Review

Benjamin Dennis Eisenhardt*

Small heat shock proteins: recent developments

Abstract: Small heat shock proteins (sHSPs) are abundantly present in many different organisms at elevated temperatures. Members of the subgroup of alpha crystallin domain (ACD)-type sHSPs belong to the large family of protein chaperones. They bind non-native proteins in an ATP-independent manner, thereby holding the incorporated clients soluble for subsequent refolding by other molecular chaperoning systems. sHSPs do not actively refold incorporated peptides therefore they are sometimes referred to as holdases. Varying numbers of sHSPs have been documented in the different domains of life and dependent on the analyzed organism. Generally, diverse sHSPs possess more sequence similarities in the conserved ACD, whereas the N- and C-terminal extensions are less conserved. Despite their designation as sHSPs, they are not solely present during heat stress. sHSPs presumably help to protect cells under various stresses, but they were also found during development, e.g., in embryonic development of higher plants which is associated with ongoing seed desiccation. The functional and physiological relevance of several different sHSPs in one organism remains still unclear, especially in plants where several highly similar sHSPs are present in the same compartment. The wide range of biotic and abiotic stresses that induce the expression of multiple sHSP genes makes it challenging to define the physiological relevance of each of these versatile proteins.

Keywords: heat stress response; small heat shock proteins.

*Corresponding author: Benjamin Dennis Eisenhardt, Institut für Mikrobiologie und Molekularbiologie, Justus-Liebig-Universität Giessen, IFZ-Interdisziplinäres Forschungszentrum, Heinrich-Buff-Ring 26-32, D-35390 Giessen, Germany, e-mail: benjamin.eisenhardt@mikro.bio.uni-giessen.de

Introduction

Years ago it was reported that organisms respond to an increase in temperature with the induction of a specific set of genes (1). High temperature can cause severe problems and is therefore often accompanied by structural and metabolic rearrangements within an organism, including membrane stiffening or change in protein composition on a cellular level (2). Temperature-induced occurrence of unfolded proteins causes a variety of different responses within a cell to maintain functionality and to circumvent harmful accumulation of protein aggregations (3). One response – the induction of the synthesis of small heat shock proteins (sHSP) – provides a transient and highly dynamic protein based environment that is able to bind thermally instable proteins (clients) in an ATP-independent way. Thereby irreversible protein denaturation and aggregation is prevented (4, 5). Few ACD-type sHSPs are found in unicellular organisms like archaea, bacteria or yeast (6–8), more than ten in humans (9, 10) and even more in plants (11–13). The general believe that bacteria have only a low number of sHSPs has been refuted by the identification of multiple sHSPs in α-proteobacteria like *Rhizobium* and *Bradyrhizobium* species (14). Proteins of the here reviewed sHSP family are characterized by their small monomeric sizes (12–45 kDa), the conserved ACD of about 80–100 residues (15–17), formation of large oligomers and an ATP-independent chaperone activity (4). The conserved ACD is flanked by a variable, not conserved N-terminal domain and a short C-terminal extension that can also be absent in some cases. In most cases the presence of sHSPs led to protection from heat-induced aggregation but not to activity preservation of thermally instable proteins (5, 18, 19). At heat stress (HS) many different proteins are produced, but not all comprise the mentioned ACD sHSP-defining characteristics. This review summarizes exemplarily chosen aspects of different methods of transcriptional and translational control, as well as structural and functional similarities and singularities of ACD-type sHSPs.

Regulation of sHSP gene expression

sHSP gene expression in eukaryotes

In eukaryotes the transcription of sHSP genes is regulated by a number of different HS transcription factors

