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Endoplasmic Reticulum Stress Response

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1. Introduction

The endoplasmic reticulum (ER) is a key organelle that serves as the gateway for newly synthesized proteins into the secretory pathway. Following synthesis, secretory proteins are exported from the ER to various cellular compartments where they fulfill their inherent biological roles. The correct trafficking of proteins through the secretory pathway is accomplished by sorting-specific motifs in the secretory proteins that are recognized by transporters that translocate the proteins to their cellular destination. As a sorting and protein-processing organelle, the ER contains protein-folding and protein-processing mechanisms for the maturation of newly-synthesized secretory proteins and a quality control mechanism to prevent abnormal proteins from reaching their final destination. Under normal conditions, the ER's processing capacity is dynamically balanced with the protein synthesis rate. Disruption of the equilibrium between the cell's secretory activity and the ER's processing and folding capacities promotes a condition that is known as ER stress. In general, perturbation of ER homeostasis by ER stress leads to the accumulation of unfolded proteins in the lumen of the organelle, which triggers a cytoprotective signaling pathway designated as the unfolded protein response (UPR). To restore ER homeostasis, activation of the UPR culminates in transient and general down-regulation of protein translation, up-regulation of ER folding functions to enhance the ER's processing capacity and induction of the ER-associated protein degradation-related quality control mechanism that ensures the disposal of unfolded and abnormal proteins. However, if the stress condition persists and UPR fails to restore ER homeostasis, a cell death signal is activated as the ultimate attempt for survival. In soybean, the ER is a prime organelle partly due to the high secretory activity of developing seeds, which accumulate high levels of storage proteins in protein bodies originating in the secretory pathway. As in any other eukaryotic organism, ER stress triggers the evolutionarily conserved UPR in soybean, but it also participates in crosstalk with several other adaptive signaling responses, such as osmotic stress-induced cell death and ER stress-induced programmed cell death.

2. Structure and constitutive function of the endoplasmic reticulum

The endoplasmic reticulum (ER) is a large, versatile and flexible organelle consisting of a high-surface-area endomembrane system into which various groups of peripheral and integral proteins are incorporated. The constitutive function of the ER is not restricted to protein synthesis and the modification and control of cellular Ca^{2+} concentration. Due to the presence of signaling proteins, the ER also acts as a regulator of signaling pathways.

There are numerous functional domains in the ER of plant cells that extend into areas in connection with other organelles in which specific products accumulate (Sparkes et al., 2009). For instance, the junction between the outer nuclear envelope and the ER forms a gated domain that controls the exchange of proteins between the two organelles. Likewise, the exportation sites to the Golgi, designated ERESs (ER exit sites), are important junctions in the secretory pathway that mediate the transport of soluble and membrane proteins from ER to Golgi.

The ER possesses three distinct sub-compartments: the rough ER, the smooth ER and the nuclear envelope, and these are interlinked by a membrane network that extends as tubules (smooth) and cisternae (rough) throughout the cytosol (Sparkes et al., 2009). The rough ER represents the entry of proteins into the secretory pathway. Soluble and membrane proteins are translocated from this pathway to their final destination: the ER, Golgi, vacuole or plasma membrane.

The rough endoplasmic reticulum (ER) is a major site of protein biosynthesis, and translation occurs on the cytosolic face of the ER via polysomes associated with the ER membrane (Vitale and Denecke, 1999). Nascent proteins are co-translationally translocated across the ER membrane through aqueous channels designated translocons. Protein synthesis initiates in the cytosol, leading to the exposure of an N-terminal peptide signal that is recognized by the ribonucleoprotein SRP (signal recognition particle). Upon binding to the signal peptide, the SRP mediates the association of the nascent ribosome-peptide-SRP complex with the ER membrane through interactions with the SR receptor and ER membrane proteins. After association with the ER membrane, the signal peptide is transferred from the SRP to the translocon, where it is positioned to drive the synthesis of the protein into the lumen of the ER. The Sec61 complex is part of the translocon and plays a key role in the translocation of newly-synthesized proteins into the lumen of the ER. In plants, homologs of Sec61 subunits have been shown to share similar functions with their yeast and mammalian counterparts (Hartmann et al., 1994). The hydrolysis of the signal peptide is carried out by specific proteases called signal peptidases, which are located on the luminal side of ER. The removal of the signal peptide from newly-synthesized proteins is crucial for the maintenance of ER homeostasis (Coleman et al., 1995). Mutants that are defective in this process, such as the maize floury2 mutant, display altered phenotypes and enhanced accumulation of molecular chaperones involved in protein folding (Fontes et al., 1991; Gillikin et al., 1997). In the case of ER integral membrane proteins that have a transmembrane segment, this hydrophobic segment functions as a stop transfer signal that interrupts the translocation process of the nascent peptide. Then, the hydrophobic residues migrate laterally to the interior of the membrane, creating transmembrane domains that anchor the protein in the ER membrane.

