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Functional diversification of ER stress responses in *Arabidopsis*

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Abstract

The endoplasmic reticulum (ER) is responsible for the synthesis of one third of the cellular proteome and is constantly challenged by physiological and environmental situations that can perturb its homeostasis and lead to the accumulation of misfolded secretory proteins, a condition referred to as ER stress. In response, the ER evokes a set of intracellular signaling processes, collectively known as the unfolded protein response (UPR), which are designed to restore biosynthetic capacity of the ER. As single cell organisms evolved into multicellular life, the UPR complexity has increased to suit their growth and development. In this review, we discuss recent advances in the understanding in the UPR, emphasizing conserved UPR elements between plants and metazoans, and highlighting unique plant-specific features.

Keywords

ER stress; unfolded protein response (UPR); *Arabidopsis thaliana*

The ER: Formidable Yet Sensitive Biosynthetic Machinery

The endoplasmic reticulum (ER) is an essential organelle for phospholipid synthesis and the Ca²⁺ storage. In addition, the ER is responsible for the synthesis and folding of one third of the cellular proteome [1]. To reach their functional state, proteins are subjected to post-translational processing, including glycosylation, disulfide-bond formation, and phosphorylation, among other modifications, before leaving the ER. The ER environment necessary for these processes is sensitive to extracellular and intracellular cues, which can negatively impact the balance between the ER protein folding and synthesis demand, and ignite a potentially cytotoxic condition known as ER stress (see Glossary) [2]. Molecular sensors associated with the ER membrane detect ER stress and are activated to restore the net protein folding capacity via an intracellular signaling network known collectively as the unfolded protein response (UPR) [2–4]. Primarily, the UPR mitigates the development of ER stress through increased ER chaperone production to aid protein folding, upregulated lipid synthesis to expand ER capacity, and repression of translation to attenuate ER protein loading. When ER stress is not resolved, UPR sensors activate irreversible pro-death

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processes in order to preserve overall organism health. These basic ER functions are broadly conserved across eukaryotes [5]. However, adaptation to specific ecological niches and utilization of secretory processes by the various taxonomic groups has led to a marked evolutionary diversification of UPR sensors and UPR signaling with overlapping and unique strategies to respond to ER stress.

We review recent advances in our understanding of the plant UPR, focusing primarily in the model plant *Arabidopsis thaliana* (Box 1), and we discuss the expanding relevance of the plant UPR in the response to environmental stressors and in the context of developmental processes. We highlight the conserved elements of UPR signaling strategies between plants and metazoans, as well as the plant-specific features that underlie a marked complexity of stress signaling in multicellular organisms.

ER Stress Sensors

In metazoans, three branches have been defined by ER membrane-associated ER-stress sensors, which initiates stress signaling pathway: the protein kinase and ribonuclease, IRE1, the transcription factor, ATF6, and protein kinase RNA-like ER kinase, PERK [2]. In plants, only IRE1 and ATF6 homologs have been identified [3] (Figure 1). IRE1 is the most conserved UPR branch with homologs in yeast, plants, and metazoans. *A. thaliana* encodes two IRE1 paralogs, that closely resemble the yeast and mammalian IRE1, IRE1A and IRE1B [6]. In mammals, there are two paralogs, IRE1 α and IRE1 β [7, 8], IRE1 α being the predominant isoform [2]. In *Arabidopsis*, IRE1B is the most widely expressed form and IRE1A is more abundant in seeds and embryos [6, 9]. Even though *Arabidopsis* paralogs have substantially overlapping functions, the evidence that unlike IRE1A, IRE1B is required for the ER-stress induced autophagy [10] supports some unique roles of these proteins. Similarly to other Brassicaceae, *A. thaliana* encodes a third structurally divergent IRE1, IRE1C [11], whose involvement in ER stress responses is yet undefined. [11] (Figure 1A, pathway 4). The *Arabidopsis* functional homologs of mammalian ATF6 (ATF6 α and ATF6 β) are the basic Leucine Zipper Protein (bZIP) 28 and 17 [12, 13]. In their respective protein families, analogously to ATF6 α , which is the main contributor to the upregulation of UPR genes [14, 15], bZIP28 is the major contributor [16]. The role of bZIP17 in the UPR is not yet clear (see below).

Inositol Requiring Enzyme 1 (IRE1)

IRE1, a type I transmembrane protein, contains an ER luminal protein-protein interaction domain and a cytosolic tail with an serine/threonine kinase and an endoribonuclease (RNase) domains (Figure 1A) [17, 18]. Models established in non-plant species envision that, in response to ER stress, IRE1 associates into homooligomers, leading first to trans-autophosphorylation of the kinase domain and a subsequent conformational change that activates the RNase domain [19, 20]. *Arabidopsis* IRE1A and B can homodimerize and autophosphorylate [6, 9, 21–23]. Although these processes have not been studied in as much detail as in other eukaryotes, it is well established that the activated RNase domain catalyzes the unconventional cytosolic splicing of a specific bZIP transcription factor (TF) mRNA at a consensus structural motif conserved across eukaryotes [24] (Figure 1A, pathway 1).

