

## Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity

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**As sessile organisms, plants are exposed to persistently changing stresses and have to be able to interpret and respond to them. The stresses, drought, salinity, chemicals, cold and hot temperatures, and various pathogen attacks have interconnected effects on plants, resulting in the disruption of protein homeostasis. Maintenance of proteins in their functional native conformations and preventing aggregation of non-native proteins are important for cell survival under stress. Heat shock proteins (HSPs) functioning as molecular chaperones are the key components responsible for protein folding, assembly, translocation, and degradation under stress conditions and in many normal cellular processes. Plants respond to pathogen invasion using two different innate immune responses mediated by pattern recognition receptors (PRRs) or resistance (R) proteins. HSPs play an indispensable role as molecular chaperones in the quality control of plasma membrane-resident PRRs and intracellular R proteins against potential invaders. Here, we specifically discuss the functional involvement of cytosolic and endoplasmic reticulum (ER) HSPs/chaperones in plant immunity to obtain an integrated understanding of the immune responses in plant cells.**

**Keywords :** chaperones, heat shock proteins, plant immunity

Heat shock proteins (HSPs) are ubiquitous proteins found in plant and animal cells. They originally were described in relation to heat shock (Ritossa, 1962) but are now known to be induced by a wide variety of stresses, including exposure to cold, UV light, wound healing, tissue remodeling, or biotic stresses (Boston et al., 1996; Lindquist and Craig, 1988; Vierling, 1991). Thus, the term “heat shock protein” is a misnomer since many stresses other than heat induce expression of *hsp* genes.

HSPs are essential components contributing to cellular homeostasis under optimal and detrimental growth conditions in prokaryotic and eukaryotic cells (Lindquist and Craig, 1988; Lindquist, 1986; Wang et al., 2004). It is well-known that HSPs are responsible for protein folding, assembly, translocation, and degradation during ordinary cellular growth and development (Lindquist and Craig, 1988; Lindquist, 1986; Wang et al., 2004). HSPs also function in the stabilization of proteins and assist protein refolding under stress conditions (Huttner and Strasser, 2012; Sitia and Braakman, 2003; Whitley et al., 1999). Most members of HSPs perform critically important chaperone functions such as three-dimensional folding of newly formed proteins and/or proteins damaged by stress within cells (Whitley et al., 1999). For this reason, many chaperones are considered as HSPs due to their nature to aggregate when denatured by heat stress.

In plants and animals, there are five major families of HSPs conservatively recognized as molecular chaperones based on their approximate molecular weights, such as HSP100, HSP90, HSP70, HSP60, and small HSP (sHSP) (Gupta et al., 2010; Kotak et al., 2007; Wang et al., 2004). Many of these HSPs are mainly located in the cytoplasm and respond to abiotic and biotic stresses (Boston et al., 1996; Vierling, 1991). In addition to the cytosol, HSPs are located in other organelles such as the ER, chloroplasts,

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mitochondria, and nucleus, suggesting that they play different and dynamic roles in protein homeostasis (Boston et al., 1996; Vierling, 1991).

Some HSPs in animals have been reported to play important roles in the immune response, including antigen presentation, activation of lymphocytes and macrophages, and activation and maturation of dendritic cells (Li et al., 2002; Tsan and Gao, 2009; Wallin et al., 2002). Many of them, if not all, are mediated by interactions between HSPs and pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) recognizing pathogen-derived conserved microbial signatures called pathogen-associated molecular patterns (PAMPs) (Chisholm et al., 2006). In addition, it has been proposed that their presence serves as a ‘danger’ signal to the host immune system at sites of tissue injury or stress where HSPs are released extracellularly (Chen et al., 1999; Williams and Ireland, 2008). For example, HSP60 is shown to be recognized by PRRs such as TLR2 and TLR4 as an endogenous ‘danger’ signal in the immune system, stimulating rapid inflammatory cytokine release (Ohashi et al., 2000; Vabulas et al., 2001).

