JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

Review

The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses☆

*Wahyu Indra Duwi Fanata, Sang Yeol Lee, Kyun Oh Lee**

Division of Applied Life Science (BK21 Program) and PMBBRC, Gyeongsang National University, 501 Jinju-daero, Jinju, 660-701, Republic of Korea

ARTICLE INFO

Keywords:
Endoplasmic reticulum stress
Unfolded protein response
Quality control
Endoplasmic reticulum-associated degradation
Apoptosis
Plants

ABSTRACT

In eukaryotic cells, proteins that enter the secretory pathway are translated on membrane-bound ribosomes and translocated into the endoplasmic reticulum (ER), where they are subjected to chaperone-assisted folding, post-translational modification and assembly. During the evolution of the eukaryotic cell, a homeostatic mechanism was developed to maintain the functions of the ER in the face of various internal and external stresses. The most severe stresses imposed on eukaryotic cells can induce ER stress that can overwhelm the processing capacity of the ER, leading to the accumulation of unfolded proteins in the ER lumen. To cope with this accumulation of unfolded proteins, the unfolded protein response (UPR) is activated to alter transcriptional programs through inositol-requiring enzyme 1 (IRE1) and bZIP17/28 in plants. In addition to transcriptional induction of UPR genes, quality control (QC), translational attenuation, ER-associated degradation (ERAD) and ER stress-induced apoptosis are also conserved as fundamental adaptive cellular responses to ER stress in plants.
This article is part of a Special Issue entitled: Translational Plant Proteomics.

Contents

☆ This article is part of a Special Issue entitled: Translational Plant Proteomics.

* *Corresponding author*. Tel.: +82 55 772 1364; fax: +82 55 759 9363.
E-mail address: leeko@gnu.ac.kr (K.O. Lee).

1874-3919/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jprot.2013.04.023

1. Introduction

In eukaryotic cells, most proteins destined for secretion, lysosomes, or the plasma membrane are translated on membrane-bound ribosomes and translocated into the rough endoplasmic reticulum (ER) through protein channels either while they are being translated on membrane-bound ribosomes or following completion of translation in the cytosol. In the ER lumen, they are subjected to a variety of post-translational modifications, chaperone-assisted folding, and/or assembly into oligomeric structures. Once they are properly folded and assembled in the ER, most client proteins are transported for further maturation and secretion. However, client proteins that fail to fold properly or assemble correctly in the ER are recognized by the QC system [1] and transported back into the cytoplasm for degradation by the ATP-dependent ubiquitin–proteasome system (UPS), in a process known as ERAD [2].

The high calcium concentration and oxidizing environment of the ER lumen relative to the cytosol are favorable for proper function of the ER chaperones required for folding disulfide bond-containing proteins [3,4]. The ER resident molecular chaperone BiP/GRP78 is the most abundant protein that assists in folding and assembly of newly synthesized proteins in the ER lumen [5]. BiP also binds misfolded proteins in the ER lumen, functioning as a sensor of ER stress and assisting in folding or targeting them for ERAD [6,7]. Calnexin (CNX) and calreticulin (CRT) are lectins that preferentially recognize and bind newly synthesized glycoproteins bearing $Glc_1Man_{5-9}GlcNAc_2$ oligosaccharides and ensure their proper folding and QC [8]. Protein disulfide isomerase (PDI) catalyzes the formation and isomerization of protein disulfide bonds [9].

A variety of environmental, physiological and pathological conditions such as loss of ER luminal calcium, inhibition of disulfide bond formation or posttranslational modification of secretory proteins, hypoxia, aging, genetic mutation, and virus or pathogen infection can lead to accumulation of unfolded and misfolded proteins in the ER lumen (ER stress). Eukaryotic cells respond to ER stress by activating an integrated signal transduction pathway called the UPR [10,11]. The UPR reestablishes cellular homeostasis or induces apoptotic programs. UPR activation triggers a series of complementary adaptive mechanisms including transient attenuation of global protein biosynthesis, transcriptional upregulation of ER chaperones, and ERAD [12]. However, prolonged UPR activation eventually triggers proapoptotic signals to protect the organism from rogue cells expressing dysfunctional or even toxic signaling molecules [12].

Transient attenuation of global protein synthesis is an important cellular response to ER stress. By transiently reducing the influx of nascent proteins into the ER lumen, eukaryotic cells can mitigate ER stress and the accumulation of unfolded proteins [12]. Temporary blocking of protein loading into the ER lumen facilitates the maintenance of protein-folding homeostasis of the stressed cells. This attenuation of global protein translation in response to ER stress is mediated by protein kinase double-stranded-RNA-dependent protein kinase (PKR)-like ER kinase (PERK) through the phosphorylation of the serine 51 residue in the α-subunit of translational initiation factor 2 in eukaryotes (eIF2α) [13,14]. The heterotrimeric translation initiation factor 2 in eukaryotes (eIF2) bound to GTP transfers methionyl-initiator $tRNA^{Met}$ to the 40S ribosomal subunit. The phosphorylated eIF2α acts as a competitive inhibitor of its guanine nucleotide exchange factor (GEF), eIF2B [15]. This inhibition of the formation of the eIF2 ternary complex, caused by phosphorylated eIF2α, leads to decreased translation initiation and ultimately reduced protein loading into the ER. Attenuation of global translation via selective inhibition of eIF2α dephosphorylation protects mammalian cells from ER stress-induced apoptosis and blocks viral replication [16,17]. Although general protein synthesis is decreased by eIF2α phosphorylation, translation of a subset of mRNAs that harbor upstream open reading frames (uORFs) in their 5′ untranslated terminal regions (UTRs) is selectively stimulated. Such mRNAs include mammalian *ATF4* and yeast *GCN4* mRNAs, members of the bZIP family of transcription factors responsible for activation of the integrated stress response (ISR), a pro-survival gene expression program [18–22]. Following a first translation initiated at the upstream uORF, under normal conditions, 40S ribosome bound to the eIF2 · GTP · Met-$tRNA_i^{Met}$ ternary complex continues scanning and resumes translation at the downstream uORF, which results in ribosome disassembly and blocks translation of mRNAs with uORFs including *ATF4* and *GCN4* [23–25] (Fig. 1A). By contrast, when eIF2α is phosphorylated in response to ER stress, the probability that ribosomes resume translation at the inhibitory uORFs is diminished and translation initiation at the downstream AUG start codon is favored (Fig. 1B).

Another common cellular strategy to mitigate ER stress is enhancing the capacity of the ER-protein folding machinery through transcriptional upregulation of ER chaperones. UPR signaling components were first identified in the yeast *Saccharomyces cerevisiae* by genetic approaches using the unfolded protein response element (UPRE) in the yeast Kar2 (BiP) promoter [26]. The UPR signaling pathway in yeast cells is mediated by inositol-requiring enzyme 1 (IRE1), an ER transmembrane kinase with an N-terminal stress-sensing domain in the lumen of the ER and C-terminal cytosolic serine/threonine kinase and endoribonuclease domains [27,28]. In response to the accumulation of unfolded proteins, BiP is released from the ER-sensing domain, which results in self-association and autophosphorylation of IRE1, and activation of the cytosolic endonuclease domain. The activated IRE1 catalyses unconventional splicing of the 252 nucleotide intron from the precursor mRNA *HAC1u* in the cytosol to produce the mature mRNA *HAC1s* encoding a basic domain/leucine zipper (bZIP) transcription factor (TF) [29–34]. HAC1s activates transcription of target genes involved in protein folding/modification/degradation, vesicle transport and phospholipid biosynthesis, and apoptosis [32,35–37].

In mammalian cells, the up-regulation of ER chaperone genes is controlled by two pathways: the IRE1 and activating TF 6 (ATF6) pathways. The IRE1-mediated pathway is an evolutionarily well-conserved signal transduction branch of the UPR that is found in most eukaryotic cells. Mammalian cells contain two yeast IRE1 homologues, namely IRE1α (ubiquitously expressed) and IRE1β (expressed only in the gut) [38,39]. In response to ER stress, protein kinase and endoribonuclease activities of IRE1α are activated through the dissociation of BiP from IRE1α and subsequent dimerization. Activated IRE1α removes an unconventional intron from unspliced *XBP1* (*uXBP1*) mRNA in the cytosol, generating a frameshift in *sXBP1*

