



Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response

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Abiotic stresses usually cause protein dysfunction. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress. Heat-shock proteins (Hsps)/chaperones are responsible for protein folding, assembly, translocation and degradation in many normal cellular processes, stabilize proteins and membranes, and can assist in protein refolding under stress conditions. They can play a crucial role in protecting plants against stress by re-establishing normal protein conformation and thus cellular homeostasis. Here, we summarize the significance of Hsps and chaperones in abiotic stress responses in plants, and discuss the co-operation among their different classes and their interactions with other stress-induced components.

Molecular chaperones are key components contributing to cellular homeostasis in cells under both optimal and adverse growth conditions. They are responsible for protein folding, assembly, translocation and degradation in a broad array of normal cellular processes; they also function in the stabilization of proteins and membranes, and can assist in protein refolding under stress conditions. A wide range of proteins has been reported to have chaperone activity. Moreover, many molecular chaperones are stress proteins and many of them were originally identified as heat-shock proteins (Hsps) [1,2]. Thus, the names of these molecular chaperones follow their early nomenclatures and are referred to here as Hsps/chaperones.

Five major families of Hsps/chaperones are conservatively recognized (Table 1): the Hsp70 (DnaK) family; the chaperonins (GroEL and Hsp60); the Hsp90 family; the Hsp100 (Clp) family; and the small Hsp (sHsp) family. Aside from these major families, there are other proteins with chaperone functions, such as protein disulfide isomerase and calnexin/calreticulin, which assist in protein folding in the endoplasmic reticulum (ER) (Table 1 footnote a). Molecular Hsps/chaperones are located in both the cytoplasm and organelles, such as the nucleus,

mitochondria, chloroplasts and ER [3–5]. Different classes of molecular chaperones appear to bind to specific non-native substrates and states. Chaperone proteins do not covalently bind to their targets and do not form part of the final product. The two best-studied families are the chaperonins and the Hsp70 family chaperones [6].

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% [7]. Elucidating the various mechanisms of plant response to stress and their roles in acquired stress tolerance is thus of great practical and basic importance. Much research is devoted to some of the major tolerance mechanisms, including ion transporters, osmoprotectants, free-radical scavengers, late embryogenesis abundant proteins and factors involved in signaling cascades and transcriptional control [7]. However, except for the sHsp family, relatively little focus has been given to the role of the many other Hsps/chaperones in plant response to abiotic stress and direct support for Hsp/chaperone function in plant abiotic stress tolerance is rather limited [7–12]. This is despite the fact that Hsps/chaperones are known to be expressed in plants not only when they experience high temperature stress but also in response to a wide range of other environmental insults, such as water stress, salinity and osmotic, cold and oxidative stress [3–5]. It is most likely, being supported by experimental data in plants and other organisms, that Hsps/chaperones play a crucial role in protecting plants against stress and in the reestablishment of cellular homeostasis.

The Hsps/chaperones Hsp60, Hsp70 and Hsp90 interact with a wide range of co-chaperone proteins that regulate their activity or aid in the folding of specific substrate proteins [6,13–15]. However, the functions of these co-chaperones are not dealt with here. Although the possible role of Hsps/chaperones has been unraveled to a great extent in other organisms, little is known in plants. Therefore, we summarize recent findings in bacteria, mammals and yeasts, and then present the available data

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Table 1. Five major classes of plant Hsps/molecular chaperones and their subfamilies, including specific examples for direct involvement of Hsps/molecular chaperones in plant tolerance to stress^{a, b}

Classes	Representative members	Intracellular localization	Major functions	Refs
Hsp70			Preventing aggregation, assisting refolding, protein import and translocation, signal transduction, and transcriptional activation	[4–6,13,14,16,18]
Subfamily: DnaK	Hsp/Hsc70 Hsp70 Bip ¹	Cytosol Chloroplast, mitochondria Endoplasmic reticulum		
Hsp110/SSE	Hsp91	Cytosol		
Chaperonin/Hsp60			Folding and assisting refolding	[4–6,13,14,17]
Subfamily: Group I Group II	Cpn60 ² CCT ³	Chloroplast, mitochondria Cytosol		
Hsp90	Hsp90		Facilitating maturation of signaling molecules, genetic buffering	[4,15,49,50,52,56]
	AtHsp90-1 AtHsp90-5 AtHsp90-6 AtHsp90-7	Cytosol Chloroplast Mitochondria Endoplasmic reticulum		
Hsp100/Clp	Hsp100 ⁴		Disaggregation, unfolding	[4,58,63,64]
Subfamily: Class I	ClpB, ClpA/C ClpD	Cytosol, mitochondria		
Class II	ClpM, ClpN ClpX, ClpY	Chloroplast Chloroplast		
sHsp			Preventing aggregation, stabilizing non-native proteins	[3–5,75–78]
Subfamily: I II III IV V VI	Hsp17.6 Hsp17.9 Hsp21 Hsp26.2 ⁵ Hsp22 Hsp23 ⁵ Hsp22.3	Cytosol Cytosol Chloroplast Endoplasmic reticulum Mitochondria Membrane		

^aAdditional groups of proteins or enzymes are also considered as molecular chaperones, including: peptidyl-prolyl *cis/trans* isomerase, which catalyzes the isomerization of peptide bonds of proline residues; protein disulfide isomerase (PDI), which catalyzes disulfide bond formation in the endoplasmic reticulum; calnexin/calreticulin, which assists the folding of glucosylated proteins in the endoplasmic reticulum. Owing to the limitations of space, these groups of chaperones are not discussed in this article and are not listed in the table.

^bExamples for direct involvement of Hsps/molecular chaperones in plant tolerance to stress:

¹Enhanced accumulation of BiP in *Nicotiana tabacum* protoplast and transgenic plants conferred tolerance to water stress [29].

²Deletion of LEN1 (Cpn60 β) triggered cell death in *Arabidopsis* [47].

³CCT α from the mangrove plant *Bruguiera sexangula* enhanced the salt and osmotic stress tolerance of *Escherichia coli* transformants [48].

⁴Hsp100 functional complementation of the temperature-sensitive yeast *hsp104* mutant cells was shown using *athsp101* and *gmhps101* cDNAs [71,72].

⁵*Zea mays* mitochondrial sHsp improved mitochondrial electron transport during salt stress, mainly by protection of the NADH:ubiquinone oxidoreductase activity (Complex I), but it failed to protect enzymes associated with Complex II [82]. A mutant of the chloroplast sHsp of *Agrostis stolonifera* grass, sHsp26.2, with a point mutation that generated a premature stop-codon (sHsp26.2m) was isolated from a heat-sensitive variant; protein product of the mutant was not accumulated upon heat stress [83].

in plants. Then, we discuss major recent findings about the five major plant Hsps/chaperones in relation to abiotic stress responses (Table 1 footnote b). In addition to their direct functions in acquired stress tolerance, Hsps/chaperones might, as a major class of stress-responsive proteins, also play a role via cross-talk with other mechanisms and function synergistically with other components to decrease cellular damage. The general aspects of Hsps/chaperones are dealt with in many previous reviews [3–6,13–18].