Recently, HSPs in plants have received considerable attention due to their novel function in innate immunity (Li et al., 2009; Liu and Howell, 2010; Nekrasov et al., 2009). Plants respond to pathogen invasion using a two-branched innate immune system consisting of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Plant PRRs transducing PTI constitute the first mode of defense against pathogen infection, like in animals (Dodds and Rathjen, 2010). Most PRRs, characterized as either receptor-like kinases (RLKs) or receptor-like proteins (RLPs), are located at the plasma membrane (Monaghan and Zipfel, 2012). Plasma membrane-resident PRRs are synthesized in the endoplasmic reticulum (ER), where they are subject to ER quality control (ER QC) (Li et al., 2009; Nekrasov et al., 2009; Park et al., 2010; Saijo, 2010). ER QC is a conserved process in eukaryotic cells that is responsible for monitoring correct folding and processing of membrane and secretory proteins (Kleizen and Braakman, 2004). Many ER proteins, including HSP70 luminal-binding protein (BiP), HSP40 ERdj3B, stromal-derived factor 2 (SDF2), calreticulin3, UDP-glucose glycoprotein glucosyl transferase, and ER retention defective 2B (ERD2B), participate in the ER QC machinery for PRR accumulation (Li et al., 2009; Liu and Howell, 2010; Nekrasov et al., 2009). The second defense system, ETI, provides a remarkable level of disease resistance (Chisholm et al., 2006; Dodds and Rathjen, 2010). ETI is triggered by R proteins acting as receptors for highly variable pathogen-derived effectors in the cytoplasm, either directly or indirectly. Most R genes

encode intracellular proteins belonging to the nucleotide-binding domain and leucine-rich repeat (NB-LRR)-containing protein family. Interaction of HSP90 with either SGT1 (suppressor of G2 allele kinetochore protein) or RAR1 (required for *Mla12* resistance) or both confers stability to R proteins, contributing to recognition of pathogen effectors (Kadota and Shirasu, 2012; Shirasu and Schulze-Lefert, 2003). HSP90 physically interacts with various R proteins such as N, RPM1 (resistance to *Pseudomonas maculicola* 1), RPS2 (resistance to *P. syringae* 2), and RPS4 (resistance to *Pseudomonas syringae* 4) (Hubert et al., 2003; Liu et al., 2004). HSP90 also interacts with the cysteine- and histidine-rich zinc-binding domain (CHORD) of RAR1 as well as the CHORD domain and SGT1 motif of SGT1 (Boter et al., 2007; Catlett and Kaplan, 2006).

In this review, we concisely discuss major recent findings regarding major plant HSPs/chaperones in relation to the immune response, particularly focusing on quality control of intracellular R proteins and plasma membrane-resident PRRs. We also cover how HSPs contribute to various types of plant immunity as well as other molecular ER chaperones.

### Cytosolic HSPs involved in plant immunity

HSPs are mainly located in the cytoplasm but involved in transferring cellular signals to the nucleus under stress conditions. Many cytosolic HSPs respond under not only abiotic stresses such as heat, drought, and salinity but also biotic stresses such as pathogen infection and insect attacks (Bhattarai et al., 2007; Breiman, 2014; Boston et al., 1996). Among HSP families, the functions of HSP90 in plant immunity are the most well characterized to date. HSP90 physically interacts with many co-chaperones, including different HSP families, to recruit and interact with diverse substrate proteins, leading to alteration of cellular processes. As a positive regulator of plant immunity, HSP90 can directly interact with R proteins, and many of its substrates are kinases and transcription factors that activate defense responses (Breiman, 2014; Sangster and Queitsch, 2005; Xu et al., 2012b). However, other HSP families such as HSP70, HSP40, and sHSPs are functional in microbial pathogenesis, in particular, during viral infections (Boevink and Oparka, 2005; Hafren et al., 2010; Soellick et al., 2000). Recently, these HSP families were reported to be involved in stability of R proteins, cell death, and positive regulation of immunity (Kim et al., 2007; Liu and Whitham, 2013; Van Ooijen et al., 2010).

