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REVIEW

Comparison and contrast of plant, yeast, and mammalian ER stress and UPR

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Abstract The endoplasmic reticulum (ER) is a well-characterized protein folding mechanism in eukaryotic organisms. Many secretory and membrane proteins are folded in the ER before they are translocated to their functional destination. Various conditions, such as biotic, abiotic, or physiological stresses, lead to the accumulation of unfolded and misfolded proteins in the ER, resulting in ER stress. In response to ER stress, cells initiate a protective response called the unfolded protein response (UPR) to maintain cellular homeostasis. Previous studies suggest that inositol-requiring kinase 1 (IRE1) is a universal ER stress sensor in yeast, mammals, and plants. IRE1-mediated splicing of UPR transducers, such as HAC1, XBP1, and bZIP60, triggers the UPR in yeast, mammals, and plants, respectively. In mammals, activated transcription factor 6 and double stranded RNA-activated protein kinase-like ER kinases are involved in the UPR. In plants, the additional UPR transducers bZIP28 and bZIP17 are activated by Golgi-localized S1P and S2P proteases. Subsequently, these UPR transducers are exported to the nucleus and upregulate the expression of UPR-responsive genes encoding BiP, calreticulin, calnexin, protein disulfide isomerase, and glucose-regulated protein 94 to decrease the amount of misfolded proteins and induce endoplasmic reticulum-associated degradation. In plants, the UPR signaling pathway plays an important role in ER homeostasis and normal biological processes; however, the molecular mechanisms of the UPR in plants remain poorly understood. This paper provides an overview of the regulatory and signaling mechanisms of the UPR across kingdoms. In addition, the emerging role of the UPR in plant physiology and defense response will be discussed.

Keywords UPR · ER stress · IRE1

Introduction

Maintaining the balance between protein folding demand and folding capacity is necessary for proper plant growth and development (Deng et al. 2013). The coordinated action of a complex orchestra of chaperones regulates the protein folding capacity of the endoplasmic reticulum (ER). The predominant protein folding catalysts are binding protein (BiP), glucose-regulated Protein 94, protein disulfide isomerase (PDI), peptidyl propyl isomerase (PPI), calnexin, and calreticulin (Del Bem 2011). During polypeptide folding, assembly, and disassembly, different chaperones increase the overall efficiency of protein folding by recognizing and stabilizing partially folded intermediates (Gupta and Tuteja 2011). Disruption of protein folding balance is caused by biotic or abiotic stresses and leads to the accumulation of misfolded or unfolded proteins in the ER, thereby creating ER stress. This results in an

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increase in cell damage followed by activation of the endoplasmic reticulum quality control system (ERQC). The accumulation of unfolded or misfolded proteins induces ER-associated degradation (ERAD). Programmed cell death (PCD) is one kind of ERAD response (Liu and Li 2014; Williams et al. 2014). However, cells can also initiate a protective response to maintain cellular homeostasis called the ER stress response or unfolded protein response (UPR) (Iwata et al. 2010). UPR upregulates the expression of genes that improve protein folding capacity or remove unfolded or misfolded proteins from the ER, and interacts at many levels with the processes of adaptive immunity in mammals or innate immunity in invertebrates (Reimold 2001; Iwakoshi 2003; Richardson et al. 2010). Although the molecular mechanisms of UPR have been described mainly in yeast and mammals, in plants, studies utilizing the N-linked glycosylation inhibitor tunicamycin (Tm) have identified analogous pathways for quality control of proteins and the UPR (Iwata et al. 2010).

Activation of the signaling pathway between the ER and nucleus that induces the transcription of genes encoding ER protein quality control molecules generally initiates from inositol-requiring kinase 1 (IRE1), which is known to play an important role in the UPR (Nagashima et al. 2011). IRE1 is an ER-resident type-1 transmembrane protein with a sensor domain on its luminal surface and an RNase domain on its cytoplasmic surface. The function of IRE1 is based on its ability to sense ER stress and mediate unconventional mRNA splicing of ERQC-related proteins, such as HAC1 in yeast or XBP1 in mammals (Moreno and Orellana 2011). Upon ER stress, IRE1 oligomers activate its RNase activity by autophosphorylation.

IRE1-mediated UPR in yeast, mammals, and plants

Gene expression profiling studies indicate that the UPR regulates a variety of genes involved in specific secretory pathway-related processes, including protein entry into the ER, folding, glycosylation, redox metabolism, protein quality control, protein degradation, lipid biosynthesis, and vascular trafficking (Martínez and Chrispeels 2003). The UPR mechanism in mammals and plants is more complex than in yeast (Deng et al. 2011).

IRE1 has important functions in the UPR in all eukaryotic cells. It was first identified in yeast as a mRNA splicing factor (Cox and Walter 1996; Sidrauski and Walter 1997), and has two homologs in mammals, IRE1α and IRE1β. IRE1 is bifunctional, as it contains two different functional domains: an ER luminal stress sensing domain and a cytosolic protein kinase and endoribonuclease domain (RNase domain) (Sidrauski and Walter 1997; Zhou et al. 2006). The RNase domain of IRE1 has mRNA splicing activity and produces active transcription factors, such as bZIP60 in plants, HAC1 in yeast, and XBP1 in mammals. The activity of the IRE1 RNase domain is dependent on the protein kinase domain and endoribonuclease activity of IRE1, which is essential for regulation of RNA stability (Welihinda and Kaufman 1996).

The luminal domain of IRE1 can be di- or oligomerized in ER-stressed cells. When the unfolded protein concentration is low in the ER, BiP associates with the luminal domain of IRE1 to maintain it in an inactive state. However, in response to ER stress, BiP disassociates from IRE1 and interacts with unfolded proteins, and the luminal domain of IRE1 forms dimers or oligomers (Li et al. 2010).

In mammalian cells, IRE1α can auto-regulate its own mRNA expression levels in the ER through endonucleolytic activity (Li et al. 2012), and IRE1β attenuates its own translation through degradation of 28S ribosomal RNA by endonucleolytic cleavage (Iwawaki et al. 2001). Human IRE1β (hIRE1β)-mediated 28S rRNA cleavage may then lead to translational repression during ER stress, which decreases the demand for protein folding in the ER. This is a rational response to relieve cells from ER stress. While mammalian IRE1α is ubiquitously expressed, expression of IRE1β is limited to the epithelium of the gastrointestinal tract (Iwata et al. 2008). The signaling pathway mediated by IRE1–XBP1 is involved in several human pathological conditions, including neurodegenerative diseases, liver dysfunction, metabolic disorders, inflammation, brain and heart ischemia, and cancer. Targeting this pathway has emerged as a promising therapeutic strategy against these diseases (Koong et al. 2006; Lipson et al. 2006; Hetz et al. 2013).