Hsp70 family

Hsp70 chaperones, together with their co-chaperones (e.g. DnaJ/Hsp40 and GrpE), make up a set of prominent cellular machines that assist with a wide range of protein-folding processes in almost all cellular compartments. Hsp70 has essential functions in preventing aggregation

and in assisting refolding of non-native proteins under both normal and stress conditions [13,14]. They are also involved in protein import and translocation processes, and in facilitating the proteolytic degradation of unstable proteins by targeting the proteins to lysosomes or proteasomes [13]. Some family members of Hsp70 are constitutively expressed and are often referred to as Hsc70 (70-kDa heat-shock cognate). These members are often involved in assisting the folding of *de novo* synthesized polypeptides and the import/translocation of precursor proteins. Other family members are expressed only when the organism is challenged by environmental assaults. Therefore, they are more involved in facilitating refolding and proteolytic degradation of non-native proteins [13,14,18]. In addition, some members of Hsp70 are involved in controlling the biological activity of folded

regulatory proteins, and might act as negative repressors of heat-shock factor (HSF) mediated transcription. (For reviews of the various Hsp70 functions, see Refs [4,13,16,18].)

Structurally, Hsp70 consists of a highly conserved N-terminal ATPase domain of 44 kDa and a C-terminal peptide-binding domain of ~25 kDa. Successive cycles of substrate binding and release are coupled to the intrinsic ATPase activity of Hsp70, which requires the participation of its cohort system, Hsp70 co-chaperones such as DnaJ/Hsp40 and GrpE [6]. Hsp70 family chaperones are considered to be the most highly conserved Hsps, with ~50% identical residues between the *Escherichia coli* homolog DnaK and the eukaryotic Hsp70. However, based on similarities in structural and functional properties, a distinct, more diverse set of proteins (including mammalian cytosolic Hsp110, yeast SSE proteins and their counterparts in the ER, and the orthologs of the mammalian Grp170) are considered to be a subfamily of the Hsp70 family [19–22]. The specific roles of individual Hsp70 proteins are likely to be determined by their location in different subcellular compartments [14,18], by the differential expression of Hsp70s at different stages of development [23] or by their interaction with specific sets of Hsp70-associated proteins [24]. For example, the cytosolic Hsc70 prevents protein aggregation, assists *de novo* protein folding and maintains the organellar precursor proteins in an import-competent stage, and the ER Bip protein, mitochondrial and chloroplastic Hsp70 proteins are involved in precursor protein import and translocation.

In plants, many Hsp70 proteins have been identified in different species [4,5]. The *Arabidopsis* genome contains at least 18 genes encoding members of the Hsp70 family, of which 14 belong to the DnaK subfamily and four to the Hsp110/SSE subfamily [25,26]. At least 12 Hsp70 members have been found in the spinach genome [27]. Expression profile analysis of the *Arabidopsis* and spinach Hsp70 genes demonstrated that members of Hsp70 chaperones are expressed in response to environmental stress conditions such as heat, cold and drought, as well as to chemical and other stresses [25–27].

The overexpression of Hsp70 genes correlates positively with the acquisition of thermotolerance [28] and results in enhanced tolerance to salt, water and high-temperature stress in plants [29–33]. However, the cellular mechanisms of Hsp70 function under stress conditions are not fully understood. The unfolded protein response (UPR) is a well-defined mechanism described in animals and in yeast, whereby the Hsp70 BiP proteins could keep the accumulated proteins unfolded, mainly in the ER, thereby avoiding protein aggregation under stress conditions [34]. Overexpression of BiP proteins in tobacco protoplasts [33] prevented the induction of the UPR-induced genes and increased cell tolerance to stress induced by tunicamycin (a potent activator of the UPR pathway), suggesting that BiP proteins might directly alleviate cells from stress. This result was confirmed by the overexpression and the repression of BiP in tobacco plants following exposure to water stress and to tunicamycin. As expected, the tobacco plants with higher BiP levels were more resistant to drought stress and to tunicamycin than the antisense and control plants [29]. These results showed that plant BiP

proteins might also be involved in the UPR pathway under stress conditions. Various members of the Hsp70 molecular chaperones have also been reported to be involved in protein import and translocation into chloroplasts and mitochondria [35], as well as in the cell-to-cell movement of proteins and viruses through the plasmodesmata [36].

Xiao-Ping Zhang and Elzbieta Glaser [37] proposed a model of interaction of plant mitochondrial and chloroplast signal peptides with the Hsp70 molecular chaperones. The cytosolic Hsc70, in cooperation with other chaperones (e.g. 14-3-3 proteins, which belong to a family of acidic soluble proteins, being a novel type of molecular chaperone proteins that modulate interactions between components of signal transduction pathways), interacts with the mitochondrial or chloroplast precursor proteins, keeping them in an unfolded, yet import competent, state. Hsc70 might be used as a motor for transporting the precursor protein through the membranes. Once protein arrives in the matrix or stroma, the mitochondrial or chloroplastic Hsp70 proteins (mHsp70 and sHsp70, respectively), together with their co-chaperones, interact with the precursor protein and allow cleavage of the leader or signal peptide by the peptidases. Cell-to-cell transport of macromolecules and viral ribonuclear complexes also requires a partial unfolding of the transferred proteins. The Hsc70 chaperone protein appears to have a structural motif that is necessary for cell-to-cell transport and was shown to interact with the plasmodesmatal translocation pathway [36].

In addition to its general chaperone functions (i.e. preventing aggregation and assisting in the refolding of non-native proteins under stress conditions), Hsp70 also plays a regulatory role in other stress-associated gene expression [28]. The interaction between Hsp70 and HSF has been suggested as a negative regulatory mechanism for HSF-mediated transcriptional activation in the heat-shock response [16]. It was suggested that the interaction between Hsp70 and HSF prevents the trimerization and binding of HSF to HSE (heat-shock element), thereby blocking the transcriptional activation of heat-shock genes by their HSFs [38]. Hsp70 is also involved in the modulation of signal transducers such as protein kinase A, protein kinase C and protein phosphatase [39]. In this respect, the Hsp70 chaperones might play a broad role by participating in modulating the expression of many downstream genes in signal transduction pathways both during stress and under normal growth conditions. Unfortunately, the role of Hsp70 in the modulation of signal transduction has not been yet studied in plants.

