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EXPERT VIEW

Multilevel regulation of endoplasmic reticulum stress responses in plants: where old roads and new paths meet

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Abstract

The sessile lifestyle of plants requires them to cope with a multitude of stresses *in situ*. In response to diverse environmental and intracellular cues, plant cells respond by massive reprogramming of transcription and translation of stress response regulators, many of which rely on endoplasmic reticulum (ER) processing. This increased protein synthesis could exceed the capacity of precise protein quality control, leading to the accumulation of unfolded and/or misfolded proteins that triggers the unfolded protein response (UPR). Such cellular stress responses are multilayered and executed in different cellular compartments. Here, we will discuss the three main branches of UPR signaling in diverse eukaryotic systems, and describe various levels of ER stress response regulation that encompass transcriptional gene regulation by master transcription factors, post-transcriptional activities including cytoplasmic splicing, translational control, and multiple post-translational events such as peptide modifications and cleavage. In addition, we will discuss the roles of plant ER stress sensors in abiotic and biotic stress responses and speculate on the future prospects of engineering these signaling events for heightened stress tolerance.

Keywords: bZIP17, bZIP28, bZIP60, endoplasmic reticulum, GCN2, IRE1, UPR.

Integrated stress response in the endoplasmic reticulum

The endoplasmic reticulum (ER) is the largest cellular organelle that controls the fundamental part of the protein homeostasis network by folding nascent peptides, packaging them, and sending them to their target locations (Chevet *et al.*, 2001; Sunkar *et al.*, 2006; Ron and Walter, 2007). The demand on the protein folding machinery can be increased by environmental conditions and endogenous factors, such as nutrient deficiency, oxidative stress, heat, or pathogen infection. When the folding demand is higher than the ER capacity, ER homeostasis becomes disrupted (Ron and Walter, 2007) while unfolded proteins begin to accumulate and aggregate, which collectively is referred to as ER stress (Walter and Ron, 2011). The cell has evolved effective mechanisms to adapt to this challenge, termed the unfolded protein response (UPR) (Kozutsumi *et al.*, 1988). The UPR aims to resolve the ER stress by attenuating protein translation, reducing the amount of misfolded proteins, and inducing expression of protein chaperones (Hetz *et al.*, 2015). If the UPR cannot alleviate the ER stress, the cell switches its signaling into pro-death mode and activates apoptotic programs (Chen and Brandizzi, 2013).

The mammalian UPR is a three-prong signaling network. One branch includes a group of four kinases that mediate phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), including GCN2 (General Control Non-repressible

2) and PERK (RNA dependent Protein Kinase like ER kinase) (Wek *et al.*, 2006). The phosphorylated eIF2α allows for translational de-repression of mRNAs containing upstream ORFs (uORFs), such as activating transcription factor 4 (ATF4) (Rutkowski and Kaufman, 2003) that regulates survival under stress, autophagy (Luhr *et al.*, 2019), apoptosis (Rozpedek *et al.*, 2016), tumor growth (Wortel *et al.*, 2017), and polysomal decrease (Hofmann *et al.*, 2012).

The other two branches of the mammalian UPR are mediated by transmembrane sensors spanning the ER lumen and cytosol: activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1, α and β isoform) (Walter and Ron, 2011). Their activation is accomplished in response to accumulation of misfolded peptides in the ER lumen, leading to BiP (binding immunoglobulin protein) dissociation from these sensors and their activation by oligomerization or export (Maurel and Chevet, 2013). In animal systems, IRE1 can be activated by direct binding to unfolded proteins in the ER lumen or by changes in membrane lipid composition (Gardner and Walter, 2011; Promlek *et al.*, 2011; Volmer *et al.*, 2013). All three animal ER stress signaling branches participate in cellular responses to various external stimuli, including the regulation of metabolism (Oyadomari *et al.*, 2008; Baird and Wek, 2012), immunity (Munn *et al.*, 2005; Bunpo *et al.*, 2010; Xia *et al.*, 2018), tumorigenesis (Dey *et al.*, 2015), and memory (Costa-Mattioli *et al.*, 2005; Sidrauski *et al.*, 2013).