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

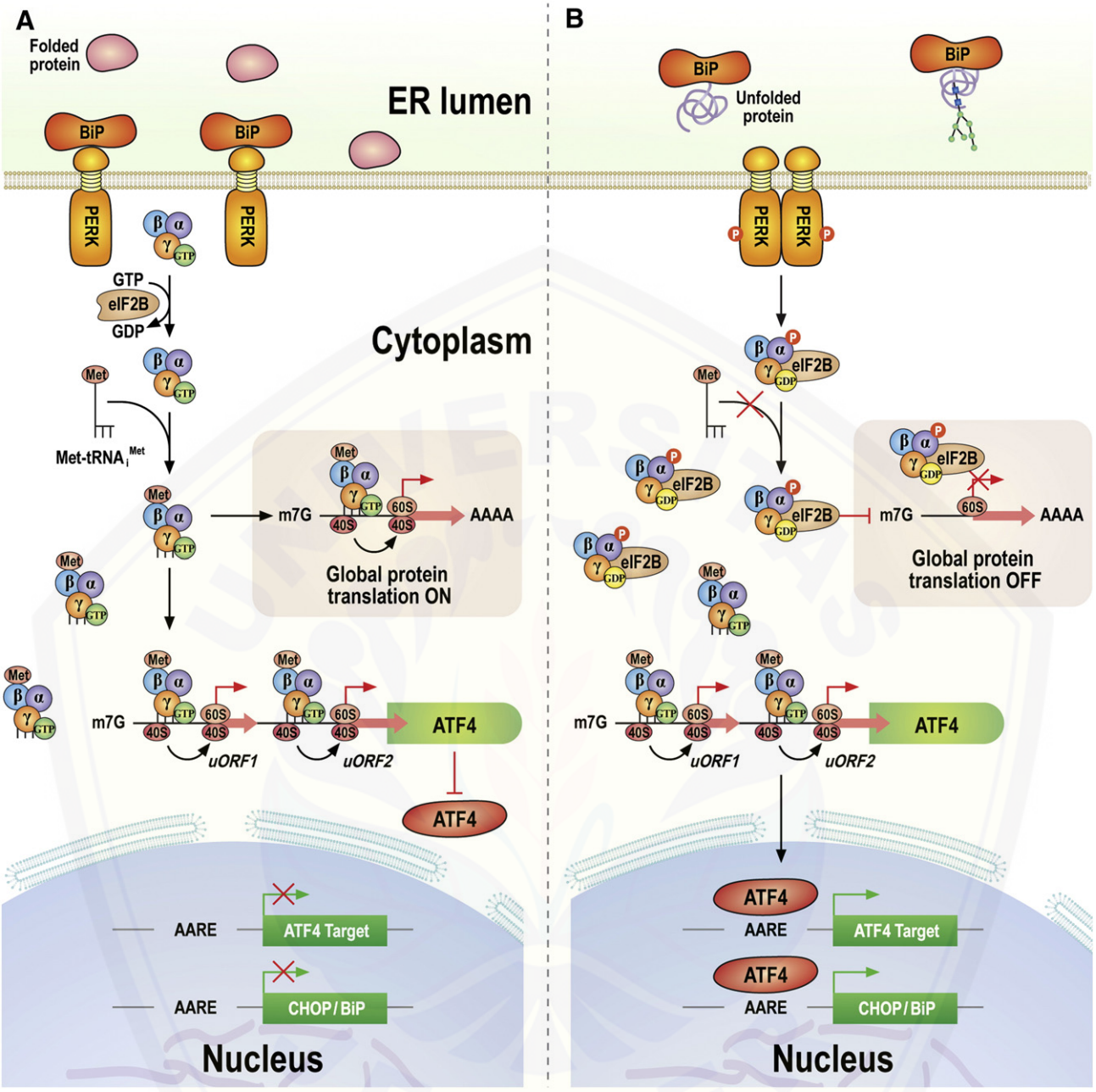

Fig. 1 – Schematic illustration of the attenuation of global protein translation. (A) Protein translation under normal conditions. PERK physically interacts with BiP and this interaction maintains the PERK in its inactive state. The inactivation of PERK contributes to the abundance of eIF2-GTP, which subsequently binds to methionyl-initiator tRNA (Met-tRNA$_i^{Met}$) to form the ternary complex eIF2-Met-tRNA$_i^{Met}$-GTP. This ternary complex delivers the initiator tRNA to the 40S ribosomal subunit and allows translation of proteins including translation at uORF1 and uORF2 of *ATF4*. As uORF2 overlaps with the ATF4 ORF, the translation of uORF2 suppresses the translation of ATF4. (B) Attenuation of global protein translation under ER stress conditions. BiP dissociates from PERK and allows its activation through oligomerization and *trans*-autophosphorylation. The active PERK phosphorylates the α-subunit of the eIF2 heterotrimeric complex and inhibits the activity of eIF2B to exchange GDP to GTP on eIF2. eIF2-GDP does not bind Met-tRNA$_i^{Met}$, resulting in inhibition of ternary complex formation. The low abundance of the ternary complex increases the likelihood of ribosomes initiating the scanning process through uORF2, which subsequently allows translation at the ATF4 start codon. As a consequence, transcription of ATF4 downstream genes is activated through the binding of ATF4 to the amino acid response element (AARE).

that allows the translation of a functional TF. sXBP1 translocates to the nucleus and controls the transcription of the genes encoding ER chaperones and ERAD components [40–42]. In response to ER stress, IRE1β induces translational repression through 28S ribosomal RNA cleavage [43]. Like the IRE1-XBP1 signaling pathway, ATF6 also transduces UPR signals from the ER to the nucleus. ATF6 is an ER membrane-bound TF activated by intramembrane proteolysis in response to ER stress. When

unfolded proteins accumulate in the ER, ATF6α and ATF6β are transported from the ER to the Golgi. Then, the cytoplasmic fragments containing the DNA-binding and transcriptional activation domains are released by regulated intramembrane proteolysis (RIP), a two-step cleavage mediated sequentially by site-1 protease (S1P) and site-2 protease (S2P) [44–46]. The free ATF6 fragment moves to the nucleus to induce genes with promoters containing an ER stress response element (ERSE). ATF6 mediates transcriptional activation of UPR- and non-UPR-target genes, including the UPR-associated proapoptotic transcriptional regulator CHOP [C/EBP homology protein, also known as GADD153 (growth arrest and DNA damage inducible protein 153)] and XBP1, which provides a positive feedback for the UPR [42,47–49].

Many chaperones and folding enzymes have been detected as abundant proteins by analyses of the ER proteome [50]. BiP, GRP94, CNX, CRT, peptidylprolyl isomerases (PPIase) and thiol disulfide oxidoreductases have also been identified by mass spectrometry (MS)-based proteomic analysis of purified rough microsomes from mouse liver [51]. However, the three ER-resident transmembrane sensors PERK, IRE1 and ATF6 have not been detected by proteomic analyses, probably because of their presence in small amounts or their low solubility. Quantitative proteomic analysis of human neuroblastoma cells revealed that ER chaperones responsible for protein folding, aminoacyl-tRNA synthetases, and proteins belonging to the Sec61 complex are increased in response to tunicamycin (TM)-induced ER stress [52]. Increased expression of UPR-associated proteins such as BiP and eIF2α has been demonstrated by proteomic analysis of cells deficient in the *ataxia-telangiectasia mutated* (ATM) gene and undergoing oxidative stress. Proteomic analysis of an oral squamous carcinoma cell (Ca9–22) showed that proteins involved in mitochondrial dysfunction and the ER stress pathway are increased by treatment with 11-dehydrosinulariolide, an active compound isolated from the soft coral *Sinularia leptoclados* [53]. These results suggest that proteomic analysis is a potent tool along with transcriptomic analysis to study UPR, especially in a high-throughput manner.

2. The UPR in plants

2.1. *Effects of internal and environmental stresses on the UPR in plants*

Internal factors, such as genetic mutations and hereditary metabolic defects, and external factors including various biotic and abiotic stresses and impaired metabolism caused by oxygen, glucose, amino acid or nutrient deprivation may also result in ER stress in plant cells. Aberrant protein synthesis, inhibition of protein glycosylation or disulfide bond formation, improper protein transfer to the Golgi, and ER Ca^{2+} depletion can also lead to the accumulation of unfolded and misfolded proteins in the ER lumen, inducing the UPR in plant cells. Inhibition of N-linked glycosylation in Arabidopsis seedlings by treatment with TM induces transcription of genes encoding ER chaperones and enzymes involved in protein glycosylation, folding, transport in the secretory pathway and UPR signaling [54]. In response to ER stress, plant cells also transduce stress signals from the ER to the nucleus for transcriptional regulation of genes involved in protein folding and degradation [55,56].

In Arabidopsis, ER stress is sensed and stress signals are transduced by the membrane-bound IRE1-like (IRE1A and IRE1B) and ATF6-like (bZIP17 and bZIP28) transducers [57–61]. Plants exposed to environmental stress activate UPR signaling components. The transcription and IRE1-mediated unconventional splicing of *bZIP60* mRNA to produce the active TF increase under conditions of salt stress, heat stress or pathogen attack in Arabidopsis [62,63]. Rice bZIP74 (also known as bZIP50) is activated by the same mechanism; its mRNA is induced by salt stress and subjected to unconventional splicing by IRE1 in the cytoplasm in response to heat and salicylic acid [64,65]. Expression of *Nicotiana tabacum bZIP60* mRNA significantly increases upon infection with the non-host pathogen *Pseudomonas cichorii*, whereas it is unaffected by infection with the compatible pathogen *Pseudomonas syringae* pv. *tabaci* [66]. Similarly, infection with Potato virus X (PVX) in *Nicotiana benthamiana* plants leads to increased transcript levels of *bZIP60* together with ER chaperone genes [67]. Salt stress induces an S1P-dependent proteolytic cleavage of Arabidopsis bZIP17 and translocation of the active TF to the nucleus [59]. Heat stress induces the transcription, RIP-dependent activation and nuclear translocation of Arabidopsis bZIP28 TF [61]. Taken together, these results suggest that various internal and environmental stresses that diminish the protein-folding capacity of the ER may result in the UPR in plants.