IRE1a (IRE1-2) and IRE1b (IRE1-1) in plants are orthologs of mammalian IRE1α and IRE1β. *Arabidopsis* IRE1a (AtIRE1a) and IRE1b (AtIRE1b) are encoded by 841 and 885 amino acid open reading frames, respectively. Both AtIRE1a and AtIRE1b are found in the peri-nuclear ER (Chen and Brandizzi 2012), but, while AtIRE1a protein is capable of autophosphorylation, AtIRE1b is not. The different functions of IRE1a and IRE1b may have consequences on their dissimilar protein kinase-activation loops. Although both IRE1a and IRE1b are responsible for mRNA splicing, IRE1b is primarily responsible for mRNA splicing in seedlings (Humbert et al. 2012). AtIRE1a is widely expressed in vascular bundles of young plants, leaves, roots, seeding, receptacles of flowers, and vascular bundles of petals, while the expression of AtIRE1b is localized to certain tissues at specific developmental stages; it is found in the apical meristem in leaf margins (where vascular bundles end), the anther before pollen is formed, the ovules at an early stage of development, and the cotyledons immediately after germination. Recent

studies reveal that, in *Arabidopsis*, the C-terminal protein kinase and RNase domains of IRE1b are important for vegetative growth under normal conditions, but that only the RNase domain is required for ER stress tolerance under stress conditions (Deng et al. 2013). IRE1 plays a significant role in plant defense and is required for pathogenesis-related 1 (PR1) protein secretion (Srivastava et al. 2013). It was reported recently that cell death was enhanced in two *Arabidopsis* mutants, *ire1a* and *ire1b*-, under ER stress conditions (Mishiba et al. 2013). In addition, AtIRE1a and AtIRE1b are specifically involved in plant root growth (Chen and Brandizzi 2012). Recently, a connection between phytohormone auxin signaling and the UPR was identified. In these studies, the auxin transporter and receptor were downregulated in response to ER stress in *Arabidopsis*. Auxin is a plant growth substance synthesized in the plant apices of shoots and roots. Auxin levels are lower in IRE1 mutants than in wild-type plants, suggesting that plant UPR has evolved a hormone-dependent strategy for coordinating ER function with physiological process (Chen et al. 2014).

Yeast UPR

Based on the results of microarray analysis, 381 of the 6607 yeast genes identified in the *Saccharomyces cerevisiae* genome are putative UPR target genes that are regulated under ER stress conditions (Travers et al. 2000). These UPR target genes are not only ER chaperones, but also proteins acting at several stages of folding, glycosylation, modification, translation, protein degradation, vesicle trafficking, vascular protein sorting, cell wall biosynthesis, and lipid metabolism (Liu et al. 2007).

The mechanism of the UPR in yeast is relatively simple compared with that in mammals and plants. In yeast, the UPR is mediated only by IRE1p (an ortholog of mammalian IRE1), whereas it can be initiated by both activated transcription factor 6 (ATF6) and PERK in mammals or bZIP28 and bZIP17 in plants as well as by IRE1. IRE1p is activated by dissociation from BiP in response to stress. The amino terminal domain of IRE1p resides in the ER lumen, and is believed to sense unusually high levels of unfolded ER proteins and eventually activate the UPR-related transcription factor, HAC1 (Patil and Walter 2001).

HAC1 mRNA contains a non-classical intron near the 3′ end of the open reading frame containing the carboxy-terminal 10 amino acids and the stop codon of the predicted protein (Fig. 1). Upon activation of the UPR in yeast, 252-nucleotide intronic sequence is spliced out from the *HAC1* mRNA by IRE1p. The 5′ and 3′ portions of the mRNA are joined by tRNA ligase (Sidrauski et al. 1996). This spliced HAC1 (HAC1 s) encodes a HAC1 protein comprised 238 amino acids, while the unspliced HAC1 (HAC1u) protein contains only 230 amino acids. The HAC1s protein contains an 18 amino acid sequence that is important for transcription factor activation; however, it is missing in the HAC1u protein (Mori 2009). Deletion of the *HAC1* intron not only allows translation of the mRNA, but also changes the sequence and properties of the encoded protein. The N-terminal DNA-binding domain has 220 amino acids common to both the HAC1s and HAC1u proteins. The DNA-binding domain is not disturbed by the splicing reaction, but the *trans*-activation domain includes the C-terminal tail. HAC1s acted as a highly active transcriptional activation domain when its C-terminal tail was fused to an unrelated DNA-binding domain, while the HAC1u tail was essentially inactive (Kawahara et al. 1997; Mori et al. 2000). Activated HAC1 (HAC1p) is translated only from *HAC1s* mRNA. Activated HAC1p binds to unfolded protein response elements (UPREs) to promote transcription of UPR target genes. UPREs are found in the promoters of several UPR target genes, such as the ER-resident chaperones KAR2, PDI1, and FKB2 (Ron and Walter 2007).

Mammalian UPR

Mammalian cells have three ER stress signaling arms: IRE1-mediated splicing activation of XBP1 mRNA, (ATF6), and double-stranded RNA-activated protein kinase (PERK).

IRE1-mediated splicing of XBP1

Upon perception of ER stress, X-box binding protein 1 (XBP1) is spliced by IRE1 to produce the bZIP transcription factor. The RNase domain of IRE1 catalyzes spliceosome-independent splicing of XBP1 mRNA. Unspliced XBP1 (XBP1u) encodes a 261 amino acid long ORF that generates a non-functional protein (Liu et al. 2007). Conversely, spliced XBP1 mRNA produces the XBP1s protein, which is 376 amino acids long with a diverse and extended amino acid sequence at the C-terminus (Kim and Jung 2014).

XBP1 can bind with co-regulator NF-Y in vitro (Yoshida et al. 2001), but it is not clear whether the activated XBP1 (XBP1s) is exported into the nucleus along with NF-Y. XBP1s regulates the expression of a variety of ER chaperones and genes that mediate protein degradation by binding to ER stress response elements (ERSEs) or UPRE (Fig. 2) (Malhotra and Kaufman 2007). XBP1s regulates the expression of a subset of UPR-inducing genes, including $p58^{IPK}$, ERdj4, H EDJ, and PDI-P5,