**HSP100.** HSPs with molecular weights of 100 to 104

kDa are classified into the HSP100 family. Under severe thermal stress conditions, HSP100 proteins maintain the functional integrity of certain key polypeptides by enabling resolubilization of non-functional protein aggregates as well as helping to degrade irreversibly damaged polypeptides (Gupta et al., 2010; Kim et al., 2007). In plants, HSP100 proteins are also widely studied for their role in heat tolerance in plants (Hong and Vierling, 2001; Lin et al., 2014; Queitsch et al., 2000). Among the various types of Arabidopsis HSP100 proteins, the cytosolic form is crucial for heat tolerance but not normal growth (Hong and Vierling, 2001). In accordance with the results of a previous study (Queitsch et al., 2000), yeast cells carrying an *hsp100* deletion can be rescued by transformation with wild-type of plant *hsp100* homologs from Arabidopsis, soybean, wheat, and tobacco. Further, HSP100 plays an important role in re-solubilizing protein aggregates via interactions with the sHSP chaperone system (Bosl et al., 2006). A positive feedback loop between HSP100 and heat stress-associated 32-KD protein (HSA32) is known to prolong the effects of heat acclimation in rice seedlings (Lin et al., 2014).

On the other hand, the function of HSP100 in plant immunity has been rarely investigated. *Clavibacter michiganensis* ssp. *sepedonicus* is the causal agent of bacterial ring rot in potato and causes a hypersensitive response (HR) in non-host plants such as tobacco. Overexpression of HSP100 in tobacco cells is known to increase survival rates compared to wild-type cells after *C. michiganensis* infection (Shafikova et al., 2013).

**HSP90.** HSP90 is the most abundant in cytosolic heat shock protein family in both eukaryotic and prokaryotic cells and is rapidly induced in response to various stress conditions. Under physiological conditions, HSP90 associates with various intracellular proteins, including calmodulin, actin, tubulin, kinases, and receptor proteins (Gupta et al., 2010; Matsumiya et al., 2009; Nguyen et al., 2009; Te et al., 2007). Plant HSP90 has been well-characterized as a core component of various protein complexes that associate with co-chaperones such as tetratricopeptide repeat (TPR)-type or non-TPR-type co-chaperones (Takahashi et al., 2003). HSP90 has been reported as a key regulator of normal growth and development in *Nicotiana benthamiana* and Arabidopsis (Liu et al., 2004; Queitsch et al., 2002; Sangster and Queitsch, 2005; Sangster et al., 2007). HSP90-silenced *N. benthamiana* plants show meristem death as well as a severely stunted growth phenotype with chlorotic leaves (Liu et al., 2004). HSP90-dependent phenotypes have also been extensively studied in Arabidopsis

with inhibited HSP90 functions (Queitsch et al., 2002; Sangster and Queitsch, 2005; Sangster et al., 2007). In these studies, lack of HSP90 caused a variety of phenotypes such as altered flowering time and morphological features. HSP90s also play essential roles in plant immunity (Kadota and Shirasu, 2012; Shirasu, 2009). The HSP90, SGT1 (suppressor of G-two allele of Skp1) containing a TPR domain, and RAR1 (required for Mla12 resistance) containing two zinc-binding modules termed cysteine- and histidine-rich domain (CHORD) form a molecular chaperone complex that is involved in plant immunity (Kadota and Shirasu, 2012; Shirasu and Schulze-Lefert, 2003; Seo et al., 2008). HSP90 activates cytosolic R proteins containing nucleotide-binding domain and leucine-rich repeat, which mediates defense against many microbial pathogens. Together with the co-chaperones RAR1 and SGT1, HSP90 modulates many cytosolic R proteins such as MLA, RPM1, RPS2, RPS4, and N by interacting directly with cytosolic R proteins (Hubert et al., 2003; Liu et al., 2004; Shirasu, 2009; Takahashi et al., 2003; Zhang et al., 2004). For example, HSP90 interacts with the LRR domain of N in tobacco (Liu et al., 2004). A co-immunoprecipitation experiment demonstrated interactions between HSP90 and RPM1 (Hubert et al., 2003). Previous studies on cytosolic HSP90s in Arabidopsis disclosed their ability to regulate the activity and stability of R proteins (Shirasu, 2009). Alteration of HSP90s is known to reduce accumulation of R proteins such as RPM1, RPS5, and RPS4 (Hubert et al., 2003; Hubert et al., 2009; Lu et al., 2003) or compromise RPM1-, RPS4-, and RPP4 (recognition of *Peronospora parasitica* 4)-mediated immune responses (Bao et al., 2014). In rice, a chaperone complex consisting of cytosolic HSP90 and its co-chaperone Hop/Sti1 participates in chitin responses and anti-fungal immunity (Chen et al., 2010). Specifically, the chaperone complex promotes delivery of OsCERK1 from the ER to the plasma membrane and mediates its maturation in a transgenic protoplast system.