Once inside the ER, the polypeptide chain may be modified by branched N-linked glycans if they have glucosylation sites for attachment of the oligosaccharide core. This process leads to the initiation of folding. The endoplasmic reticulum (ER) provides a folding environment that facilitates the proper folding and assembly of secretory proteins, which are prerequisites for their movement through the secretory apparatus. A set of ER-resident proteins that includes molecular chaperones and folding enzymes associates with newly synthesized polypeptides to promote the proper folding and assembly of oligomeric secretory proteins (see below). The folding of glycoproteins is facilitated by the calnexin/calreticulin system of molecular chaperones, whereas the folding of many other proteins is adenosine triphosphate (ATP)-dependent in a process mediated by ER-resident

members of the heat shock protein family. The tertiary structure of secretory proteins is stabilized by intra- and inter-chain disulfide (S) bonds, which are catalyzed by the protein disulfide isomerase (PDI).

Proteins that have not acquired the correct conformation must be identified and transported back to the cytosol or to the vacuoles for degradation in a process that is mediated by the ER-associated protein degradation (ERAD) machinery. This process of protein folding as well as unfolded protein identification and targeting for degradation underlies the mechanism of quality control in the organelle that ensures that abnormal proteins do not reach their final destination.

One of the most dynamic domains of ER is the exportation site to the Golgi that is designated the ERES (ER exit site). This important junction in the secretory pathway mediates the transport of soluble and membrane proteins from the rough ER to the Golgi apparatus through the COPII complex, which is a type of vesicle coat protein that transports proteins in anterograde transport (Lee & Miller, 2007). COPII refers to the specific coat protein complex that initiates the budding process on the ER membrane. The coat consists of large protein subcomplexes that are made of four different protein subunits. The transport may be bidirectional, and retrograde movement from Golgi to ER is mediated by COPI. This retrograde transport promotes the endocytosis of extracellular molecules and the recycling of proteins and membranes to maintain the integrity of different compartments.

During the process of protein export from ER to Golgi, the exported proteins are recruited on the cytosolic side of the ER membrane by the COPII complex. The assembly of the complex is activated by interactions of GTPases with GEFs (guanine-nucleotide exchange factor) to control the process of budding vesicles off from the ER membrane. The exit of this complex is balanced by the entry of the COPI complex into the ER. COPI is a protein that coats vesicles transporting proteins from the *cis* end of the Golgi complex back to the rough ER, where they were originally synthesized. The coat consists of large protein subcomplexes that are made of seven different protein subunits.

The vacuole is an organelle that may also be a direct target of proteins from the ER, which are transported to the vacuoles via a Golgi apparatus-independent pathway. For instance, maize endosperm protein bodies emerge directly from the ER by the accumulation of zeins (the maize storage proteins) as an insoluble matrix in the organelle (Herman and Larkins, 1999). Zeins belong to the class of seed proteins known as prolamins. In many cereals, prolamins are sequestered in the ER and form protein bodies that may remain in the ER or be delivered directly to the vacuole for storage (Chrispeels & Herman, 2000). In soybean, the biogenesis of seed protein bodies is predominantly via Golgi-mediated transport of storage proteins to vacuoles. Nevertheless, there is also compelling evidence for a Golgi-independent form of protein body biogenesis in soybean seeds (Mori et al. 2004).

The ER is also the storage site for the calcium that is used in many signal transduction cascades. Finally, if the cell encounters adverse physiological conditions that ultimately affect the environment of the ER, protein folding will be hampered and unfolded proteins will accumulate. A signal transduction response called the unfolded protein response (UPR) is initiated in the ER to protect the cell until normal conditions are restored.

2.1 Endoplasmic reticulum-resident molecular chaperones

The protein concentration in the lumen of the ER is estimated to be 100 mg/mL. Such a high concentration is believed to promote protein aggregation, which is particularly facilitated by the exposure of hydrophobic regions of newly synthesized proteins that otherwise would

not be exposed in their native conformation. As a consequence, the ER contains a set of resident proteins called reticuplasmins, including enzymes and chaperones, that function in the synthesis, folding and assembly of newly synthesized proteins in the ER.