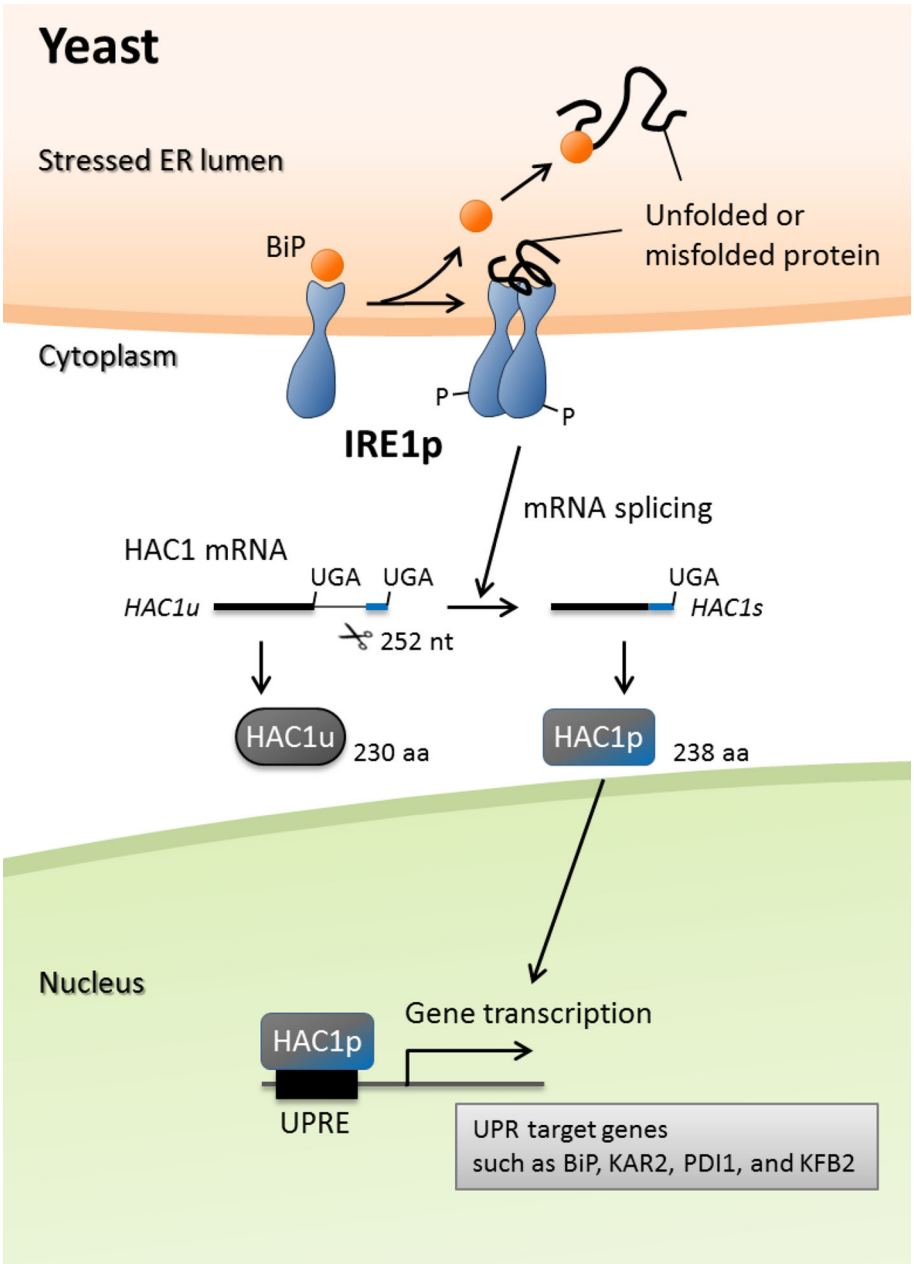

Fig. 1 A schematic illustration of the unfolded protein response in yeast. Under normal conditions, ER membrane-localized yeast IRE1 is bound to BiP at the N-terminal region located in the ER lumen, with the C-terminus facing into the cytosol. Upon the accumulation of unfolded proteins in the ER lumen, IRE1 is released from BiP and activated by autophosphorylation and dimerization. Activated IRE1 splices HAC1 mRNA and removes 253 nucleotides including stop codon. *HAC1s* ligated by tRNA ligase possesses a longer ORF than unspliced HAC1 mRNA (*HAC1u*). HAC1p is exported to the nucleus and binds with the UPRE region of UPR-responsive genes

which were identified in mouse embryonic fibroblasts (MEFs) and participate in protein folding quality control and protein degradation (Li et al. 2010). In addition, XBP1 s indirectly controls organelle biosynthesis of the ER and Golgi by enhancing the activity of specific enzymes related to phospholipid biosynthesis (Iwata et al. 2010). Activation of XBP1 upregulates expression of the DNAJ family protein $p58^{IPK}$, which negatively regulates the expression of PERK, one of the major branches of the mammalian UPR (Yan et al. 2002). The effects of XBP1 on organelle biosynthesis can also be observed in other organelles, including lysosomes and mitochondria, as evidenced by increased cell size (Iwata et al. 2010). Analysis of gene expression in neurons indicated that XBP1s may control distinct sets of genes in different cell types (Kakiuchi et al. 2006).

Activated transcription factor (ATF6)

ATF6 is a type-2 transmembrane protein that contains a bZIP transcription factor domain in the cytosolic region of the protein. Generally, ATF6 is synthesized as an inactive precursor and retained in the ER by association with BiP/GRP78 (Harding et al. 2001). In response to stress conditions, ATF6 disassociates from BiP/GRP78 and is transported to the Golgi apparatus, where proteolytic processing occurs via