**HSP70.** The HSP70 family represents one of the most highly conserved classes of heat shock proteins. In animals and plants, HSP70 functions as a chaperone for newly synthesized proteins to prevent their accumulation as aggregates as well as to ensure proper protein folding during their transfer to their final location. HSP70 has various functions in microbial pathogenesis. In particular, HSP70 appears to regulate viral reproduction and movement, which ultimately promotes viral infection (Boevink and Oparka, 2005; Hafren et al., 2010). Cytoplasmic HSP70 enhances infection of *N. benthamiana* by *Tobacco mosaic virus*, *Potato virus X*, and *Watermelon mosaic virus* (Chen

et al., 2008). Recently, the coat protein (CP) of *Potato virus A* was shown to interact with *N. tabacum* HSP70, which is important for viral infection (Hafren et al., 2010), whereas CP of *Tomato yellow leaf curl virus* was shown to be involved in recruiting host plant HSP70 during viral infection (Gorovits et al., 2013). However, HSP70 is also an important molecular chaperone that plays critical roles in biotic stress responses. The cytosolic/nuclear heat shock cognate 70 (HSC70) chaperone, a highly homologous to HSP70, regulates Arabidopsis immune responses together with SGT1. Cytoplasmic HSP70 is required for the *Phytophthora infestans* INF1-mediated hypersensitive response (HR) and non-host resistance to *Pseudomonas cichorii* in *N. benthamiana* (Kanzaki et al., 2003). In addition, cytoplasmic *Capsicum annuum* HSP70 (CaHSP70) significantly accumulates in pepper leaves, inducing the HR by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) infection. CaHSP70 silencing in pepper was recently shown to increase susceptibility to *Xcv* as well as alter the cell death response to *Xcv* infection (Kim and Hwang, 2015).

**HSP40.** HSP40/DnaJ, also termed J-domain-containing protein (J-protein) is a co-chaperone component of the HSP70 system known to increase HSP70 affinity for clients (Kampinga and Craig, 2010). HSP40 contains a conserved 70-amino acid J-domain that interacts with the nucleotide-binding domain (NBD) of HSP70. Similar to HSP70, the function of HSP40 in viral pathogenesis has been well studied in various virus-plant interactions. For examples, the CP of *Potato virus Y* interacts with DnaJ-like protein (HSP40), which is important for cell-to-cell movement (Hofius et al., 2007). Similarly, the movement protein (MP) of *Tomato spotted wilt virus* interacts with DnaJ-like protein (Soellick et al., 2000). Recently, HSP40 was demonstrated to function in plant immunity, as overexpression of HSP40 causes HR-like cell death and silencing of HSP40 enhances susceptibility to *Soybean mosaic virus* in soybean (Liu and Whitham, 2013). The functions of HSP70 and HSP40 in plant immunity have been generally identified as chaperones in microbial pathogenesis, particularly, in viral movement. Both HSP chaperones interact with viral MP or CP that binds viral nucleic acids or virions and facilitate viral movement from cytosol (or nucleus) to membrane or plasmodesmata between two plant cells, leading to more rapid or severe symptom development (Chen et al., 2008; Hafren et al., 2010; Hofius et al., 2007). In these days, several HSP70 and HSP40 was demonstrated as positive regulators in plant immunity. Although overexpression or knockdown of these HSPs enhance resistance and susceptibility to pathogen infections, respectively, most of fine

mechanisms remains unclear.