2.1.1 ER members of the HSP family: Binding Protein (BiP) and Hsp94.

The molecular chaperone BiP (GRP78) is a 78-KDa protein that belongs to the HSP70 (Heat shock protein 70 kDa) family and represents one of the best characterized molecular chaperones of the ER. BiP has been shown to play a dynamic role in the regulation of various ER-supported processes, including regulation of molecular chaperone levels, translocation of secretory proteins into the ER lumen, catalysis of protein folding, targeting of incorrectly folded proteins for degradation and perception of ER stress (Hendershot, 2004). BiP binds and protects newly synthesized proteins when they are in an unfolded state, but it is released from them before maturation to allow folding of the substrate protein.

BiP harbors an N-terminal ATPase domain and a domain for substrate binding at the carboxyl-terminus. Therefore, like all Hsp70 family members, BiP binds and hydrolyzes adenosine triphosphate (ATP), which controls its chaperone function (Gething, 1999). Upon binding to ATP, Hsp70 undergoes a conformational change ("open" configuration), which allows it to associate with unfolded substrates. The presence of a DnaJ co-factor in the complex catalyzes the rapid hydrolysis of ATP to adenosine diphosphate (ADP), stabilizing the complex formed between Hsp70 and the unfolded protein substrate. The next step in the Hsp70 adenosine triphosphatase (ATPase) cycle is the exchange of ADP by ATP in the nucleotide binding cleft, which "reopens" the Hsp70 protein and releases the unfolded protein.

Soybean has four BiP genes: soyBiPA, soyBiPB, soyBiPC and soyBiPD, which are differentially expressed in different organs (Kalinski et al, 1995; Figueiredo et al., 1997; Cascardo et al., 2001). *soyBiPD* is expressed in all organs, whereas the expression of *soyBiPB* is restricted to leaves. *soyBiPA* transcripts are detected in leaves, roots and seeds, and *soyBiPC* RNA is confined to leaves, seeds and pods. Like all plant BiPs, conditions that promote the accumulation of unfolded proteins in the ER, such as treatment with tunicamycin or AZC (L-azetidine-2-carboxylic acid), strongly induce soybean BiPs. Soybean *BiP* expression has also been shown to respond to a variety of abiotic and biotic stress conditions, including water stress, fungus infestation, insect attack, nutritional stress, and elicitors of the plant-pathogenesis response (Carolino et al., 2003).

Soybean BiP exists in interconvertible phosphorylated and non-phosphorylated forms, and the equilibrium can be shifted to either direction in response to different stimuli (Cascardo et al., 2000). In contrast to tunicamycin treatment, water stress stimulates phosphorylation of BiP species in cultured soybean cells and stressed soybean leaves. Although the tunicamycin-induced BiP forms are unmodified and osmotic stress-induced BiP forms are phosphorylated, both treatments cause the conversion of oligomeric BiP to the monomeric forms (Carolino et al., 2003). In mammalian cells, modification of BiP is associated with its oligomerization, and it is generally accepted that the non-phosphorylated form is the biologically active BiP species in the folding pathway because tunicamycin-induced BiP or BiP bound to nascent proteins is unmodified (Freiden et al., 1992). Thus, the modification of plant BiP protein in response to water stress differs from the usual pattern of post-translational modifications of eukaryotic BiPs. The simplest explanation for these results is

that phosphorylation of soybean BiP by osmotic stress may serve as a distinct regulatory function, and because it is not restricted to the oligomeric form of BiP, it may occur at different sites. This argument is supported by the observation that despite its phosphorylation state, the soybean BiP isoform from water-stressed leaves exhibits protein-binding activity and associates with a water stress-induced 28 kDa polypeptide (Cascardo et al., 2000).

Soybean BiP has also been shown to associate detectably with normal storage proteins *in vitro* (Gillikin et al., 1995). However, direct evidence for a role of plant BiP in storage protein folding and maturation includes the demonstration that BiP associates co-translationally with rice prolamin storage proteins (Li et al., 1993) and binds to exposed sites on monomers of phaseolin but not to the trimeric form of the bean protein (Foresti et al., 2003). Thus, the BiP binding site is buried in the mature bean protein, supporting the notion that BiP action precedes and contributes to the maturation of legume storage proteins.