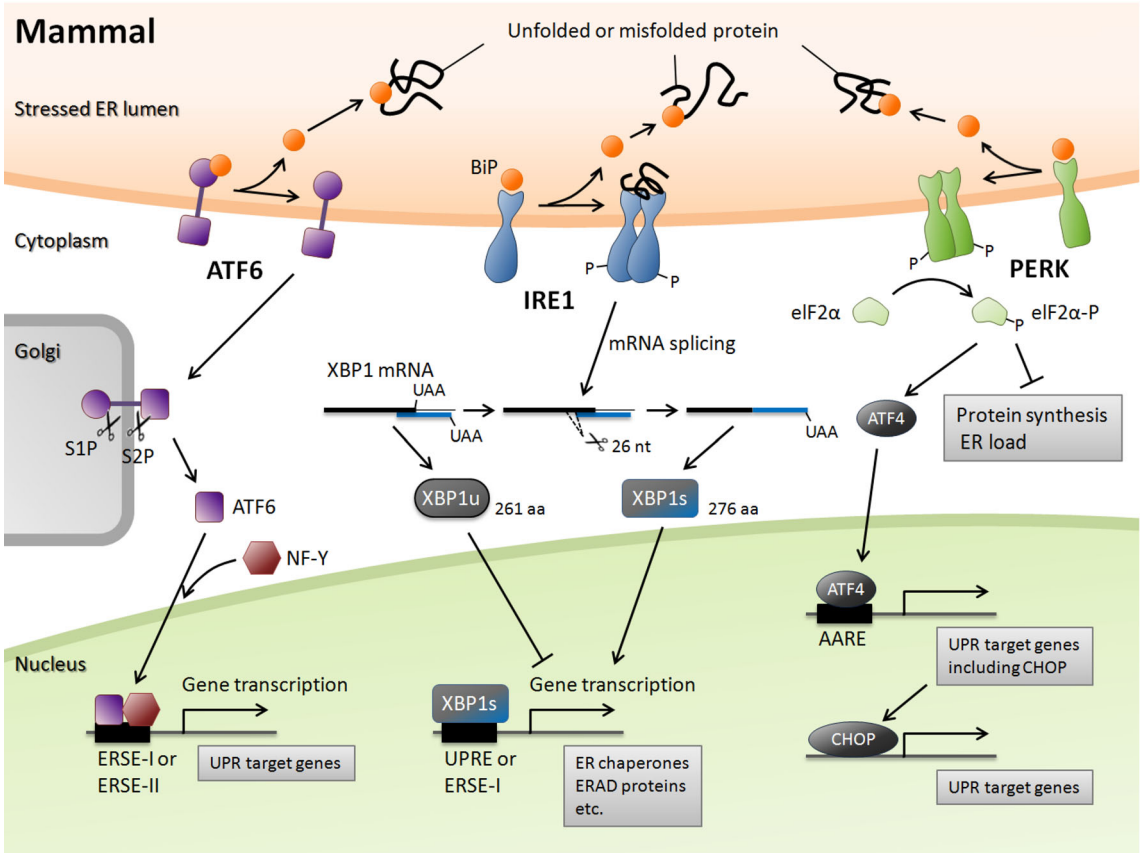

Fig. 2 A schematic illustration of the unfolded protein response in mammals. IRE1 splices 26 nucleotides in XBP1 mRNA in the mammalian UPR. ATF6 is activated in the Golgi by S1P and S2P. The N-terminal domain of ATF6 is translocated to the nucleus for the induction of UPR gene expression. PERK activates UPR genes and functions in translational attenuation

serine protease site I protease (S1P) in the C-terminal region and metalloprotease S2P in the intra-membrane region of ATF6 (Fig. 2). The N-terminal domain of ATF6 contains bZIP a DNA-binding domain and transcription activation domain and is exported to the nucleus where it binds to the upstream promoter region cis-elements of ER stress-responsive genes, such as BiPs, to activate their transcription, and upregulates many UPR-responsive genes related to protein folding and ERAD. These promoter-localized cis-elements are also called ERSEs (Yoshida et al. 1998). There are three types of ERSEs: ERSE-1 (CCAAT-N9-CCACG), ERSE-2 (ATTGG–NCCACG), and XBP1-BS or UPRE (GA-TGACGT-T/G). Mammalian UPRE (TGACGTG-T/G) is the specific cis-acting regulatory element bound by XBP1 and is referred to as the XBP1 binding site. ERSE-1 (CCAAT-N9-CCACG) is recognized by both ATF6 and XBP1 in vitro (Yoshida et al. 1998), and ERSE-2 (ATTGG-N-CCACG) is also a target of ATF6 (Kokame et al. 2001). Together with the transcription factor NF-Y, stress-activated ATF6 binds to cis-elements in the promoter region, resulting in the transcription of downstream genes. In mammals, NF-Y is a heterotrimeric complex comprised three conserved subunits: NF-YA, NF-YB, and NF-YC (Gusmaroli et al. 2001). ATF6 upregulates BiP, PDI, ER degradation-enhancing alpha-mannosidase-like protein1(EDEM1) expression (Chakrabarti et al. 2011).

Double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK)

PERK is a type-1 ER transmembrane protein that contains an ER luminal stress sensor and cytosolic protein kinase domain. PERK is activated in response to ER stress and inhibits normal protein translation in the ER of mammalian cells by inactivating eukaryotic initiation factor (eIF2α) via serine 51 phosphorylation (Iwata et al. 2010). The three-subunit eIF2α protein is essential for translation initiation of eukaryote genes, including GTP-dependent start site recognition (Merrick 2004). Phosphorylation of the α subunit of eIF2 blocks the exchange of GDP to GTP, and, as a result, initiation of translation is blocked (Gebauer and Hentze 2004). Eventually, the inhibitory effect on translation resulting from phosphorylation of eIF2α helps alleviate ER stress by decreasing the amount of unfolded

proteins (Fig. 2; Ye et al. 2000). In addition, eIF2α phosphorylation increases the translation of specific mRNAs, such as activating transcription factor 4 (ATF4), that contain inhibitory regulator sequences within their 5′ untranslated region (UTR) that prevent translation in unstressed cells (Schröder and Kaufman 2005).

Activation of ATF4 by PERK upregulates a subset of UPR genes that function preferentially in amino acid import, glutathione biosynthesis, and resistance to oxidase reactions (Harding et al. 2003). ATF4 binds to AARE (C/EBT-ATF) (TT-G/T-CATCA), an element that was discovered in the promoter of the transcription factor C/EBP homologous protein (CHOP) (Bruhat et al. 2002). CHOP is a 29 kDa protein composed of an N-terminal transcriptional activation domain and a C-terminal bZIP domain. ATF4 induces the expression of CHOP, which is associated with apoptotic cell death (Harding et al. 2000; Bruhat et al. 2002). PERK is also essential for the normal function of secretory cells in the pancreas and skeletal system in mammals (Zhang et al. 2002).

UPR in plants

The membrane-associated ER stress transducers bZIP60, bZIP28, and bZIP17 were identified in *Arabidopsis* (AtbZIP60, AtbZIP28, and AtbZIP17) and are upregulated in response to different stress conditions.

IRE1-mediated splicing of basic leucine zipper 60 (bZIP60)

In Arabidopsis, the ER stress transducer AtbZIP60, an ortholog of HAC1 in yeast and XBP1 in mammals, has been studied in greater depth compared with other transducers. AtbZIP60 mRNA encodes a 295-amino-acid protein with an N-terminal bZIP domain and a C-terminal putative transmembrane domain (TMD) (Urade 2009). Under stress conditions, a 23-nucleotide mRNA fragment of the C-terminal TMD-encoding region is cleaved out by IRE1 (Fig. 3). During proteolytic release, IRE1 attaches to the *bZIP60* mRNA hairpin loop. Activated bZIP60 translated from the spliced mRNA is exported to the nucleus where it binds to UPREs or ERSEs in the promoter regions of UPR-related genes. The expression of ER stress-responsive genes, such as members of the BiP family, was less strongly induced in the *bzip60* mutant than in the wild-type under ER stress conditions. Additionally, overexpression of bZIP60 increased the full length bZIP60 protein, but the BiP gene was not actively transcribed in the absence of ER stress (Iwata et al. 2008). In maize (*Zea mays*), ZmbZIP60, an ortholog of AtbZIP60, is spliced by IRE1 in response to heat stress, and active ZmbZIP60 is targeted to the nucleus where it mediates the upregulation of 22 *BiP*-like genes (Li et al. 2012). There are two other AtbZIP60 orthologs in tobacco: NtbZIP60 (*Nicotiana tabacum*) and NbbZIP60 (*Nicotiana benthamiana*). However, NtbZIP60 is upregulated only in response to ER stress, whereas other bZIP60s are activated both as part of the plant ER stress response and during anther cell development (Iwata et al. 2008). Expression of bZIP60 in cultured cells also increases their tolerance to salt, drought, and cold stress (Tang and Page 2013). The plant-specific transcription factor NAC103 is induced by ER stress and is dependent on bZIP60 to activate expression of the UPR downstream genes, calreticulin and calnexin (Sun et al. 2013). Exogenous salicylic acid (SA) also activates BiP3 and the UPR-related gene bZIP60, but in an IRE1–bZIP60 pathway-independent manner (Nagashima et al. 2014).