UPR in plants: the next frontier for plant stress signaling

While UPR has been a topic of active investigation in mammals and yeast for over three decades (Kozutsumi *et al.*, 1988), it only recently has come to light as a complex stress response mechanism in plants (Koizumi *et al.*, 2001; Liu *et al.*, 2007*b*; Iwata *et al.*, 2008; Lageix *et al.*, 2008). Plant cells contain signaling pathways functionally corresponding to the three arms of the mammalian UPR (Korner *et al.*, 2015; Bao and Howell, 2017; Park and Park, 2019) (Fig. 1). The most conserved arm is IRE1, equipped with an N-terminal ER lumen sensor domain and a C-terminal kinase/endonuclease domain. The genomes of Arabidopsis, rice, soybean, maize, and *Nicotiana attenuata* contain two homologs, IRE1a and IRE1b, that share high sequence similarity and conserved domain architecture (Li *et al.*, 2012; Wakasa *et al.*, 2012; Silva *et al.*, 2015; Xu *et al.*, 2019). Recently, it was reported that a third IRE1-like gene, designated IRE1c, exists in Arabidopsis (Mishiba *et al.*, 2019, Preprint). Upon ER stress, IRE1 dimerizes, trans-autophosphorylates, and unconventionally splices mRNA for the bZIP60 transcription factor (TF) in a process termed regulated IRE1-dependent splicing (RIDS; see below). In Arabidopsis, the spliced form of AtbZIP60 is an active TF that translocates into the nucleus to drive expression of ER stress-responsive genes (Deng *et al.*, 2011; Moreno *et al.*, 2012). The IRE1 substrates from yeast and mammals, HAC1 and XBP1, also belong to the bZIP family and, akin to bZIP60, display conserved double stem–loop structures with specific nucleotide signatures required for mRNA processing (Back *et al.*, 2005). Recently, Arabidopsis bZIP60 was also demonstrated to move from local to systemic tissues to propagate the UPR signal in distal parts of the plant (Lai *et al.*, 2018*b*).

The second arm involves the membrane-associated TFs AtbZIP17 and AtbZIP28 (Srivastava *et al.*, 2014; Kim *et al.*, 2018). Their precursor forms are C-terminally anchored at the ER membrane, and upon ER stress translocate to the Golgi apparatus where they undergo sequential proteolytic cleavage by site-specific proteases S1P and S2P. Resulting N-termini, containing the bZIP and transcriptional activation domains, are transported to the nucleus, where they function as active TFs and up-regulate expression of stress response genes (Liu *et al.*, 2007*a*, *b*; Che *et al.*, 2010; Howell, 2013; Kim *et al.*, 2018) (Fig. 1). Similar to the IRE1 branch, there is a considerable degree of conservation between plant AtbZIP17/28 and mammalian ATF6 that also contains a bZIP domain and is activated by ER stress by cleavage from the membrane (Hillary and FitzGerald, 2018).

Along with the well-established AtIRE1/AtbZIP60- and AtbZIP17/28-driven ER stress-mediating pathways, recently the eIF2α phosphorylation was shown to be another cornerstone of cellular stress activation in plants, further emphasizing the resemblance between the mechanisms of the UPR signaling in the animal and plant kingdoms (Korner *et al.*, 2015; Liu *et al.*, 2019). Plant genomes (*Arabidopsis thaliana* and *Nicotiana benthamiana*) encode a single copy of a GCN2 kinase that responds to a variety of ER stress-related abiotic and biotic stimuli such as amino acid starvation, the herbicide glyphosate (Faus *et al.*, 2015), UV and cold stress, wounding, and salicylic acid (SA) (Lageix *et al.*, 2008), as well as pathogen infection (Monaghan and Li, 2010; Liu *et al.*, 2015*a*; Li *et al.*, 2018). Recently, AtGCN2 was shown to control abscisic acid (ABA) homeostasis and stomatal immunity, and regulate translation of a TF, TBF1, through uORF reinitiation, akin to mammalian ATF4 and yeast GCN4 (Pajerowska-Mukhtar *et al.*, 2012; Liu *et al.*, 2019). While plants do not possess a bona fide homolog of the main mammalian ER stress sensor PERK, and the plant GCN2 activation mechanism remains unknown, the connection of AtGCN2 signaling to the ER stress responses is strengthened by its downstream target AtTBF1, which is the master regulator of secretory pathway-related genes during pathogen infection (Pajerowska-Mukhtar *et al.*, 2012) and binds to their promoters through the *TL1 cis*-regulatory motif (Wang *et al.*, 2005).