Glucose-regulated protein 94, also designated endoplasmin or Hsp94, is also a member of the heat shock family, and it is an abundant ER molecular chaperone. It seems to be encoded by a single gene in the Arabidopsis genome, the SHEPHERD (SHD) gene. Arabidopsis SHD is induced by ER stressors and exhibits chaperone activity with very specific substrate binding activity (Martınez & Chrispeels, 2003; Klein et al., 2006). At-SHD is implicated in the synthesis of clavata proteins; thus, null alleles of SHD display defects in meristem size control similar to those of clavata mutants (Ishiguro et al., 2002). Glycine max Hsp94 is also represented by a single gene in the soybean genome, but there is no information with respect to the function of soybean GRP94.

2.1.2 Calnexin/Calreticulin system

Calnexin and calreticulin are lectins that function as components of the major protein folding machinery of the ER and specifically bind glycoproteins that carry monoglucosylated N-linked glycans (Parodi, 2000). Calreticulin is a soluble protein in the ER lumen, whereas calnexin is a type 1 membrane protein. In eukaryotes, a large fraction of secretory proteins are glycosylated in the ER by attachment of a Glc3Man9GlcNac2 oligosaccharide core that has 14 monosaccharide moieties. The three glucoses in the most external portion of the oligosaccharide are numbered G12, G13 and G14. As the protein enters into the folding/unfolding cycle, the removal of G14 and G13 is initiated by the action of glycosidases I and II. Glycosidase I is a membrane glycoprotein tightly associated with the translocon, and it hydrolyses α-1,2 glycosidic bonds to remove the G14 of oligosaccharide chains on secretory proteins. In plants, the relevance of this hydrolysis for protein folding has been demonstrated by the incapacity of phaseolin to assemble into the mature trimeric protein if the hydrolysis of G14 is blocked (Lupattelli et al., 1997). Glycosidase II hydrolyses α-1,3 glycosidic bonds to release G13 from the oligosaccharide core on the protein. After removal of G13 and G14, the glycoproteins associate with calnexin and calreticulin for proper folding and protection against misaggregation. The recognition of folding glycoproteins by calnexin and calreticulin is mediated by protein-linked monoglucosylated oligosaccharides. The glycoproteins are released from their calreticulin/calnexin anchor through the action of glycosidase II, which releases G12 to leave an unglycosylated oligosaccharide on the protein. Monoglucosylated glycans are then recreated by UDP-Glc:glycoprotein glucosyltransferase (GT), a soluble luminal enzyme, and they are thus recognized again by the lectins if they are linked to improperly folded protein

moieties. The deglucosylation-reglucosylation cycle continues until proper folding is achieved. In this system, GT behaves as a sensor of glycoprotein conformation by specifically glucosylating N-linked glycans in misfolded glycoproteins and thus retaining them in the calnexin/calreticulin chaperone cycle.

As ER-resident molecular chaperones, both calreticulin and calnexin from soybean are induced by ER stressors, such as tunicamycin and AZC (Irsigler et al., 2007). Under hyperosmotic conditions, soybean calnexin is up-regulated and accumulates in the plasma membrane (Nouri & Komatsu, 2010).

2.1.3 PDI family

Protein disulfide isomerases (PDIs) also play important roles in the folding of nascent polypeptides. More specifically, they are involved in the formation of disulfide bonds on secretory proteins in the ER. PDIs are represented by a small family in soybean with at least two copies: GmPDIS-1and GMPDIS-2 (Wadahama et al., 2007). Their domain structures contain two thioredoxin-like domains, a and a′, and an ERp29c domain. In cotyledon cells, both proteins are distributed to the endoplasmic reticulum and protein storage vacuoles. Data from coimmunoprecipitation and crosslinking experiments indicate that GmPDIS-1 associates with proglycinin, a precursor of the seed storage protein glycinin, in the cotyledon. Levels of GmPDIS-1, but not of GmPDIS-2, increase in cotyledons with the accumulation of glycinin during seed development and under ER-stress conditions. In contrast, GmPDIS-2, but not GmPDIS-1, is induced by osmotic stress (Irsigler et al., 2007).

2.2 The endoplasmic reticulum-associated protein degradation system (ERAD)

Quality control in the ER is associated with two fundamental aspects of protein metabolism: the retention of incompletely or incorrectly folded proteins in the ER and the targeting of irreversibly misfolded proteins for degradation. This quality control mechanism increases the efficiency of correctly folded proteins, prevents the export of defective proteins, restores the amino acid pool and maintains the homeostasis of the endomenbrane system. Defective proteins are eliminated by the ERAD (ER-associated degradation) system, which recognizes terminally unfolded proteins, retrotranslocates them to the cytosol, promotes their ubiquitinization and degrades them.