Proteolytic activation of basic leucine zipper 28 (bZIP28)

bZIP28 and bZIP17 are type-2 ER stress transducers in plants. The structure and mode of action of bZIP28 and bZIP17 are similar to those of the mammalian ER stress transducer, ATF6. bZIP28 and bZIP17 are composed of a cytosolic N-terminal bZIP domain, TMD, and C-terminal domain.

Under normal conditions, AtbZIP28 localizes to the ER membrane and binds with BiP. BiP binds to the C-terminal tail of bZIP28 and interacts independently with different areas of the luminal facing tail. In Tm-treated Arabidopsis seedlings, bZIP28 is transported from the ER to the nucleus via the Golgi apparatus (Wang et al. 2007; Srivastava et al. 2014). A broad range of genes involved in ER protein folding and secretion require bZIP28 for full induction, including BiP3, HSP90-like protein, CNX, DNA J domain-containing proteins, and PDI. As with mammalian ATF6, bZIP28 disassociates from BiP in response to Tm-induced stress and translocates to the Golgi apparatus. It is then cleaved by canonical subtilisin-like serine protease (S1P) in the C-terminal region and undergoes putative S2P cleavage at the TMD region (Fig. 3). Recent studies demonstrate that bZIP28 has two S1P cleavage sites at amino acid positions 375 and 573, identified as $RVLM^{373}$ and $RRIL^{573}$, respectively. $RRIL^{573}$ is critical for biological function, while the $RVLM^{373}$ site is less important (Sun et al. 2015). After proteolytic cleavage, activated bZIP28 is exported to the nucleus where it binds to cis-elements on UPR-responsive genes, resulting in the upregulation of target genes. Like mammalian ATF6, bZIP28 forms a transcriptional complex with the NF-YA4/NF-YB3/NF-YC2 trimer at the ERSE-1 region of the target genes (Liu and Howell 2010). There are two ERSE-1 elements in *BiP3*. Both are required for full function, and mutation in

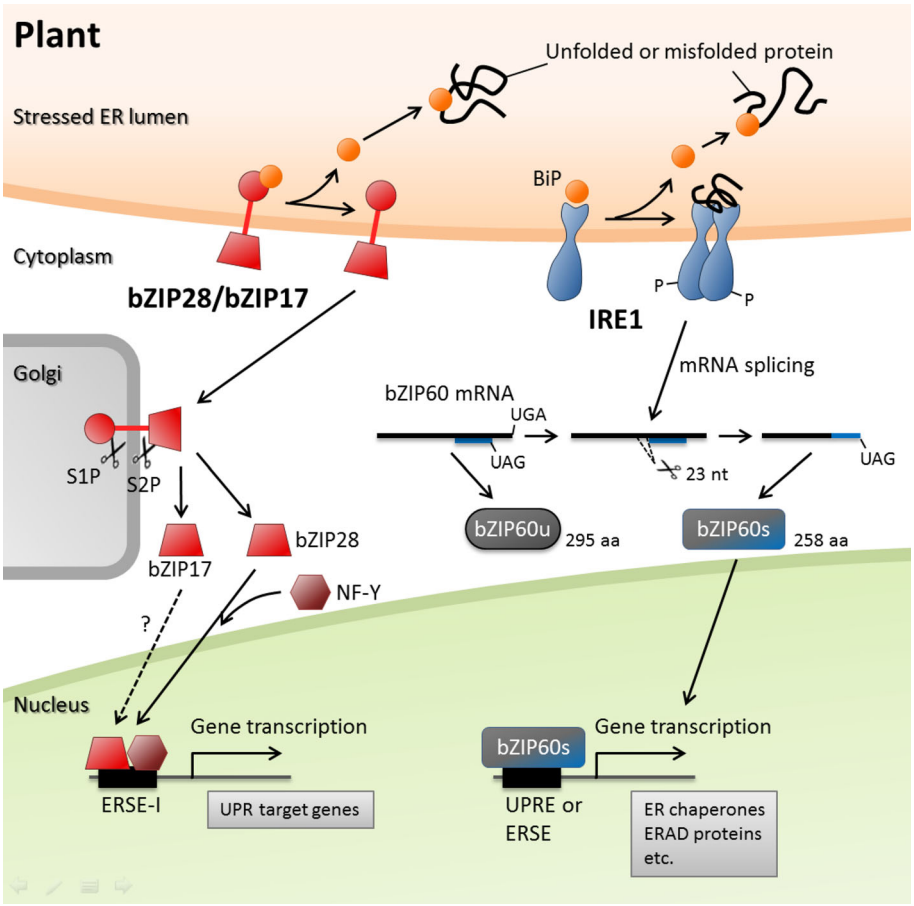

Fig. 3 A schematic illustration of the unfolded protein response in plants. IRE1 splices 23 nucleotides in bZIP60 mRNA in the plant UPR. bZIP28 and bZIP17 are activated in the Golgi by S1P and S2P. The N-terminal domains of bZIP28 and bZIP17 are translocated to the nucleus, where bZIP28, together with NF-Y, induces the expression of UPR genes. The function of bZIP17 in the UPR is not clear. No homolog of PERK has been identified in plants

one of the ERSE-1 elements resulted in a partial increase in transcriptional activity (Iwata et al. 2009). The *Arabidopsis* NF-Y subunits are also known as heme-activator proteins (HAPs): HAP29 (NF-YA), HAP3 (NF-YB), and HAP5 (NF-YC) (Edwards et al. 1998; Gusmaroli et al. 2001). All of the NF-Y subunits are essential for embryo development, plastid biogenesis, flowering time regulation, and biotic stress tolerance (Nelson et al. 2007). In addition, UPR genes can be upregulated even in the absence of stress by overexpression of active bZIP28 (Liu et al. 2007).

It was recently shown that bZIP28 can be activated by SA (Nagashima et al. 2014). However, recent studies suggest that bZIP17 and bZIP60 also play a role in the response to salt stress, but this response goes through a signaling pathway different than that triggered by the UPR (Henriquez-Valencia et al. 2015).