Regulated IRE1-dependent splicing (RIDS)

In plants, IRE1 splices two known mRNA orthologs: AtbZIP60 in Arabidopsis and OsbZIP50 in rice (Korner *et al.*, 2015). AtbZIP60 was once thought to have been cleaved by proteolytic processing (Iwata *et al.*, 2008); however, it does not have the canonical S1P cleavage site and its activation does not require S1P or S2P. Consequently, it was demonstrated that it is subject to processing by IRE1 (Deng *et al.*, 2011; Nagashima *et al.*, 2011; Moreno *et al.*, 2012). Splicing of AtbZIP60us mRNA (unspliced form) removes a 23 nt long intron, and tRNA ligase RLG1 catalyzes ligation of the resulting fragments

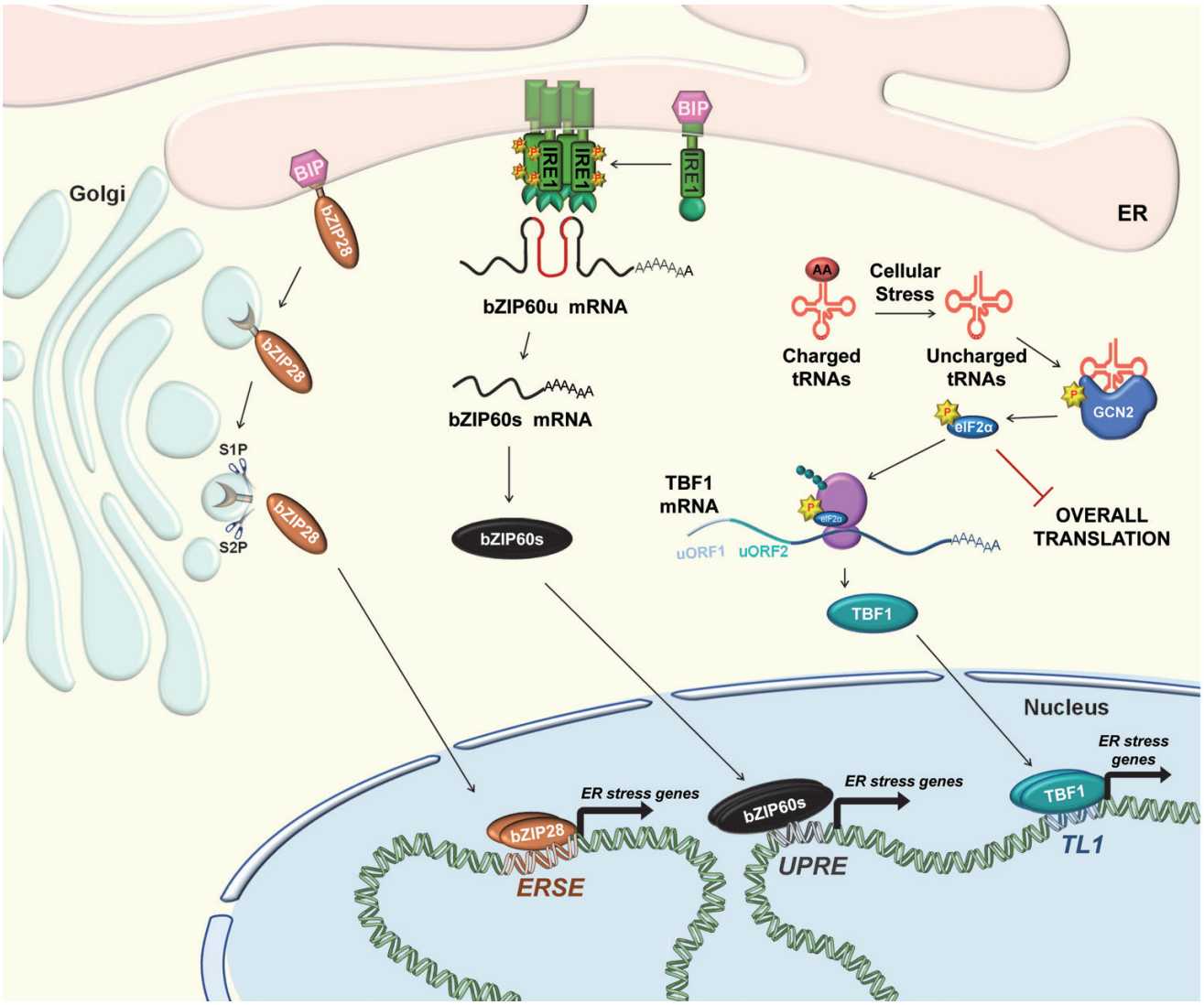

Fig. 1. Three arms of ER stress response in plants. Accumulation of misfolded proteins causes BiP to dissociate from bZIP28, leading to its translocation to the Golgi where it is proteolytically cleaved by site-1 and site-2 proteases. The freed cytoplasmic domain enters the nucleus as a fully functional transcription factor and activates several ER stress genes through binding to their *ERSE cis*-regulatory motifs. Following ER stress, BiP also dissociates from IRE1, which enables it to oligomerize and trans-autophosphorylate. Thus activated, IRE1 cleaves cytoplasmic mRNA bZIP60u, which when spliced (bZIP60s) and translated enters the nucleus and activates several ER stress genes through binding to their *UPRE cis*-regulatory motifs. Cellular stress also causes a build-up of uncharged tRNAs, which are sensed and bound by GCN2, leading to its autophosphorylation. Thus activated, GCN2 phosphorylates eIF2α to inhibit overall translation while specifically de-repressing translation of target mRNA TBF1 to form a transcription factor that enters the nucleus and activates several ER stress genes through binding to their *TL-1 cis*-regulatory motifs.

(Nagashima *et al.*, 2016), forming the bZIP60s (spliced) form. The reading frame of AtbZIP60s shifts, which eliminates a putative transmembrane domain normally present in AtbZIP60us (Deng *et al.*, 2011) and instead encodes a nuclear localization signal (Parra-Rojas *et al.*, 2015). AtbZIP60us and AtbZIP60s are not detectable under basal conditions when tagged with green fluorescent protein (GFP), even though the AtbZIP60us transcript was shown to be present (Parra-Rojas *et al.*, 2015). The AtbZIP60s protein became detectable following ER stress or treatment with a proteasome inhibitor, suggesting rapid degradation of AtbZIP60us protein under basal conditions (Parra-Rojas *et al.*, 2015), both of which are reminiscent of the regulation of XBP1 (Yoshida *et al.*, 2001).