(Hsfs). They bind to specific consensus sequences in front of HS-induced genes, so-called heat shock elements (HSE). In particular, in plants a complex world of different Hsfs has emerged (20–22). As an immobile organism in a varying habitat, a plant has to cope with and adapt to special challenges, such as day-to-night temperature differences or the changing availability of resources like light or water. These circumstances could have promoted the functional diversification of Hsfs and sHSPs in plants. Despite their designation as sHSPs, these proteins are not solely present at HS. Expression is induced at the levels of transcription and translation in response to various stresses like osmotic, cold and salt stress, amino acid analogues and pathogen attack (12, 23). Independent from any occurring stress, a development dependent presence of selected sHSPs is also observed. Regulated by specific Hsfs and a modified HSE in case of seed maturation (24–26), some sHSPs are produced during the development of the petals, pollen and seeds (25, 27, 28). In general the complex Hsf world shows a great plasticity in interactions with one another, which specifies the response (26, 29, 30). The well-studied example of three tomato Hsfs (Hsf A1, A2, B1) illustrates the functional diversification and cooperation of plant Hsfs. All together, these form a triad for subsequent responses at different stress phases. HsfA1 is the master regulator of the heat shock response (HSR). HsfA1/B2 heterooligomers trigger the HSR, but HsfB1 has a dual function. It can maintain the HSR alone, or during the recovery phase it restores, in cooperation with housekeeping transcription factors, the housekeeping gene transcription. A subsequent HS during the recovery phase induces heterooligomer formation of HsfA1 and HsfA2, which leads to a rapid recovery of the HSR (20, 22, 31, 32). In particular, during repeated cycles of HS, interactions between specific sHSPs and Hsfs might influence and coordinate the HSR by modulating the intracellular localization and activator function of Hsfs (Figure 1). In tomato Hsp17.4-CII functions as co-regulator and cytoplasmic retention factor of HsfA2, thereby the sHSP exerts a repressive effect on the transcriptional activator activity of the bound Hsf (33). However, only a few cases of interactions between sHSPs and Hsfs are documented. Interactions between Hsp70 or Hsp90 and Hsfs – especially at the beginning and end of the HSR – are more frequently described (31, 34, 35). In animals, sHSPs are associated with a variety of different HS-independent responses comparable to plants. In contrast to plants, animals developed more specialized tissues and cell types, so the development of more complex regulatory concepts should not be surprising, but it appears that compared to plants the multiplicity of Hsfs and sHSPs is much smaller in other
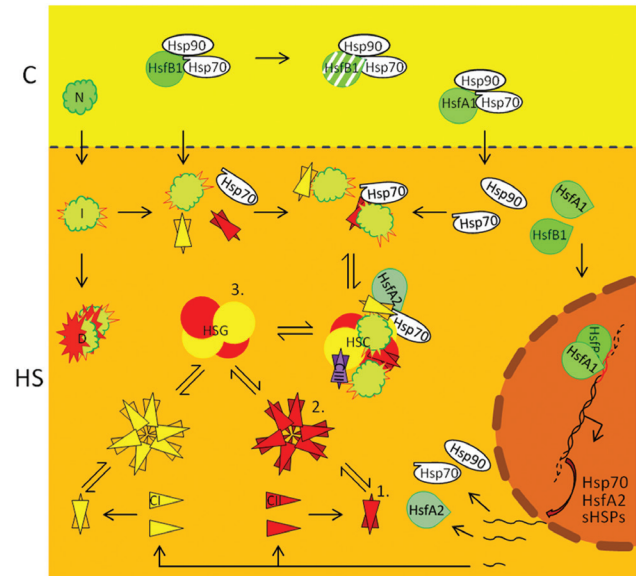

Figure 1 Schematic model of HSG and HSC formation and their influence on acclimation.

The HSR was extensively studied in tomatoes. Under control conditions (C) Hsp70 binds to HsfA1 (the major regulator of the HSR) and turns it inactive. Hsp90 binds and marks HsfB1 for rapid degradation. At HS, thermally instable proteins lose their native structure (N), whereby increasing amounts of intermediate products (I) start to attract Hsp70 and Hsp90. These high molecular weight chaperones try to prevent the aggregation of denatured proteins (D) and the released Hsfs can now activate the expression of HS regulated genes. New produced sHSPs and Hsp70 support in aggregation protection. sHSPs are highly dynamic proteins, so it is unclear whether they incorporate clients as (1) dimer, (2) oligomer or (3) in granular form. There is some evidence that at least for class I the dimeric form binds to unfolding proteins and further assembles in the other two structures, which presumably serve as storage forms. sHSPs of class II were described to be impaired in their dissociation into dimers. Thus, differences to the simplified scheme presented here are possible. The incorporation of clients in HSGs promotes the formation of HSCs. These structures exert an indirect regulative function by storage of the HsfA1/HsfB1 dependently produced HsfA2. Under recovery conditions Hsp70 and Hsp101 refold the incorporated proteins and the HSEs occupied by Hsfs are cleared. Hsp90 promotes rapid degradation of HsfB1 (to some extent HsfB1 also contributes in restoring the transcription of housekeeping genes) and Hsp70 binds to HsfA1 again. At a repeated HS during the recovery, HsfA2 assists HsfA1 (again released from Hsp70) in rapid transcriptional reactivation of HS-dependent genes.

organisms, including humans (36). Yeast comprises one Hsf and animals normally comprise three-to-four Hsfs, and their activity is often regulated by interactions of the Hsf with high molecular weight chaperones, e.g., Hsp70 (37–39). Thus, the basic mode of HSR regulation in animals seems comparable to plants even though differences are found. In animals, all Hsfs are constitutively present and they are often regulated by phosphorylation (40). In

plants, the appearance of some sHsfs is HS-induced, and it was believed for a long time that phosphorylation of Hsfs is a unique feature to animals. However, recent findings described phosphorylation-dependent localization and stability of HsfA2 in *Arabidopsis* (41).