Together with a specific tRNA ligase [25–28], the splicing of the mRNA of *HAC1* in yeast [29, 30], *XBPI* in metazoans [31, 32], and *bZIP60* in *A. thaliana* [33–35] leads to the production of an active TF. More specifically, the bZIP60 mRNA splicing leads a frameshift that eliminates an ER anchor, thus allowing for nuclear translocation and transcriptional modulation of UPR target genes [33] (discussed below).

IRE1 RNase activity is also required for a conserved process known as regulated IRE1-dependent decay (RIDD), which degrades mRNAs by site-specific endoribonucleolytic cleavage [36–38] (Figure 1A, pathway 2). Generally, RIDD contributes to pro-life processes by reducing ER client mRNA abundance in ER stress conditions [2, 39]. However, RIDD activity against targeted non-ER client mRNAs has expanded the downstream effects of IRE1 activation in a species-specific manner. In metazoans, under prolonged or severe ER stress, RIDD promotes apoptosis by degrading pre-micro RNAs (miRNAs) and mRNAs of pro-survival proteins, such as miR-17, miR-34a, miR-96, and miR125b which normally repress the translation of caspase-2 (CASP2) [39, 40]. In Arabidopsis, IRE1 RIDD activity is required for the upregulation of autophagy in response to ER stress through degradation the mRNA of proteins involved in autophagy such as β -glucosidase 21 as well as pathogenesis-related protein 14 [23]. Whether RIDD is necessary for plant survival is yet unknown.

IRE1 also executes RNase-independent functions. For example, mammalian IRE1 α functions as a platform for the binding assembly of other proteins, a concept referred as UPRosome [41]. This scaffold function enables a connection between the UPR and other signaling pathways. The most representative example is the TNFR-associated factor 2 (TRAF2) binding, which triggers the activation of JNK pathway and autophagy [42]. In plants, no IRE1 interactors have been identified yet, leaving open the question on whether plant IRE1 may have acquired RNase-independent functions *in vivo*. The evidence that recombinant IRE1 phosphorylates model substrates *in vitro* also raises the possibility of kinase-dependent function(s) of IRE1 [43, 44] (Figure 1A, pathway 3).

Activating Transcription Factor 6 (ATF6) and Basic Leucine Zipper Proteins 17 and 28 (bZIP17 and bZIP28)

ATF6 α and ATF6 β (herein referred to as ATF6), bZIP17, and bZIP28 are type II transmembrane proteins with a cytosolic N-terminal portion that contains the bZIP TF domain and an ER luminal C-terminus with amino acid signals for ER retention [12, 13, 45]. In ER stress conditions, ATF6/bZIP17/bZIP28 translocate to the Golgi where they are activated via regulated intermembrane proteolysis (RIP) mediated by proteases (Figure 1B, pathways 1 and 2), which release the active TF domain into the cytosol for nuclear translocation [12, 46]. Although the main characteristics of trafficking and activation are conserved across these TFs, there are a number of important distinctions between mammals and plants. For example, the ER-to-Golgi transport of mammalian ATF6 and plant bZIP28 is mediated by the coat protein complex II (COPII) [47–49]. In physiological growth, the COPII assembly process is initiated by the activation of the GTPase SAR1 by the ER membrane protein, SEC12. In ATF6, two Golgi localization signals (GLSs) have been identified in the ER luminal domain as being sufficient for translocation [48, 50]. This supports a model whereby a yet-unknown cargo receptor is necessary to link the luminal

ATF6 GLSs and the cytoplasmic COPII machinery for transport to the Golgi under ER stress conditions [48]. Conversely, bZIP28 interacts directly with SAR1 and SEC12 and this interaction is enhanced in ER stress conditions [49]. bZIP28 translocation to the Golgi requires two dibasic motifs proximal to the bZIP28 transmembrane domain [49]. How bZIP28 transduces the ER stress signal from its luminal domain to the cytosolic domain where the dibasic motifs are localized is still unknown. Once in the Golgi membrane, it has been widely assumed that bZIP28 is sequentially cleaved by S1P and S2P proteases similar to ATF6 and bZIP17 since all of them contain a canonical S1P recognition motif in the C-terminal domain [12, 51]. However, the findings that in ER stress bZIP28 exhibits similar processing in an *s1p* mutant and wild-type [52] indicate that bZIP28 is first cleaved by yet-unknown proteases that may act redundantly with S1P. The bZIP17 translocation from the ER to the Golgi has not been yet explored. The conservation of the dibasic motifs in its sequence makes it likely that its activation is similar to bZIP28.

