR does not solely function in cellular protection against unfolding and aggregation of ER client proteins. In other words, the UPR is a cellular system to cope with a wide variety of impairments or shortage of ER function, which is linked to protein unfolding and/or membrane-lipid aberrancy.

Furthermore, the UPR is likely to play a role even under normal culturing conditions, in which cells exhibit faint HAC1-mRNA splicing. Even in such a situation, IRE1-gene knock-out retains cellular growth, albeit slightly. According to Bicknell et al. (2007), the weak UPR under non-stress conditions is not absolutely required, but is helpful for cytokinesis, during which the ER may have to be functionally enhanced and/or massively expanded.

THE UPR IN OTHER FUNGAL SPECIES AND ANIMALS

Although Ire1 is ubiquitously conserved in eukaryotic species, only some (but not all) fungal species analyzed so far bear HAC1 orthologue. Blockage of the UPR machinery in pathogenic fungi, including the plant pathogenic fungus Ustilago maydis, impairs cell-wall integrity, hyphal development and virulence (Wimalasena et al. 2008, Richie et al. 2009, Heimel et al. 2013) probably through damage of the protein-secretory pathway. This observation demonstrates a role of the UPR in natural life.

Similarly to fungal HAC1 mRNA, the transcript of the metazoan XBP1 gene is spliced by Ire1 in ER-stressed cells and then translated into bZIP-family transcription-factor protein (Figure 7, the XBP1s protein (s for spliced), Yoshida et al. 2001; Calfon et al. 2002). Unlike the case for S. cerevisiae HAC1 mRNA, the unspliced form of XBP1 mRNA (XBP1u; u for unspliced) is effectively translated into a protein, which acts as an inhibitor of XBP1s protein (Yoshida et al. 2006). Intriguingly, the nascent XBP1u peptide is transported to the ER surface as a ribosome-nascent chain-mRNA complex (Yanagitani et al. 2009, Yanagitani et al. 2011, Kanda et al. 2016). According to Yanagitani et al. (2009), this phenomenon contributes to recruitment of the XBP1u mRNA to the ER surface and thus increases the efficiency of its splicing by Ire1.

The regulated Ire1-dependent decay of mRNA (RIDD), which was initially found in a study of Drosophila melanogaster (Hollien and Weissman 2006), is a cellular event in which Ire1 does not work in UPR transcriptional induction. In various eukaryotic organisms excluding S. cerevisiae, Ire1 cleaves mRNAs encoding ER client proteins in ER-stressed cells (Figure 7). The benefit of this phenomenon is believed to be reduction of client protein load in the ER. Since fusion yeast Schizosaccharomyces pombe does not carry bZIP-family transcription-factor mRNA that serves as a target of Ire1-dependent splicing (Kimmig et al. 2012), cleavage of mRNAs not being accompanied with their splicing seems to be the sole job of Ire1 in S. pombe. According to Kimmig et al. (2012), ER stress-dependent induction of BiP in S. pombe is caused by stabilization of BiP mRNA, which is caused by Ire1-dependent
removal of the 3'-untranslated region (UTR) that carries a destabilization signal.

Probably in order to reduce client protein load into the ER, metazoan cells carry PERK, which is an ER-located type-I transmembrane kinase (Figure 7, Harding et al. 1999). The structure of luminal domain of PERK exhibits significant similarity to that of Ire1, and thus PERK likely functions in sensing ER stress through a similar mechanism to that of Ire1. Upon ER stress, PERK phosphorylates and inactivates eukaryotic translation initiation factor 2a, leading to an attenuation of global protein synthesis. It should also be noted that this translational blockage causes translational induction of a subset of mRNAs, including that encoding ATF4, which carry unique 5'UTR structures (Harding et al. 2000).