AtAGB1, the only G-protein β-subunit encoded by the Arabidopsis genome, was also demonstrated to act in concert with AtIRE1a and AtIRE1b to control two independent UPR pathways (Chen and Brandizzi, 2012). While *atagb1* mutants have shown a distinct sensitivity to tunicamycin, a potent inhibitor of *N*-linked glycosylation, this phenotype was further enhanced in *ire1a ire1b agb1* triple mutants. So far, AGB1 has only been implicated in the plant UPR, although the large numbers of often redundant G-protein complex members may hinder investigation of their involvement in the mammalian UPR (Chen and Brandizzi, 2013).

Regulated IRE1-dependent decay (RIDD)

IRE1 is also known to engage in cleavage and bulk degradation of certain mRNAs in a process called regulated IRE1-dependent decay (RIDD) (Hollien and Weissman, 2006;

Coelho and Domingos, 2014). In mammals, RIDD cleaves mRNAs at an XBP1-like consensus site but with an activity divergent from XBP1 mRNA splicing (Maurel *et al*., 2014). The exact function and nature of the degraded mRNAs is not completely understood, though it is known that RIDD is integral in the switch between pro-survival and pro-death IRE1 functions (Abdullah and Ravanan, 2018). While IRE1 splicing activity fully promotes pro-survival through activation of genes that help alleviate ER stress (Chen and Brandizzi, 2013), RIDD can tip the scales of cell fate depending on which specific mRNAs are degraded. While the cell is still in the pro-survival state, IRE1 degrades mRNAs encoding ER-resident proteins, decreasing the protein folding load in the ER. Recently, a study in Arabidopsis showed that many mRNAs encoding secretory pathway proteins that are known to be degraded upon treatment with tunicamycin are not degraded in *ire1a ire1b* double mutants. However, these targets are degraded in *bzip60* plants, indicating that this process is independent of RIDS (Mishiba *et al*., 2013). Defects in AtIRE1 lead to enhanced cell death as measured by ion leakage assays and inhibition of germination by tunicamycin. However, mammalian IRE1α can also contribute to pro-death signaling through degradation of mRNAs of ER chaperones such as BiP, which decreases the protein folding capacity (Han *et al*., 2009). AtIRE1b RNase activity is also required for ER stress-induced autophagy in Arabidopsis through RIDD-mediated degradation of mRNAs that interfere with its induction (Bao *et al*., 2018).