**Small HSP (sHSP).** Similar to other HSPs, sHSPs function as molecular chaperones, preventing undesired protein-protein interactions and assisting refolding of denatured proteins (Gupta et al., 2010). sHSP confers a protective function by preventing thermal aggregation of proteins through binding to non-native forms (van Montfort et al., 2001). HSP20, a representative sHSP, maintains denatured proteins in a folding-competent state and allows subsequent ATP-dependent disaggregation through the HSP70/90 chaperone system (Kotak et al., 2007; Liberek et al., 2008). Similar to HSP70/HSP40, a number of sHSPs are associated with viral infection (Verchot, 2012), whereas there have been several reports on their role in plant disease resistance. An HSP20 member is known to specifically interact with I-2, which confers resistance to *Fusarium oxysporum* (Simons et al., 1998) by accumulation of I-2. Another HSP20 from *Nicotiana tabacum* (NtsHSP) was shown to be involved in disease resistance in plants (Maimbo et al., 2007). Disease symptoms caused by *Ralstonia solanacearum* are enhanced in *NtsHsp*-silenced plants.

### Endoplasmic reticulum (ER) HSPs involved in plant immunity

The ER is a cellular organelle with important functions in eukaryotic cells. It connects to other cellular compartments such as the nucleus, Golgi, mitochondria, and plasma membrane and is therefore a major site of protein passage from other organelles to the plasma membrane and extracellular space. Importantly, the ER has numerous quality control (ER QC) mechanisms to assure that properly folded proteins exit the ER and reach their final destinations, such as the plasma membrane, vacuoles, or apoplast. The ER operates the ER QC that identifies permanently misfolded proteins and retranslocates them to the cytoplasm for proteasomal degradation. These pathways that orchestrate destruction of aberrant proteins are collectively termed ER-associated degradation (ERAD) (Huttner and Strasser, 2012; Liu and Howell, 2010; Vitale and Boston, 2008). Many proteins within the ER, including HSPs and chaperones, are critical to ER function, including protein folding modes or extracellular release and cell-surface localization of proteins. In plants, recent genetic evidence indicates that HSPs/chaperones play critical roles in biogenesis, maturation, and stabilization of PRRs through ER QC pathways (Haweker et al., 2010; Li et al., 2009; Liu and Howell, 2010; Lu et al., 2009; Nekrasov et al., 2009; Saijo et al., 2009, 2010). We will further discuss how such HSPs/chap-

erones contribute to accumulation of PRRs at the plasma membrane and regulate plant immunity.

**Binding protein (BiP).** The immunoglobulin binding protein (BiP) was discovered as one of glucose-regulated proteins (Grps). In 1977, Ira Pastan and his colleagues observed that two proteins with molecular sizes of 78 and 94 kDa were strongly induced in chicken embryo fibroblasts cultured in glucose-free medium (Lee, 2001; Shiu et al., 1977). These proteins were subsequently identified as Grp78 (also referred to as BiP) and Grp94 (also known as gp96).

BiP, an abundant HSP70 in the ER, has diverse functions but is best known for its central role in ER stress and the ‘unfolded protein response (UPR)’, which are essential to the development and health of mammalian cells (Gupta and Tuteja, 2011; Kleizen and Braakman, 2004). The intrinsic adenosine triphosphatase (ATPase) activity of BiPs regulates binding and release from their substrates. In previous reports, BiP was shown to interact with the growing nascent chain of substrates containing N-linked glycans, promoting their translocation into the ER (Molinari and Helenius, 2000). BiP is also involved in ER QC, in which unassembled and/or misfolded proteins are selectively retained in the ER (Gupta and Tuteja, 2011; Kleizen and Braakman, 2004). In addition, BiP targets permanently misfolded proteins for ERAD, a complex process through which the misfolded proteins are selected and ultimately degraded by the ubiquitin-proteasome system (Gupta and Tuteja, 2011; Kleizen and Braakman, 2004).