Basic leucine zipper 17 (bZIP17)

Activation of AtbZIP17 is controlled by heat stress in a manner similar to the regulatory mechanism that controls the UPR (Moreno and Orellana 2011). Under normal ER conditions, the AtbZIP17-GFP fusion protein is localized to the ER. However, in response to salt stress, the fusion protein relocates from the ER to the nucleus. The C-terminal tail of AtbZIP17 is inserted into the ER lumen of the plant cell. Under stress conditions, AtbZIP17 is transported to the Golgi apparatus before being exported to the nucleus. In the Golgi, the C-terminal tail of AtbZIP17 is cleaved by a Golgi-localized protease (AtS1P) and the N-terminus of AtbZIP17 enters the nucleus to activate stress-responsive genes (Liu et al. 2008). Following salt treatment in Arabidopsis seedlings, an 84 kDa myc-tagged AtbZIP17 protein was processed to a 46 kDa protein, consistent with cleavage in or near the TMD (Schütze et al. 2008).

Golgi-localized AtS1P, which is related to mammalian S1P (Liu et al. 2007), has a prodomain structure with an N-terminal signal peptide that targets it to the secretory pathway and a sub-terminal prodomain responsible for activation of proenzymes. AtS1P is responsible for the salt-sensitive phenotype. However, although BiP genes are considered ER stress markers, they are not induced significantly by AtbZIP17-dependent signaling under salt stress conditions (Sun et al. 2013).

IRE1-induced autophagy/ERAD in yeast, mammals, and plants

Under ER stress conditions, autophagy may help remove damaged organelles and abnormal proteins (Bernales et al. 2006). Depletion of IRE1 or HAC1 blocks autophagy as well as Atg proteins, which are induced by ER stress. IRE1- or HAC1-depleted cells are more sensitive to ER stress than Atg-deficient cells, suggesting that IRE1/HAC1 signaling is involved in the induction of autophagy to promote cell survival in yeast (Bernales et al. 2006; Yorimitsu et al. 2006). In mammalian cells, IRE1α is a key regulator of macroautophagy, possibly through activation of the JNK pathway under ER stress conditions. Activation of autophagy in response to ER stress in MEFs is dependent on the kinase domain of IRE1α, but, interestingly, is not affected by the RNAse/XBP1 signaling pathway (Lisbona and Hetz 2009). On the other hand, knockdown of XBP1 leads to an increase in basal autophagy in Drosophila cells as well as neuronal cells and the central nervous system of mice, even in the absence of stress (Arsham and Neufeld 2009; Hetz et al. 2009).

Protein–protein interaction data show that IRE1α physically interacts with ubiquitin-specific protease (USP) and ERAD components (Nagai et al. 2009). XBP1 induces the ERAD components, such as ER degradation-enhancing α-mannosidase-like protein 1 (EDEM1), under ER stress conditions. Impairment of ERAD activity by XBP1 deficiency is associated with enhanced autophagy in neurons. In this scenario, accumulation of abnormally folded proteins in the ER due to impaired ERAD activity may operate as a signal to induce autophagy (Matus et al. 2009). Recent data show that autophagosome formation occurred in Arabidopsis seedlings in response to ER stress. Arabidopsis seedlings harboring knockdown mutations in IRE1b failed to form autophagosomes in response to ER stress, suggesting that IRE1b is a key component in the signaling pathway that connect ER stress to autophagy (Liu et al. 2012). Interestingly, autophagosome formation was not blocked by knockout of bZIP60, suggesting that a function of IRE1b other than its RNA-splicing capacity connects ER stress to autophagy.

Concluding remarks and future perspectives

UPR is a compound response mediated by multiple signal transducers in a pathway essential for the survival of organisms under stress conditions and the recovery of ER homeostasis. Under stress conditions, UPR genes are upregulated in yeast, mammals, and plants (summarized in Table 1). In plants, IRE1-mediated splicing of the bZIP60 nucleotide sequence is different in monocot and dicot plants. Activation of the IRE1-mediated apoptosis signal-regulating kinase1 (ASK1)-JNK signaling cascade and degradation of mRNA encoding secretory proteins through regulated IRE1-dependent decay (RIDD) are responsible for decreasing the unfolded protein load under ER stress conditions. In plants, bZIP60, bZIP28, and bZIP17 are activated to induce the expression of ER stress-responsive genes, but it remains unclear which UPR genes are induced by each individual UPR sensor. IRE1 is a universal UPR sensor in yeast, mammals, and plants. In plants, activation of bZIP60 and bZIP28 occurs in a process similar to that responsible for activation of mammalian XBP1 and ATF6, respectively. The IRE1-mediated cleavage position in

Table 1 Comparison summary of the UPR in yeast, mammals, and plants

UPR arms	Yeast	Mammal	Plant
IRE1-mediated mRNA splicing of HAC1, XBP1, and bZIP60	HAC1 spliced by IRE1p binds to UPRE to upregulate UPR gene expression Spliced HAC1 is longer than unspliced HAC1	XBP1 spliced by IRE1α binds to UPRE or ERSE-I to upregulate UPR gene expression Spliced XBP1 is longer than unspliced XBP1	bZIP60 splicing by IRE1 binds to UPRE or ERSE to upregulate UPR gene expression Spliced bZIP60 is shorter than unspliced bZIP60
Proteolytic activation of ATF6, bZIP28, and bZIP17 by S1P and S2P	–	ATF6 cleaved by S1P and S2P at Golgi binds to ERSE-I or ERSE-II together with NF-Y	bZIP28 and bZIP17 cleavage by S1P and S2P at the Golgi bZIP28 translocates to the nucleus and binds to ERSE-I to upregulate UPR gene expression
PERK-mediated phosphorylation of eIF2α and translational control	–	eIF2α phosphorylated by PERK decreases global protein translation to reduce ER unfolded protein load and control translation of UPR genes. ATF4 activated by phosphorylated eIF2α binds to AARE to upregulate CHOP	–

bZIP60 in plants is still unclear at present. In addition, a homolog of PERK, part of the mammalian ER stress response, has not yet been identified in plants (Fig. 2). However, the kinase AtGCN2IS was identified in plants in response to plant starvation, and is capable of phosphorylation activity similar to that of mammalian elF2α (Zhang et al. 2008). Cleavage of *Arabidopsis* bZIP60 mRNA by SA treatment and *agb1-2* mutant plant (G-protein beta subunit null mutation) show less cell death in response to ER stress, suggesting that plants may possess an IRE1a/IRE1b-independent UPR pathway (Iwata and Koizumi 2005; Wang et al. 2007; Nagashima et al. 2014). However, the mechanisms of ERAD, ERQC, and ER stress-induced autophagy/PCD are mostly unknown in plants. Current research will delineate the function of the UPR in relation to plant defense systems and other biological processes in the plant ER.

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Compliance with ethical standards

Conflict of interest All authors discussed and agreed on the contents of the paper and have no conflicts of interest to declare.