sHSP gene expression in prokaryotes

The transcription of HS-activated genes in prokaryotes often underlies negative regulating sequence elements, such as CIRCE or HAIR elements which control and influence the expression of heat shock genes (42). The CIRCE system (controlling inverted repeat of chaperone expression) is one of the most widely distributed negative *cis* acting DNA elements in heat shock regulation. HrcA (heat regulation at CIRCE) binds as a repressor to CIRCE and inhibits transcription of HS-dependent genes (43–45). The inhibitory effect of HrcA itself depends on GroESL and during HS the depletion of the GroE pool by denatured proteins renders HrcA inactive, leading to elevated transcription of CIRCE-controlled heat shock genes (46–48). The prokaryotic transcription of genes is regulated by sigma factors, exchangeable RNA polymerase subunits, which specifically recruit the polymerase for gene transcription. In the well-studied *Eschericha coli* model, protein quality control is regulated by sigma32 (σ^{32}), also known as heat shock sigma factor RpoH (49–51). In several α-proteobacteria, two or more paralogs of RpoH exist, suggesting functional divergence of this alternative transcription factor (52). In *Rhodobacter*, an exemplarily chosen member of this bacterial class, HS response underlies the specialized sigma factor RpoH(I), whereas the close paralog RpoH(II) controls the oxidative stress response (53, 54). In *E. coli*, DnaK (Hsp70), GrpE (Hsp24) and DnaJ (Hsp40) sequester RpoH under non-stress conditions and DnaK promotes RpoH degradation by the FtsH protease. HS-induced unfolding of thermally instable proteins leads to the recruitment of DnaK from the bound sigma factor (51, 55). This chaperone-mediated negative feedback control allows the alternative transcription factor to sense the cellular folding state. The response is further indirectly modulated by the RNA binding protein Hfq (host factor required for replication of the RNA phage Qβ), which is also involved in stress acclimation (56, 57). Hfq associates with small regulatory RNAs (sRNAs) to promote their base-pairing with target mRNAs, thereby the sRNA-mRNA pairing affects the translation rate and lifetime of the targeted transcript (58). In this case, it controls the translation efficiency of several HS-dependent gene products including *dnaK* mRNA translation thereby regulating the DnaK level within the cell (57). In addition to the already complex network, the amount of RpoH in a cell is directly regulated by the *rpoH* mRNA itself in a temperature-dependent mechanism. The *rpoH* mRNA and also some sHSP gene products belong to a group of RNA thermometers, which respond at high temperature with exposure of the ribosomal binding site. The highly structured mRNA unfolds at high temperatures and provides ribosomal subunits access to the Shine Dalgarno sequence for translation initiation (59–61). Other examples are the Hsp17 thermometer (62) or so-called ROSE (repression of heat shock gene expression) elements in rhizobial species, which control heat shock gene product translation in a similar way (48, 63, 64). As described for *Agrobacterium tumefaciens*, it is also possible that several occurring sHSP genes are regulated differently in a replicon dependent manner. While the sHSP gene on the linear chromosome turned out to be regulated by RpoH, transcripts of the sHSP genes on the *A. tumefaciens* plasmids were under the control of ROSE sequences in their 5′ untranslated region (65). In summary, this tight and optimized regulation of the cytoplasmic stress response is important for unicellular organisms to cope with suddenly occurring stress and recovery conditions in an adequate way.

Localization and physiological relevance

Most organisms except plants do not possess an extended ACD-type sHSP family though exceptions are found. To date, 10 different sHSPs were described for humans and even more for some other vertebrates like birds or fish (9, 66). These sHSPs were found in various cell types, from eye lenses to sperm cells (10) and also in diverse compartments from cytosol, medial golgi apparatus to mitochondrial membranes (67, 68). Nevertheless, the existence of highly similar sHSPs in one cellular compartment forming a superfamily with distinguishable subfamilies [Figure 2; (69–71)] is a unique feature of plants (12, 72, 73). In particular, the diversity of plant sHSPs displays that these proteins can be localized virtually to all organelles of a cell, such as the chloroplasts, mitochondria, nucleus, endoplasmatic reticulum and peroxisomes (11, 72, 74). Although the cytosolic groups of sHSPs do often not possess any familiar nuclear localization signal (NLS), studies indicated that these sHSPs are distributed between cell plasma and nucleus (12). So far, the physiological function of normally cytosolic sHSPs in the nucleus is often
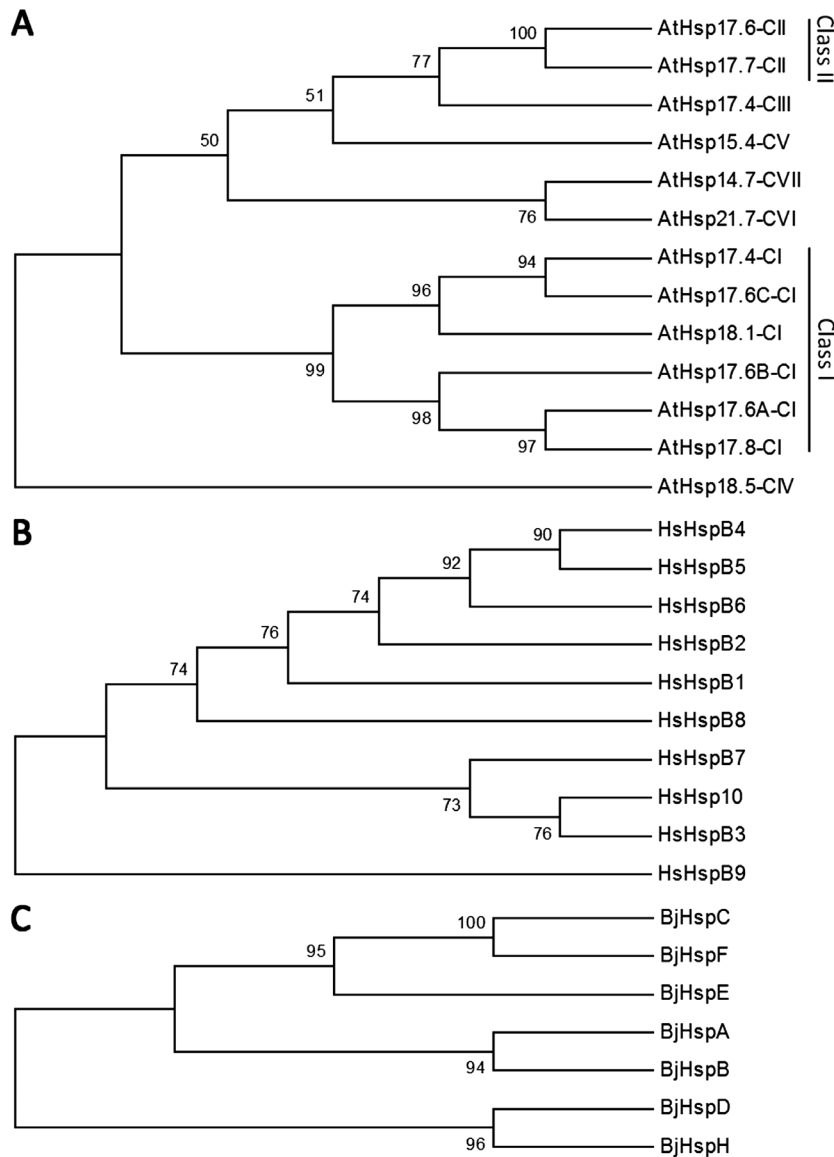
A