As a chaperone that monitors folding conditions in the ER lumen, BiP interacts with a wide range of plasma membrane-resident proteins, particularly PRRs. In animals, BiPs have been shown to interact with various cell surface receptors such as nicotinic acetylcholine receptor,  $\gamma$ -aminobutyric acid type A receptor,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor, and epidermal growth factor receptor (EGFR) (Cai et al., 1998; Fleck, 2006). Whereas overexpression of human BiP inhibits translocation of EGFR to the cell surface (Cai et al., 1998), it has no effect on AMPA receptor expression (Vandenberghe et al., 2005). It has also been shown that Arabidopsis BiP physically interacts with a mutant form of cell surface brassinosteroid (BR) receptor for steroid hormones but not wild-type BR receptor (Hong et al., 2008; Jin et al., 2007). Consistent with mammalian BiPs, these results suggest that plant BiPs also prevent export of structurally perturbed cell surface receptors.

BiP has also been demonstrated to participate in plant immunity as a central regulator. The ER participates in at

least three different processes in plant immunity, and compelling evidence has linked BiP to all three ER-supported immunity functions (Carvalho et al., 2014; Eichmann and Schafer, 2012). First, the ER functions as a surveillance system for proper glycosylation and folding of membrane-resident PRRs (Li et al., 2009; Liebrand et al., 2012; Nekrasov et al., 2009; Saijo et al., 2009). The involvement of ER QC and ERAD in XA21-mediated immunity has been demonstrated through isolation of an *in vivo* XA21 protein complex (Park et al., 2010). An approximately 75-kDa protein co-immunoprecipitated with XA21 was previously identified as OsBiP3 through LC-MS/MS sequencing. Accordingly, overexpression of rice BiP3 regulates XA21 PRR-mediated innate immunity, but not NB-LRR protein Pi5-mediated innate immunity, by specifically controlling the processing and stability of XA21 (Park et al., 2010, 2014). A BiP-containing ER protein complex is indispensable for proper biogenesis of EF-Tu receptor (EFR) PRR, demonstrating conserved involvement of ER QC and PRR function in the monocotyledon as well as dicotyledon (Nekrasov et al., 2009). Secondly, plant immunity depends on the efficient production and secretion of defense-related proteins (Moreno et al., 2012; Wang et al., 2005). BiP2-silencing Arabidopsis attenuates pathogenesis-related protein 1 (PR1) secretion, a valid marker of salicylic acid (SA)-regulated immunity, upon treatment with SA analogs and impairs resistance against bacterial pathogens (Wang et al., 2005). Thirdly, BiP plays a role in pathogen-induced hypersensitive cell death, although with contrasting results (Xu et al., 2012a; Ye et al., 2011). BiP2 silencing is associated with delay of non-host hypersensitive cell death caused by *Xanthomonas oryzae* pv. *oryzae* (Xu et al., 2012a), whereas BiP overexpression alleviates cell death induced by ectopic expression of TGBp3, an 8-kDa membrane-embedded protein from *Potato virus X* (Ye et al., 2011). These conflicting results could be due to versatile functions of BiP in ER stress response. Recently, dual function of BiP was reported in modulating cell deaths (Carvalho et al., 2014). BiP positively regulates the cell death signaling through a yet undefined mechanism that is activated by SA signaling and related to ER functioning. By contrast, BiP’s negative regulation of cell death may be linked to its ability to attenuate the UPR activation caused by overexpression of viral protein.

**Grp94 (gp96).** The 94-kDa glucose-regulated protein (Grp94) is an HSP90 protein family member found in the ER (Shiu et al., 1977). Grp94 has approximately 50% homology with its cytosolic counterpart, HSP90. Grp94 is known to be expressed in all cell types and is transcription-

ally co-regulated with another important ER resident chaperone, BiP (also known as Grp78), in HeLa cells (Liu and Lee, 1991). Expression of *Grp94* is induced under various stress conditions, including interferon (Anderson et al., 1994), glucose starvation (Shiu et al., 1977), overexpression of misfolded proteins (Ramakrishnan et al., 1995) and ER stress connected with inflammatory diseases (Guo and Li, 2014).