References

Arsham AM, Neufeld TP (2009) A genetic screen in Drosophila reveals novel cytoprotective functions of the autophagy-lysosome pathway. PLoS One 4(6):e6068

Bernales S, McDonald KL, Walter P (2006) Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. PLoS Biol 4(12):e423

Bruhat A, Averous J, Carraro V, Zhong C, Reimold AM, Kilberg MS, Fafournoux P (2002) Differences in the molecular mechanisms involved in the transcriptional activation of the CHOP and asparagine synthetase genes in response to amino acid deprivation or activation of the unfolded protein response. J Biol Chem 277(50):48107–48114

Chakrabarti A, Chen AW, Varner JD (2011) A review of the mammalian unfolded protein response. Biotechnol Bioeng 108(12):2777–2793

Chen Y, Brandizzi F (2012) AtIRE1A/AtIRE1B and AGB1 independently control two essential unfolded protein response pathways in Arabidopsis. Plant J 69(2):266–277

Chen Y, Aung K, Rolčík J, Walicki K, Friml J, Brandizzi F (2014) Inter-regulation of the unfolded protein response and auxin signaling. Plant J 77(1):97–107

Cox JS, Walter P (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell 87(3):391–404

Del Bem LE (2011) The evolutionary history of calreticulin and calnexin genes in green plants. Genetica 139(2):255–259

Deng Y, Humbert S, Liu J-X, Srivastava R, Rothstein SJ, Howell SH (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 108(17):7247–7252

Deng Y, Srivastava R, Howell SH (2013a) Endoplasmic reticulum (ER) stress response and its physiological roles in plants. Int J Mol Sci 14(4):8188–8212

Deng Y, Srivastava R, Howell SH (2013b) Protein kinase and ribonuclease domains of IRE1 confer stress tolerance, vegetative growth, and reproductive development in Arabidopsis. Proc Natl Acad Sci USA 110(48):19633–19638

Edwards D, Murray JA, Smith AG (1998) Multiple genes encoding the conserved CCAAT-box transcription factor complex are expressed in Arabidopsis. Plant Physiol 117(3):1015–1022

Gebauer F, Hentze MW (2004) Molecular mechanisms of translational control. Nat Rev Mol Cell Biol 5(10):827–835

Gupta D, Tuteja N (2011) Chaperones and foldases in endoplasmic reticulum stress signaling in plants. Plant Signal Behav 6(2):232–236

Gusmaroli G, Tonelli C, Mantovani R (2001) Regulation of the CCAAT-Binding NF-Y subunits in Arabidopsisthaliana. Gene 264(2):173–185

Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 6(5):1099–1108

Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD, 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(6):1153–1163

Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 11(3):619–633

Henriquez-Valencia C, Moreno AA, Sandoval-Ibañez O, Mitina I, Blanco-Herrera F, Cifuentes-Esquivel N, Orellana A (2015) bZIP17 and bZIP60 regulate the expression of BiP3 and other salt stress responsive genes in an UPR-independent manner in Arabidopsis thaliana. J Cell Biochem 116(8):1638–1645

Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, Martinez G, Cuervo AM, Brown RH, Glimcher LH (2009) XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Genes Dev 23(19):2294–2306

Hetz C, Chevet E, Harding HP (2013) Targeting the unfolded protein response in disease. Nat Rev Drug Discov 12(9):703–719

Humbert S, Zhong S, Deng Y, Howell SH, Rothstein SJ (2012) Alteration of the bZIP60/IRE1 pathway affects plant response to ER stress in Arabidopsis thaliana. PLoS One 7(6):e39023

Iwakoshi NN (2003) Plasma cell differentiation and the Unfolded Protein Response intersect at the transcription factor XBP-1. Nat Immunol 4:321–329

Iwata Y, Koizumi N (2005) An Arabidopsis transcription factor, AtbZIP60, regulates the endoplasmic reticulum stress response in a manner unique to plants. Proc Natl Acad Sci USA 102(14):5280–5285

Iwata Y, Fedoroff NV, Koizumi N (2008) Arabidopsis bZIP60 is a proteolysis-activated transcription factor involved in the endoplasmic reticulum stress response. Plant Cell 20(11):3107–3121

Iwata Y, Yoneda M, Yanagawa Y, Koizumi N (2009) Characteristics of the nuclear form of the Arabidopsis transcription factor AtbZIP60 during the endoplasmic reticulum stress response. Biosci Biotechnol Biochem 73(4):865–869

Iwata Y, Nishino T, Takayama S, Koizumi N (2010a) Characterization of a plant-specific gene induced by endoplasmic reticulum