2.2. *Major UPR signaling pathways in plants*

Arabidopsis IRE1A and IRE1B share key structural features with their yeast and mammalian counterparts. They contain N-terminal sensor domains facing the lumen of the ER, transmembrane domains near the middle of the proteins, and cytosol-facing C-terminal putative kinase and ribonuclease domains. Amino acid sequence comparison indicates that the C-terminal kinase and ribonuclease domains are highly conserved (30–40%) among the IRE1 homologues in different kingdoms [68]. Like its yeast and mammalian counterparts, Arabidopsis IRE1 plays a central role in the ER stress response by catalyzing unconventional splicing of *bZIP60* mRNA to produce the active TF (Fig. 2) [60,62]. The unspliced *bZIP60* mRNA (*bZIP60u*) encodes bZIP60 TF with a C-terminal transmembrane domain (TMD), which tethers the protein in the ER membrane under unstressed conditions. In response to ER stress, IRE1 catalyzes unconventional splicing of the 23-nucleotide intron from *bZIP60u* to produce the spliced *bZIP60* mRNA (*bZIP60s*), which encodes an active bZIP60 TF with a different open reading frame (ORF) in the C-terminal region. The active bZIP60 TF is recruited to the nucleus to activate transcription of UPR-associated genes in Arabidopsis [62]. The unconventional splicing of *bZIP60* mRNA is detected in *ire1a* and *ire1b* single mutants, but not in the *ire1a ire1b* double mutant [60]. This indicates that Arabidopsis IRE1A and IRE1B are functionally redundant in the unconventional splicing of *bZIP60* mRNA. IRE1 in rice also catalyzes unconventional splicing of the 20-nucleotide intron from unspliced rice *bZIP74* mRNA (*bZIP74u*) [65]. This process generates a C-terminal amino acid frame shift that not only removes the TMD of bZIP74u but also produces an additional putative bipartite nuclear localization signal (NLS) RRKR mediating the effective nuclear localization of

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

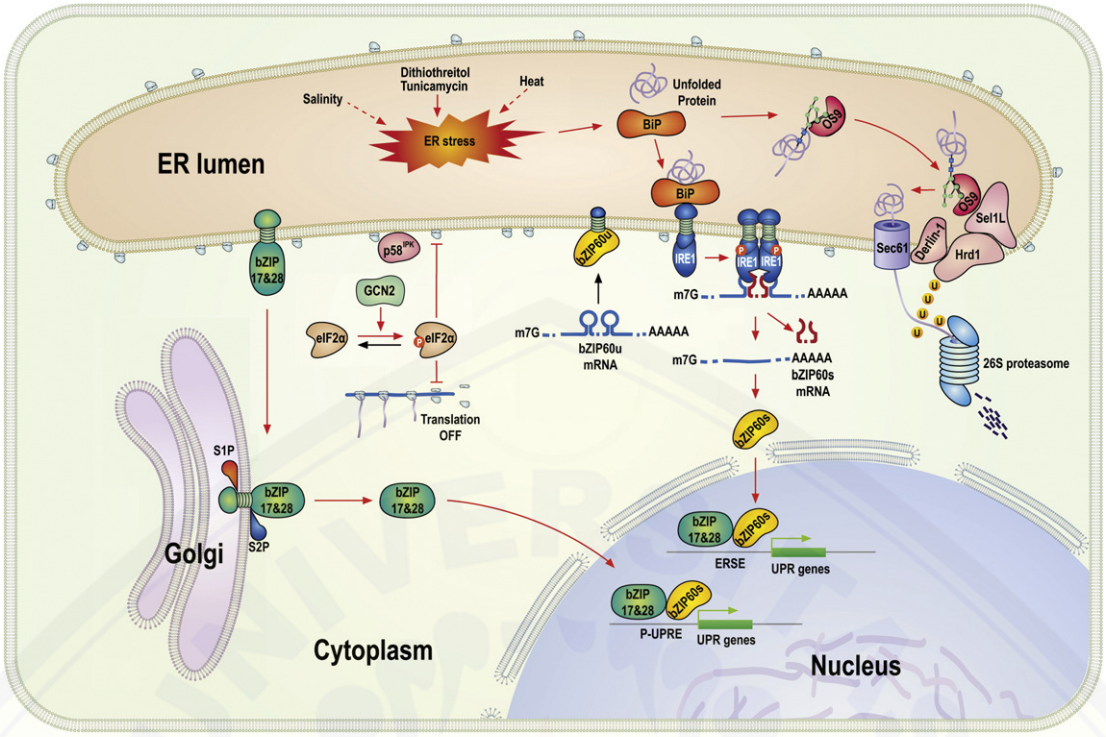

Fig. 2 – Schematic illustration of the core elements and UPR signaling network in plants. Under conditions that promote ER stress, plant IRE1 is activated by autophosphorylation and dimerization. The active IRE1 unconventionally splices *bZIP60* mRNA, creating a frameshift that replaces the C-terminal region including the trans-membrane domain (TMD) and contributing to the relocation of bZIP60 from the ER membrane to the nucleus. ER stress also induces proteolytic cleavage of bZIP17 and bZIP28 by S1P and S2P, releasing the cytoplasm-facing N-terminal domain containing the transcriptional activation and bZIP domain from the ER membrane and allowing it to target to the nucleus. These ER stress-activated TFs bind to ERSE and P-UPRE to induce the expression of UPR-associated genes encoding ER chaperones and ERAD proteins. ER chaperones assist in correct protein folding during ER stress, and ERAD proteins mediate the disposal of permanently unfolded proteins. ERAD is initialized when the N-glycan of a permanently misfolded protein is recognized by OS9 and associates with Sel1L/Hrd3. The efficient disposal of the terminally misfolded protein requires Hrd1, an E3 ubiquitin ligase. Finally, the Hrd1-Sel1L/Hrd3-OS9 complex disposes of the misfolded protein through ubiquitination and subsequent proteasomal degradation in the cytosol. Derlin-1 has also been identified in plants, but the mechanism by which it contributes to the ERAD remains to be elucidated. Plant genomes also encode proteins that are involved in eIF2α phosphorylation-mediated protein translational control such as GCN2 and p58^{IPK}. However, the functional mechanism of these proteins during ER stress remains obscure.

the active bZIP74 TF (bZIP74s). The additional NLS obtained after the C-terminal amino acid frame shift is also found in some other bZIP60/bZIP74 orthologues in plants [64,65]. However, transiently expressed Arabidopsis bZIP60ΔC-GFP lacking the C-terminal region localizes to the nucleus [69]. Thus, further studies are needed to determine whether the additional NLS of bZIP60/bZIP74 orthologs is essential for nuclear localization in plants.

The Arabidopsis *bzip60* mutant shows markedly reduced induction of many ER stress-responsive genes when compared to wild type (WT) plants [69]. A number of bZIP60-responsive genes contain *cis*-elements responsible for the ERSE and the plant-specific unfolded protein response element (P-UPRE) [69]. Thus, it seems likely that active bZIP60 TF binds to the *cis*-elements in the promoters of downstream genes to activate their transcription, leading to the ER stress response. The truncated bZIP60 activates not only transcription of *BiP1/2* and *BiP3*, but also its own transcription [70,71], indicating that the transcription factor is its own activator in a positive feedback loop. Together, these results reveal that IRE1-mediated unconventional splicing and nuclear translocation of the active bZIP TFs that are found in yeast and mammals as well are also conserved in plants.

bZIP28 and bZIP17 are ER-membrane localized TFs that have been identified as signal transducers from the ER to the nucleus under ER- and salt-stress conditions [58]. bZIP28 and bZIP17 were identified based on their domain structures, which are similar to that of mammalian ATF6. They contain an N-terminal bZIP domain, a transmembrane segment, and a canonical S1P cleavage site (RXXL or RXLX) on the C-terminal side of the transmembrane segment. Like mammalian ATF6, in response to ER stress, both bZIP28 and bZIP17 are translocated from the ER through the Golgi, where the cytoplasm-facing N-terminal regions are released by RIP to be active TFs (Fig. 2). The active TFs move into the nucleus where they activate the transcription of downstream genes including UPR-associated genes [72].