Recently, the functional relationship between Grp94 and PRR has been intensively studied in animals (Liu, 2014). Toll-like receptors (TLRs) are a major family of PRRs mainly expressed by cells of the innate immune system. Currently, more than 13 TLRs have been cloned in mammals, and each TLR is involved in the recognition of a unique set of PAMPs (Kawai and Akira, 2010). Grp94 is now considered to be a master molecular chaperone of TLRs in the ER, and the function of most TLRs is dependent on the integrity of Grp94 in the ER (Liu and Li, 2008; Yang et al., 2007). In the absence of Grp94, TLRs fail to translocate to the cell surface or endosomes and are instead retained in the ER. Furthermore, macrophage-specific Grp94-deficient mice are highly susceptible to acute infection by the Gram-positive bacterial pathogen *Listeria monocytogenes*, indicating that the functions of macrophages based on TLR signaling are mediated by Grp96 (Yang et al., 2007). The functional involvement of Grp94 in plant immunity remains unclear. However, highly conserved ER QC in plants and mammals (Hong et al., 2008; Nekrasov et al., 2009; Sitia and Braakman, 2003; Saijo et al., 2009) suggests that plant Grp94 as a molecular chaperone also participates in the translocation of membrane-resident PRRs.

**HSP40.** Similar to their cytosolic counterparts, ER-resident HSP40s function as molecular co-chaperones defined by the presence of a highly conserved J-domain of about 70 amino acids, which regulates the activity of HSP70 proteins (Rug and Maier, 2011). ER-resident HSP40 is often denoted as ERdj (or ERj) in mammalian systems. In mammals, seven ERdjs (ERdj1 to 7) have been identified with widely different concentrations (Qiu et al., 2006). ERdjs appear to play dual roles of increasing BiP affinity for clients as well as regulating delivery of clients to BiP (Guo and Snapp, 2013). ERdjs all belong to the HSP40 family and bind to BiP via their conserved J-domains, particularly the HPD amino acid motif (Shen and Hendershot, 2005). Among these seven members, ERdj3B is likely involved in immunity. Mammalian ERdj3 forms a complex with unfolded proteins and multiple chaperones, including Grp94 (Meunier et al., 2002), which is the unique and obligatory

master chaperone for TLRs (Yang et al., 2007).

In contrast to animal systems, there is little information on the function of ERdjs in plants. Five types of ERdjs (P58, ERdj2, ERdj2A, ERdj3B, and ERdj7) have been identified (Ohta and Takaiwa, 2014). In Arabidopsis, ERdj3B forms a complex with SDF2 (Nekrasov et al., 2009). SDF2 proteins are conserved throughout plants and animals. Upon UPR activation and ER stress, transcription of SDF2 genes is significantly enhanced in humans and Arabidopsis, suggesting an evolutionarily conserved role (Fukuda et al., 2001; Schott et al., 2010). The precise molecular functions of ERdj3B and SDF2 proteins still need to be established. However, recent reports in plants suggest that SDF2 play an important role in the immune response with co-chaperones. SDF2 acts in a multi-protein complex with ERdj3 and BiP in the ER (Meunier et al., 2002). *Aterdj3b* mutants are susceptible to the bacterial pathogen *Pseudomonas syringae* (Nekrasov et al., 2009). Mutation of *sdj2-2* causes retention and degradation of EFR in the ER. These previous reports suggest that AtERdj3B forms a complex with BiP and SDF2 to mediate proper accumulation of EFR at the plasma membrane.

In rice, SDF2 is co-purified with XA21, suggesting its role in biogenesis and functionality of XA21 receptor (Park et al., 2013). SDF2 silencing lowers XA21-mediated resistance, indicating a critical role for ER homeostasis in the PRR pathway (Park et al., 2010; Park et al., 2013; Park et al., 2014). These results suggest that rice ERdj3b is also involved in resistance against pathogens directly and/or indirectly.