B

C

Figure 2 The cytosolic sHSP group of plants.
Comparing (A), (B) and (C) illustrates the unique feature of plants by possessing several highly related sHSPs in one compartment (cytosol) that can be sorted in different classes (CI–CVII). In other species with several sHSPs (e.g., humans or Bradyrhizobium) this phenomenon is absent. Nucleotide sequences (codons) were aligned with clustalW and the evolutionary history was inferred using the maximum parsimony (MP) method. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The MP tree was obtained using default settings. Evolutionary analyses were conducted in MEGA5. The two major cytosolic sHSP classes are marked. At, *Arabidopsis thaliana*; Hs, *Homo sapiens*; Bj, *Bradyrhizobium japonicum*.

unclear. However, several sHSPs with a typical NLS are known (CIII in Figure 2), which are specifically targeted to the nucleus (11, 72, 75). This multiplicity of sHSPs in one organism, especially in plants, complicates a clear separation of the *in vivo* function of single sHSPs. Nevertheless, sHSPs appeared to be beneficial for different aspects of life, in particular under protein misfolding and other cell metabolism destabilizing conditions. As described for the golgi localized Hsp-16.1 of *Caenorhabditis elegans*, sHSPs

are associated with acclimation processes and can protect from heat-stroke associated neurodegeneration (68). In addition to acclimatization, members of this group of proteins were reported to be involved in several other protection mechanisms, such as during the encystment and diapauses in *Artemia franciscana* (76–78). In humans, the well-studied αA- and αB-crystallins (CRYAA and CRYAB) prevent from cataract formation [summarized in (79, 80)]. Mutations in these and other human sHSPs are associated

with a wide range of diseases, the majority of which are neuropathies and myopathies (81–84). Besides possible interactions with elements of the cytoskeleton (85–87), different members of the chaperone family are found to be present at sites of inflammation (88, 89) and cellular defects like ischemic acute renal failure (90) or excito-toxic lesions (91). As a general protective effect, it was reported that high levels of sHSPs extend the life span of some organisms (92–94). In *Synechocystis* a sHSP (Hsp17) was found to be involved in membrane stabilization (95, 96), in *Chlamydomonas* Hsp-22 in photosystem protection (97) and AtHsp17.8-CI in *Arabidopsis* facilitated the protein reallocation from the cytosol to the outer chloroplast membrane (98). sHSPs with methionines as redox sensor motif (e.g., AtHsp25.3-P in *Arabidopsis*) were described in chloroplasts (99). Chloroplast-localized sHSPs often show a temperature-dependent membrane association (100, 101), maybe acting in a comparable way as described for Hsp17 from *Synechocystis* (102). In general, sHSPs can interact with a wide variety of proteins *in vivo*, thereby preserving a wide range of cellular functions (103). In plants they assist during several developmental pro-cesses, such as seed development (25), and it is assumed that they confer enhanced desiccation tolerance to the embryo (104). In prokaryotes, only a lower number of different sHSPs within an organism are usually detected (with exceptions), however wide effects on different phys-iological functions were found. In *E. coli* sHSPs were first described associated to inclusion bodies (105), therefore named 'inclusion body associated proteins' (IbpA/IbpB). But besides their responsiveness to unfolded proteins during heterologous protein expressions, both proteins are involved in heat and oxidant protection mechanisms (106, 107). In addition, sHSPs with unusual functions are known. In *Bacillus subtilis*, an ACD-containing sHSP (CotM) was identified as a spore coat protein (108, 109). The plant pathogen *A. tumefaciens* uses HspL as virulence promoting factor (110–112). Thus sHSPs exert pleiotropic effects throughout the life and it cannot be excluded that new functions will be discovered. Schematic structures of some of the described ACD-containing sHSPs are sum-marized in Figure 3. Table 1 summarizes the accession numbers of all discussed sHSPs.