Previously, it was reported that the direct binding of ATF6 to the CCACG box of the ERSE element occurs only when NF-Y, a heterotrimeric complex composed of NF-YA, NF-YB, and NF-YC, bind to the CCAAT box [49]. Similarly, bZIP28 assembles

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

a larger transcriptional activation complex by interaction with the heterotrimeric CCAAT binding factors composed of subunits NF-YA4, NF-YB3 and NF-YC2 [72]. Although base substitutions in the CCAAT box in ERSE-I reduce promoter activity induced by TM treatment, bZIP28 alone can bind to CACG in vitro, and knockout of *NF-YA4* does not affect the induction of *BiP3* by TM, indicating possible functional redundancy of the *NF-YA* family in Arabidopsis [72]. In a yeast two-hybrid assay, bZIP28 interacts with itself, bZIP17 and bZIP60. Although bZIP60 exhibits a much weaker ability to homodimerize, it also interacts with bZIP17 and bZIP28 to make heterodimers [72]. This indicates that bZIP28, bZIP17 and bZIP60 interact with each other to make a larger transcriptional activation complex in plants. Nevertheless, it is still unclear whether bZIP28 and bZIP17 are functionally redundant [61]. Arabidopsis *bZIP17* and its homologue in *Nicotiana tabacum*, TGA1b, are induced by salt stress and activated by RIP to induce salt stress-responsive genes containing the TGACG element in their promoters [73–75]. However, our analysis revealed that double mutation of *bZIP28* and *bZIP17* is lethal at some point during gametogenesis or embryogenesis. Therefore, functional redundancy of bZIP28 and bZIP17 is supported by the genetic analysis, and it is possible that salt stress can influence the UPR in plants.

2.3. *Translational attenuation of global protein biosynthesis*

Plants respond with changes in their patterns of gene expression and protein biosynthesis when exposed to various types of stress. Regulation of gene expression at the level of translation initiation allows an immediate response to stress. In response to ER stress, global protein biosynthesis by ribosomes is inhibited by phosphorylation of eIF2α to relieve the overloaded ER machinery and to translate TFs that trigger downstream stress adaptation responses selectively in eukaryotic cells (Fig. 2). Several serine/threonine eIF2α kinases that respond to different stress signals have been identified in mammals. These include PKR, which is activated during viral infection, PERK, which is activated during the UPR, heme-regulated inhibitor kinase (HRI), which is activated by heme-deficiency, and general control non-depressible 2 (GCN2), which is activated during amino acid starvation [12,76–79].

Whereas no apparent plant orthologues of PKR, PERK and HRI have been identified, a GCN2 homologue that is activated by various stresses including amino acid starvation, UV irradiation, wounding, cold shock, salicylic acid, methyl jasmonate and I-aminocyclopropane-I-carboxylic acid (ACC) has been cloned in Arabidopsis [80–83]. Expression of the Arabidopsis GCN2 in yeast *gcn2* mutants complements the growth inhibition of the mutants in the presence of amino acid biosynthesis inhibitors [80]. Arabidopsis GCN2 interacts with uncharged tRNA and functions as an eIF2α kinase using both Arabidopsis eIF2α homologues as direct substrates in vitro, and its activity results in strong inhibition of global protein synthesis in plants [81–84]. However, additional studies are required to elucidate whether the eIF2α phosphorylation is relevant to selective translation of the TFs that trigger downstream stress adaptation responses in plants.

$P58^{IPK}$ is a member of the tetratricopeptide repeat (TPR)- and J-domain-containing protein families. $P58^{IPK}$, transcriptionally activated by ER stress, is known to interact with the kinase domain of PERK and inhibit its activity [85,86]. However, it has also been reported that $P58^{IPK}$ plays an essential role in the recruitment of Hsp70 chaperones to the cytosolic face of the ER protein translocation channel Sec61 for translational control [87]. A $P58^{IPK}$ homologue has been cloned in Arabidopsis and its role in plant survival during viral infection has been demonstrated [88]. However, further studies are needed to elucidate the functional relevance and underlying molecular mechanisms of $P58^{IPK}$ in plant responses to ER stress.

2.4. *Folding and quality control (QC) in the ER*

N-glycosylation is an important co- and post-translational modification affecting the physicochemical properties of proteins in the secretory pathway [89]. N-linked glycosylation appears to function in protein folding, QC and ERAD [90–92]. In eukaryotic cells, glycoproteins cotranslationally translocated in an unfolded state through the Sec61 translocon into the ER lumen are rapidly modified by covalent addition of the preassembled oligosaccharide precursor ($Glc_3Man_9GlcNAc_2$) to the Asn residue in the sequence Asn-X-Ser/Thr (where X is not proline) of the nascent polypeptide [93]. Subsequently, the glucose (Glc) residues of the fourteen-sugar oligomannose glycan ($Glc_3Man_9GlcNAc_2$) are trimmed by α-glucosidase I (GI) and α-glucosidase II (GII) in the ER [94,95]. Two genes encoding GI and three genes encoding an α- and two β-subunits of GII have been identified in Arabidopsis [96,97].

If the glycoproteins are not correctly folded, however, they are reglucosylated in the ER with a Glc unit by the action of UDP-Glc: glycoprotein glucosyltransferase (UGGT), which specifically recognizes misfolded glycoproteins [98,99]. A UGGT homologue is found in Arabidopsis and the *ebs1-1* mutant with a defect in UGGT allows *bri1-9*, a weak mutant caused by ER retention of a structurally defective brassinosteroid (BR) receptor BRI1, to escape QC and reach the cell surface as a functional receptor [98]. This indicates that UGGT in Arabidopsis is also involved in the retention of misfolded glycoproteins in the ER. Misfolded proteins with monoglucosylated intermediate N-glycans are retained in the ER where they bind to the membrane-bound CNX and to its soluble homologue CRT, which serve as molecular chaperones [100]. In the QC system, PDI also interacts with CNX and CRT to facilitate the folding process [101]. Glycoproteins with native conformations are liberated from the folding machinery through the removal of the innermost of Glc by GII and delivered to subsequent organelles of the secretory pathway. By contrast, glycoproteins are subject to continuous deglucosylation and reglucosylation cycles as long as they remain misfolded or incompletely folded in the ER [102,103]. Essential components of the QC system appear to have been conserved during land plant evolution. Nevertheless, additional studies are needed to elucidate their specific molecular mechanisms in plants.

2.5. *ER-associated degradation (ERAD)*

If the QC system fails to prevent protein misfolding, causing an overload of unfolded proteins in the ER, cells activate a defense mechanism called ERAD. ERAD is a misfolded protein clearance pathway that facilitates maintenance of ER functions by reducing levels of misfolded proteins in the ER via the

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

ubiquitin–proteasome pathway in eukaryotes. In yeast (*S. cerevisiae*), ERAD substrates and elimination pathways are classified into three categories based on the topologies of the lesions that cause the misfolding [104]. Secretory and ER luminal proteins that contain lesions in the ER lumen are degraded through the ERAD-L pathway. Membrane proteins that contain lesions in the ER lumen, within the membrane, or in the cytoplasm are the substrates of ERAD-L, ERAD-M or ERAD-C, respectively [104]. The process of ERAD can be subdivided into three steps: (i) recognition of substrates, (ii) retro-translocation, and (iii) degradation by the 26S proteasome. Polyubiquitination is essential for the retro-translocation of misfolded substrates from the ER into the cytosol [105]. In yeast, the ER membrane-resident ubiquitin ligase (E3) Hrd1p/Der3p forms a complex predominantly with the ubiquitin-conjugating enzymes (E2s) Ubc7p and Ubc1p to mediate both retrograde transport and ubiquitination of substrate molecules [106]. ERAD-L and ERAD-M appear to be dependent on Hr1p and Hrd3p, whereas ERAD-C is mediated by Doa10, an ER membrane-resident ubiquitin ligase (E3) with an unusual RING finger [107–110].

Prolonged residence of misfolded proteins in the ER leads to exposure of the terminal α1,6-mannose residue on their N-glycans, which is mediated by the action of the α1,2-specific exomannosidase Htm1 [111,112]. The exposed terminal α1,6-mannose residue together with polypeptide regions surrounding the N-glycosylation site serves as the degradation signal recognized by the mannose-6-phosphate receptor homology (MRH) domain of Yos9 [112]. Similarly, ERAD substrates in mammalian cells are recognized by OS-9 and XTP3-B via the action of ER degradation–enhancing α-mannosidase-like lectins (EDEMs) [113–116]. Sel1L, the mammalian homologue of yeast Hrd3p, which is an ER membrane protein with a large luminal domain, seems to mediate the dislocation of ERAD substrates from the ER [117]. BLAST searches of the plant genome databases identified two, three and two degradation in ER protein (Derlin) genes in corn, Arabidopsis and rice, respectively [118]. The plant Derlin genes are classified into two groups (Derlin-1 and Derlin-2) according to the similarity in their amino acid sequences [118].

Molecular components of ERAD including the homologues of EDEMs, OS9, Hrd1, Hrd3/Sel1L and Derlin-1 are also found in plant genomes [54–56,118]. However, little is known about the molecular pathways of ERAD in plants and their underlying molecular mechanisms. The accumulation of misfolded glycoproteins in the ER and subsequent degradation were first observed in plant cells when an assembly-defective phaseolin was expressed in transgenic tobacco [119]. A genetic screen for suppressors of the ERAD-associated dwarf phenotype of *bri1-9*, caused by ER retention of a structurally defective BRI1, resulted in identification of the EMS-mutagenized *bri1* suppressor 5 (*EBS5*) encoding an Arabidopsis homologue of the yeast Hrd3/mammalian Sel1L protein [120]. The ERAD-associated dwarf phenotype of *bri1-5* can be also suppressed by the *ebs5-1* mutation [120]. ERAD of bri1-5 seems to be mediated by the terminal α1,6-mannose residue on the glycan. Addition of class I α-mannosidase inhibitor kifunensine or inhibition of α1,6-mannose attachment to the C-branch of N-glycan by the mutation of *ALG12* enhances protein stability of bri1-5 and suppresses the dwarf phenotype of *bri1-5* [121,122]. Suppression of the dwarf phenotype and enhanced protein stability are also observed when Hrd1 homologues are depleted by introduction of the *hrd1a hrd1b* double mutation in the *bri1-9* background [123]. Arabidopsis OS9 has been identified as interacting with Hrd3/Sel1L as well as with bri1-5 and bri1-9 and loss of OS9 function suppresses the dwarf phenotypes of both *bri1-5* and *bri1-9* [124]. Taken together, these results strongly indicate the presence of conserved Hrd1–Hrd3/Sel1L–OS9 machinery in plants for the dislocation of terminally misfolded proteins from the ER (Fig. 2).