Several homologs of ERAD-associated genes have been identified in soybean, such as genes encoding polyubiquitin, a ubiquitin conjugating enzyme, the alpha subunit of the proteasome, CDC48 and Derlin (Irsigler et al., 2007). However, the functions of these soybean homologs have not been demonstrated experimentally. Further evidence that a quality control mechanism operates in legumes is the demonstration that bean seed storage glycoproteins (*Phaseolus lunatus*) associate irreversibly with BiP and accumulate in the ER if glucosylation is blocked by treatment with tunicamycin (Sparvoli et al., 2000).

3. ER stress and the unfolded protein response

The ER is the gateway for secretory proteins, which are synthesized by ER-associated polyribosomes and interact with molecular chaperones as they enter the lumen of the organelle. The ER-resident molecular chaperones facilitate the folding and assembly of newly synthesized proteins into a competent conformation that allows their translocation further in the secretory pathway (Liu & Howell, 2010). However, if the secretory proteins

fail to acquire a native conformation, they are targeted for degradation in a process mediated by the ER-associated degradation system (ERAD). Therefore, under normal conditions, the rate of protein synthesis is balanced with the rate of protein processing and folding within the lumen of the ER. Any condition that disrupts the homeostasis of the organelle and the proper folding and maturation of secretory proteins promotes the accumulation of unfolded proteins in the lumen of the organelle, which results in ER stress. Cells respond to ER stress by activating a protective signaling cascade designated the unfolded protein response (UPR), which allows information about the folding status of proteins in the lumen of the ER to be transmitted to the nucleus and cytosol. In mammals, ER stress is sensed and the UPR is transduced by three distinct classes of ER transmembrane proteins: PERK, ATF6 and Ire1p. Upon activation, these receptors act in concert to transiently attenuate protein synthesis, up-regulate ER folding capacity and degrade misfolded proteins. However, if the stress persists and the UPR is unable to restore ER homeostasis, a cell death signal is activated as an ultimate attempt for survival.

Components of the plant UPR that are the proximal sensors include Ire1p homologs and ATF6-related proteins, designated AtbZIP28 and AtbZIP60 (Urade, 2009; Liu & Howell, 2010). Two Ire1p homologs were found in Arabidopsis (AtIre1-1 e AtIre1-2), and one was found in rice (OsIre1). Plant Ire1p homologs contain an Ire1p-like receptor configuration with a stress sensor luminal domain at the N-terminus, a transmembrane segment, and a kinase domain and a ribonuclease domain at the C-terminus. AtIre1-1 and AtIre1-2 are type I transmembrane proteins with a one-pass transmembrane segment in their central portion. AtIre1 is located in the nuclear envelope and in regions of the ER contiguous with the nucleus.

Three lines of evidence indicate that the C-terminal domains of plant Ire1p homologs are functional (Urade, 2009; Liu & Howell, 2010). First, the C-terminal domains of plant Ire1p homologs display high sequence conservation with the kinase/endonuclease C-terminal portion of yeast Ire1 and contain the DLKPQN motif, which resembles motives found in Ser/Thr kinases. In addition, AtIRE1 and OsIre1 exhibit autophosphorylation activity in vitro. Finally, mutation of the lysine residue in the AtIre1-1 DLKPQN motif totally abolishes the autophosphorylation activity of the receptor. In spite of having a ribonuclease domain at the C-terminus, its putative ribonuclease activity has not been demonstrated in plants.

The N-terminal domains of AtIre1-1, AtIre1-2 and OsIre1 function as ER stress sensors (Urade, 2009). The N-terminal domains of plant Ire1p homologs have been functionally characterized by complementation assays in yeast. In these experiments, the N-terminal domain of plant Ire1p, when fused to the C-terminal domain of yeast Ire1, was able to perceive ER stress in tunicamycin-treated yeast cells. Although the N-terminal domains of plant IRE1 homologs can functionally replace yeast IRE1, downstream components of the IRE1 signaling pathway have yet to be identified in plants.

AtbZIP60 and AtbZIP28 are ER stress-induced leucine zipper (bZIP) transcription factors that are anchored to the ER membrane under normal conditions and may serve as ER stress sensors and transducers, such as the mammalian ATF6 transducer (Liu & Howell, 2010). ATF6 is a transcription factor that is anchored to the ER membrane under normal conditions, and it has a C-terminal ER stress-sensing domain oriented to the ER lumen. In response to ER stress, ATF6 is translocated to the Golgi, where it is specifically cleaved by S1P and S2P proteases to release its N-terminal transcription factor domain. The released ATF6 domain is targeted to the nucleus, where it activates the coordinated up-regulation of a set of genes encoding ER chaperones, folding enzymes and ERAD components. Likewise,

the plant ATF6 homolog AtbZIP28 is located in the ER membrane under normal conditions. However, under stress conditions, AtbZIP28 undergoes regulated intermembrane proteolysis by S1P and S2P proteases. The released bZIP domain is translocated to the nucleus, where it acts in concert with the heterotrimeric NF-Y complex to activate UPR genes. The NF-Y complex is composed by the transcriptional factors NF-YA4, NF-YB3 and NF-YC2.