Chaperonins (Hsp60)

Chaperonins (Hsp60; the term chaperonin was first suggested [40] to describe a class of molecular chaperones that are evolutionarily homologous to *E. coli* GroEL) are a class of molecular chaperones found in prokaryotes and in the mitochondria and plastids of eukaryotes [4,13]. Major examples of this class of Hsps/chaperones include the prokaryotic GroEL and the eukaryotic equivalent Hsp60. Chaperonins are further classified into two subfamilies: the GroE chaperonins (Group I) are found in bacteria, mitochondria and chloroplasts (e.g. GroE and chCpn60); the CCT chaperonins [chaperonins containing *t*-complex

polypeptide 1 (TCP1; Group II) are found in Archaea (e.g. trigger factor 55 and the thermosomes) and in the cytosol of eukaryotes (e.g. the TCP-1 ring complex TriC) [17]. Chaperonins play a crucial role by assisting a wide range of newly synthesized and newly translocated proteins to achieve their native forms [6,14]. The structure and function of chaperonins, especially Group I chaperonins, have been extensively studied [6,13,14,17,41]. In prokaryotes, Group I chaperonin (e.g. GroEL of *E. coli*) consists of two distinct family members, chaperonin 60 (Cpn60) and chaperonin 10 (Cpn10) as co-chaperone, which function together in an ATP-dependent manner. They are double-ring assemblies composed of back-to-back stacked rings of identical or closely related rotationally symmetrical subunits [6]. Owing to the high conservation of the primary sequence among Group I chaperonins, it is generally accepted that organellar chaperonins function similarly to the bacterial ones. However, recent studies indicate that plastid chaperonins possess unique structural and functional properties that distinguish them from their bacterial homologs [42]. Plant chloroplast chaperonins consist of two distinct polypeptides, Cpn60 α and Cpn60 β , which share only ~50% amino acid identity [4]. Another difference that characterizes the chloroplast chaperonins relates to the structure of their co-chaperonin. The chloroplast co-chaperonin is composed of two GroES-like domains that make up a molecular mass of approximately double the size (20 kDa) of the bacterial Cpn10 (GroES) [43]. In addition to the double domain co-chaperonins, *Arabidopsis* genome contains at least one normal size Cpn10 [44].

In contrast to Group I, Group II chaperonins form eight- or nine-member rings, each member is encoded by related but distinct genes, and they are independent of a general co-chaperone (although a protein cofactor, prefoldin, has been identified). It has been shown that CCT chaperonins assist in the folding of tubulin and actin [41].

Seven *Arabidopsis* genomic sequences have been identified as having the potential to encode plastid Cpn60 proteins [44]. Two of these appear to encode Cpn60 α subunits, and four to encode Cpn60 β subunits. The seventh genomic sequence seems to be a Cpn60 β subunit pseudogene. Nine *Arabidopsis* sequences are predicted to encode proteins similar to CCT protein subunits α , β , γ , δ , ϵ , ζ , η and θ , with two of them corresponding to CCT- ζ [44].

Functional characterization of plant chaperonins is limited. It is generally agreed that they are important in assisting plastid proteins such as Rubisco [4,40]. It has been reported that a mutated species of *Arabidopsis* chloroplast Cpn60 α exhibits defects in chloroplast development and, subsequently, in the proper development of the plant embryo and seedling [45]. Antisense Cpn60 β -transgenic tobacco plants showed drastic phenotypic alterations, including slow growth, delayed flowering, stunting and leaf chlorosis [46]. The deletion of *LEN1* (encoding Cpn60 β) triggers cell death in *Arabidopsis*, which leads to the establishment of activated systemic acquired resistance, a broad-spectrum plant resistance mechanism normally triggered by necrotic lesions resulting from pathogen infection [47]. CCT α from the

mangrove plant *Bruguiera sexangula*, a Group II chaperonin, enhances salt- and osmotic-stress tolerance of *E. coli* transformants [48].

Hsp90 family

Hsp90 is distinct from many other molecular chaperones in that most of its known substrates to date are signal transduction proteins such as steroid hormone receptors and signaling kinases [49]. The major role of Hsp90 is to manage protein folding [14,15] but it also plays a key role in signal-transduction networks, cell-cycle control, protein degradation and protein trafficking [49–51]. In addition, it might also play a role in morphological evolution and stress adaptation in *Drosophila* and *Arabidopsis* [52,53]. A recent report [54] showed that Hsp90 interacts with the 26S proteasome and plays a principal role in its assembly and maintenance. Hsp90 is one of the major species of molecular chaperones that requires ATP for its functions. It is among the most abundant proteins in cells: 1–2% of total cellular protein [14]. To fulfill its cellular roles, Hsp90 acts as part of a multichaperone machine together with Hsp70 and co-operates with a cohort of co-chaperones, including Hip (Hsp70 interacting protein), Hop (Hsp70/Hsp90 organizing protein), p23 and Hsp40 (a DnaJ homolog), the immunophilins FKBP51/54 and FKBP52, and Cdc37/p50. GmHop-1, a co-chaperone homologous to the mammalian Hop protein, was isolated from soybean (*Glycine max*) and its transcripts were detected under normal growth conditions but their levels increased upon stress [55].

In plants, cytosol-, ER- and plastid-localized Hsp90 genes have been isolated from several plant species, sharing 63–71% amino acid identities with Hsp90 of yeast and animal origin [56]. In the *Arabidopsis* genome, the Hsp90 family includes seven members: AtHsp90-1 to AtHsp90-4 constitute the cytoplasmic subfamily; AtHsp90-5, AtHsp90-6 and AtHsp90-7 are predicted to be localized to the plastid, mitochondria and ER, respectively [56].

Although Hsp90 chaperones are constitutively expressed in most organisms, their expression increases in response to stress in both prokaryotes and eukaryotes. Expression of Hsp90 in *Arabidopsis* is developmentally regulated and responds to heat, cold, salt stress, heavy metals, phytohormones and light and dark transitions [56,57].

Interestingly, decreasing the levels of functional Hsp90 in *Drosophila* by genetic mutation or by treatment with the Hsp90 inhibitor geldanamycin causes developmental abnormalities and morphological changes [52]. This work was the first to link Hsp90 functions with morphological evolution, a process that often requires the effects of independent genetic changes. It was suggested in this work that Hsp90 acts as a 'buffer' to sustain the functions of those mutated proteins that participate in the signaling pathways of development and morphogenesis. The 'buffering' effect of Hsp90 thus allows the existence of mutated developmental and morphological controlling proteins. Under normal physiological conditions, the expression of genetic variations that are hidden by the Hsp90 'buffering' effect is suppressed or silenced. When the organism encounters environmental assaults under which chaperones

might be needed mainly for the organism viability (i.e. Hsp90 surveillance is impaired), the existing hidden mutations/variants from the Hsp90 'buffer' system are then exposed. From this work, the authors suggested a powerful evolutionary mechanism through which Hsp90 plays an important role in ensuring genetic stability at normal physiological conditions, while permitting the accumulation of mutations that could manifest under stress conditions. The notion that Hsp90 functions as 'buffer' of morphological evolution was further demonstrated in *Arabidopsis*, in which reducing Hsp90 function by treatment with geldanamycin produced an array of morphological phenotypes, presumably because of the release of genetic variations that are normally buffered by Hsp90 [53]. It appears that Hsp90 chaperones accompany other signaling proteins that control plant growth and development. Thus, Hsp90 might provide genetic buffering in *Arabidopsis* and contribute to the evolutionary adaptation of this plant, as is the case with *Drosophila* [52].