Structure and molecular function

The ACD-type sHSPs were originally summarized as a group of small HS-induced proteins (15–45 kDa) with an ACD and an ability to form high molecular weight
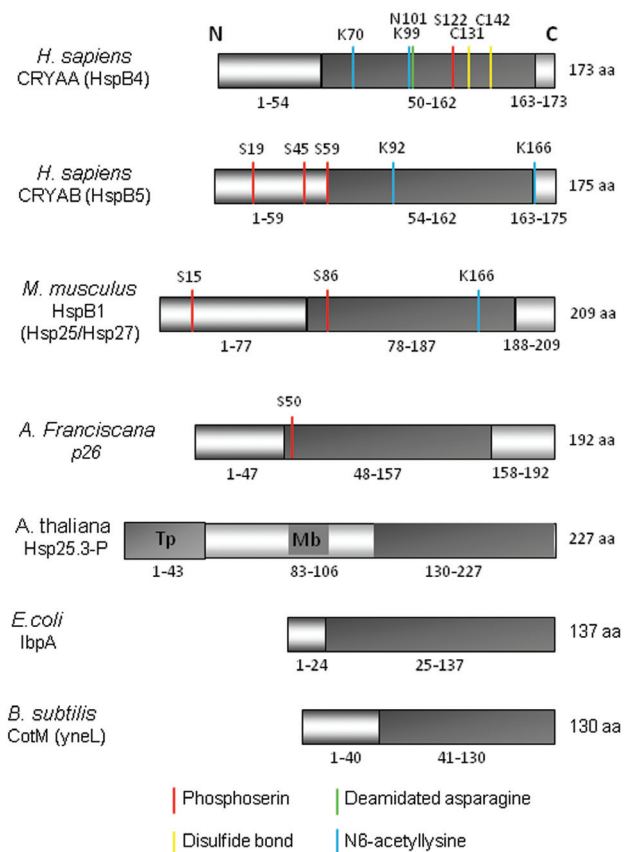

Figure 3 Comparative schematic composition of ACD-type sHSPs from different organisms.
Indicated amino acid positions (aa) for shown domains and modi-fications were taken from following protein accession numbers: *Homo sapiens* αA crystallin (CRYAA/P02489), *Homo sapiens* αB crystallin (CRYAB/P02511), *Mus musculus* HspB1 (P14602), *Artemia franciscana* p26 (O44112), *Arabidopsis thaliana* HSP25.3-P (P31170), *Escherichia coli* IbpA (P0C054), *Bacillus subtilis* CotM (Q45058). Zn^{2+} binding histidine residues of αA/B crystallin are not shown. Tp, transit peptide; Mb, methionine bristle.

complexes up to 150–800 kDa in size. sHSP oligomers are dynamic structures that often underlie temperature- or post-translational modification-dependent structural rearrangements. With few exceptions, dimers act as basic structural subunits, which further assemble into an oligo-meric structure composed of an even number of subunits like 12, 24 and 36mers (113–115). Nonamers with a trimer of trimers or oligomeric structures with tetramers as sub-units were also discovered, but are more unusual (116, 117). For quite a number of sHSPs from different organ-isms (plants, animals and prokaryotes) structural data is available, even though the crystallization of full length sHSPs is difficult. The highly dynamic N-terminal domain complicates stable positioning of sHSPs, a prerequi-site for the crystallization process and subsequent data

Table 1 Summary of discussed sHSPs.