AtbZIP60 is a type II transmembrane transcriptional factor. It also undergoes regulated proteolysis by an unknown mechanism that is not dependent on the S1P and S2P proteases (Urade, 2009). Expression of a truncated form of AtbZIP60 harboring the bZIP domain up-regulates UPR target genes under normal conditions, indicating that the regulated release of AtbZIP60 from the membrane may underlie the transduction mechanism of an ER stress signal.

Comprehensive genome-wide evaluations of ER stress-induced changes in gene expression have provided evidence that the UPR operates in a similar fashion in both soybean and Arabidopsis (Irsigler et al., 2007). Inducers of ER stress, such as tunicamycin and AZC, promote the up-regulation of a class of genes that functions in (i) protein folding, (ii) ERAD and (iii) translational regulation. In the protein folding category, the up-regulated genes include ER-resident molecular chaperones such as BiP, calreticulin, calnexin, and the folding catalyst protein disulfide isomerase (PDI). ERAD-associated genes that are up-regulated by ER stress in soybean include those encoding polyubiquitin, ubiquitin conjugating enzyme, the alpha subunit of the proteasome, CDC48 and Derlin. Examples of ER stress-induced genes that are potential regulators of protein translation are a translational inhibitor protein (AW508686), eukaryotic translation initiation factor 3 subunit 10 (AW317679), translation elongation factor 1-gamma (AI960794) and a series of up-regulated ribosomal protein genes. These genomic analyses suggested that soybeans, like Arabidopsis and mammals, have evolved at least three different mechanisms that mediate UPR: (1) transcriptional induction of genes encoding chaperones and vesicle trafficking proteins, (2) attenuation of genes that encode secretory proteins, and (3) upregulation of the ER-associated protein degradation (ERAD) system for rapid disposal of unfolded proteins in the ER.

As further evidence that the UPR operates in soybean, the promoters of soybean BiP genes contain functional ER stress cis-acting elements (ERSEs), and soybean BiP functions as a regulator of the UPR as it does in mammals and yeast (Buzeli et al., 2002; Costa et al., 2008). Nevertheless, the roles of plant BiP in stress perception and in the mechanism of signal propagation remain to be elucidated. Overexpression of soybean BiP inhibits activation of the UPR and attenuates ER stress in response to tunicamycin, a potent inducer of ER stress (Alvim et al., 2001; Costa et al., 2008). Soybean BiP has also been demonstrated to confer tolerance to drought in transgenic soybean and tobacco plants (Alvim et al., 2001; Valente et al., 2009). The apparent increase in drought tolerance mediated by BiP has not been associated with typical short-term and long-term avoidance responses or with typical tolerance mechanisms. BiP overexpression confers resistance to drought through an unknown mechanism that may be related to ER function and cross-talk with the osmotic signaling response.

4. Integration of ER stress and the osmotic stress response: the NRP-mediated cell death response.

The potential of the endoplasmic reticulum (ER) stress response to accommodate adaptive pathways and its integration with other environmentally induced responses has been the