Hsp100/Clp family

The Hsp100/Clp family chaperones are members of the large AAA ATPase superfamily with a broad spectrum of diverse functional properties [58–61]. Interestingly, rather than the regular chaperone function of preventing protein aggregation and misfolding, the Hsp100/Clp family functions in protein disaggregation and/or protein degradation. The removal of non-functional but potentially harmful polypeptides arising from misfolding, denaturation or aggregation is important for the maintenance of cellular homeostasis.

Members of the Hsp100 family were first described as components of the two-subunit bacterial Clp protease system [62], which consists of regulatory ATPase/chaperones (such as ClpA and ClpX) and proteolytic (ClpP) subunits. The family is further divided into two major classes and eight distinct subfamilies within these classes. Members of the first class (A–D) contain two nucleotide-binding domains (also called ATP-binding domains), whereas those in the second class (M,N,X,Y) have only one nucleotide-binding domain [58]. Hsp100/Clp proteins are typically hexameric rings.

The mechanism for rescuing proteins from aggregation also involves the cooperation of another ATP-dependent chaperone system, the Hsp70. The Hsp100/Clp family solubilizes the aggregated protein and releases it in a state that can be refolded with the assistance of the Hsp70 system [63,64]. When associated with ClpP, Hsp100 proteins perform dual chaperone and regulatory activities, thereby influencing the eventual fate of selected protein substrates, which are either fully degraded [65] or unfolded and released [66].

Hsp100/Clp proteins have been reported in many plant species, such as *Arabidopsis*, soybean, tobacco, rice, maize (*Zea mays*), Lima bean (*Phaseolus lunatus*) and wheat [61,67,68]. Like many other Hsps/chaperones, Hsp100/Clp family chaperones are often constitutively expressed in plants, but their expression is developmentally regulated and is induced by different environmental assaults, such as heat, cold, dehydration, high salt or dark-induced etiolation [61,68–70]. Sharon J. Keeler and co-workers

[68] demonstrated the association of the expression of cytosolic, as well as the chloroplastic, Hsp100 with heat response in Lima bean. Genetic evidence indicating a role for this family of proteins in thermoprotection was obtained earlier [71,72]. In these studies, functional complementation of the temperature-sensitive yeast *hsp104* mutant cells was shown using *athsp101* and *gmhps101* cDNAs. Further evidence was achieved by using mutational [10] and ectopic over- and underexpression [69,73] approaches. In a recent report [73], rice HSP100 protein production was correlated with the disappearance of protein granules in yeast cells. It was further shown that the dissolution of electron-dense granules by Hsp101 takes place during the post-stress phase, implying a role for Hsp100 in the recovery of cell stress.

sHsp family

The sHsps are low-molecular-mass Hsps (12–40 kDa). In plants, sHsps form a more diverse family than other Hsps/chaperones with respect to sequence similarity, cellular location and functions [3,5]. sHsps are synthesized ubiquitously in prokaryotic and eukaryotic cells in response to heat and other stresses, and some sHsps are expressed during certain developmental stages [3,4]. sHsps share a conserved 90-amino-acid C-terminal domain called the α -crystallin domain (ACD), related to a domain from the vertebrate α -crystallin proteins of the eye lens [3,5]. It was shown that the ACDs are organized as trimers of dimers, forming a dodecamer double ring as revealed by the crystal structure of the wheat Hsp16.9 [74].

The sHsps are not themselves able to refold non-native proteins [75–78]. They have a high capacity to bind non-native proteins, probably through hydrophobic interaction [75–79], and to stabilize and prevent non-native aggregation, thereby facilitating their subsequent refolding by ATP-dependent chaperones such as the DnaK system or ClpB/DnaK [75–78,80]. Recent findings showed that the sHsp 18.1 isolated from *Pisum sativum*, as well as Hsp 16.6 from *Synechocystis* sp. PCC6803 under *in vitro* conditions, binds to unfolded proteins and allows further refolding by Hsp70/Hsp100 complexes [80]. However, the mechanism of this refolding process is poorly understood.

Among the five conserved families of Hsps (Hsp70, Hsp60, Hsp90, Hsp100 and sHsp), sHsps are the most prevalent in plants [5]. For example, in plants, there are six recognized families, many of which are highly expressed [3]. Plants synthesize multiple sHsps encoded by six nuclear multigene families; each gene family encodes proteins found in a distinct cellular compartment (i.e. the cytosol, chloroplast, ER and mitochondrion) [3]. In *Arabidopsis*, 13 different sHsps are grouped into six classes based on their intracellular localization and sequence relatedness. Six open reading frames encoding multidomain proteins that contain one or more regions with homology to the ACD containing proteins were also identified [81]. The high diversification of plant sHsps probably reflects a molecular adaptation to stress conditions that are unique to plants. Plant sHsps respond to a wide range of environmental stresses, including heat, cold, drought, salinity and oxidative stress. Increasing data suggest a strong correlation between sHsp accumulation

and plant tolerance to stress. Maize mitochondrial sHsps (msHsp) were shown to improve mitochondrial electron transport during salt stress, mainly by protection of the NADH:ubiquinone oxidoreductase activity (Complex I), although they failed to protect enzymes associated with Complex II [82]. The importance of the chloroplast sHsps was reported in *Agrostis stolonifera* grass, from which sHsp26.2 was isolated in a heat-tolerant variant. An identical sHsp, sHsp26.2m, with a point mutation that generated a premature stop codon isolated from the heat-sensitive variant failed to accumulate upon heat stress [83]. Moreover, it was recently found that another class of stress-associated proteins, SP family proteins [84], shares some of the features of sHsps, yet have additional characteristics and functions. The abundance of sHsps in plants and their functional characteristics of binding and stabilizing denatured proteins suggest that sHsps play an important role in plant-acquired stress tolerance [3,7,85]. Ongoing research on the functional and structural aspects, and cross-talk with other chaperone systems, should provide further elucidation of their functions.

Hsp/chaperone network

The protective effects of Hsps/chaperones can be attributed to the network of the chaperone machinery, in which many chaperones act in concert. During stress, many enzymes and structural proteins undergo deleterious structural and functional changes. Therefore, maintaining proteins in their functional conformations, preventing aggregation of non-native proteins, refolding of denatured proteins to regain their functional conformation and removal of non-functional but potentially harmful polypeptides (arising from misfolding, denaturation or aggregation) are particularly important for cell survival under stress. Thus, the different classes of Hsps/chaperones cooperate in cellular protection and play complementary and sometimes overlapping roles in the protection of proteins from stress (Figure 1). It was demonstrated *in vitro* that sHsps bind to non-native proteins and prevent their aggregation, thus providing a reservoir of substrates for subsequent refolding by members of the Hsp70/Hsp100 chaperone families. This has been shown with IbpA and IbpB from *E. coli* [77,80,86] and with Hsp16.6 from *Synechocystis* sp. PCC6803 [80]. The cooperation between different classes of chaperones was confirmed by using plasmid-controlled chaperone expression in thermosensitive *E. coli* mutants [86]. Another mechanism proposed that protein aggregates can be efficiently resolubilized by Hsp100/Clp family chaperones and are then refolded by the assistance of the Hsp70 system; the final refolding of solubilized proteins into the native form might be completed by members of the Hsp60 family (GroEL–GroES) [87]. Similar observations have been also reported with plant chaperones. For example, Hsp18.1 from pea (*Pisum sativum*) can stably bind heat-denatured protein and maintain it in a folding-competent state for the further refolding by Hsp70/Hsp100 complexes [78,80]. Additional examples of chaperone networks are presented in the legend to Figure 1.