2.6. *ER stress-induced apoptosis*

Transmembrane sensors recognize unfolded proteins in the ER, and trigger the UPR to maintain ER function and folding capacity. However, if the adaptive response fails to resolve the overload of unfolded or misfolded proteins, the excessive and prolonged ER stress leads to ER stress-induced apoptosis to protect the organism from rogue cells. To combat the deleterious effects of protein aggregates in the organism, it is likely that plant cells also are equipped with the ER stress-induced apoptosis system. In mammals, the transmembrane sensors PERK, ATF6 and IRE1 that initiate the UPR are also required to trigger proapoptotic signals during excessive and prolonged ER stress. However, it seems that they activate proapoptotic signals via induction of intermediating signaling molecules such as CCAAT/-enhancer-binding protein homologous protein (CHOP) and c-Jun NH_2-terminal kinase (JNK) rather than direct induction of the downstream proapoptotic molecules [125]. As a proapoptotic TF, CHOP mediates ER stress-induced apoptosis via upregulation of growth arrest and DNA damage-inducible protein (GADD34) and via down-regulation of the anti-apoptotic protein B-cell lymphoma 2 (Bcl2) [18,126]. GADD34 is a protein phosphatase 1 (PP1)-interacting protein that facilitates PP1 dephosphorylation of eIF2α and allows cells to escape from translational shutoff during ER stress [127].

The Arabidopsis heterotrimeric G protein appears to play a role in ER stress-induced apoptosis as well as in plant development [128]. *AGB1* encodes the Gβ subunit of heterotrimeric G protein, which forms a stable heterodimer with the Gα subunit in Arabidopsis. The short root phenotype of the Arabidopsis *ire1a ire1b* double mutant is further enhanced by *agb1* loss-of-function mutations [129]. However, induction of UPR genes was lower in *ire1a ire1b* double mutant but higher in *agb1* mutants compared to WT [129]. These results suggest that IRE1A, IRE1B and AGB1 are independently involved in the control of plant UPR pathways, which possibly initiate ER stress-induced apoptosis under excessive and prolonged ER stress conditions. However, further studies are required to clarify whether the phenotypes shown by the mutants are relevant to ER stress-induced apoptosis in plants.

In mammals, overexpression of Bcl2 or loss of Bcl-2–associated X protein (Bax) and Bcl-2 homologous antagonist/killer (Bak) block ER stress-induced cytochrome *c* release and apoptosis [130,131]. Whereas overexpression of the ER-targeted Bcl2 blocks ER stress-induced cytochrome *c* release and apoptosis, expression of the ER-targeted Bak in *bax bak* double mutant cells induces apoptosis, indicating that the ER stress-induced apoptosis signal is relayed from the ER to mitochondria via the Bcl2 family of proteins [132–134]. Although homologues of Bcl-2 family proteins have not been identified in plants and yeast, Bax inhibitor 1 (BI1),

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

which is a suppressor of Bax-induced cell death, is widely conserved across different kingdoms [135].

In Arabidopsis, expression of *BI1* is strongly increased before the initiation of TM-induced cell death [136]. Nevertheless, alterations in *BI1* gene expression levels do not cause a significant effect on the expression of ER chaperone genes such as *BiP2*, *PDI*, *CRT1*, and *CNX1* [136]. Compared with WT plants, Arabidopsis *BI1* mutants *bi1-1* and *bi1-2* exhibit hypersensitivity to TM with progressed apoptosis [136]. Conversely, overexpression of BI1 leads to reduced sensitivity of Arabidopsis seedlings to TM [136]. Thus, BI1 plays a crucial role as a survival factor during ER stress-induced cell death in Arabidopsis [136].

Plants also contain the evolutionarily conserved Bcl-2–associated athanogene (BAG) family that performs diverse cellular functions in processes ranging from proliferation to growth arrest and cell death in yeast and in mammals. The Arabidopsis genome contains seven BAG family homologues. Arabidopsis BAG7, localized in the ER, interacts with BiP2 in vivo [137]. Loss-of-function mutant *bag7* results in accelerated TM-induced cell death, indicating that BAG7 plays a role in ER stress-induced apoptosis [137].

N-rich proteins (NRP-A and NRP-B), synergistically upregulated by TM and polyethylene glycol treatments, were identified as new components of the integrated stress-induced apoptosis process in soybean [138]. Expression of NRPs is positively regulated by a transcription factor, *Glycine max* Early Responsive to Dehydration 15 (ERD15), which is induced by ER stress and osmotic stress [139]. Overexpression of NRPs results in increased caspase-3-like activity as well as promotes DNA fragmentation and leaf senescence in soybean [138]. NAC domain-containing protein NAC6 was identified as a downstream component of the integrated stress-induced NRP-mediated apoptotic pathway in soybean [140]. Increased levels of *NRP-A* and *NRP-B* transcripts induce the expression of the *NAC6* in soybean [140]. Enhanced expression of BiP prevented NRP- and NAC6-mediated apoptosis, whereas silencing of endogenous BiP accelerated the onset of leaf senescence mediated by NRPs and NAC6. These results indicate that an ER-resident molecular chaperone, BiP, functions as a negative regulator in stress-induced NRP-mediated apoptosis [141] (Fig. 3). However, little is known about how the commitment to cell death is made and about the mechanisms that are responsible for ER stress-induced apoptosis in plants.

3. Conclusions and future perspectives

It appears that external factors including abiotic stress [62,63], biotic stress [66] and plant hormones [64,65], as well as internal factors such as genetic mutations and hereditary metabolic defects lead to induction of the UPR in plants. Throughout evolution, plants have evolved fundamental adaptive cellular responses termed the UPR, ERAD and ER stress-induced apoptosis to cope with various internal and environmental stresses. Although further studies are required to elucidate their detailed mechanisms in plant cells, key factors in the plant UPR pathways may be used in engineering multi-stress tolerant plants. Indeed, several UPR components have been employed to improve the stress tolerance of plants. Overexpression of BiP confers drought tolerance in soybean and tobacco [142,143]. Arabidopsis plants overexpressing full-length *bZIP60* exhibit enhanced salt tolerance [63]. Expression of the bZIP17 active form under the stress-inducible *RD29A* promoter confers salt stress tolerance in Arabidopsis [75]. Thus, these key factors in the plant UPR pathways are valuable research targets not only to achieve a better understanding of ER stress and UPR signaling, but also for crop improvement and stress tolerance in plants and plant-like algae (green algae).

The UPR is a fundamental adaptive cellular response to various internal and environmental stresses in plants. It is evident that phosphorylated eIF2α, a marker of translational attenuation, is upregulated in response to ER stress in plants. This finding indicates that assessment of the translational attenuation by measuring the level of phosphorylated eIF2α can be effective to determine the stress state of plants. This will be useful not only to examine plant stress at the cellular level under various environmental or agronomic conditions, but also to monitor internal stress that could be caused by transgenes that are introduced into plants for various purposes.

Although results from yeast and animal studies provided many insights into the nature of UPR in plants, a subset of the mechanisms underlying UPR in plant cells is still not well

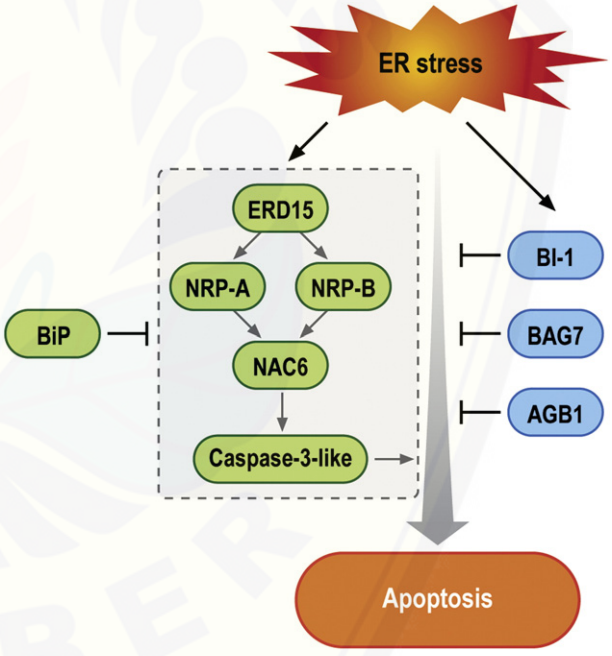

Fig. 3 – Schematic illustration of ER stress-induced apoptosis in plants. ER stress-induced apoptosis is negatively regulated by BI1, AGB1 and BAG7 in Arabidopsis. Transcription of *BI1* is upregulated in response to ER stress, and BI1 negatively regulates apoptosis during development. Although their transcriptional levels are not changed during ER stress, BAG7 and AGB1 proteins also function as negative regulators of apoptosis. ER stress upregulates transcription of *ERD15*, which leads to transcriptional induction of *NRP-A* and *NRP-B* and in turn induction of *NAC6* expression in soybean. BiP inhibits apoptosis by negatively regulating the integrated signal transduction pathway. Green indicates regulators of ER stress-induced apoptosis found in soybean. Blue denotes regulators of ER stress-induced apoptosis found in Arabidopsis.