Despite the possibility that bZIP17 and bZIP28 may be activated by similar proteolytic machineries, they appear to participate in different stress responses [13, 53]. A recent whole-transcriptome analysis of various double mutants combining *bzip17*, *bzip28*, and *bzip60* backgrounds supports that bZIP28 and bZIP60 are the major contributors to the transcriptional regulation of the UPR canonical genes [16]. Unlike bZIP28, under salt stress, bZIP17 upregulates only the most ER stress responsive of the three members of the ER-resident chaperone family Binding Protein (BiP) in *Arabidopsis*, *BIP3* [54]. The mechanisms underlying the specificity of the stress responses mediated by these bZIP-TFs are unknown. The fact that they control only partially overlapping genes supports that the specificity of their responses is likely mediated by the control of distinct gene subsets.

How is ER Stress Detected?

Despite an advanced understanding of the downstream UPR signalling, at least at a transcriptional level, the molecular mechanisms by which the UPR regulators sense misfolded proteins in the ER lumen are not completely understood, especially in plants. In mammals, BiP plays a fundamental role in the activation of ATF6 and IRE1 by binding their luminal domain in non-stress conditions and releasing them in stress conditions [41]. In particular, BiP binds to the luminal domain of ATF6, masking the GLSs and leading to ATF6 retention in the ER [50]. In presence of unfolded proteins, BiP dissociates from ATF6 [50]. The BiP-ATF6 complex is stable, even in the presence of ATP, which is necessary for BiP function [55]. Dissociation of the ATF6-BiP complex requires a reducing agent and ATP working synergistically [48]. In *Arabidopsis*, BiP binds to the luminal intrinsically disorder regions in the bZIP28 C-terminus and is released in ER stress [56]. Because alteration of BiP expression affect bZIP28 activation, it has been proposed that BiP binding to bZIP28 may be controlled by competition of misfolded proteins for BiP and consequent dissociation of bZIP28 from this chaperone [56] (Figure 1B, pathway 1). Experimental validation of this model may help define whether the activation of bZIP28 is similar to ATF6. The interaction between bZIP17 and BiP has not been investigated yet (Figure 1B, pathway 2).

In yeast, BiP binds IRE1 in non-stress conditions; however, in stress conditions, dissociation of BiP and direct interaction with unfolded proteins lead to IRE1 clustering and activation

[57, 58] (Figure 1A). In mammals, BiP binds IRE1 α , keeping it in a monomeric inactive state in non-stress conditions [59]. Upon ER stress, BiP is released from IRE1 α allowing homodimerization. Unfolded proteins may also directly bind to IRE1 α and induce allosteric changes triggering its oligomerization [60]. However, the interaction between IRE1 and BiP has not been directly established in plants yet.

Activation of IRE1 and ATF6 may also depend on activity of ER chaperones and isomerases, suggesting multiple layers of regulation [41]. For example, the molecular chaperone Hsp47 directly interacts with IRE1 α , displacing BiP and facilitating IRE1 α oligomerization [61]. Additionally, the ER resident oxidoreductase ERp18 associates with ATF6 α to ensure optimal trafficking and activation following release from BiP [62]. To date, a unique interaction between bZIP28 and the putative GTPase-activating protein Bcl-2-associated athanogene (BAG7) has been established in a yeast-2-hybrid analysis [63]; BAG7 is a member of a conserved ER chaperone family, which is required for ER stress survival [64]. The relevance of the bZIP28-BAG7 interaction in ER stress is yet to be determined; nonetheless, its occurrence suggests that plants may have evolved unique mechanisms to suit the activation of the ER stress sensors or mechanisms that are yet to be discovered in other species to suit the activation of the ER stress sensors.

An alternative UPR activation mechanism independent of unfolded protein accumulation has been described for IRE1, PERK, and ATF6 [65–67]. In this model, the UPR regulators detect ER membrane disequilibrium independently from the ER luminal domain. It has been recently reported that, similar to yeast and metazoan IRE1, an *Arabidopsis* IRE1B lacking the luminal sensor domain is activated in conditions known to increase fatty acid saturation levels in plant membranes, but not in tunicamycin (Tm) or dithiothreitol (DTT) treatment (chemical inducers of unfolded protein accumulation) [11]. While the physiological significance of this mechanism is still unknown, testing a functional correlation with stress and physiological situations that alter the lipid composition in the plant ER will be interesting to test for mechanisms underlying UPR activation independently from misfolded proteins and ER chaperones.

Integration of Transcriptional Activities of the UPR

Upon activation and nuclear translocation, bZIP28 and bZIP60 regulate the expression of target genes by binding directly to conserved ER stress *cis*-elements within their promoter, which include the ER stress response element-I (ERSE-I, 5'-CCAAT-N₁₀-CACG-3') and the unfolded protein response element-1 (UPRE-I, 5'-TGACGTGR-3') [68] (Figure 2). This interaction is assisted by the CCAAT-box-binding complex (conserved between metazoans and plants) which is composed of three nuclear Y factor (NF-Y) subunits: NF-YA, NF-YB, and NF-YC (Figure 2, pathway 1). Among the 36 NF-Y factors in *Arabidopsis*, NF-YA4, NF-YB3, and NF-YC2 interact with the nuclear-localized bZIP28 at the ERSE-I *cis*-element on promoters of UPR target genes [68]. The interaction of bZIP60 with NF-Ys has not been experimentally established yet. The evidence that bZIP28 and bZIP60 form homo- and heterodimers [68] raises the question of whether the binding of bZIP28 to the target promoters may be modulated by bZIP60. In order to increase the expression of canonical UPR targets, bZIP60 and bZIP28 interact with the core components of the COMPASS-like

complex Ash2 and WDR5 (in mammals, BRE2 and SWD3, respectively), to direct H3K4me3 deposition onto the promoters of UPR genes and facilitate the formation of the transcription preinitiation complex, which is critical to gene-specific transcription [69] (Figure 2, pathway 2).