In vertebrate cells, a considerable part of UPR transcriptional induction is governed by ATF6 (Yoshida et al. 1998), which is believed to sense ER stress independently of Ire1 or PERK. As shown in Figure 7, ATF6 is expressed as a type-II transmembrane protein located in the ER, while ER stress induces its transport to the Golgi apparatus, where the Site-1 and Site-2 proteases cleave it (Haze et al. 1999, Ye et al. 2000). Subsequently, the cleaved fragment of ATF6 is transported to the nuclei and works as a transcription factor. Since XBP1s, ATF4 and cleaved ATF6 proteins work differently in transcriptional induction in the nuclei (Maly and Papa 2014), their coordination is likely to be a key event for cellular adaptation to ER stress.

It is likely that cells that secrete high levels of protein are intrinsically ER stressed probably because of their excessive ER protein load, which leads to requirement of cellular systems that guard cells against ER stress even in the absence of an external ER-stressing stimulus. Knockout of IRE1a gene, which encodes the major parologue of mammalian Ire1, causes embryonic lethality in mouse, possibly because this knockout impairs growth-factor secretion from the placenta (Iwawaki et al. 2009). Moreover, according to Harding et al. (2001), PERK-gene knockout mouse exhibits dysfunction of the pancreas and suffers from diabetes. It is also widely accepted that, in metazoan species, chronically and highly ER-stressed cells are eliminated by apoptosis (Maly and Papa 2014). Since the UPR and related cellular events are tightly linked to various aspects of mammalian pathology, chemicals controlling the UPR are intriguing targets for clinical drug discovery (Maly and Papa 2014).

THE UPR IN PLANTS

Koizumi et al. (2001) exhibited the transcriptional induction of genes encoding ER-located chaperones in ER-stressed Arabidopsis thaliana seedlings, indicating a UPR in plant cells. As described in Koizumi et al. (2001), Okushima et al. (2002) and Ruberti et al. (2015), plants carry one or more Ire1 isoforms. Like yeast HAC1 and metazoan XBP1 mRNAs, bZIP-family transcription-factor mRNA, which in the case of A. thaliana encodes bZIP60, is spliced by IRE1 (Deng et al. 2011, Nagashima et al. 2011, Ruberti et al. 2015).

Intriguingly, in order to yield an active transcription-factor protein only after being spliced by IRE1, bZIP60 mRNA employs a strategy different from those of HAC1 and XBP1 mRNAs. According to Iwata et al. (2008), the unspliced form of
bZIP60 mRNA is translated into a transmembrane protein, which cannot be transported into the nucleus. IRE1-gene knockout studies showed its involvement in normal vegetative growth and immunity in plants, indicating roles for the ER stress-responsive cellular machinery in natural life (Chen and Brandizzi 2012, Moreno et al. 2012, Deng et al. 2013). Since the IRE1-gene knockout phenotypes are more severe than those of bZIP60-gene knockout in *A. thaliana* (Deng et al. 2013, Mishiba et al. 2013), there seems likely to be a role(s) of *A. thaliana* IRE1 other than in bZIP60-mRNA splicing. Mishiba et al. (2013) exhibited the RIDD in *A. thaliana* cells.

Some other bZIP-family transcription factors are also upregulated upon ER stress and promote UPR transcriptional induction together with the bZIP60-like transcription factors (Ruberti et al. 2015). For example, according to Liu et al. (2007) and Srivastava et al. (2012), *A. thaliana* bZIP28, similarly to ATF6 of vertebrate cells, is produced as an ER-located transmembrane protein, which, upon ER stress, is transported to the Golgi apparatus and cleaved to become an active transcription-factor fragment resulting in UPR evocation.

CONCLUSION AND PERSPECTIVE

As described above, transcriptional induction of ER chaperone genes upon ER accumulation of unfolded proteins was initially reported in a study using mammalian cultured cells (Kozutsumi et al. 1988), and today is called as the UPR. Although the following studies over 20 years seem to have mostly uncovered molecular mechanism for progression of *S. cerevisiae* UPR, greater number of issues remain obscure for metazoan and plant UPR, probably and partly because the mechanisms of the UPR in those organisms are more complicated than those in yeast and fungal cells. For instance, while mammalian IRE1a is likely to change its main roles from splicing of XBP1 mRNA (cytoprotective) to the RIDD (often proapoptotic) depending on stressing conditions (Han et al. 2009, Upton et al. 2012), the molecular basis controlling such substrate specificity of IRE1a has not yet been disclosed. Moreover, physiological and pathological roles of the UPR in metazoan and plant bodies appear to be intriguing research topics which should be addressed also in future.