Cross-talk between Hsps/chaperones and other stress-response mechanisms

Abiotic stress evokes multiple responses that involve a series of physiological, biochemical and molecular events. Acquired stress tolerance in plant is often a result of various stress-response mechanisms that act coordinately or synergistically to prevent cellular damage and to re-establish cellular homeostasis [7,88]. The ubiquitous Hsp/chaperone system plays pivotal roles in cells, both under normal growth conditions and when stressed. An increasing number of studies suggest that the Hsps/chaperones interact with other stress-response mechanisms (Figure 2). Several examples of possible cross-talk are discussed below. Osmolytes, or compatible solutes, are a group of low-molecular-weight organic compounds that accumulate in organisms in response to osmotic stress. A study performed in yeast [89] showed that trehalose suppresses the aggregation of denatured proteins, maintaining them in a partially folded state from which they can be reactivated by molecular chaperones. In *E. coli*, it was suggested that the studied osmolytes (glycine, betaine, glycerol, proline and trehalose) can also act as ‘chemical chaperones’ by increasing the stability of native proteins and assisting in the refolding of unfolded

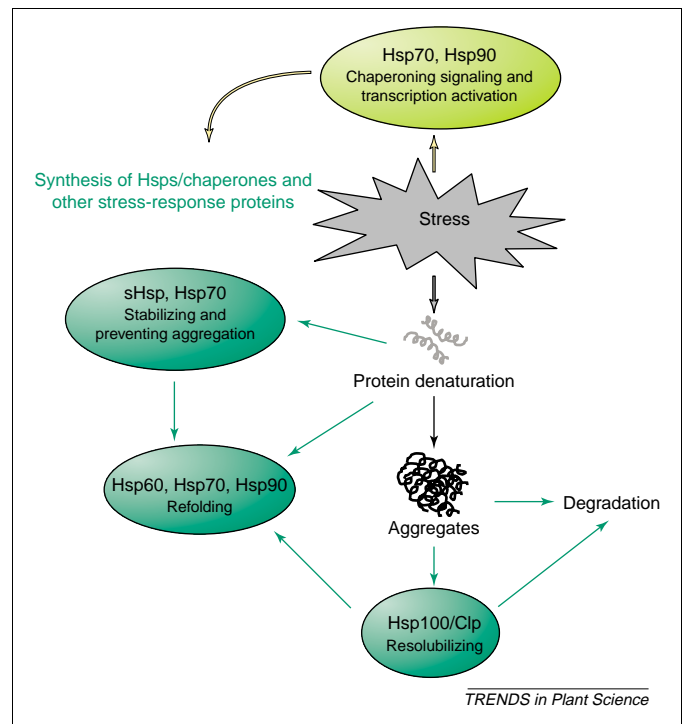


Figure 1. The heat-shock protein (Hsp) and chaperone network in the abiotic stress response. Different classes of Hsps/chaperones play complementary and sometimes overlapping roles in protecting proteins from stress. Abiotic stress in plants often causes dysfunction/denaturation of structural and functional proteins. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress conditions. To maintain cellular homeostasis, some members of the Hsp/chaperone families [e.g. small Hsp (sHsp) and Hsp70] stabilize protein conformation, prevent aggregation and thereby maintain the non-native protein in a competent state for subsequent refolding, which is achieved by other Hsps/chaperones (e.g. Hsp60, Hsp70 and Hsp90). When denatured or misfolded proteins form aggregates, they can be resolubilized by Hsp100/Clp followed by refolding, or degraded by protease. Some Hsps/chaperones (e.g. Hsp70, Hsp90) accompany the signal transduction and transcription activation that lead to the synthesis of other members of Hsps/chaperones [e.g. those controlled by heat-shock factor (HSFs)] and other stress-response proteins (e.g. antioxidants).

polypeptides [90]. Trehalose and an sHsp, p26 from the primitive crustacean *Artemia franciscana*, can act synergistically *in vitro* during and after thermal stress [91]. Interference of high concentrations of trehalose with chaperone-mediated protein folding was documented in all three studies mentioned above. It was suggested that cells specifically control protein stability and chaperone-mediated disaggregation and refolding by modulating the intracellular levels of different osmolytes [90]. These studies give new insight into the cross-talk between Hsps/chaperones and other stress-protective mechanisms.

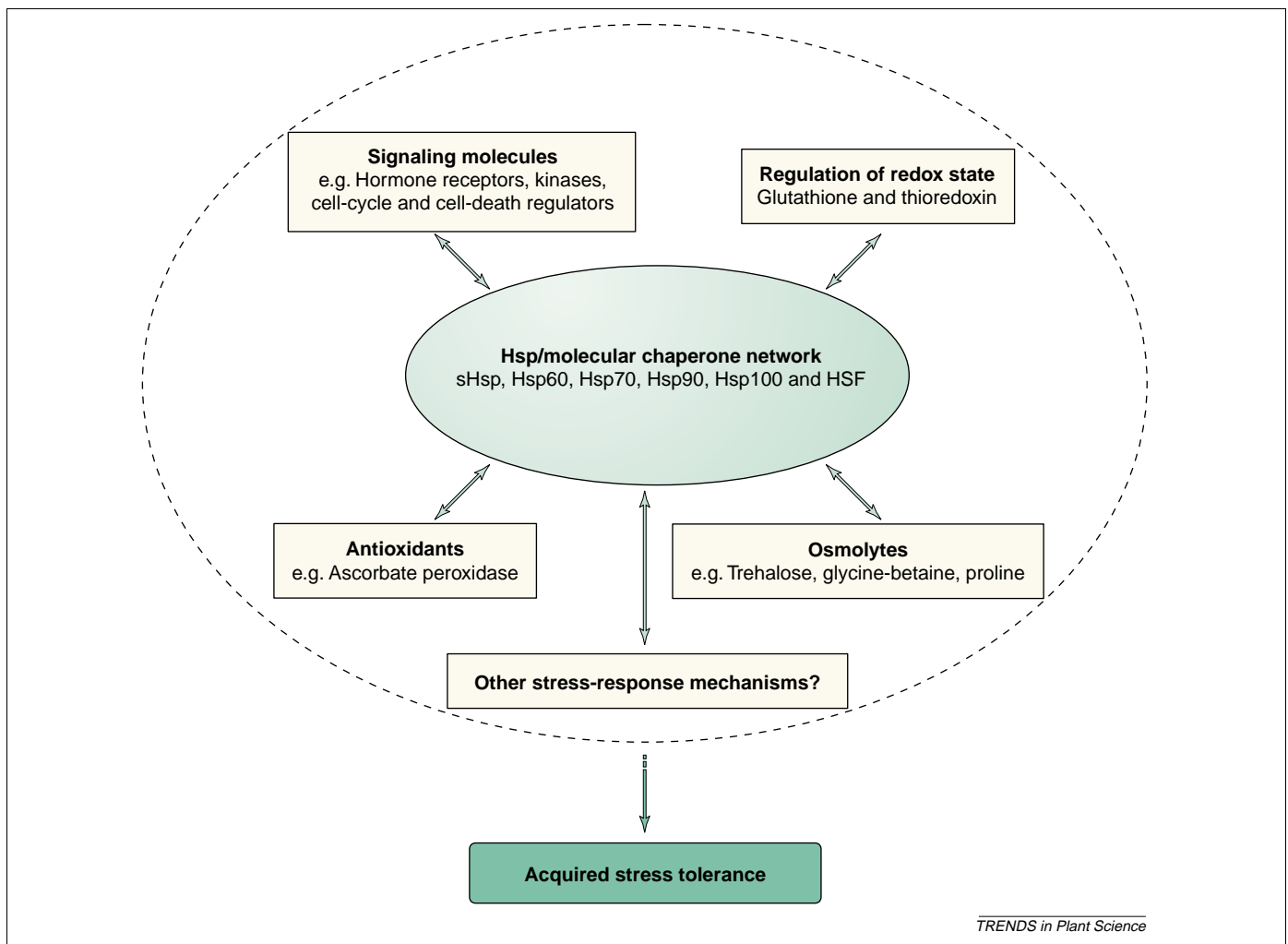
In addition to osmolytes, chaperones of the Hsp90 and Hsp70 families and their co-chaperones were also found to interact with a growing number of signaling molecules, including nuclear hormone receptors, tyrosine- and serine/threonine kinases, and cell-cycle and cell-death regulators, demonstrating that they play a key role in cellular signal transduction networks [92]. The redox status of thiol-containing molecules is important to cellular functions such as the synthesis and folding of proteins, and the regulation of the structure and activity of enzymes, receptors and transcription factors. In mammalian cells, the