Post-translational modifications of ER stress signaling components

Early experimental work in mammals showed that mutation of catalytic residues in the IRE1 kinase domain disrupts RNase function, and inhibition of kinase activity leads to a loss of splicing *in vivo* (Shamu and Walter, 1996; Tirasophon *et al*., 1998). Mechanistic understanding of how phosphorylation affects the RNase activity of IRE1 in plants is of crucial importance for future agricultural interventions as it would allow tailoring IRE1 variants with altered ability to splice AtbZIP60, offering a strategy to fine-tune UPR during drought and pathogen infection. A site-specific mutation N820A in AtIRE1b leads to the abolishment of RNase activity (Deng *et al*., 2013), and three mutations within the kinase domain (D608N, K610N, and D628A) blocked autophosphorylation and AtbZIP60 splicing (Fig. 2A). Complementation analyses revealed that both kinase and RNase domains were required for normal vegetative growth, while a functional RNase domain was required for root elongation and shoot growth under stress conditions. Similarly, mutation K442A in AtIRE1a.1 (the only splice variant of AtIRE1a described to date) was reported to reduce its autophosphorylation activity (Noh *et al*., 2002). Intriguingly, in the subsequent iterations of the Arabidopsis genome annotation effort, it transpired that AtIRE1b occurs in three splicing variants, AtIRE1b.1, AtIRE1b.2, and AtIRE1b.3, that differ at their N-termini but are identical in their cytosol

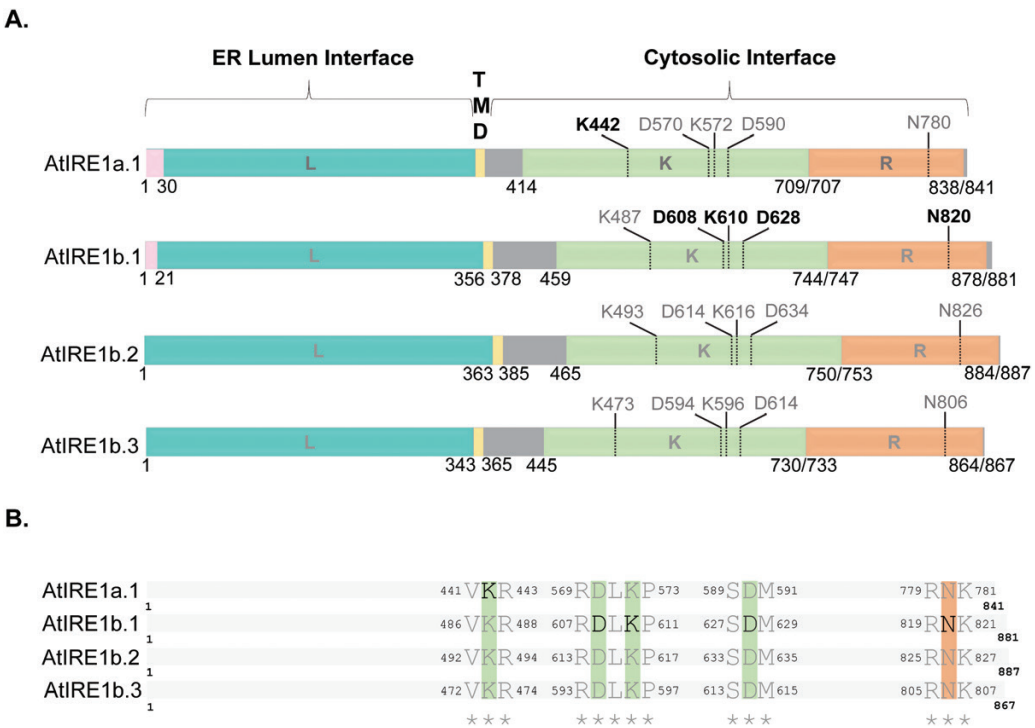

Fig. 2. Conserved domain architecture of IRE1 proteins in Arabidopsis. (A) Amino acid sites K442 for AtIRE1a.1, and D680, K610, D628, and N820 for IRE1b.1, experimentally determined to be critical for function, are shown in black. Predicted conserved sites on other AtIRE1 protein variants are labeled in gray. Signal peptides are marked in pink; no signal peptides were predicted in AtIRE1b.2 and AtIRE1b.3. Luminal domains (L) are shown in teal, transmemberane domains (TMD) in yellow, kinase domains (K) in green, and RNase domains (R) in orange. Gray shading corresponds to protein regions of unclassified domain nature. Numbers indicate predicted start and stop sites of individual domains as predicted by the SMART database (http://smart.embl-heidelberg.de/), InterPro (https://www.ebi.ac.uk/interpro/), and SignalP-5.0 (http://www.cbs.dtu.dk/services/SignalP/). With the exception of the luminal and transmembrane domains of AtIRE1a.1, all other regions have been predicted with high confidence. (B) A partial multiple sequence alignment showing a prediction of critical AtIRE1 amino acids. The experimentally characterized conserved amino acid residues are shown in black.