Name	Synonym	Organism	Accesion number (protein)	Length (aa)	MW (kDa)	Localization
AtHsp17.4-CI	–	*A. thaliana*	P19036	156	17.4	c/n
AtHsp17.6A-CI	–	*A. thaliana*	Q9XIE3	155	17.6	c/n
AtHsp17.6B-CI	–	*A. thaliana*	Q9ZW31	153	17.6	c/n
AtHsp17.6C-CI	Hsp17.6	*A. thaliana*	P13853	157	17.6	c/n
AtHsp17.8-CI	–	*A. thaliana*	Q9LNW0	157	17.8	c/n
AtHsp18.1-CI	Hsp18.2	*A. thaliana*	P19037	161	18.1	c/n
AtHsp17.6-CII	Hsp17.6-II	*A. thaliana*	P29830	155	17.6	c/n
AtHsp17.7-CII	Hsp17.6A	*A. thaliana*	O81822	156	17.7	c/n
AtHsp17.4-CIII	–	*A. thaliana*	Q9SYG1	155	17.4	c/n
AtHsp18.5-CIV	AtHsp18.5-CI(r)	*A. thaliana*	O64564	162	18.5	c/n
AtHsp15.4-CV	AtHsp15.4-CI(r)	*A. thaliana*	O49710	134	15.4	c/n
AtHsp21.7-CVI	AtHsp21.7-CI(r)	*A. thaliana*	Q9FIT9	192	21.7	c/n
AtHsp14.7-CVII	AtHsp14.2-P(r)	*A. thaliana*	Q6NLV0	131	14.7	c/n
AtHsp25.3-P	Hsp21	*A. thaliana*	P31170	227	25.3	p
TaHsp17.6B-CI		*T. aestivum*	Q41560	151	16.9	c
LpHsp16.1-CIII		*L. peruvianum*	Q94EN7	144	16.1	n/c
Afp26		*A. franciscana*	O44112	192	20.8	–
CeHsp-16.1		*C. elegans*	P34696	145	16.3	gmc
Tsp36	R-Tso2	*T. saginata*	Q7YZT0	314	35.6	c
MmHspB1	Hsp25	*M. musculus*	P14602	209	23.0	c/n
HsHspB1	Hsp25/27	*H. sapiens sapiens*	P04792	205	22.8	c/n
HsHspB4	CRYAA	*H. sapiens sapiens*	P02489	173	19.9	c/n
HsHspB5	CRYAB	*H. sapiens sapiens*	P02511	175	20.2	c/n
ScHsp26		*S. cerevisiae*	P15992	214	23.9	c/n
BsCotM	yneL	*B. subtilis*	Q45058	130	15.2	sc
MjHsp16.5		*M. jannaschii*	Q57733	147	16.5	c
SsHsp17		S. PCC 6803	L8AFE3	146	16.6	c/m
XaHspA		*Xanthomonas*	ADI78883	158	17.7	–
MtHspX	Acr, Nox16	*M. tuberculosis*	P0A5B7	144	16.2	c, cw
RsHsp20		*R. sphaeroides*	Q3IWA3	177	19.0	–
BjHspA		*B. japonicum*	P70917	152	17.2	–
EcIbpA		*E. coli*	P0C054	137	15.8	c, om
EcIbpB		*E. coli*	P0C058	142	16.1	c, om

c, cytoplasm; n, nucleus; m, membrane; p, plastids; gmc, golgi medial cisterna; sc, spore coat; cw, cell wall; om, outer membrane; –, not defined.

acquisition. In many cases (e.g., *Methanocaldococcus jannaschii* Hsp16.5; *Xanthomonas citri* pv. *citri* HspA, Human Hsp27 and αB-crystallin) structures were solved without the N-terminus. The dodecameric Hsp16.9 structure from *Triticum aestivum* (118) consists of only six complete subunits (residues 2–151), while the other six subunits are missing the first 42 N-terminal residues [summarized in (119)]. However the existing data increased our knowledge regarding structural and functional aspects of different sHSPs. sHSPs belong to the large family of protein chaperones. Incorrect folded proteins are recognized and bound by sHSPs in an ATP-independent manner. The incorporated protein stays protected in high molecular weight complexes for subsequent refolding by other members of the molecular chaperone network. For client reconstitution, ATP-dependent high molecular weight chaperoning machineries are needed (120, 121), for example, members of the Hsp70/40 class (DnaK/DnaJ in prokaryotes). In the case of improperly refolded clients, a linkage to a degradation or recycling pathway would be reasonable, but was not clearly identified up to now (119). Normally ACD-type sHSPs are holdases and cannot refold incorporated clients on their own. Few exceptions were described where certain sHSPs seem to have refolding function, but these examples are rare (122). A detailed *in silico* examination found characteristic differences in segment lengths between plants, animals and bacteria (123). Generally the ACD of sHSPs seems to be a platform for flexible arms that capture clients to maintain their solubility. Taking a look at the different structural sections, the highly variable and often hydrophobic N-terminus appeared to be necessary for client binding, the

β-sheeted ACD for the dimer assembly and the C-terminal extension with the basic IXI/V motif (118) responsible for oligomer formation. But it is not possible to generalize that the N-terminus is the major client recognition and protection site and the C-terminus is responsible for the control of the oligomer size. In many cases all sections have overlapping functions (124, 125). The exact mechanism of how clients are bound and protected is not completely solved until now. At HS, different proteins bound on different sites at the N-terminal sHSP arm (126) and the proteins did not show any commonality in sequence or structure that could be a signature for a sHSP binding target (103). Based on the available data of sHSP structures, different concepts of mechanistic modus operandi are discussed. It is unclear whether heat-induced oligomer dissociation into dimers is indispensable for client recognition and protection (127) or not (113). Comparing different analyses might suggest that the functional relevance of heat-induced dissociation may vary between sHSPs and the examined organism – but at least for most of the analyzed plant sHSPs, oligomer dissociation seems to be a prerequisite for client protection (127, 128). Exceptions might be dimeric sHSPs without a dominant oligomeric structure such as AtHsp17.8-CI or AtHsp18.5-CV in *Arabidopsis thaliana*. As already described for AtHsp17.8-CI, it has (besides its chaperone activity) an additional function in protein reallocation processes within the cell and perhaps the dimeric structure facilitates this function (18, 98). The functional and physiological relevance of the presence of multiple cytosolic and nuclear localized similar proteins in plants remains a matter of debate. Aspects of cooperative function was discussed, but mainly additive not cooperative client protection was measured (129). At finding a clear connection between the oligomeric state of sHSPs and their client protection capacity, interesting results were obtained, because *in vitro* and *in vivo* observations were inconsistent with one another. A screening for mutations of *Synechocystis* Hsp17 that reduced the ability of the protein to provide thermo tolerance *in vivo* identified two groups of alterations. One group of mutations destabilized the oligomer and reduced the *in vitro* chaperone activity. The other group of mutations, especially in the N-terminus, had little effect on the oligomer stability or chaperone activity *in vitro*. As these mutations still failed to provide thermo tolerance *in vivo*, the results indicate a previously unrecognized function of the N-terminus that is not adequately analyzed by current biochemical *in vitro* measurements. Therefore, it was concluded that some *in vitro* client protection measurements are not directly comparable to the *in vivo* situation (130). Changes in structure or protection