REFERENCES

Aragon, T., van Anken, E., Pincus, D., Serafimova, I. M., Korenykh, A. V., Rubio, C. A., and Walter, P. (2009) Messenger RNA targeting to endoplasmic reticulum stress signalling sites. *Nature* 457: 736-740.

Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P., and Ron, D. (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat Cell Biol* 2: 326-332.

Bicknell, A. A., Babour, A., Federovitch, C. M., and Niwa, M. (2007) A novel role in cytokinesis reveals a housekeeping function for the unfolded protein response. *J Cell Biol* 177: 1017-1027.

Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G., and Ron, D. (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415: 92-96.

Carrara, M., Prischi, F., Nowak, P. R., Kopp, M. C., and Ali, M. M. (2015) Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling. *eLife* 4: e03522.

Casagrande, R., Stern, P., Diehn, M., Shamu, C., Osario, M., Zuniga, M., Brown, P. O., and Ploegh, H. (2000) Degradation of proteins from the ER of *S. cerevisiae* requires an intact unfolded protein response pathway. *Mol Cell* 5: 729-735.

Chawla, A., Chakrabarti, S., Ghosh, G., and Niwa, M. (2011) Attenuation of yeast UPR is essential for survival and is mediated by IRE1 kinase. *J Cell Biol* 193: 41-50.

Chen, Y. and Brandizzi, F. (2012) AtIRE1A/AtIRE1B and AGB1 independently control two essential unfolded protein response pathways in *Arabidopsis*. *Plant J* 69: 266-277.

Cox, J. S., Chapman, R. E., and Walter, P. (1997) The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. *Mol Biol Cell* 8: 1805-1814.

Cox, J. S., Shamu, C. E., and Walter, P. (1993) Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 73: 1197-1206.

Cox, J. S. and Walter, P. (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* 87: 391-404.

Credle, J. J., Finer-Moore, J. S., Papa, F. R., Stroud, R. M., and Walter, P. (2005) On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc Natl Acad Sci USA* 102: 18773-18784.

Deng, Y., Humbert, S., Liu, J. X., Srivastava, R., Rothstein, S. J., and Howell, S. H. (2011) Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in *Arabidopsis*. *Proc Natl Acad Sci USA* 108: 7247-7452.

Deng, Y., Srivastava, R., and Howell, S. H. (2013) Protein kinase and ribonuclease domains of IRE1 confer stress tolerance, vegetative growth, and reproductive development in *Arabidopsis*. *Proc Natl Acad Sci USA* 110: 19633-19638.

Di Santo, R., Aboulhouda, S., and Weinberg, D. E. (2016) The fail-safe mechanism of post-transcriptional silencing of unspliced *HAC1* mRNA. *eLife* 5: e20069.

Fordyce, P. M., Pincus, D., Kimmig, P., Nelson, C. S., El-Samad, H., Walter, P., and DeRisi, J. L. (2012) Basic leucine zipper transcription factor Hac1 binds DNA in two distinct modes as revealed by microfluidic analyses. *Proc Natl Acad Sci USA* 109: E3084-E3093.

Friedlander, R., Jarosch, E., Urban, J., Volkwein, C., and Ali, M. M. (2012) A novel role in cytokinesis reveals a housekeeping function for the unfolded protein response. *J Cell Biol* 193: 41-50.

Gardner, B. M. and Walter, P. (2011) Unfolded proteins are
Ire1-activating ligands that directly induce the unfolded protein response. *Science* 333: 1891-1894.

Gonzalez, T. N., Sidrauski, C., Dorfler, S., and Walter, P. (1999) Mechanism of non-spliceosomal mRNA splicing in the unfolded protein response pathway. *EMBO J* 18: 3119-3132.