interfaces. Luminal domains of mammalian IRE1 are known to interact with numerous ER chaperones and co-chaperones, such as DNA-J and heat shock proteins, to form diverse complexes that can signal specific types and/or intensities of ER stress (Amin-Wetzel *et al.*, 2017). Previous studies indicate that AtBiP1 and AtBiP2 may be primarily involved in biotic stress signaling, while AtBiP3 is implicated in heat stress (Wang *et al.*, 2005; Zhang *et al.*, 2017). It would be intriguing to address whether sequence variation within plant IRE1 luminal domains can cause preferential interactions with specific chaperones, providing a stress-specific response, and whether it could be exploited to manipulate ER stress perception and sensitivity of crop plants. All AtIRE1 variants appear to contain the conserved residues involved in the activity of kinase and RNase domains (Fig. 2A, B), although their functionality remains to be tested. Further experimentation on these conserved residues could open up promising directions for tailoring the levels of ER signaling in crops, such as avoiding developmentally costly, spurious UPR activation.

Similar to IRE1, the GCN2 kinase is another ER stress sensor that is primarily controlled via phosphoregulation. *GCN2* encodes a protein kinase with a conserved N-terminal kinase domain and a C-terminal region homologous to histidyl-tRNA synthetase (HisRS) (Zhang *et al.*, 2003). The GCN2 HisRS domain senses cellular stress by binding to uncharged tRNAs (Dong *et al.*, 2000; Hao *et al.*, 2005), which in turn stimulates its kinase activity, leading to di- or tetramerization, autophosphorylation on two threonine residues, and downstream phosphorylation of eIF2α (Hinnebusch, 2005; Wek *et al.*, 2006). Yeast GCN2 is thought to be kept inactive via phosphorylation at Ser577, which depends on TORC1 activity (Castilho *et al.*, 2014). The mechanisms of GCN2 autophosphorylation or the presence of additional GCN2 kinases/phosphatases have not yet been elucidated in plants, although it has been suggested that the TOR pathway is not involved in crosstalk with AtGCN2 (Lageix *et al.*, 2008).

Another arm of the UPR that is activated through post-translational regulation is ATF6 in animals and its two plant equivalents bZIP17 and bZIP28. Under unstressed conditions, ATF6 and its functional homologs reside in the ER membrane, where BiP is bound to their luminal domains. Dissociation of BiP from ATF6 unmasks Golgi localization signals, initiating the Golgi translocation (Shen *et al.*, 2002; Srivastava *et al.*, 2013). It is unknown whether bZIP28 translocates through this or another mechanism, since the Golgi localization signals are not found in bZIP28 (Srivastava *et al.*, 2014) and, instead, BiP binds to intrinsically disordered regions on bZIP28's luminal domain (Srivastava *et al.*, 2013).

Once at the Golgi, ATF6 is cleaved first by site-1 protease (S1P), which reduces the size of the luminal domain and enables more efficient sequential cleavage at the transmembrane domain by site-2 protease (S2P) (Shen and Prywes, 2004; Sun *et al.*, 2015). It was assumed that the process was very similar to bZIP28, and Sun *et al.* even demonstrated that the S1P RRIL^{573} cleavage site is necessary for full activation of bZIP28 (Sun *et al.*, 2015). However, Iwata *et al.* showed through genetic knockouts that S2P proteases, but not S1P proteases, were necessary for the activation of bZIP28 and claimed that the results of Sun *et al.* could have stemmed from a change in secondary structure or protein interactions affecting the ability of bZIP28 to sense ER stress or to translocate to the Golgi, but not necessarily because bZIP28 was cleaved at the RRIL^{573} site (Iwata *et al.*, 2017). Regardless of the exact method of proteolytic cleavage, the liberated cytosolic domains of bZIP28 and ATF6 are able to translocate to the nucleus and bind to ER stress element (ERSE) *cis*-regulatory motifs to regulate ER stress genes (Yamamoto *et al.*, 2004; Liu and Howell, 2010).

Roles of ER stress signaling in plant development and stress

The vast majority of work dedicated to plant stress responses has focused on understanding the response mechanisms of plants against individual types of stresses. However, in their natural environment, plants are simultaneously exposed to multiple types of stress, including both abiotic and biotic factors. The ER deciphers and processes diverse inputs from inside and outside the cell, ensuring an optimal, integrated output response.