This process may also be regulated by a variety of other nuclear factors, allowing the integration of the UPR with multiple physiological signals. For example, Arabidopsis plants grown under high light conditions were found to be more sensitive to ER stress [70] (Figure 2, pathway 3). Interrogation of the causative mechanism demonstrated that Elongated Hypocotyl 5 (HY5), another bZIP TF that acts downstream of photoreceptors to mediate light dependent growth programs [71], competes with bZIP28 for the binding to the G-box element (CACGTG) within ERSE-I on promoters of UPR target genes. Consistent with the negative role of HY5 in the UPR, a HY5 loss-of-function mutant shows higher expression of UPR marker genes and enhanced tolerance to ER stress [70]. These results provide a physiological context of the UPR in light responses.

Other examples of interactors of the UPR TFs involved in other signaling pathways are found in the response to salicylic acid (SA), a plant chemical hormone induced by pathogen attack, which was also demonstrated to activate the UPR [72]. The nuclear localized constitutive expresser of pathogenesis-related genes-5 (CPR5) protein is a known regulator of plant growth and defense by negatively modulating SA signaling [73] (Figure 2, pathway 4). In ER stress conditions, CPR5 physically interacts with bZIP28 and bZIP60 to repress their functions [72, 74]. Thus, absence of functional CPR5 reduces plant ER stress tolerance, supporting a molecular link of the pathogen signaling with plant the UPR. bZIP60 and bZIP28 were also shown to physically interact with the SA receptor Nonexpressor of Pathogenesis Related 1 (NPR1) [75] (Figure 2, pathway 5). NPR1 forms homooligomers in the cytosol through inter-protein disulfide bridges which are reduced under SA treatment, allowing nuclear localization [76]. In the nucleus, NPR1 binds to the TGA2 clade of bZIP family TF, inducing the expression of pathogen response genes, including Pathogenesis-Related 1 (PR1) [77]. ER stress can also cause cytosol reduction in a SA-independent fashion [75]. In these conditions, nuclear-translocated NPR1 binds to bZIP60 and bZIP28 independently of SA [75]. Such an interaction was found to repress the transcriptional induction of UPR genes; consistently with this, an *NPR1* loss-of-function mutant is more resistant to ER stress [75]. Therefore, the plant UPR does acts in coordination with *cis*-, *trans*-regulatory factors, and co-regulators of other signaling pathways. In the context of multicellularity, this indicates either that the ER stress resolution integrates clues from other signaling pathways or that critical elements of other pathways have assumed multifunctionality.

From the Cell to the Whole Organism

The first phase in the UPR is the activation of pro-survival strategies. If these adaptive responses fail, the UPR signalling turns into pro-death process (Box 2). The mechanisms responsible for the transition from pro-survival to pro-death response are not well established, especially in plants. However, since the UPR is critical for the cell-fate decisions, its amplitude and duration must be tightly regulated. One of the known regulators

of the activity of the mammalian IRE1 α is the ER transmembrane protein BAX inhibitor 1 (BI1). This integral membrane protein plays a dual role in ER stress: physically interacts with IRE1 α , inhibiting the XBP1 (homologous to bZIP60 in plants) splicing and therefore attenuating the pro-survival role of the IRE1 α /XBP1 pathway [78]. However, under chronic ER stress, BI1 also blocks the activation of the proapoptotic member of Bcl-2 family, Bcl-2 Associated X (BAX) [79][80]. Interestingly, BAX also interacts with the cytosolic domain of IRE1 α activating its signalling pathway [81].

Although the anti-apoptotic role of BI1 was thought to be conserved in plants [82, 83], the *Arabidopsis* BI1 was found not to modulate IRE1 splicing activity as supported by the evidence that a loss of BI1 does not affect IRE1-mediated ER stress signaling [84]. In contrast, BI1 was found to negatively regulate the bZIP28 pro-survival signaling during ER stress recovery [84], indicating that the IRE1 signaling modulation may have acquired specific features in plants. However, the mechanism involved in the ER-phagy to selectively phagocyte ER to remove misfolded proteins and restore the ER homeostasis seems to be at least partially conserved between mammalian cells and plants, but seems to be different from yeast [85, 86]. In mammals, this mechanism involves SEC62, a component of the translocon complex [87]. During ER stress recovery, SEC62 functions as an ER-phagy receptor through its C-terminal domain acting as a bridge between the membrane of a phagophore and the autophagic cargoes and promoting their delivery to autolysosomes [88]. *A. thaliana* encodes one SEC62, which contains an extra transmembrane domain in its C-terminal compared to its orthologs [86, 89]. Although the roles of SEC62 in protein post-translational translocation have not been yet investigated in *Arabidopsis*, a recent study has shown that SEC62 might function as an ER-phagy receptor during ER stress also in plants [86]. Although the translocon domains and ER-phagy receptor function may be conserved [86, 89], its unique protein structure supports the possibility that SEC62 may have additional plant-specific functions.