sHsps are known to be involved not only in protection against stress but also in the modulation of other cellular functions (such as apoptosis and differentiation) via their participation in the regulation of cellular redox states [93].

Although most of these studies were carried out in organisms other than plants, similar cross-talk mechanisms might operate in plants. For example, heat-shock transcription-factor-dependent expression of antioxidants ascorbate peroxidases in *Arabidopsis* [94] suggested that HSFs might be involved not only in Hsp synthesis but also in oxidative stress regulation of antioxidant gene expression. Furthermore, it was shown in *Arabidopsis* that several Hsp genes were upregulated under high light stress, implicating Hsps in the antioxidative response in addition to their chaperone function [95]. However, the involvement of Hsps as regulators of cellular redox states and in other stress-response mechanisms in plants remain to be established.

Concluding remarks

Individual members of each class of Hsps/chaperones have particular functions, but the co-operation between different Hsp/chaperone networks appears to be a central principle of



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Figure 2. Suggested cross-talk between the heat shock protein (Hsp)/chaperone network and other stress-response mechanisms in plants. Acquired stress tolerance in plants is often a result of various stress-response mechanisms that might act coordinately or synergistically to prevent cellular damage and to re-establish homeostasis [7]. There might be cross-talk between Hsps/chaperones and other stress-response mechanisms in plants. For example, the Hsps/chaperones can play a role in stress signal transduction and gene activation [92] as well as in regulating cellular redox state [93]. They also interact with other stress-response mechanisms such as osmolytes [90,91] and antioxidants [94,95].

the integrated Hsp/chaperone machinery. Under normal growth conditions or during and after stress, the fate of a particular denatured or non-native protein is determined by the entire Hsp/chaperone system. Many questions about this issue have still to be answered. For example, how does a specific chaperone recognize its particular substrate (a nascent peptide or a malfunction protein) in a crowded cellular environment? How and when is the decision on the fate of each denatured/non-native protein determined? What is the fate of a denatured/non-native protein: is it stabilized or protected from aggregation, refolded, or eventually aggregated and targeted for degradation? Is there a central determinant of the Hsp/chaperone network?

We are still far from understanding how Hsps/chaperones, as regulatory molecules, participate in stress sensing, signal transduction and transcription activation of stress genes. This is true for many organisms but especially for abiotic stress tolerance in plants. Most of the current research in plants and in other organisms is devoted to detecting changes in Hsps/chaperones (under- or over-expression) during or following stress. Also, in many cases, conclusions about the function of Hsps/ chaperones under stress are based on *in vitro* assays, mainly because of the lack of appropriate mutants in which a specific Hsp/chaperone is not expressed. Future research should be devoted to creating plant mutants lacking a specific, or several Hsps/chaperones and to apply results from available mutants (e.g. Refs [10–12]). Moreover, studies in which changes in one or more stress response mechanisms in plants are investigated simultaneously with changes in Hsps/chaperones and structural changes seem to be especially important in this respect. We anticipate that active research into the cross-talk between Hsps/chaperones and other stress-response mechanisms in plants will provide a further understanding of acquired stress tolerance.