Among the abiotic stress factors, high temperature is one of the leading causes of ER stress and the UPR (Fig. 3). Several studies reported heat-induced bZIP60 splicing by IRE1 in Arabidopsis (Deng *et al.*, 2011, 2016), maize (Li *et al.*, 2012), and rice (OsbZIP50). Overexpression of BhbZIP60, an AtbZIP60

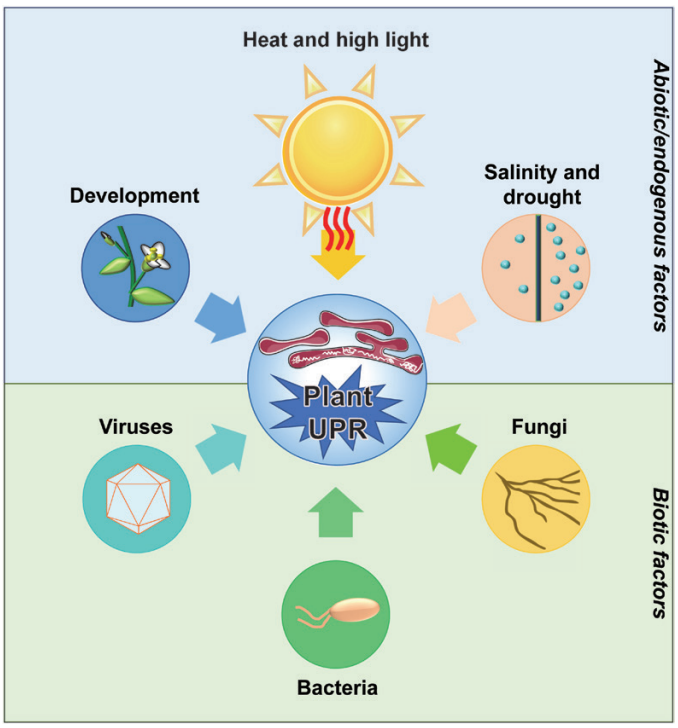

Fig. 3. Stress-mediated activation of the unfolded protein response (UPR). Abiotic and biotic stresses as well as developmental processes trigger the UPR in Arabidopsis. Abiotic stresses (such as heat, high light intensity, salinity, and drought) and biotic stresses (infection with bacterial, fungal, or viral phytopathogens), as well as normal developmental processes can lead to excessive accumulation of unfolded proteins in the ER or cause imbalances in amino acid supply, collectively leading to the activation of one or more arms of the UPR.

homolog from a resurrection plant *Boea hygrometrica*, resulted in heightened tolerance to drought and mannitol stresses (Wang *et al.*, 2017). Overexpression of bZIP60 yielded increased salt tolerance in Arabidopsis (Fujita *et al.*, 2007). In the laboratory, bZIP60 splicing can be induced chemically by DTT or tunicamycin (Nagashima *et al.*, 2011; Li *et al.*, 2012; Moreno *et al.*, 2012).

The AtIRE1/AtbZIP60 pathway was also reported to influence plant responses to pathogen infection (Fig. 3). *atire1a atire1b* and at*bzip60* loss-of-function plants are more susceptible to *Pseudomonas syringae* pv. *maculicola* strain ES4326 (Moreno *et al.*, 2012). Several studies demonstrated that the AtIRE1/AtbZIP60 pathway is involved in anti-viral defenses, such as against *Turnip mosaic virus* (TuMV) in Arabidopsis (Zhang *et al.*, 2015), *Rice black streak dwarf virus* (RBSDV) and *Garlic virus X* (GarVX) in *N. benthamiana* (Sun *et al.*, 2013; Lu *et al.*, 2016), as well as potexvirus, potyvirus, TuMV, and *Plantago asiatica mosaic virus* (PIAMV) in Arabidopsis, *N. benthamiana*, and potato (Gaguancela *et al.*, 2016). Very recently, the IRE1/bZIP60 pathway was also shown to be required for *N. attenuata* resistance to a fungal pathogen *Alternaria alternata* (Xu *et al.*, 2019). AtIRE1 and AtbZIP60 also play important roles in vegetative and reproductive developmental processes, notably root growth and pollen development (Chen and Brandizzi, 2012; Deng *et al.*, 2013, 2016).

The AtGCN2 pathway is stimulated by a variety of ER stress-related stimuli such as amino acid starvation, the herbicide glyphosate, UV and cold stress, wounding, and SA (Lageix *et al.*, 2008), infection with pathogens *Bemisia tabaci* (Li *et al.*, 2018), *Hyaloperonospora arabidopsidis*, *Golovinomyces cichoracearum*, *Pectobacterium carotovorum* subsp. *carotovorum* (Liu *et al.*, 2015*a*), and various strains of *Pseudomonas syringae* (Monaghan and Li, 2010; Liu *et al.*, 2019), as well as developmental processes such as seed germination (Liu *et al.*, 2015*b*) (Fig. 3).