stress in Arabidopsis thaliana. Biosci Biotechnol Biochem 74(10):2087–2091
Iwata Y, Sakiyama M, Lee M-H, Koizumi N (2010b) Transcriptomic response of Arabidopsis thaliana to tunicamycin-induced endoplasmic reticulum stress. Plant Biotechnol 27(2):161–171
Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, Kimata Y, Tsuru A, Kohno K (2001) Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. Nat Cell Biol 3(2):158–164
Kakiuchi C, Ishiwata M, Hayashi A, Kato T (2006) XBP1 induces WFS1 through an endoplasmic reticulum stress response element-like motif in SH-SY5Y cells. J Neurochem 97(2): 545–555
Kawahara T, Yanagi H, Yura T, Mori K (1997) Endoplasmic reticulum stress-induced mRNA splicing permits synthesis of transcription factor Hac1p/Ern4p that activates the unfolded protein response. Mol Biol Cell 8(10):1845–1862
Kim HS, Jung G (2014) Reactive oxygen species increase HEPN1 expression via activation of the XBP1 transcription factor. FEBS Lett 588(23):4413–4421
Kokame K, Kato H, Miyata T (2001) Identification of ERSE-II, a new cis-acting element responsible for the ATF6-dependent mammalian unfolded protein response. J Biol Chem 276(12): 9199–9205
Koong AC, Chauhan V, Romero-Ramirez L (2006) Targeting XBP-1 as a novel anti-cancer strategy. Cancer Biol Ther 5(7):756–759
Li H, Korennykh AV, Behrman SL, Walter P (2010) Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proc Natl Acad Sci USA 107(37):16113–16118
Li Y, Humbert S, Howell SH (2012) ZmbZIP60 mRNA is spliced in maize in response to ER stress. BMC Res Notes 5:144
Lipson KL, Fonseca SG, Ishigaki S, Nguyen LX, Foss E, Bortell R, Rossini AA, Urano F (2006) Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. Cell Metab 4(3):245–254
Lisbona F, Hetz C (2009) Turning off the unfolded protein response: an interplay between the apoptosis machinery and ER stress signaling. Cell Cycle 8(11):1641–1644
Liu JX, Howell SH (2010) bZIP28 and NF-Y transcription factors are activated by ER stress and assemble into a transcriptional complex to regulate stress response genes in Arabidopsis. Plant Cell 22(3):782–796
Liu Y, Li J (2014) Endoplasmic reticulum-mediated protein quality control in Arabidopsis. Front Plant Science 5:162
Liu JX, Srivastava R, Che P, Howell SH (2007a) 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(12):4111–4119
Liu JX, Srivastava R, Che P, Howell SH (2007b) Salt stress responses in Arabidopsis utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. Plant J 51(5):897–909
Liu JX, Srivastava R, Howell SH (2008) Stress-induced expression of an activated form of AtbZIP17 provides protection from salt stress in Arabidopsis. Plant Cell Environ 31(12):1735–1743
Liu Y, Burgos JS, Deng Y, Srivastava R, Howell SH, Bassham DC (2012) Degradation of the endoplasmic reticulum by autophagy during endoplasmic reticulum stress in Arabidopsis. Plant Cell 24(11):4635–4651
Malhotra JD, Kaufman RJ (2007) The endoplasmic reticulum and the unfolded protein response. Semin Cell Dev Biol 18(6):716–731
Martínez IM, Chrispeels MJ (2003) Genomic analysis of the unfolded protein response in Arabidopsis shows its connection to important cellular processes. Plant Cell Online 15(2):561–576
Matus S, Nassif M, Glimcher LH, Hetz C (2009) XBP-1 deficiency in the nervous system reveals a homeostatic switch to activate autophagy. Autophagy 5(8):1226–1228
Merrick WC (2004) Cap-dependent and cap-independent translation in eukaryotic systems. Gene 332:1–11
Mishiba K, Nagashima Y, Suzuki E, Hayashi N, Ogata Y, Shimada Y, 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(14):5713–5718
Moreno AA, Orellana A (2011) The physiological role of the unfolded protein response in plants. Biol Res 44(1):75–80
Mori K (2009) Signalling pathways in the unfolded protein response: development from yeast to mammals. J Biochem 146(6): 743–750
Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T (2000) mRNA splicing-mediated C-terminal replacement of transcription factor Hac1p is required for efficient activation of the unfolded protein response. Proc Natl Acad Sci 97(9):4660–4665
Nagai A, Kadowaki H, Maruyama T, Takeda K, Nishitoh H, Ichijo H (2009) USP14 inhibits ER-associated degradation via interaction with IRE1α. Biochemical Biophys Rescommun 379(4):995–1000
Nagashima Y, Mishiba K, Suzuki E, Shimada Y, Iwata Y, Koizumi N (2011) Arabidopsis IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor. Sci Rep 1:29
Nagashima Y, Iwata Y, Ashida M, Mishiba K-I, Koizumi N (2014) Exogenous salicylic acid activates two signaling arms of the unfolded Protein response in Arabidopsis. Plant Cell Physiol 55:1772–1778
Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. Proc Natl Acad Sci 104(42):16450–16455
Patil C, Walter P (2001) Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. Curr Opin Cell Biol 13(3):349–355
Reimold AM (2001) Plasma cell differentiation requires the transcription factor XBP-1. Nature 412:300–307
Richardson CE, Kooistra T, Kim DH (2010) An essential role for XBP-1 in host protection against immune activation in C. elegans. Nature 463(7284):1092–1095
Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 8(7):519–529
Schröder M, Kaufman RJ (2005) The mammalian unfolded protein response. Annu Rev Biochem 74:739–789
Schütze K, Harter K, Chaban C (2008) Post-translational regulation of plant bZIP factors. Trends Plant Sci 13(5):247–255
Sidrauski C, Walter P (1997) The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell 90(6):1031–1039
Sidrauski C, Cox JS, Walter P (1996) tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. Cell 87(3):405–413
Srivastava R, Deng Y, Shah S, Rao AG, Howell SH (2013) BINDING PROTEIN is a master regulator of the endoplasmic reticulum stress sensor/transducer bZIP28 in Arabidopsis. Plant Cell 25(4):1416–1429
Srivastava R, Deng Y, Howell SH (2014) Stress sensing in plants by an ER stress sensor/transducer, bZIP28. Front Plant Sci 5:59
Sun L, Yang ZT, Song ZT, Wang MJ, Lu SJ, Liu JX (2013) The plant-specific transcription factor gene NAC103 is induced by bZIP60 through a new cis-regulatory element to modulate the unfolded protein response in Arabidopsis. Plant J 76(2):274–286
Sun L, Zhang S-S, Lu S-J, Liu J-X (2015) Site-1 protease cleavage site is important for the ER stress-induced activation of

membrane-associated transcription factor bZIP28 in Arabidopsis. Sci China Life Sci 58(3):1–6

Tang W, Page M (2013) Transcription factor AtbZIP60 regulates expression of $Ca2^{+}$-dependent protein kinase genes in transgenic cells. Mol Biol Rep 40(3):2723–2732

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101(3):249–258

Urade R (2009) The endoplasmic reticulum stress signaling pathways in plants. BioFactors 35(4):326–331

Wang S, Narendra S, Fedoroff N (2007) Heterotrimeric G protein signaling in the Arabidopsis unfolded protein response. Proc Natl Acad Sci USA 104(10):3817–3822

Welihinda AA, Kaufman RJ (1996) The unfolded protein response pathway in Saccharomyces cerevisiae. Oligomerization and trans-phosphorylation of Ire1p (Ern1p) are required for kinase activation. J Biol Chem 271(30):18181–18187

Williams B, Verchot J, Dickman MB (2014) When supply does not meet demand-ER stress and plant programmed cell death. Front Plant Sci 5:211

Yan W, Frank CL, Korth MJ, Sopher BL, Novoa I, Ron D, Katze MG (2002) Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. Proc Natl Acad Sci USA 99(25):15920–15925

Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, Goldstein JL (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell 6(6):1355–1364

Yorimitsu T, Nair U, Yang Z, Klionsky DJ (2006) Endoplasmic reticulum stress triggers autophagy. J Biol Chem 281(40): 30299–30304

Yoshida H, Haze K, Yanagi H, Yura T, 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(50):33741–33749

Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 107(7):881–891

Zhang P, McGrath B, Li SA, Frank A, Zambito F, Reinert J, Gannon M, Ma K, McNaughton K, Cavener DR (2002) The PERK eukaryotic initiation factor 2α kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. Mol Cell Biol 22(11): 3864–3874

Zhang Y, Wang Y, Kanyuka K, Parry MA, Powers SJ, Halford NG (2008) GCN2-dependent phosphorylation of eukaryotic translation initiation factor-2α in Arabidopsis. J Exp Bot 59(11): 3131–3141

Zhou J, Liu CY, Back SH, Clark RL, Peisach D, Xu Z, Kaufman RJ (2006) The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. Proc Natl Acad Sci USA 103(39):14343–14348