efficiency do not only depend on heat-induced dissociation events, but cold-induced changes in protection capabilities were also observed in different organisms (131, 132). In addition, the oligomeric structure of sHSPs is in many organisms often regulated by post-translational modifications. In animals and humans the phosphorylation of sHSPs resulted in different visible or measurable responses, which presumably are all connected to a modification-dependent change of the dissociation and re-association behavior of the oligomer. Very early it was found that αA- and αB-crystallin undergo a large variety of post-translational modifications, such as deamidation, racemization, phosphorylation, acetylation, glycation and age-dependent truncation (summarized in (133) and in Figure 2). Some histidine residues involved in Zn^{2+} binding were newly identified (134), as earlier studies showed an increase of activity after Zn^{2+} addition (135). For the αA-crystallin, homo- and cAMP pathway-dependent phosphorylations were described (136–138). In the murine model system, non-phosphorylated Hsp25 monomers were active in inhibiting actin polymerization, while phosphorylated Hsp25 monomers and non-phosphorylated multimeric Hsp25 particles were inactive (139). Phosphorylation and other modifications were not found in plants or prokaryotes up to now. The redox-sensing chloroplast-localized sHSPs are one exception (140). These sHSPs share an oxidant-accessible region formed by methionines, referred to as 'methionine bristle' (141). Only sulfoxidation – not phosphorylation or heat treatment – leads to changes in structure and abolished the chaperone-like activity of the tested sHSP (99, 140, 142, 143). The exchange of the methionines to sulfoxidation-resistant leucines residues leads to a loss of redox-sensing capabilities but no decrease in chaperone function. The methionines are exclusively required as sensors but not for client protection (144).

Heat shock granules

Heat shock granules (HSG) are large cytosolic complexes of up to 1–2 MDa molecular weight that are present in all tissues during heat shock (145). So far this phenomenon has only been described in plants and is therefore presumably a unique feature of plants. They are formed in the presence of certain cytosolic sHSP members, whereat proteins of the cytosolic class II family seem to be a prerequisite (75, 146). In the cytosol of plants, several sHSPs are present under HS conditions. The classification of sHSP subfamilies in plants is based on differences and

similarities in their N-terminal sequences. sHSPs with high similarities in their N-terminal sequences are summarized in same class (see also Figure 2). There are many different cytosolic sHSP classes distinguishable, whereby class I comprises the most members (e.g., 6 in *A. thaliana*) and class II is often associated to HSG formation. These irregular predominantly globular-shaped cytosolic particles with up to 40 nm in size are composed of class I and class II sHSPs, and can further assemble in larger cytosolic heat shock complexes (HSCs), involving sHSP classes I-III as well as high molecular weight HSPs, e.g., members of the Hsp70 and Hsp40 family (12). HSG may build up a basic core complex, whereby sHSPs provide binding and incorporation of misfolded cell proteins, which are stored in HSCs for subsequent refolding by high molecular weight HSPs during recovery (schematic summary of HSG and HSCs is presented in Figure 1). Other proteins such as α- and β-tubulins were also found to be associated with HSCs, but partial digestion analysis revealed that these proteins, in contrast to sHSPs and to some extent Hsp70, are probably not integral part of the complexes and are therefore hypersensitive (susceptible) to proteolysis (147). Furthermore, HSG and HSC may be indirectly involved in transcriptional control by storage of Hsfs (32). Surprisingly, neither ubiquitin nor ubiquitin-protein conjugates were detected in these structures. This does not exclude that refolded proteins undergo cellular quality control mechanisms as usual, when they are released from the granules, or a missing linkage to the degradation systems will be found in future. Earlier findings suggested that HSG are able to incorporate and protect untranslated mRNAs (145), but comparative studies on messenger ribonucleo protein (mRNPs) homeostasis, which depends on rapid transitions between different functional states (translated mRNPs, untranslated mRNPs, mRNPs under degradation), clearly highlighted that mRNP-dependent cytosolic aggregates are different to HS-induced HSG. In plant cells, different types of cytoplasmic aggregates, such as stress granules (SGs) or processing bodies (PBs), are present in addition to HSG. On one hand, neither SGs (stalled mRNPs accumulated in cytosolic aggregates) nor PBs (sites of mRNP processing) contained sHSPs. On the other hand, HSG had no mRNA incorporated (148, 149). As class I proteins are not able to form granules on their own, HSG formation represents a specific assembly process that depends on formation of class II sHSP oligomers as a prerequisite for the auto-aggregation (146, 147).