More broadly, plant-specific features are reflected in the connection between reactive oxygen species (ROS) production and UPR in plants [90, 91]. The ER provides a unique oxidizing environment for disulfide bond formation, a major source of ROS by-products within cells. In yeast and in mammalian cells, increasing concentrations of cellular ROS negatively impacts UPR efficiency [92–95]. Indeed, low ROS levels are maintained in ER stress responses via specific mechanisms, such as a PERK-dependent expression of antioxidant proteins [96]. Similarly, ROS is also produced during ER stress response in plants [97] via the activity of NADPH/respiratory burst oxidase protein-D (RBOHD) and -F (RBOHF), the *Arabidopsis* homologs of mammalian ROS-producing NADPH oxidase enzymes [91]. Contrasting with the effects of plasma membrane NADPH oxidases in mammalian models, which promote apoptosis [98], RBOHD and RBOHF promote cell survival and extend pro-survival UPR activation [91]. Given that NADPH oxidases are known to mediate rapid systemic signaling in plants [99] and that low concentrations of ROS induce ER-related gene expression [100], it is plausible that RBOHD and RBOHF modulate UPR activation in a systemic manner during ER stress.

Evidence for systemic propagation of UPR signals in plants was also demonstrated by a transduction of spliced *bZIP60* and *BiP3* mRNA from roots subjected to ER stress

conditions to untreated shoots [101]. Systemic induction was attributed to cell-to-cell translocation of spliced bZIP60, either protein, mRNA, or both, through plasmodesmata (Figure 3). The involvement of bZIP28, whose transcripts can be translocated under certain conditions [102], and of other UPR regulators in the systemic stress signals in plants has yet to be specifically evaluated. However, the occurrence of a non-cell autonomous UPR supports the hypothesis that a systemic UPR signaling likely plays a fundamental role in coordinating whole-body responses to ER stress in plants [101].

The UPR in Plant Physiology

In mammals, the UPR is connected to devastating diseases (cancer, diabetes mellitus, and neurodegenerative diseases, among others) [41]. In plants, the UPR components have been implicated in the response to a large number of stresses, such as pathogen attack, heat, or hormones, probably through perturbation of protein and lipid homeostasis (recently reviewed in [103]) (Figure 3). In addition to responses to environmental stresses, the UPR contributes to physiological development of multicellular organisms (Table 1). In unicellular organisms, such as yeast and algae [37, 104, 105], the loss of IRE1 is viable. However, in *D. melanogaster*, the loss of IRE1 is lethal at larval stage [106], and in mice, the loss of IRE1 α causes embryonic lethality [107], similar to the concomitant loss of both ATF6 isoforms [108]. In *A. thaliana*, the *ire1a* mutant is viable, but a complete *ire1b* knockout mutant seems to be lethal since a full loss-of-function allele was not identified [109, 110]. Of note, the *ire1b* allele used in functional studies to date is a functional knockdown [110]. IRE1C may be involved in male gametophyte development, and a triple mutant lacking the three IRE1 isoforms, including IRE1C, is lethal [11].

For the other UPR arm, a *bzip17 bzip28* knockout mutant is viable [16] while the triple mutants *ire1a ire1b bzip28* and *bzip17 bzip28 bzip60*, which lack components of both UPR arms, are lethal [16, 111]. Even being viable, multiple high-order mutants involving one, such as *ire1a ire1b* [110] and *bzip17 bzip28* [16], or both UPR arms, like *ire1a ire1b bzip17* [112], have impairment of primary root elongation, adult vegetative growth, and male gametophyte development, supporting a role of these proteins in physiological growth and development. Whole genome transcriptomic analyses of *ire1a ire1b bzip17* [112] and *bzip17 bzip28* [16] have shed some light on how the UPR arms may participate in root development. The *ire1a ire1b bzip17* transcriptome analysis showed an up-regulation of genes encoding secreted proteins, suggesting a possible role of RIDD activity in promoting physiological development [112]. Such bZIP60 splicing-independent function of IRE1 is supported by the evidence that a *bzip60* knockout does not have phenotypic alterations in non-stressed conditions in contrast to mice and *Drosophila* where XBP1 plays an important role in development [106, 113]. However, this does not exclude the possibility that IRE1 may modulate plant development through other mechanisms, such as phosphorylation of other targets through its kinase domain.