## Conclusions and perspectives

The involvement of cytosolic and ER-resident HSPs in protein folding processes might have general significance in plants. HSPs also play an essential and regulatory role in the innate immune response in plant cells. Genetic and proteomic research has led to the isolation of various subcellular HSPs, which are critical for innate immunity in different plant species. However, we are still far from understanding exactly how HSPs/chaperones participate in PAMP sensing, translocation of immune receptors, signal transduction, and transcriptional activation of stress genes. Increasing evidence points that fine control of both the quantity and quality of membrane-resident PRRs and cytosolic R proteins occurs. In addition to their critical role in cellular homeostasis, HSPs/chaperones should perform an active role in the fine control through yet undefined mechanisms that is regulated by cellular stress responses. It was proposed that evolution of new recognition specificities for emerging

**Table 1.** Summary of HSPs in plant immunity

Classes	Molecular size (kDa)	Proposed functions	Location	Members	References
HSP100	100–104	protein folding, disaggregation	Cytosol, mitochondria, chloroplast	HSP100, Clp	(Bosl et al., 2006; Hong and Vierling, 2001)
HSP90	82–90	Genetic buffering, Regulation of receptors, Protein translocation	Cytosol, ER, nucleus, mitochondria, chloroplast	HSP90, Grp94/gp96	(Bao et al., 2014; Hubert et al., 2009; Hubert et al., 2003; Kadota and Shirasu, 2012; Queitsch et al., 2002)
HSP70	68–75	Assisting refolding, Preventing aggregation	Cytosol, ER, nucleus, mitochondria, chloroplast	HSP70, BiP/Grp78	(Kampinga and Craig, 2010; Kanzaki et al., 2003; Park et al., 2010)
HSP40	35–54	Co-chaperone	Cytosol, ER	HSP40, ERdj	(Guo and Snapp, 2013; Ohta and Takaiwa, 2014; Shen and Hendershot, 2005)
sHSP	15–30	Preventing aggregation, Co-chaperone	Cytosol, ER, mitochondria, chloroplast	HSP20	(Kotak et al., 2007; Liberek et al., 2008)

pathogens may result in proteins that detect novel ligands but have not been selected for high stability, thus requiring extra “buffering” (Nekrasov et al., 2009; Queitsch et al., 2002). In support of this hypothesis, recent reports demonstrated the client-specific affinity of HSPs/chaperones for receptor proteins. For example, *Arabidopsis* ER QC components such as calreticulin3, UDP-glucose:glycoprotein glycosyltransferase, SDF2, BiP, and ERdj3b are essential for EFR but not (or at least less) FLS2 biogenesis (Li et al., 2009; Nekrasov et al., 2009; Saijo, 2010). Although EFR and FLS2 are structurally similar and carry numerous putative glycosylation sites, they may have different glycosylation states that could impact their dependence on the ER-QC machinery (Nekrasov et al., 2009). Similarly, although rice brassinosteroid insensitive 1 (OsBRI1), a brassinosteroids receptor, shows overall structural similarity with XA21, BiP3-overexpressing rice does not show accumulation of XA21, which compromises XA21-mediated immunity but not OsBRI1-mediated signaling (Park et al., 2010). Therefore, future research identifying which client immune receptors are matched with which HSPs/chaperones for their biogenesis/glycosylation and signal transduction will help our understanding of plant innate immunity.

In animals, HSPs such as HSP60, HSP70, HSP90, and Grp94 are able to induce the innate immune response directly and are thus considered as danger- or damage-associated molecular patterns (DAMPs) (Wallin et al., 2002). However, there is some evidence against HSPs as DAMPs. It was proposed that HSP should be called DAMPERs rather than DAMPs, as HSPs have a dampening effect on immune activation as well as the capacity to promote immune homeostasis (van Eden et al., 2012). In

plants, receptors involved in recognizing DAMPs include plasma membrane-resident RLKs. For example, systemin, an 18-amino-acid peptide released from tomato, and *AtPep1*, a 23-amino-acid peptide from *Arabidopsis*, are recognized by the RLK-encoding BRI1/SR160 and PEPR1 genes, respectively. Although it is clear that HSPs are key components for quality control of PRRs and R proteins in the innate immune response, none have been reported in plant cells as DAMPs/DAMPERs so far. Future studies will be needed to reveal the possible interactions between HSPs and receptors that trigger innate immunity.

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