Unlike the other two branches of the UPR, which in general have been associated with a variety of biotic and abiotic stresses (Qiang *et al.*, 2012; Verchot, 2016; Fan *et al.*, 2018), the AtbZIP17/AtbZIP28 pathway has mainly been implicated in heat stress. Heat induces an increase in AtbZIP28 transcripts as well as stimulating its proteolytic cleavage and nuclear translocation (Gao *et al.*, 2008). In addition, the *atbzip28* mutant displays a marked sensitivity to heat stress (Gao *et al.*, 2008). AtbZIP17 was reported to be activated in response to salt and osmotic stress (Liu *et al.*, 2007*b*) (Fig. 3).

While not directly implicated in any specific biotic stress, AtbZIP28 has been shown to be linked to PAMP (pathogen-associated molecular pattern)-triggered immunity. Bacterial elicitor flg22 induces an accumulation of ER chaperone transcripts by down-regulating WRKY7, WRKY11, and

Box 1. Key developments in understanding the function of ER stress signaling in plant development and stress responses

- In a recent study, Liu *et al.* (2019) showed that bacterial infection activates the Arabidopsis General Control Non-derepressible 2 (GCN2) kinase pathway, leading to AtGCN2-mediated phosphorylation of eIF2α and uORF-mediated translational de-repression of the transcription factor TBF1, which regulates ER stress responses and immunity. This study provides the first line of evidence that plants contain the eIF2α-mediated signaling arm of the UPR. AtGCN2 also plays a role in abscisic acid homeostasis and stomatal immunity by regulating stomatal closure and reopening in response to pathogenic bacteria or their toxin coronatine, respectively.
- Bao *et al.* (2019). recently showed that the UPR is required for normal growth and development of plants. In their study, the triple mutant *ire1a ire1b bzip17* showed impaired growth under normal growth conditions, and was also defective in stress signaling pathways. They found that mobilization of bZIP17 into the nucleus is required for normal growth and development.
- Lai *et al.* (2018*a*) showed that NPR1 (Non-Expressor of PR1 genes 1; a master transcriptional immune co-regulator and one of the salicylic acid receptors) negatively regulates the transcriptional regulation of bZIP60 and bZIP28. They also demonstrated that NPR1 can translocate to the nucleus and physically interact with bZIP60 and bZIP28, helping alleviate ER stress.
- Through micro-grafting experiments, Lai *et al.* (2018*b*) demonstrated that Arabidopsis UPR signaling has both local (cell-autonomous) and systemic (non-cell-autonomous) components. Spliced bZIP60 can translocate from roots into shoots and participate in systemic propagation of UPR signaling.
- Bao *et al.* (2018) established that the functional endonuclease domain of IRE1b and RNase activity, but not its kinase domain and bZIP60 splicing, are required for activation of autophagy. They also showed that, upon ER stress, the RNase activity directs a promiscuous splicing and degradation of mRNAs encoding secreted proteins in a process termed RIDD (regulated IRE1-dependent decay). They identified and tested 12 gene targets of RIDD and concluded that three of them (*BGLU21*, *ML*, and *PR-14*) are involved in down-regulating autophagy by overexpression.
- Kim *et al.* (2018) studied the roles of bZIP17 in Arabidopsis development using genetic and genomic approaches. Unlike bZIP28 and bZIP60, bZIP17 is not a major UPR activator, but it functions in concert with bZIP28 to control genes required for development, especially root elongation and stress maintenance.

WRKY17 TFs that repress the expression of AtbZIP28, which then up-regulates those ER chaperone genes (Arrano-Salinas *et al.*, 2018).

Concluding remarks

The field of plant UPR has enjoyed a rapid expansion during the past decade. Multiple signaling aspects have been elucidated, novel players have been discovered, and additional plant species have joined Arabidopsis as models to study ER stress, as highlighted in Box 1. These findings highlight the need to further explore the multifaceted interactions of ER signaling. The next challenge will be to identify connections and cross-talk between the pathways to understand how the plant cells integrate the abundance of qualitative and quantitative, biotic and abiotic stimuli to respond with an appropriate pro-survival or pro-death program. The ultimate goal is to extrapolate this knowledge for translational studies to develop and deploy crops with superb ER stress tolerance and yield performance in the field.

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