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

understood. eIF2α and its kinase GCN2, which are probably relevant to attenuation of global protein synthesis, have been identified in plants. However, questions remain about whether additional kinases for phosphorylation of eIF2α exist, and whether a TF similar to ATF4 and GCN4 is necessary to activate ISR in plants. Questions also remain regarding UPR, ERAD and ER stress-induced apoptosis in plants. Key questions include whether any negative regulators of the UPR that play a role in recovery from ER stress exist in plants, and whether caspase activity is necessary for ER stress-induced apoptosis in plants. Studies on the underlying mechanisms of the components and their functional networks are required to unravel the complexities of the UPR, ERAD and ER stress-induced apoptosis in plants.

Acknowledgments

The authors would like to acknowledge the support given by the Next-Generation BioGreen Program (SSAC, PJ008109), the Rural Development Administration, the TDPAF (609004-5), the Ministry for Food, Agriculture, Forestry and Fisheries, and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Republic of Korea (2012R1A1A2001074).

REFERENCES

[1] Ellgaard L, Helenius A. Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 2003;4:181–91.
[2] Bonifacino JS, Weissman AM. Ubiquitin and the control of protein fate in the secretory and endocytic pathways. Annu Rev Cell Dev Biol 1998;14:19–57.
[3] Higa A, Chevet E. Redox signaling loops in the unfolded protein response. Cell Signal 2012;24:1548–55.
[4] Hwang C, Sinskey AJ, Lodish HF. Oxidized redox state of glutathione in the endoplasmic reticulum. Science 1992;257:1496–502.
[5] Gething MJ, Sambrook J. Protein folding in the cell. Nature 1992;355:33–45.
[6] Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2000;2:326–32.
[7] Shen J, Chen X, Hendershot L, Prywes R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Dev Cell 2002;3:99–111.
[8] Williams DB. Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. J Cell Sci 2006;119:615–23.
[9] Wilkinson B, Gilbert HF. Protein disulfide isomerase. Biochim Biophys Acta 2004;1699:35–44.
[10] Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature 1988;332:462–4.
[11] Harding HP, Calfon M, Urano F, Novoa I, Ron D. Transcriptional and translational control in the mammalian unfolded protein response. Annu Rev Cell Dev Biol 2002;18:575–99.
[12] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007;8:519–29.
[13] Prostko CR, Brostrom MA, Brostrom CO. Reversible phosphorylation of eukaryotic initiation factor 2 alpha in response to endoplasmic reticular signaling. Mol Cell Biochem 1993;127–128:255–65.
[14] Ron D. Translational control in the endoplasmic reticulum stress response. J Clin Invest 2002;110:1383–8.
[15] Holcik M, Sonenberg N. Translational control in stress and apoptosis. Nat Rev Mol Cell Biol 2005;6:318–27.
[16] Tsaytler P, Harding HP, Ron D, Bertolotti A. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. Science 2011;332:91–4.
[17] Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, et al. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science 2005;307:935–9.
[18] Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, et al. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev 2004;18:3066–77.
[19] Ameri K, Harris AL. Activating transcription factor 4. Int J Biochem Cell Biol 2008;40:14–21.
[20] Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 2003;11:619–33.
[21] Dever TE, Feng L, Wek RC, Cigan AM, Donahue TF, Hinnebusch AG. Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast. Cell 1992;68:585–96.
[22] Hinnebusch AG. Evidence for translational regulation of the activator of general amino acid control in yeast. Proc Natl Acad Sci U S A 1984;81:6442–6.
[23] Mueller PP, Hinnebusch AG. Multiple upstream AUG codons mediate translational control of GCN4. Cell 1986;45:201–7.
[24] Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 2000;6:1099–108.
[25] Vattem KM, Wek RC. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc Natl Acad Sci U S A 2004;101:11269–74.
[26] Mori K, Sant A, Kohno K, Normington K, Gething MJ, Sambrook JF. A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins. EMBO J 1992;11:2583–93.
[27] Cox JS, Shamu CE, Walter P. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell 1993;73:1197–206.
[28] Mori K, Ma W, Gething MJ, Sambrook J. A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. Cell 1993;74:743–56.
[29] Cox JS, Walter P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell 1996;87:391–404.
[30] Kawahara T, Yanagi H, Yura T, Mori K. Endoplasmic reticulum stress-induced mRNA splicing permits synthesis of transcription factor Hac1p/Ern4p that activates the unfolded protein response. Mol Biol Cell 1997;8:1845–62.
[31] Kawahara T, Yanagi H, Yura T, Mori K. Unconventional splicing of HAC1/ERN4 mRNA required for the unfolded protein response. Sequence-specific and non-sequential cleavage of the splice sites. J Biol Chem 1998;273:1802–7.
[32] Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T. mRNA splicing-mediated C-terminal replacement of transcription factor Hac1p is required for efficient activation of the unfolded protein response. Proc Natl Acad Sci U S A 2000;97:4660–5.
[33] Shamu CE, Walter P. Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the

endoplasmic reticulum to the nucleus. EMBO J 1996;15:3028–39.
[34] Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. Cell 1997;90:1031–9.
[35] Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T. Palindrome with spacer of one nucleotide is characteristic of the cis-acting unfolded protein response element in *Saccharomyces cerevisiae*. J Biol Chem 1998;273:9912–20.
[36] Ng DT, Spear ED, Walter P. The unfolded protein response regulates multiple aspects of secretory and membrane protein biogenesis and endoplasmic reticulum quality control. J Cell Biol 2000;150:77–88.
[37] Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 2000;101:249–58.
[38] Tirasophon W, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes Dev 1998;12:1812–24.
[39] Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D. Cloning of mammalian Ire1 reveals diversity in the ER stress responses. EMBO J 1998;17:5708–17.
[40] Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol 2003;23:7448–59.
[41] Yoshida H, Matsui T, Hosokawa N, Kaufman RJ, Nagata K, Mori K. A time-dependent phase shift in the mammalian unfolded protein response. Dev Cell 2003;4:265–71.
[42] Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 2001;107:881–91.
[43] Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, Kimata Y, et al. Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. Nat Cell Biol 2001;3:158–64.
[44] Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell 1999;10:3787–99.
[45] Okada T, Haze K, Nadanaka S, Yoshida H, Seidah NG, Hirano Y, et al. A serine protease inhibitor prevents endoplasmic reticulum stress-induced cleavage but not transport of the membrane-bound transcription factor ATF6. J Biol Chem 2003;278:31024–32.
[46] Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell 2000;6:1355–64.
[47] Adachi Y, Yamamoto K, Okada T, Yoshida H, Harada A, Mori K. ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. Cell Struct Funct 2008;33:75–89.
[48] Yoshida H, Haze K, Yanagi H, Yura T, Mori K. 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 1998;273:33741–9.
[49] Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M, et al. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol Cell Biol 2000;20:6755–67.
[50] Chen X, Karnovsky A, Sans MD, Andrews PC, Williams JA. Molecular characterization of the endoplasmic reticulum: insights from proteomic studies. Proteomics 2010;10:4040–52.
[51] Chen X, Walker AK, Strahler JR, Simon ES, Tomanicek-Volk SL, Nelson BB, et al. Organellar proteomics: analysis of pancreatic zymogen granule membranes. Mol Cell Proteomics 2006;5:306–12.
[52] Bull VH, Thiede B. Proteome analysis of tunicamycin-induced ER stress. Electrophoresis 2012;33:1814–23.
[53] Liu CI, Wang RY, Lin JJ, Su JH, Chiu CC, Chen JC, et al. Proteomic profiling of the 11-dehydrosinulariolide-treated oral carcinoma cells Ca9-22: effects on the cell apoptosis through mitochondrial-related and ER stress pathway. J Proteomics 2012;75:5578–89.
[54] Iwata Y, Fedoroff NV, Koizumi N. Arabidopsis bZIP60 is a proteolysis-activated transcription factor involved in the endoplasmic reticulum stress response. Plant Cell 2008;20:3107–21.
[55] Kamauchi S, Nakatani H, Nakano C, Urade R. Gene expression in response to endoplasmic reticulum stress in *Arabidopsis thaliana*. FEBS J 2005;272:3461–76.
[56] Martinez IM, Chrispeels MJ. Genomic analysis of the unfolded protein response in Arabidopsis shows its connection to important cellular processes. Plant Cell 2003;15:561–76.
[57] Che P, Bussell JD, Zhou W, Estavillo GM, Pogson BJ, Smith SM. Signaling from the endoplasmic reticulum activates brassinosteroid signaling and promotes acclimation to stress in Arabidopsis. Sci Signal 2010;3:ra69.
[58] Liu JX, Srivastava R, Che P, Howell SH. An endoplasmic reticulum stress response in Arabidopsis is mediated by proteolytic processing and nuclear relocation of a membrane-associated transcription factor, bZIP28. Plant Cell 2007;19:4111–9.
[59] Liu JX, Srivastava R, Che P, Howell SH. Salt stress responses in Arabidopsis utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. Plant J 2007;51:897–909.
[60] Nagashima Y, Mishiba K, Suzuki E, Shimada Y, Iwata Y, Koizumi N. Arabidopsis IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor. Sci Rep 2011;1:29.
[61] Gao H, Brandizzi F, Benning C, Larkin RM. A membrane-tethered transcription factor defines a branch of the heat stress response in Arabidopsis thaliana. Proc Natl Acad Sci U S A 2008;105:16398–403.
[62] Deng Y, Humbert S, Liu JX, Srivastava R, Rothstein SJ, Howell SH. 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 U S A 2011;108:7247–52.
[63] Fujita M, Mizukado S, Fujita Y, Ichikawa T, Nakazawa M, Seki M, et al. Identification of stress-tolerance-related transcription-factor genes via mini-scale Full-length cDNA Over-eXpressor (FOX) gene hunting system. Biochem Biophys Res Commun 2007;364:250–7.
[64] Hayashi S, Wakasa Y, Takahashi H, Kawakatsu T, Takaiwa F. Signal transduction by IRE1-mediated splicing of bZIP50 and other stress sensors in the endoplasmic reticulum stress response of rice. Plant J 2012;69:946–56.
[65] Lu SJ, Yang ZT, Sun L, Song ZT, Liu JX. Conservation of IRE1-regulated bZIP74 mRNA unconventional splicing in rice (*Oryza sativa* L.) involved in ER stress responses. Mol Plant 2012;5:504–14.
[66] Tateda C, Ozaki R, Onodera Y, Takahashi Y, Yamaguchi K, Berberich T, et al. NtbZIP60, an endoplasmic reticulum-localized transcription factor, plays a role in the defense response against bacterial pathogens in *Nicotiana tabacum*. J Plant Res 2008;121:603–11.