In contrast, both transcriptional analyses support that bZIP17 may regulate plant development through the upregulation of non-canonical UPR genes associated with regulation of hormone-dependent cell growth or chloroplast functions [16, 112]. The strong phenotype of a *bzip17 bzip28* compared to the respective single mutants indicates that, as

discussed above, bZIP17 plays only partially redundant roles with bZIP28 modulating the expression of these non-canonical UPR genes. However, *ire1a ire1b bzip28* is lethal [111], suggesting that a bZIP28 loss is not completely compensated by bZIP17. This might be due to a lower expression level of bZIP17 compared to bZIP28 (<http://bar.utoronto.ca>). Alternatively, bZIP17 and bZIP28 may have largely overlapping functions in normal conditions of growth but control a few distinct genes that are critical in physiological UPR.

The molecular mechanisms harnessed by the ER stress sensors to participate in physiological processes are yet undiscovered. Studies performed in *ire1a ire1b* and *ire1a ire1b bzip17* mutants complemented with RNase or protein kinase defective versions of IRE1 or an inactive version of bZIP17 suggested that the ER stress sensors may be activated in a similar manner in ER stress [111, 112]. Nevertheless, the stimuli that trigger their activation are not clear. During pollen development and polar pollen tube growth, the participation of the UPR components has been proposed to be associated with a heavy demand of protein secretion, which may elicit the UPR by exceeding the capacity of the protein folding machinery [111, 114, 115]. Whether this is the same situation in root development is an open question. It cannot be excluded that a functional UPR is required for the synthesis of growth-related factors (e.g., plasma membrane receptors) that are required for organ growth. The possibility that the plant UPR has developed specific activation mechanisms depending on the plant tissue or the developmental stage should be explored deeply in the future. Understanding the integration of these mechanism with the response to multiple stimuli will become even more critical in the developing global climate crisis, considering that heat activates the UPR in plants [33, 53].

Concluding Remarks

In plants, the UPR plays a key role in mediating the response to multiple abiotic and biotic stresses [103], and developmental processes, supporting that the UPR signaling is intertwined with processes that control organismal defense and growth. Despite a broad conservation of core UPR mechanisms amongst eukaryotes, recent advances in our understanding of the integration of these mechanisms in plant specific contexts, such as the interaction of bZIP28 and bZIP60 with HY5 [70] or NPR1 [75], or the pro-survival role of ROS signaling [91], provide evidence that plants have evolved unique strategies to respond to ER stress situations and utilize UPR mechanisms for adaptation to in the environment. Together these findings raise new questions that have to be investigated to better understand the relevance and regulation of UPR signaling in the context of the whole organism during the response to environmental threats and basal developmental programming (see Outstanding Questions).

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Glossary

Canonical unfolded protein response (UPR) genes

Genes regulated by UPR with their expression associated with ER stress response

Endoplasmic reticulum (ER) client mRNAs

mRNAs that are delivered to the ER for synthesis of polypeptides through ribosomes

ER-phagy

A type of selective autophagy through specific domains of the ER are degraded and recycled via a specific mechanism of autophagy

ER stress

Potential lethal condition caused by an accumulation of excessive levels of unfolded or misfolded proteins in the ER, which can be caused by environmental and physiological conditions

ER stress recovery

The process of growth restoration and reduction of ER stress triggered by ER stress inducers

Noncanonical UPR genes

Genes regulated by UPR but their expression associated primarily with physiological growth or development

Phagophore

A double membrane vesicle that encloses and delivers cytoplasmic materials for degradation through autophagy

Plasmodesmata

Narrow channels that connect neighboring plant cells. They function as cytoplasmic bridges that enable intracellular communication and transportation of cellular materials

Regulated intramembrane proteolysis (RIP)

In the context of the UPR, RIP is a process for the activation of membrane tethered UPR bZIP transcription factors through translocation from ER to the Golgi for proteolytic cleavage by S1P and S2P. RIP is a conserved process in animals and plants

Regulated IRE dependent decay (RIDD)

The process of site-specific endonucleolytic cleavage of mRNAs via the IRE1 ribonuclease activity

Systemic signaling

Intercellular long-distance signal transduction throughout an organism

Tunicamycin (Tm) and Dithiothreitol (DTT)

Tm and DTT are chemical inducers of the UPR that lead to accumulation of unfolded secretory proteins through interfering with their N-linked glycosylation and proper disulfide bonds formation, respectively

Unfolded protein response (UPR)

An intracellular signaling process triggered by ER stress. The UPR is designed to restore ER function as well as to ignite programmed cell death processes if ER stress is unresolved

UPRosome

A dynamic protein platform provided by IRE1 α where regulators assemble to regulate the kinetic, amplitude and tissue specificity of UPR

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Box 1.**Investigation of the UPR in *Arabidopsis thaliana* – tools**