Halbleib, K., Pesek, K., Covino, R., Hofbauer, H. F., Wunnnicke, D., Hanelt, I., Hummer, G., and Ernst, R. (2017) Activation of the Unfolded Protein Response by Lipid Bilayer Stress. *Mol Cell* 67: 673-684 e678.

Han, D., Lerner, A. G., Vande Walle, L., Upton, J. P., Xu, W., Hagen, A., Backes, B. J., Oakes, S. A., and Papa, F. R. (2009) IRE1 alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* 138: 562-575.

Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., and Ron, D. (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 6: 1099-1108.

Harding, H. P., Zeng, H., Zhang, Y., Jungries, R., Chung, P., Plesken, H., Sabatini, D. D., and Ron, D. (2001) Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. *Mol Cell* 7: 1153-1163.

Harding, H. P., Zhang, Y., and Ron, D. (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397: 271-274.

Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999) Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 10: 3787-3799.

Heimel, K., Freitag, J., Hampel, M., Ast, J., Bolker, M., and Kamper, J. (2013) Crosstalk between the unfolded protein response and pathways that regulate pathogenic development in *Ustilago maydis*. *Plant Cell* 25: 4262-4277.

Hiller, M. M., Finger, A., Schweiger, M., and Wolf, D. H. (1996) ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. *Science* 273: 1725-1728.

Hollien, J. and Weissman, J. S. (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* 313: 104-107.

Ishiwata-Kimata, Y., Yamamoto, Y. H., Takizawa, K., Kohno, K., and Kimata, Y. (2013a) F-actin and a type-II myosin are required for efficient clustering of the ER stress sensor Ire1. *Cell Struct Funct* 38: 135-143.

Ishiwata-Kimata, Y., Promlek, T., Kohno, K., and Kimata, Y. (2013b) BiP-bound and nonclustered mode of Ire1 evokes a weak but sustained unfolded protein response. *Genes Cells* 18: 288-301.

Iwata, Y., Fedoroff, N. V., and Koizumi, N. (2008) *Arabidopsis* bZTP60 is a proteolysis-activated transcription factor involved in the endoplasmic reticulum stress response. *Plant Cell* 20: 3107-3121.

Iwawaki, T., Akai, R., Yamanaka, S., and Kohno, K. (2009) Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. *Proc Natl Acad Sci USA* 106: 16657-16662.

Kanda, S., Yanagitani, K., Yokota, Y., Esaki, Y., and Kohno, K. (2016) Autonomous translational pausing is required for XBP1u mRNA recruitment to the ER via the SRP pathway. *Proc Natl Acad Sci USA* 113: E5886-E5895.

Kawahara, T., Yanagi, H., Yura, T., and Mori, K. (1998) Unconventional splicing of HAC1/ERN4 mRNA required for the unfolded protein response. Sequence-specific and non-sequential cleavage of the splice sites. *J Biol Chem* 273: 1802-1807.

Kawai, N., Kimata, Y., and Izawa, S. (2017) Acetic acid causes endoplasmic reticulum stress and induces the unfolded protein response in *Saccharomyces cerevisiae*. *Front Microbiol* 8: 1192.

Kimata, Y., Ishiwata-Kimata, Y., Ito, T., Hirata, A., Suzuki, T., Oikawa, D., Takeuchi, M., and Kohno, K. (2007) Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. *J Cell Biol* 179: 75-86.

Kimata, Y., Ishiwata-Kimata, Y., Yamada, S., and Kohno, K. (2006) Yeast unfolded protein response pathway regulates expression of genes for anti-oxidative stress and for cell surface proteins. *Genes Cells* 11: 59-69.

Kimata, Y., Kimata, Y. I., Shimizu, Y., Abe, H., Faricasanu, I. C., Takeuchi, M., Rose, M. D., and Kohno, K. (2003) Genetic evidence for a role of BiP/Kar2 that regulates Ire1 in response to accumulation of unfolded proteins. *Mol Biol Cell* 14: 2559-2569.