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

[67] Ye C, Dickman MB, Whitham SA, Payton M, Verchot J. The unfolded protein response is triggered by a plant viral movement protein. Plant Physiol 2011;156:741–55.
[68] Koizumi N, Martinez IM, Kimata Y, Kohno K, Sano H, Chrispeels MJ. Molecular characterization of two Arabidopsis Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases. Plant Physiol 2001;127: 949–62.
[69] Iwata Y, Koizumi N. An Arabidopsis transcription factor, AtbZIP60, regulates the endoplasmic reticulum stress response in a manner unique to plants. Proc Natl Acad Sci U S A 2005;102:5280–5.
[70] Seo PJ, Kim SG, Park CM. Membrane-bound transcription factors in plants. Trends Plant Sci 2008;13:550–6.
[71] Urade R. The endoplasmic reticulum stress signaling pathways in plants. Biofactors 2009;35:326–31.
[72] Liu JX, Howell SH. 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 2010;22:782–96.
[73] Katagiri F, Lam E, Chua NH. Two tobacco DNA-binding proteins with homology to the nuclear factor CREB. Nature 1989;340:727–30.
[74] Schindler U, Beckmann H, Cashmore AR. TGA1 and G-box binding factors: two distinct classes of Arabidopsis leucine zipper proteins compete for the G-box-like element TGACGTGG. Plant Cell 1992;4:1309–19.
[75] Liu JX, Srivastava R, Howell SH. Stress-induced expression of an activated form of AtbZIP17 provides protection from salt stress in Arabidopsis. Plant Cell Environ 2008;31: 1735–43.
[76] Clemens MJ, Elia A. The double-stranded RNA-dependent protein kinase PKR: structure and function. J Interferon Cytokine Res 1997;17:503–24.
[77] Samuel CE, Kuhen KL, George CX, Ortega LG, Rende-Fournier R, Tanaka H. The PKR protein kinase–an interferon-inducible regulator of cell growth and differentiation. Int J Hematol 1997;65:227–37.
[78] Deng J, Harding HP, Raught B, Gingras AC, Berlanga JJ, Scheuner D, et al. Activation of GCN2 in UV-irradiated cells inhibits translation. Curr Biol 2002;12:1279–86.
[79] Kimball SR. Regulation of translation initiation by amino acids in eukaryotic cells. Prog Mol Subcell Biol 2001;26: 155–84.
[80] Zhang Y, Dickinson JR, Paul MJ, Halford NG. Molecular cloning of an arabidopsis homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation. Planta 2003;217:668–75.
[81] Lageix S, Lanet E, Pouch-Pelissier MN, Espagnol MC, Robaglia C, Deragon JM, et al. Arabidopsis eIF2alpha kinase GCN2 is essential for growth in stress conditions and is activated by wounding. BMC Plant Biol 2008;8:134.
[82] Sormani R, Delannoy E, Lageix S, Bitton F, Lanet E, Saez-Vasquez J, et al. Sublethal cadmium intoxication in *Arabidopsis thaliana* impacts translation at multiple levels. Plant Cell Physiol 2011;52:436–47.
[83] Zhang Y, Wang Y, Kanyuka K, Parry MA, Powers SJ, Halford NG. GCN2-dependent phosphorylation of eukaryotic translation initiation factor-2alpha in Arabidopsis. J Exp Bot 2008;59: 3131–41.
[84] Li C, Wang L, Huang K, Zheng L. Endoplasmic reticulum stress in retinal vascular degeneration: protective role of resveratrol. Invest Ophthalmol Vis Sci 2012;53:3241–9.
[85] Barber GN, Thompson S, Lee TG, Strom T, Jagus R, Darveau A, et al. The 58-kilodalton inhibitor of the interferon-induced double-stranded RNA-activated protein kinase is a tetratricopeptide repeat protein with oncogenic properties. Proc Natl Acad Sci U S A 1994;91: 4278–82.
[86] Yan W, Frank CL, Korth MJ, Sopher BL, Novoa I, Ron D, et al. Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. Proc Natl Acad Sci U S A 2002;99:15920–5.
[87] Oyadomari S, Yun C, Fisher EA, Kreglinger N, Kreibich G, Oyadomari M, et al. Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. Cell 2006;126:727–39.
[88] Bilgin DD, Liu Y, Schiff M, Dinesh-Kumar SP. P58(IPK), a plant ortholog of double-stranded RNA-dependent protein kinase PKR inhibitor, functions in viral pathogenesis. Dev Cell 2003;4:651–61.
[89] Ruiz-Canada C, Kelleher DJ, Gilmore R. Cotranslational and posttranslational N-glycosylation of polypeptides by distinct mammalian OST isoforms. Cell 2009;136:272–83.
[90] Molinari M, Helenius A. Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells. Nature 1999;402:90–3.
[91] Sitia R, Braakman I. Quality control in the endoplasmic reticulum protein factory. Nature 2003;426:891–4.
[92] Spiro RG. Role of N-linked polymannose oligosaccharides in targeting glycoproteins for endoplasmic reticulum-associated degradation. Cell Mol Life Sci 2004;61: 1025–41.
[93] Hardt B, Aparicio R, Bause E. The oligosaccharyltransferase complex from pig liver: cDNA cloning, expression and functional characterisation. Glycoconj J 2000;17:767–79.
[94] Boisson M, Gomord V, Audran C, Berger N, Dubreucq B, Granier F, et al. Arabidopsis glucosidase I mutants reveal a critical role of N-glycan trimming in seed development. EMBO J 2001;20:1010–9.
[95] Soussilane P, D'Alessio C, Paccalet T, Fitchette AC, Parodi AJ, Williamson R, et al. N-glycan trimming by glucosidase II is essential for Arabidopsis development. Glycoconj J 2009;26: 597–607.
[96] Burn JE, Hurley UA, Birch RJ, Arioli T, Cork A, Williamson RE. The cellulose-deficient Arabidopsis mutant rsw3 is defective in a gene encoding a putative glucosidase II, an enzyme processing N-glycans during ER quality control. Plant J 2002;32:949–60.
[97] Gillmor CS, Poindexter P, Lorieau J, Palcic MM, Somerville C. Alpha-glucosidase I is required for cellulose biosynthesis and morphogenesis in Arabidopsis. J Cell Biol 2002;156: 1003–13.
[98] Jin H, Yan Z, Nam KH, Li J. Allele-specific suppression of a defective brassinosteroid receptor reveals a physiological role of UGGT in ER quality control. Mol Cell 2007;26:821–30.
[99] Saijo Y, Tintor N, Lu X, Rauf P, Pajerowska-Mukhtar K, Haweker H, et al. Receptor quality control in the endoplasmic reticulum for plant innate immunity. EMBO J 2009;28:3439–49.
[100] Molinari M, Eriksson KK, Calanca V, Galli C, Cresswell P, Michalak M, et al. Contrasting functions of calreticulin and calnexin in glycoprotein folding and ER quality control. Mol Cell 2004;13:125–35.
[101] Moremen KW, Molinari M. N-linked glycan recognition and processing: the molecular basis of endoplasmic reticulum quality control. Curr Opin Struct Biol 2006;16:592–9.
[102] Hebert DN, Garman SC, Molinari M. The glycan code of the endoplasmic reticulum: asparagine-linked carbohydrates as protein maturation and quality-control tags. Trends Cell Biol 2005;15:364–70.
[103] Jessop CE, Chakravarthi S, Garbi N, Hammerling GJ, Lovell S, Bulleid NJ. ERp57 is essential for efficient folding of glycoproteins sharing common structural domains. EMBO J 2007;26:28–40.
[104] Vashist S, Ng DT. Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. J Cell Biol 2004;165:41–52.