The availability of a high-quality simple reference genome, genetic tractability, and ample resources for genetics and genomics studies have made *A. thaliana* a powerful experimental platform for investigating the UPR in multicellular organisms. First, functional homologs of nearly all components of metazoan UPR are redundantly encoded in multiple isoforms, allowing generation of a large germplasm library, including viable whole body, loss-of-function mutations of critical UPR regulators, which can be lethal in other model organisms [106–108, 113]. These mutants also provide an opportunity to interrogate the functional and genetic interactions of these conserved UPR transducers *in vivo* through the generation of high-order mutants. Second, its relatively simple organs (e.g., roots and shoots) and comprehensive methods for phenotyping well-established through previous studies (for examples see [84, 91]) allow *in vivo* investigation of physiological changes controlled by single or multiple UPR components in a large-scale with a high reproducibility. Third, various effects of ER stress, including adaptive, persistent, and resolution stages, can be analyzed in *A. thaliana* by applying adjusted dosages of ER stress inducers to the growth media [116]. Together, such molecular and physiological flexibility of *A. thaliana* in response to ER stress enables to decipher the functional complexity of the UPR in plants but also other organisms for conserved processes.

Box 2.**Unique contributions of the ER stress sensors to cell-fate under temporary or chronic ER stress**

ER stress activates the UPR leading to the nuclear translocation of the TFs (i.e., bZIP60, bZIP28, and bZIP17) to coordinate multiphasic gene expression events. These can be broadly grouped as pro-survival, transition, and pro-death phases, and lead to cell fate determination through intracellular signaling tuning. At the onset of ER stress, the UPR activates the adaptive pro-survival signaling programs to restore ER homeostasis through induction of genes that encode ER-resident chaperones and foldases to reduce protein-folding load, downregulates the expression of extracellular proteins to reduce secretory proteins translocation to the ER, and increases the expression of effector molecules that function in ER protein quality control to expand the folding capacity [3, 4, 24, 117]. Interestingly, the early phase of ER stress also decreases the expression of genes encoding oxidative stress response (e.g. peroxidase) via RIDD [118]. In conditions of chronic ER stress, which exceed the adaptive response of the ER, the UPR transitions to an irreversible pro-death phase through the induction of the expression of UPR genes associated with programmed cell death and autophagy [3, 4, 24, 117]. The understanding of the mechanisms of UPR signaling transition from pro-survival to pro-death phase are still largely unknown, especially in plants. IRE1 and bZIP28/bZIP60 are essential to activate the pro-survival signaling. This is supported by the evidence that the Arabidopsis *ire1a ire1b* and *bzip28 bzip60* mutant are lethal in chronic ER stress conditions [35, 38, 110, 111, 119]. Nonetheless the single *bzip28* and *bzip60* single mutants are viable in the same conditions [33, 111, 119], suggesting that bZIP28 and bZIP60 share redundant pro-survival functions but also that the pro-survival function of IRE1 is independent of bZIP60. Furthermore, bZIP28 and bZIP60 exhibit tissue-specific roles in adaptive UPR and during ER stress recovery, as supported by the evidence that in ER stress recovery conditions, the primary root growth defects of *bzip28* and *bzip60* single mutants are exacerbated in the *bzip28 bzip60* double mutant [84]. These results indicate a complexity of the UPR signaling in plants whose foundations are yet largely unexplored.

Highlights

The UPR is an essential signaling pathway that, although fundamentally conserved among eukaryotes, has assumes some unique features in plants.

The plant ER stress sensors IRE1/bZIP60 and bZIP28/17 (homologs of IRE1 α /XBP1, and ATF6 α / β , respectively) activate conserved UPR pathways, but have diversified functions in response to stress stimuli.

Plant UPR TFs coordinate with nuclear co-regulators of other signaling pathways to modulate ER stress outputs.

Genes conserved between metazoans and plants such as BI-1 and NADPH oxidases have developed unique functions in ER stress responses in plants.

The UPR contributes to plant cell processes at least through the upregulation of non-canonical UPR genes via bZIP28 and bZIP17 regulation.

Outstanding Questions

To what extent are the RNase-independent functions of IRE1 conserved in plants? Could IRE1-kinase domain phosphorylate substrates with functional relevance for plant development or ER stress response? Have plant IRE1s evolved unique signaling activities? Are the activation requirements of plant IRE1 similar to yeast or mammals?

How IRE1C is activated? Which are the mechanisms that keep IRE1C in an inactive state? Has IRE1C a functional role in response to ER stress or other environmental stressors besides its role in plant development?

How may environmental stresses activate the ER stress sensors? Which are the specific activation mechanisms of UPR sensors in response to biotic and abiotic stresses? How does the plant UPR integrate responses to multiple stimuli?

What is the specific role of the systemic UPR in regulating whole-body responses to external and internal stimuli?

How does the UPR participate in the development of different tissues in plants? How is the UPR dependent regulation of plant development altered during stress situations?