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References

- 1 Lindquist, S. (1986) The heat-shock response. *Annu. Rev. Biochem.* 55, 1151–1191
- 2 Lindquist, S. and Craig, E.A. (1988) The heat-shock proteins. *Annu. Rev. Genet.* 22, 631–677
- 3 Waters, E.R. *et al.* (1996) Evolution, structure and function of the small heat shock proteins in plants. *J. Exp. Bot.* 47, 325–338
- 4 Boston, R.S. *et al.* (1996) Molecular chaperones and protein folding in plants. *Plant Mol. Biol.* 32, 191–222
- 5 Vierling, E. (1991) The roles of heat shock proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42, 579–620
- 6 Bukau, B. and Horwich, A.L. (1998) The Hsp70 and Hsp60 chaperone machines. *Cell* 92, 351–366
- 7 Wang, W.X. *et al.* (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218, 1–14
- 8 Burke, J.J. *et al.* (2000) Isolation of *Arabidopsis* mutants lacking components of acquired thermotolerance. *Plant Physiol.* 123, 575–587
- 9 Burke, J.J. (2001) Identification of genetic diversity and mutations in higher plant acquired thermotolerance. *Physiol. Plant.* 112, 167–170
- 10 Hong, S.W. and Vierling, E. (2000) Mutants of *Arabidopsis thaliana* defective in the acquisition of tolerance to high temperature stress. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4392–4397
- 11 Hong, S.W. and Vierling, E. (2001) Hsp101 is necessary for heat tolerance but dispensable for development and germination in the absence of stress. *Plant J.* 27, 25–35
- 12 Hong, S.W. *et al.* (2003) *Arabidopsis* hot mutants define multiple functions required for acclimation to high temperatures. *Plant Physiol.* 132, 757–767
- 13 Hartl, F.U. (1996) Molecular chaperones in cellular protein folding. *Nature* 381, 571–580
- 14 Frydman, J. (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu. Rev. Biochem.* 70, 603–647
- 15 Buchner, J. (1999) Hsp90 & Co. – a holding for folding. *Trends Biochem. Sci.* 24, 136–141
- 16 Morimoto, R.I. (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.* 12, 3788–3796
- 17 Ranson, N.A. *et al.* (1998) Chaperonins. *Biochem. J.* 333, 233–242
- 18 Miernyk, J.A. (1997) The 70kDa stress-related proteins as molecular chaperones. *Trends Plant Sci.* 2, 180–187
- 19 Easton, D.P. *et al.* (2000) The Hsp110 and Grp170 stress proteins: newly recognized relatives of the Hsp70s. *Cell Stress Chaperones* 5, 276–290
- 20 Mukai, H. *et al.* (1993) Isolation and characterization of SSE1 and SSE2, new members of the yeast HSP70 multigene family. *Gene* 132, 57–66
- 21 Lee-Yoon, D. *et al.* (1995) Identification of a major subfamily of large Hsp70-like proteins through the cloning of the mammalian 110-kDa heat shock protein. *J. Biol. Chem.* 270, 15725–15733
- 22 Chen, X. *et al.* (1996) The 170 kDa glucose regulated stress protein is a large HSP70-, HSP110-like protein of the endoplasmic reticulum. *FEBS Lett.* 380, 68–72
- 23 Sung, D.Y. *et al.* (2001) Plant Hsp70 molecular chaperones: protein structure, gene family, expression and function. *Physiol. Plant.* 113, 443–451
- 24 May, T. and Soll, J. (2000) 14-3-3 proteins form a guidance complex with chloroplast precursor proteins in plants. *Plant Cell* 12, 53–64
- 25 Lin, B.L. *et al.* (2001) Genomic analysis of the Hsp70 superfamily in *Arabidopsis thaliana*. *Cell Stress Chaperones* 6, 201–208
- 26 Sung, D.Y. *et al.* (2001) Comprehensive expression profile analysis of the *Arabidopsis* Hsp70 gene family. *Plant Physiol.* 126, 789–800
- 27 Guy, C.L. and Li, Q.B. (1998) The organization and evolution of the spinach stress 70 molecular chaperone gene family. *Plant Cell* 10, 539–556
- 28 Lee, J.H. and Schöffl, F. (1996) An Hsp70 antisense gene affects the expression of HSP70/HSC70, the regulation of HSF and the acquisition of thermotolerance in transgenic *Arabidopsis thaliana*. *Mol. Gen. Genet.* 252, 11–19
- 29 Alvim, F.C. *et al.* (2001) Enhanced accumulation of BiP in transgenic plants confers tolerance to water stress. *Plant Physiol.* 126, 1042–1054
- 30 Sung, D.Y. and Guy, C.L. (2003) Physiological and molecular assessment of altered expression of Hsc70-1 in *Arabidopsis*. Evidence for pleiotropic consequences. *Plant Physiol.* 132, 979–987
- 31 Sugino, M. *et al.* (1999) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytice* acquires resistance to salt stress in transgenic tobacco plants. *Plant Sci.* 146, 81–88
- 32 Ono, K. *et al.* (2001) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* enhances the high-temperature tolerance of tobacco during germination and early growth. *Plant Sci.* 160, 455–461
- 33 Leborgne-Castel, N. *et al.* (1999) Overexpression of BiP in tobacco alleviates endoplasmic reticulum stress. *Plant Cell* 11, 459–470
- 34 Kaufman, R.J. (1999) Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev.* 13, 1211–1233
- 35 Huang, S. *et al.* (1999) Mitochondrial unfold precursor proteins by unraveling them from their N-termini. *Nat. Struct. Biol.* 6, 1132–1138
- 36 Aoki, K. *et al.* (2002) A subclass of plant heat shock cognate 70 chaperones carries a motif that facilitates trafficking through plasmodesmata. *Proc. Natl. Acad. Sci. U. S. A.* 10, 16342–16347
- 37 Zhang, X.P. and Glaser, E. (2002) Interaction of plant mitochondrial and chloroplast signal peptides with the Hsp70 molecular chaperone. *Trends Plant Sci.* 7, 14–21
- 38 Kim, B.H. and Schöffl, F. (2002) Interaction between *Arabidopsis* heat shock transcription factor 1 and 70 kDa heat shock proteins. *J. Exp. Bot.* 53, 371–375
- 39 Ding, X.Z. *et al.* (1998) Overexpression of HSP-70 inhibits the