Kimata, Y., Oikawa, D., Shimizu, Y., Ishiwata-Kimata, Y., and Kohno, K. (2004) A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. *J Cell Biol* 167: 445-456.

Kimmig, P., Diaz, M., Zheng, J., Williams, C. C., Lang, A., Aragon, T., Li, H., and Walter, P. (2012) The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *eLife* 1: e00048.

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

Koizumi, N., Martinez, I. M., Kimata, Y., Kohno, K., Sano, H., and Chrispeels, M. J. (2001) Molecular characterization of two *Arabidopsis* Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases. *Plant Physiol* 127: 949-962.

Korenykh, A. V., Egea, P. F., Korostelev, A. A., Finer-Moore, J., Zhang, C., Shokat, K. M., Stroud, R. M., and Walter, P. (2009) The unfolded protein response signals through high-order assembly of Ire1. *Nature* 457: 687-693.

Kozutsumi, Y., Segal, M., Normington, K., Gething, M. J., and Sambrook, J. (1988) The presence of malformed proteins in the endoplasmic reticulum signals the induction of glucose-
regulated proteins. *Nature* 332: 462-464.

Le, Q. G., Ishiwata-Kimata, Y., Kohno, K., and Kimata, Y. (2016) Cadmium impairs protein folding in the endoplasmic reticulum and induces the unfolded protein response. *FEMS Yeast Res* 16: fow049.

Lee, K. P., Dey, M., Neculai, D., Cao, C., Dever, T. E., and Sicheri, F. (2008) Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. *Cell* 132: 89-100.

Liu, J. X., Srivastava, R., Che, P., and Howell, S. H. (2007) An endoplasmic reticulum stress response in *Arabidopsis* is mediated by proteolytic processing and nuclear relocation of a membrane-associated transcription factor, bZIP28. *Plant Cell* 19: 4111-4119.

Maly, D. J. and Papa, F. R. (2014) Druggable sensors of the unfolded protein response. *Nat Chem Biol* 10: 892-901.

Mathuranyanon, R., Tsukamoto, T., Takeuchi, A., Ishiwata-Kimata, Y., Tatschuya, Y., Kohno, K., and Kimata, Y. (2015) Tight regulation of the unfolded protein sensor Ire1 by its intramolecularly antagonizing subdomain. *J Cell Sci* 128: 1762-1772.

Mishiba, K., Nagashima, Y., Suzuki, E., Hayashi, N., Ogata, Y., Shimada, Y., and Koizumi, N. (2013) 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* 110: 5713-5718.

Miyagawa, K., Ishiwata-Kimata, Y., Kohno, K., and Kimata, Y. (2014) Ethanol stress impairs protein folding in the endoplasmic reticulum and activates Ire1 in *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem* 78: 1389-1391.

Moreno, A. A., Mukhtar M. S., Blanco F., Boatwright J. L., Kariyone T., Kimata, Y., Iwata, M., Sant, A., Kohno, K., Normington, K., Gething, M. J., and Oikawa, D., Kimata, Y., Takeuchi, M., and Kohno, K. (2005) An essential dimer-forming subregion of the endoplasmic reticulum stress sensor Ire1. *Biochem J* 391: 135-142.

Okamura, K., Kimata, Y., Higashio, H., Tsuru, A., and Kohno, K. (2000) Dissociation of Kar2/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. *Biochem Biophys Res Commun* 279: 445-450.

Okushima, Y., Koizumi, N., Yamaguchi, Y., Kimata, Y., Kohno, K., and Sano, H. (2002) Isolation and characterization of a putative transducer of endoplasmic reticulum stress in *Oryza sativa*. *Plant Cell Physiol* 43: 532-539.

Papa, F. R., Zhang, C., Shokat, K., and Walter, P. (2003) Bypassing a kinase activity with an ATP-competitive drug. *Science* 302: 1533-1537.

Pincus, D., Chevalier, M. W., Aragon, T., van Anken, E., Vidal, S. E., El-Samad, H., and Walter, P. (2010) BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. *PLoS Biol* 8: e1000415.