subject of studies in recent years (Liu & Howell, 2010). The most well-characterized ER-based integrative pathway in plants is the ER stress and osmotic stress integrated response that is mediated by N-rich proteins and transduces a cell death signal (Figure 1; Costa et al., 2008). This integrative pathway was first identified by genome-wide approaches and expression profiling studies, which revealed the existence of a modest overlap of the ER stress- and osmotic stress-induced transcriptomes in soybean seedlings treated with PEG (an inducer of osmotic stress) or tunicamycin and AZC, potent inducers of ER stress (Irsigler et al., 2007). In fact, these global expression-profiling analyses revealed that only a small
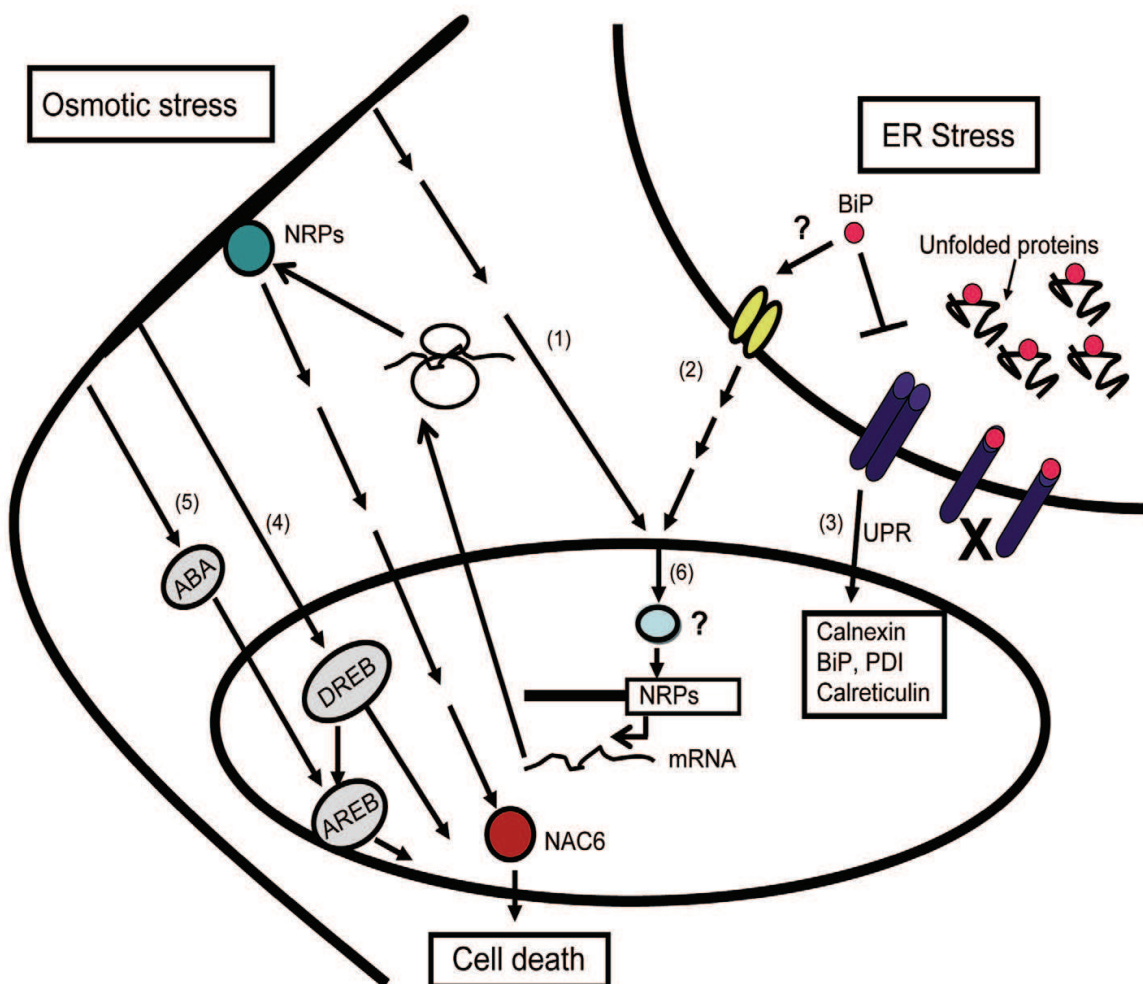
Fig. 1. Osmotic- and ER-stress integrating pathway.
ER stress and osmotic stress activate two independent signaling pathways (1 e 2), which converge upon *NRP-A* and *NRP-B* expression to activate an osmotic- and ER-stress integrating pathway, also called the integrated pathway (6). The ER-stress signaling branch (2) of the integrated pathway is distinct from the UPR (3). However, whether the osmotic-stress signaling branch (1) of this integrated pathway shares components with the ABA-dependent (4) and ABA-independent response (5) to drought is unknown at present. Enhanced accumulation of the membrane-associated NRPs activates a cascade to induce the expression of the nuclear transactivator *NAC6* that in turn promotes cell death. BiP, an ER-resident molecular chaperone, regulates the UPR. Whether BiP also modulates the integrated pathway remains to be determined.

proportion of the up-regulated genes (only 5.5%) represented a shared response that integrates the two signaling pathways. These co-regulated genes have been considered to be downstream targets of the integrated pathway based on similar induction kinetics and a synergistic response to the combination of osmotic and ER stress-inducing treatments. Genes in this integrated pathway with the strongest synergistic induction encode proteins with diverse roles, such as plant-specific development and cell death (DCD) domain-containing proteins (NRP-A and NRP-B), a ubiquitin-associated (UBA) protein homolog and NAC domain-containing proteins (GmNAC6). This integrated pathway diverges from the molecular chaperone-inducing branch of the unfolded protein response (UPR) in several ways. While UPR-specific targets are inversely regulated by ER and osmotic stresses, the co-regulated target genes require both signals for full activation. Furthermore, BiP (binding protein) overexpression in soybean prevents activation of the UPR by ER stress inducers but does not affect the induction of the integrated target genes.