- phosphorylation of HSF1 by activating protein phosphatase and inhibiting protein kinase C activity. *FASEB J.* 12, 451–459
- 40 Hemmingsen, S.M. *et al.* (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* 26, 330–334
 - 41 Gutsche, I. *et al.* (1999) Group II chaperonins: new TriC(k)s and turns of a protein folding machine. *J. Mol. Biol.* 293, 295–312
 - 42 Levy-Rimler, G. *et al.* (2002) Type I chaperonins: not all are created equal. *FEBS Lett.* 529, 1–5
 - 43 Bertsch, U. *et al.* (1992) Identification, characterization, and DNA sequence of a functional 'double' GroES-like chaperonin from chloroplasts of higher plants. *Proc. Natl. Acad. Sci. U. S. A.* 89, 8696–8700
 - 44 Hill, J.E. and Hemmingsen, S.M. (2001) *Arabidopsis thaliana* type I and II chaperonins. *Cell Stress Chaperones* 6, 190–200
 - 45 Apuya, N.R. *et al.* (2001) The *Arabidopsis* embryo mutant *schlepperless* has a defect in the *chaperonin-60* gene. *Plant Physiol.* 126, 717–730
 - 46 Zabaleta, E. *et al.* (1994) Antisense expression of chaperonin 60 β in transgenic tobacco plants leads to abnormal phenotypes and altered distribution of photoassimilates. *Plant J.* 6, 425–432
 - 47 Ishikawa, A. *et al.* (2003) Deletion of a chaperonin 60 β gene leads to cell death in the *Arabidopsis* lesion initiation 1 mutant. *Plant Cell Physiol.* 44, 255–261
 - 48 Yamada, A. *et al.* (2002) The role of plant CCT α in salt- and osmotic-stress tolerance. *Plant Cell Physiol.* 43, 1043–1048
 - 49 Young, J.C. *et al.* (2001) Hsp90: a specialized but essential protein-folding tool. *J. Cell Biol.* 154, 267–273
 - 50 Richter, K. and Buchner, J. (2001) Hsp90: chaperoning signal transduction. *J. Cell. Physiol.* 188, 281–290
 - 51 Pratt, W.B. *et al.* (2001) Hsp90-binding immunophilins in plants: the protein movers. *Trends Plant Sci.* 6, 54–58
 - 52 Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature* 396, 336–342
 - 53 Queitsch, C. *et al.* (2002) Hsp90 as a capacitor for phenotypic variation. *Nature* 417, 618–624
 - 54 Imai, J. *et al.* (2003) The molecular chaperone Hsp90 plays a role in the assembly and maintenance of the 26S proteasome. *EMBO J.* 22, 3557–3567
 - 55 Zhang, Z. *et al.* (2003) Characterization of a plant homolog of Hop, a co-chaperone of Hsp90. *Plant Physiol.* 131, 525–535
 - 56 Krishna, P. and Gloor, G. (2001) The Hsp90 family of proteins in *Arabidopsis thaliana*. *Cell Stress Chaperones* 6, 238–246
 - 57 Milioni, D. and Hatzopoulos, P. (1997) Genomic organization of Hsp90 gene family in *Arabidopsis*. *Plant Mol. Biol.* 35, 955–961
 - 58 Schirmer, E.C. *et al.* (1996) Hsp100/Clp proteins: a common mechanism explains diverse functions. *Trends Biochem. Sci.* 21, 289–296
 - 59 Patel, S. and Latterich, M. (1998) The AAA team: related ATPases with diverse functions. *Trends Cell Biol.* 8, 65–71
 - 60 Neuwald, A.F. *et al.* (1999) AAA+: a class of chaperone-like ATPases associated with the assembly, operation and disassembly of protein complexes. *Genome Res.* 9, 27–43
 - 61 Agarwal, M. *et al.* (2001) *Arabidopsis thaliana* Hsp100 proteins: kith and kin. *Cell Stress Chaperones* 6, 219–224
 - 62 Gottesman, S. *et al.* (1990) Conservation of the regulatory subunit for the Clp ATP-dependent protease in prokaryotes and eukaryotes. *Proc. Natl. Acad. Sci. U. S. A.* 87, 3513–3517
 - 63 Glover, J.R. and Lindquist, S. (1998) Hsp104, Hsp70 and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94, 73–82
 - 64 Goloubinoff, P. *et al.* (1999) Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13732–13737
 - 65 Beuron, M.R. *et al.* (1998) At sixes and sevens: characterization of the symmetry mismatch of the ClpAP chaperone-assisted protease. *J. Struct. Biol.* 123, 248–259
 - 66 Weber-Ban, E.U. *et al.* (1999) Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. *Nature* 401, 90–93
 - 67 Adam, Z. *et al.* (2001) Chloroplast and mitochondrial proteases in *Arabidopsis*. A proposed nomenclature. *Plant Physiol.* 125, 1912–1918
 - 68 Keeler, S. *et al.* (2000) Acquired thermotolerance and expression of the HSP100/ClpB genes of Lima bean. *Plant Physiol.* 123, 1121–1132
 - 69 Queitsch, C. *et al.* (2000) Heat stress protein 101 plays a crucial role in thermotolerance in *Arabidopsis*. *Plant Cell* 12, 479–492
 - 70 Adam, Z. and Clarke, A.K. (2002) Cutting edge of chloroplast proteolysis. *Trends Plant Sci.* 7, 451–456
 - 71 Schirmer, E.C. *et al.* (1994) An *Arabidopsis* heat shock protein complements a thermotolerance defect in yeast. *Plant Cell* 6, 1899–1909
 - 72 Lee, Y.R.J. *et al.* (1994) A soybean 101-kD heat shock protein complements a yeast HSP 104 deletion mutant in acquiring thermotolerance. *Plant Cell* 6, 1889–1897
 - 73 Agarwal, M. *et al.* (2003) Molecular characterization of rice *HSP101*: complementation of yeast *hsp104* mutation by disaggregation of protein granules and differential expression in indica and japonica rice types. *Plant Mol. Biol.* 51, 543–553
 - 74 Van Montfort, R.L. *et al.* (2001) Crystal structure and assembly of a eukaryotic small heat shock protein. *Nat. Struct. Biol.* 8, 1025–1030
 - 75 Ehrnsperger, M.S. *et al.* (1997) Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. *EMBO J.* 16, 221–229
 - 76 Lee, G.J. *et al.* (1997) A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. *EMBO J.* 16, 659–671
 - 77 Veinger, L. *et al.* (1998) The small heat-shock protein IbpB from *E. coli* stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. *J. Biol. Chem.* 273, 11032–11037
 - 78 Lee, G.J. and Vierling, E. (2000) A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein. *Plant Physiol.* 122, 189–198
 - 79 Reddy, G.B. *et al.* (2000) Temperature-dependent chaperone activity and structural properties of human α A- and α B-crystallins. *J. Biol. Chem.* 275, 4565–4570
 - 80 Mogk, A. *et al.* (2003) Refolding of substrates bound to small Hsps relies on a disaggregation reaction mediated most efficiently by ClpB/DnaK. *J. Biol. Chem.* 278, 31033–31042
 - 81 Scharf, K.D. *et al.* (2001) The expanding family of *Arabidopsis thaliana* small heat stress proteins and a new family of proteins containing α -crystallin domains (ACD proteins). *Cell Stress Chaperones* 6, 225–237
 - 82 Hamilton, E.W. III and Heckathorn, S.A. (2001) Mitochondrial adaptations to NaCl. Complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. *Plant Physiol.* 126, 1266–1274
 - 83 Wang, D. and Luthe, D.S. (2003) Heat sensitivity in a bentgrass variant. Failure to accumulate a chloroplast heat shock protein isoform implicated in heat tolerance. *Plant Physiol.* 133, 319–327
 - 84 Wang, W.X. *et al.* (2002) Characterization of SP1, a stress-responsive, boiling-soluble, homo-oligomeric protein from Aspen (*Populus tremula* L.). *Plant Physiol.* 130, 865–875
 - 85 Sun, W. *et al.* (2002) Small heat shock proteins and stress tolerance in plants. *Biochim. Biophys. Acta* 1577, 1–9
 - 86 Mogk, A. *et al.* (2003) Small heat shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. *Mol. Microbiol.* 50, 585–595
 - 87 Peres Ben-Zvi, A. and Goloubinoff, P. (2001) Mechanisms of disaggregation and refolding of stable protein aggregates by molecular chaperones. *J. Biol. Chem.* 276, 84–93
 - 88 Wang, W.X. *et al.* (2001) Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. *Acta Hort.* 560, 285–292
 - 89 Singer, M.A. and Lindquist, S. (1998) Multiple effects of trehalose on protein folding *in vitro* and *in vivo*. *Mol. Cell* 1, 639–648
 - 90 Diamant, S. *et al.* (2001) Chemical chaperones regulate molecular chaperones *in vitro* and in cells under combined salt and heat stresses. *J. Biol. Chem.* 276, 39586–39591
 - 91 Viner, R.I. and Clegg, J.S. (2001) Influence of trehalose on the molecular chaperone activity of p26, a small heat shock/ α -crystallin protein. *Cell Stress Chaperones* 6, 126–135
 - 92 Ellen, A.A. *et al.* (2002) Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J. Cell Sci.* 115, 2809–2816
 - 93 Arrigo, A.P. (1998) Small stress proteins: chaperones that act as regulators of intracellular redox state and programmed cell death. *Biol. Chem.* 379, 19–26
 - 94 Panchuk, I.I. *et al.* (2002) Heat stress- and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in *Arabidopsis*. *Plant Physiol.* 129, 838–853
 - 95 Rossel, J.B. *et al.* (2002) Global changes in gene expression in response to high light in *Arabidopsis*. *Plant Physiol.* 130, 1109–1120