Promlek, T., Ishiwata-Kimata, Y., Shido, M., Sakuramoto, M., Kohno, K., and Kimata, Y. (2011) Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. *Mol Biol Cell* 22: 3520-3532.

Richie, D. L., Hartl, L., Aimanianwa, V., Winters, M. S., Fuller, K. M., Miley, M. D., White, S., McCarthy, J. W., Latgé, J.-P., and Feldmesser, M. (2009) A role for the unfolded protein response (UPR) in virulence and antifungal susceptibility in *Aspergillus fumigatus*. *PLoS Pathog* 5: e1000258.

Rose, M. D., Misra, L. M., and Vogel, J. P. (1989) KAR2, a karyogamy gene, is the yeast homolog of the mammalian BiP/GRP78 gene. *Cell* 57: 1211-1221.

Ruberti, C., Kim, S. J., Stefano, G., and Brandizzi, F. (2015) Unfolded protein response in plants: one master, many questions. *Curr Opin Plant Biol* 27: 59-66.

Rubio, C., Pincus, D., Korennykh, A., Schuck, S., El-Samad, H., and Walter, P. (2011) Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. *J Cell Biol* 193: 171-184.

Ruegsegger, U., Leber, J. H., and Walter, P. (2001) Block of *HAC1* mRNA translation by long-range base pairing is
released by cytoplasmic splicing upon induction of the unfolded protein response. *Cell* 107: 103-114.

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

Sidrauski, C., Cox, J. S., and Walter, P. (1996) tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. *Cell* 87: 405-413.

Sidrauski, C. and Walter, P. (1997) The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* 90: 1031-1039.

Srivastava, R., Chen, Y., Deng, Y., Brandizzi, F., and Howell, S. H. (2012) Elements proximal to and within the transmembrane domain mediate the organelle-to-organelle movement of bZIP28 under ER stress conditions. *Plant J* 70: 1033-1042.

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

Upton, J. P., Wang, L., Han, D., Wang, E. S., Huskey, N. E., Lim, L., Truitt, M., McManus, M. T., Ruggero, D., Goga, A., Papa, F. R., and Oakes, S. A. (2012) IRE1a cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. *Science* 338: 818-822.

van Anken, E., Pincus, D., Coyle, S., Aragon, T., Osman, C., Lari, F., Gomez Puerta, S., Korenykh, A. V., and Walter, P. (2014) Specificity in endoplasmic reticulum-stress signaling in yeast entails a step-wise engagement of HAC1 mRNA to clusters of the stress sensor Ire1. *eLife* 3: e05031.

Wimalasena, T. T., Enjalbert, B., Guillemette, T., Plumridge, A., Budge, S., Yin, Z., Brown, A. J., and Archer, D. B. (2008) Impact of the unfolded protein response upon genome-wide expression patterns, and the role of Hac1 in the polarized growth, of Candida albicans. *Fungal Genet Biol* 45: 1235-47.

Yanagitani, K., Imagawa, Y., Iwawaki, T., Hosoda, A., Saito, M., Kimata, Y., and Kohno, K. (2009) Cotranslational targeting of XBPI protein to the membrane promotes cytoplasmic splicing of its own mRNA. *Mol Cell* 34: 191-200.

Yanagitani, K., Kimata, Y., Kadokura, H., and Kohno, K. (2011) Translational pausing ensures membrane targeting and cytoplasmic splicing of XBPIu mRNA. *Science* 331: 586-589.

Ye, J., Rawson, R. B., Komuro, R., Chen, X., Dave, U. P., Prywes, R., Brown, M. S., and Goldstein, J. L. (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 6: 1355-1364.

Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998) Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. *J Biol Chem* 273: 33741-33749.

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

Yoshida, H., Oku, M., Suzuki, M., and Mori, K. (2006) pXBPI(U) encoded in XBPI pre-mRNA negatively regulates unfolded protein response activator pXBPI(S) in mammalian ER stress response. *J Cell Biol* 172: 565-575.

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