NRP-A and NRP-B share a highly conserved C-terminal DCD (development and cell death) domain in addition to a high number of asparagine residues at their more divergent N-termini. This structural organization places NRP-A and NRP-B in subgroup I of the plant-specific DCD-containing proteins. NRPs are critical mediators of ER and osmotic stress-induced cell death in soybean (Costa el al., 2008). The cell death response mediated by NRPs resembles a programmed cell death event. Overexpression of NRPs in soybean protoplasts induces caspase-3-like activity and promotes extensive DNA fragmentation. Furthermore, transient expression of NRPs in plants causes leaf yellowing, chlorophyll loss, malondialdehyde production, ethylene evolution, and induction of the senescence marker gene CP1.

Like NRPs, GmNAC6, another target of the integrated pathway, is strongly induced by cycloheximide, a potent inducer of cell death in soybean suspension cells, and it promotes cell death when ectopically expressed in tobacco leaves (Pinheiro et al., 2009). GmNAC6 belongs to the class of NAC (NAM, ATAF1, ATAF2 and CUC2) domain-containing proteins, which comprise a large family of plant-specific transcription factor genes represented by at least 101 sequences in the soybean genome that are clustered into 15 different sub-families. Members of this family are involved in development and stress response.

The NAC transfactors are organized into a general structure that consists of a highly conserved N-terminal domain involved in DNA binding (called the NAC domain) and a C-terminal region highly divergent in sequence and length that functions as the activation domain (Hegedus et al., 2003). The NAC domain contains nearly 160 amino acid residues divided into five subdomains (A–E) of conserved blocks intercalated with heterogeneous blocks or gaps. In the ER and osmotic stress-induced cell death response, GmNAC6 acts downstream of NRPs as it is strongly induced by ectopic expression of NRPs in soybean protoplasts (unpublished), and it promotes cell death when expressed *in planta* (Pinheiro et al., 2009).

In mammalian cells, ER stress has been shown to trigger both ER stress-specific apoptotic pathways and shared PCD signaling pathways elicited by other death stimuli (Malhotra & Kaufman, 2007). The NRP-mediated PCD signaling fits the concept of a shared pathway induced by ER stress and other stimuli, which is consistent with the localization of NRPs either as cytosolic or membrane-associated proteins. This localization of NRPs, their rapid response to programmed cell death inducers along with their capacity to induce caspase-3-like activity in soybean protoplasts are all consistent with a model in which NRPs act as adaptor proteins that activate upstream initiator caspases. Several cell surface death

receptors have been identified in mammalian cells as the mediators of the extrinsic cell death-inducing pathway (Ashkenazi & Dixit, 1998). Upon activation by ligand binding, the cytoplasmic domain of these death receptors recruits adaptor proteins to activate caspase cascades that result in morphological changes associated with apoptosis. Identification of the downstream targets of NRPs and the regulators of their induction will be crucial to elucidating the underlying mechanism of this ER- and osmotic stress-induced cell death signaling pathway that transduces a PCD signal into multiple hallmarks of leaf senescence.

5. Acknowledgements

6. References

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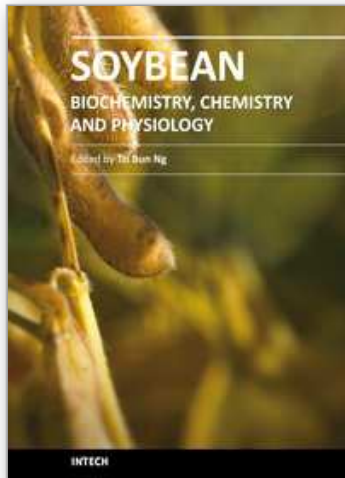
Soybean - Biochemistry, Chemistry and Physiology
Edited by Prof. Tzi-Bun Ng

Soybean is an agricultural crop of tremendous economic importance. Soybean and food items derived from it form dietary components of numerous people, especially those living in the Orient. The health benefits of soybean have attracted the attention of nutritionists as well as common people.

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