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

[105] Hirsch C, Gauss R, Horn SC, Neuber O, Sommer T. The ubiquitylation machinery of the endoplasmic reticulum. Nature 2009;458:453–60.
[106] Plemper RK, Bordallo J, Deak PM, Taxis C, Hitt R, Wolf DH. Genetic interactions of Hrd3p and Der3p/Hrd1p with Sec61p suggest a retro-translocation complex mediating protein transport for ER degradation. J Cell Sci 1999;112(Pt 22): 4123–34.
[107] Bernasconi R, Galli C, Calanca V, Nakajima T, Molinari M. Stringent requirement for HRD1, SEL1L, and OS-9/XTP3-B for disposal of ERAD-LS substrates. J Cell Biol 2010;188: 223–35.
[108] Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. Cell 2006;126:361–73.
[109] Deng M, Hochstrasser M. Spatially regulated ubiquitin ligation by an ER/nuclear membrane ligase. Nature 2006;443: 827–31.
[110] Ravid T, Kreft SG, Hochstrasser M. Membrane and soluble substrates of the Doa10 ubiquitin ligase are degraded by distinct pathways. EMBO J 2006;25:533–43.
[111] Clerc S, Hirsch C, Oggier DM, Deprez P, Jakob C, Sommer T, et al. Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. J Cell Biol 2009;184:159–72.
[112] Quan EM, Kamiya Y, Kamiya D, Denic V, Weibezahn J, Kato K, et al. Defining the glycan destruction signal for endoplasmic reticulum-associated degradation. Mol Cell 2008;32:870–7.
[113] Molinari M, Calanca V, Galli C, Lucca P, Paganetti P. Role of EDEM in the release of misfolded glycoproteins from the calnexin cycle. Science 2003;299:1397–400.
[114] Oda Y, Hosokawa N, Wada I, Nagata K. EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. Science 2003;299:1394–7.
[115] Hirao K, Natsuka Y, Tamura T, Wada I, Morito D, Natsuka S, et al. EDEM3, a soluble EDEM homolog, enhances glycoprotein endoplasmic reticulum-associated degradation and mannose trimming. J Biol Chem 2006;281:9650–8.
[116] Xie W, Kanehara K, Sayeed A, Ng DT. Intrinsic conformational determinants signal protein misfolding to the Hrd1/Htm1 endoplasmic reticulum-associated degradation system. Mol Biol Cell 2009;20:3317–29.
[117] Mueller B, Lilley BN, Ploegh HL. SEL1L, the homologue of yeast Hrd3p, is involved in protein dislocation from the mammalian ER. J Cell Biol 2006;175:261–70.
[118] Kirst ME, Meyer DJ, Gibbon BC, Jung R, Boston RS. Identification and characterization of endoplasmic reticulum-associated degradation proteins differentially affected by endoplasmic reticulum stress. Plant Physiol 2005;138:218–31.
[119] Pedrazzini E, Giovinazzo G, Bielli A, de Virgilio M, Frigerio L, Pesca M, et al. Protein quality control along the route to the plant vacuole. Plant Cell 1997;9:1869–80.
[120] Su W, Liu Y, Xia Y, Hong Z, Li J. Conserved endoplasmic reticulum-associated degradation system to eliminate mutated receptor-like kinases in Arabidopsis. Proc Natl Acad Sci U S A 2011;108:870–5.
[121] Hong Z, Jin H, Tzfira T, Li J. Multiple mechanism-mediated retention of a defective brassinosteroid receptor in the endoplasmic reticulum of Arabidopsis. Plant Cell 2008;20: 3418–29.
[122] Hong Z, Jin H, Fitchette AC, Xia Y, Monk AM, Faye L, et al. Mutations of an alpha1,6 mannosyltransferase inhibit endoplasmic reticulum-associated degradation of defective brassinosteroid receptors in Arabidopsis. Plant Cell 2009;21:3792–802.
[123] Liu L, Cui F, Li Q, Yin B, Zhang H, Lin B, et al. The endoplasmic reticulum-associated degradation is necessary for plant salt tolerance. Cell Res 2011;21:957–69.
[124] Huttner S, Veit C, Schoberer J, Grass J, Strasser R. Unraveling the function of *Arabidopsis thaliana* OS9 in the endoplasmic reticulum-associated degradation of glycoproteins. Plant Mol Biol 2012;79:21–33.
[125] Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 2006;7:880–5.
[126] Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, et al. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 1998;12: 982–95.
[127] Brush MH, Weiser DC, Shenolikar S. Growth arrest and DNA damage-inducible protein GADD34 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. Mol Cell Biol 2003;23: 1292–303.
[128] Wang S, Narendra S, Fedoroff N. Heterotrimeric G protein signaling in the Arabidopsis unfolded protein response. Proc Natl Acad Sci U S A 2007;104:3817–22.
[129] Chen Y, Brandizzi F. AtIRE1A/AtIRE1B and AGB1 independently control two essential unfolded protein response pathways in Arabidopsis. Plant J 2012;69:266–77.
[130] Distelhorst CW, McCormick TS. Bcl-2 acts subsequent to and independent of Ca2+ fluxes to inhibit apoptosis in thapsigargin- and glucocorticoid-treated mouse lymphoma cells. Cell Calcium 1996;19:473–83.
[131] Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 2001;292:727–30.
[132] Boya P, Cohen I, Zamzami N, Vieira HL, Kroemer G. Endoplasmic reticulum stress-induced cell death requires mitochondrial membrane permeabilization. Cell Death Differ 2002;9:465–7.
[133] Hacki J, Egger L, Monney L, Conus S, Rosse T, Fellay I, et al. Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2. Oncogene 2000;19: 2286–95.
[134] Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu QC, Yuan J, et al. Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. J Cell Biol 2003;162:59–69.
[135] Huckelhoven R. BAX Inhibitor-1, an ancient cell death suppressor in animals and plants with prokaryotic relatives. Apoptosis 2004;9:299–307.
[136] Watanabe N, Lam E. BAX inhibitor-1 modulates endoplasmic reticulum stress-mediated programmed cell death in Arabidopsis. J Biol Chem 2008;283:3200–10.
[137] Williams B, Kabbage M, Britt R, Dickman MB. AtBAG7, an Arabidopsis Bcl-2-associated athanogene, resides in the endoplasmic reticulum and is involved in the unfolded protein response. Proc Natl Acad Sci U S A 2010;107: 6088–93.
[138] Costa MD, Reis PA, Valente MA, Irsigler AS, Carvalho CM, Loureiro ME, et al. A new branch of endoplasmic reticulum stress signaling and the osmotic signal converge on plant-specific asparagine-rich proteins to promote cell death. J Biol Chem 2008;283:20209–19.
[139] Alves MS, Reis PA, Dadalto SP, Faria JA, Fontes EP, Fietto LG. A novel transcription factor, ERD15 (Early Responsive to Dehydration 15), connects endoplasmic reticulum stress with an osmotic stress-induced cell death signal. J Biol Chem 2011;286:20020–30.
[140] Faria JA, Reis PA, Reis MT, Rosado GL, Pinheiro GL, Mendes GC, et al. The NAC domain-containing protein, GmNAC6, is a downstream component of the ER stress- and osmotic stress-induced NRP-mediated cell-death signaling pathway. BMC Plant Biol 2011;11:129.

Please cite this article as: Wahyu Indra Duwi Fanata , et al, The unfolded protein response in plants: A fundamental adaptive cellular response to internal and external stresses, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.023

[141] Reis PA, Rosado GL, Silva LA, Oliveira LC, Oliveira LB, Costa MD, et al. The binding protein BiP attenuates stress-induced cell death in soybean via modulation of the N-rich protein-mediated signaling pathway. Plant Physiol 2011;157: 1853–65.
[142] Alvim FC, Carolino SM, Cascardo JC, Nunes CC, Martinez CA, Otoni WC, et al. Enhanced accumulation of BiP in transgenic plants confers tolerance to water stress. Plant Physiol 2001;126:1042–54.
[143] Valente MA, Faria JA, Soares-Ramos JR, Reis PA, Pinheiro GL, Piovesan ND, et al. The ER luminal binding protein (BiP) mediates an increase in drought tolerance in soybean and delays drought-induced leaf senescence in soybean and tobacco. J Exp Bot 2009;60:533–46.