Figure 1. Mechanisms of activation of the ER stress sensors in plants

(A) In plants, IRE1A and IRE1B have a luminal domain and cytosolic kinase and RNase domains. By similarity with yeast and metazoans, it has been proposed that the interaction between BiP and the luminal domain of IRE1 may keep the latter in an inactive monomeric state. Upon the accumulation of unfolded proteins, BiP dissociates from IRE1, leading to IRE1 homodimerization and auto-transphosphorylation as well as promoting activation of the RNase domain. In plants, the RNase domain catalyzes two processes: the unconventional splicing of bZIP60 mRNA producing the translation of an active TF, bZIP60 (pathway 1), and the selective degradation of mRNAs (RIDD) (pathway 2), which has been associated with autophagy. If the IRE1-kinase domain phosphorylates other functional substrates besides itself has not been yet established (pathway 3). The recent identification of IRE1C (pathway 4), which lacks the luminal domain, raises questions about its mechanism of activation and its functional implication in the response to stressors (Outstanding questions).

(B) The activation of bZIP28 is mediated by its interaction with BiP (pathway 1). An accumulation of unfolded proteins causes the dissociation of BiP from the bZIP28 luminal domain leading to its Golgi translocation. Once in the Golgi, bZIP28 is cleaved first by unknown protease(s), possibly by S1P, and then by S2P. This process releases the active TF from the membrane anchor for nuclear translocation. Although the activation mechanism of bZIP17 may be similar to bZIP28 (pathway 2), the interacting proteins that retain bZIP17 in

the ER under normal conditions has not been described yet. CT, cytoplasm; LM, ER lumen; N, nucleus.

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Figure 2. Nuclear regulation of the UPR TFs binding to *cis*-elements in the plants.

In the nucleus, the active forms of bZIP28 and bZIP60 bind to the *cis*-elements responsive to ER stress (e.g. ER Stress responsible Element-1, ERSE-I and Unfolded Protein Response Element-1, UPRE-I). Their transcriptional activity is enhanced (blue dotted lines) or inhibited (red dotted lines) by interactioning with other transcription regulators associated with other biological pathways. The COMPASS-like complex increases the levels of H3K4me3 in the promoters of UPR target genes, causing gene expression modulation. CP, chloroplast; ER; endoplasmic reticulum; G, Golgi; MT, mitochondria; VC, vacuole; ER, endoplasmic reticulum.

Figure 3. Mechanisms for the systemic signaling of UPR in the whole plant.

Abiotic (such as heat, salt or light) and biotic (pathogen attack) stresses in combination with physiological processes can elicit the UPR activation. For the whole plant adaptation, plants can evoke a long-distance signaling to communicate the occurrence of ER stress in a tissue to other tissue a systemic by the ROS molecules produced during the ER stress response and also by the cell-to-cell translocation of active bZIP60 TF and the spliced bZIP60 mRNA (sbZIP60) through the plasmodesmata.

Table 1.

Physiological consequences of the genetic manipulation of UPR components in several organisms.

	Mutant	Organism	Phenotype	Reference
Single	<i>ire1a</i>	<i>A. thaliana</i>	No obvious phenotype	[35, 111]
	<i>ire1b^a</i>	<i>A. thaliana</i>	Lethal ^a	[109, 110]
	<i>ire1b^b</i>		No obvious phenotype ^b	[35, 110, 111]
	<i>ire1</i>	<i>S. cerevisiae</i>	Viable	[104]
		<i>S. pombe</i>		[37]
	<i>ire1</i>	<i>C. reinhardtii</i>	Viable	[105]
	<i>ire1</i>	<i>D. melanogaster</i>	Lethal at early larval stages	[106]
	<i>ire1a</i>	<i>M. musculus</i>	Embryonic lethal.	[107]
			Impaired development of blood vessels of the placenta.	
	<i>bzip60</i>	<i>A. thaliana</i>	No obvious phenotype	[111]
	<i>xbp1</i>	<i>D. melanogaster</i>	Lethal at early larval stages	[106]
	<i>xbp1</i>	mice	Embryonic lethal.	[113]
			Impaired liver development.	
	<i>bzip28</i>	<i>A. thaliana</i>	No obvious phenotype	[111]
	<i>bzip17</i>	<i>A. thaliana</i>	No obvious phenotype	[111]
Double	<i>ire1a ire1b^b</i>	<i>A. thaliana</i>	Impaired primary root development	[110, 111]
	<i>bzip17 bzip28</i>	<i>A. thaliana</i>	Severe dwarfism, retarded germination and scarcely elongated root.	[16]
	<i>atf6a atf6β</i>	<i>M. musculus</i>	Embryonic lethal	[108]
Triple	<i>ire1a ire1b^b ire1c</i>	<i>A. thaliana</i>	Lethal	[11]
	<i>ire1a ire1b^b bzip60</i>	<i>A. thaliana</i>	Impaired primary root development	[111]
	<i>ire1a ire1b^b bzip17</i>	<i>A. thaliana</i>	Impaired shoot and root development and delayed flowering.	[112]
	<i>ire1a ire1b^b bzip28</i>	<i>A. thaliana</i>	Lethal	[111]
	<i>bzip17bzip28 bzip60</i>	<i>A. thaliana</i>	Lethal	[16]