Summary and outlook

The field of sHSP research is still developing. Besides the observation that the presence of sHSPs may be beneficial for an organism to cope with unfavorable conditions, the exact cellular function of several sHSPs still has to be elucidated. How clients are recognized and bound, as well as the role of multiple sHSPs in the same compartment of one organism, is not understood in detail. The fact that most of the tested sHSPs act as holdases *in vitro* does not necessarily explain the advantage of multiple in parallel up-regulated sHSPs in one location of a cell. Functional redundancy or cooperation may be a possible explanation, as well as more specified not yet identified functions of single sHSPs. In plants, they might have a buffer function in acclimated cells comparable to compatible compounds during osmotic stress conditions, but the efforts for an organism to produce so many sHSPs under HS seems to be not worth the cost. We could expect that more efficient systems should have evolved, if sHSPs should just perform buffering. Perhaps new technical approaches (e.g., mass spectroscopy based analysis of sHSP/client interactions, NMR structures of full length sHSP monomers) help to add new details in the emerging picture of sHSP function. sHSP are already used as stabilizers in molecular biological processes and their use is discussed for clinical therapies (150, 151). Thereby the oligomeric sHSP structure shall serve as a nano cage for drug delivery. Nonetheless, more insights in sHSP structure and function are needed to fulfill all the ambitious expectations.

References

1. Ritossa F. A new puffing pattern induced by temperature shock and DNP in Drosophila. Cell Mol Life Sci 1962; 18: 571–3.

2. Raison JK, Roberts JKM, Berry JA. Correlations between the thermal stability of chloroplast (thylakoid) membranes and the

composition and fluidity of their polar lipids upon acclimation of the higher plant, Nerium oleander, to growth temperature. BBA-Rev Biomembranes 1982; 688: 218–28.

3. Taylor JP, Hardy J, Fischbeck KH. Toxic proteins in neurodegenerative disease. Science 2002; 296: 1991–5.

4. Jakob U, Gaestel M, Engel K, Buchner J. Small heat shock proteins are molecular chaperones. J Biol Chem 1993; 268: 1517–20.

5. Lee GJ, Roseman AM, Saibil HR, Vierling E. A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J 1997; 16: 659–71.

6. Kappe G, Leunissen JA, de Jong WW. Evolution and diversity of prokaryotic small heat shock proteins. Prog Mol Subcell Biol 2002; 28: 1–17.

7. Inglis DO, Arnaud MB, Binkley J, Shah P, Skrzypek MS, Wymore F, Binkley G, Miyasato SR, Simison M, Sherlock G. The Candida genome database incorporates multiple Candida species: multispecies search and analysis tools with curated gene and protein information for Candida albicans and Candida glabrata. Nucleic Acids Res 2011; 40: D667–74.

8. Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence 2013; 4: 119–28.

9. Kappe G, Franck E, Verschuure P, Boelens WC, Leunissen JA, de Jong WW. The human genome encodes 10 α-crystallin-related small heat shock proteins: HspB1-10. Cell Stress Chaperones 2003; 8: 53–61.

10. Fontaine JM, Rest JS, Welsh MJ, Benndorf R. The sperm outer dense fiber protein is the 10th member of the superfamily of mammalian small stress proteins. Cell Stress Chaperones 2003; 8: 62–9.

11. Scharf KD, Siddique M, Vierling E. 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 2001; 6: 225–37.

12. Siddique M, Gernhard S, von Koskull-Doring P, Vierling E, Scharf KD. The plant sHSP superfamily: five new members in Arabidopsis thaliana with unexpected properties. Cell Stress Chaperones 2008; 13: 183–97.

13. Waters ER, Aevermann BD, Sanders-Reed Z. Comparative analysis of the small heat shock proteins in three angiosperm genomes identifies new subfamilies and reveals diverse evolutionary patterns. Cell Stress Chaperones 2008; 13: 127–42.

14. Munchbach M, Nocker A, Narberhaus F. Multiple small heat shock proteins in rhizobia. J Bacteriol 1999; 181: 83–90.

15. Waters ER, Vierling E. The diversification of plant cytosolic small heat shock proteins preceded the divergence of mosses. Mol Biol Evol 1999; 16: 127–39.

16. Plesofsky-Vig V, Brambl R. Phylogeny of the α-crystallin-related heat-shock proteins. J Mol Evol 1992; 35: 537–45.

17. Caspers GJ, Leunissen JA, de Jong WW. The expanding small heat-shock protein family, and structure predictions of the conserved 'α-crystallin domain'. J Mol Evol 1995; 40: 238–48.

18. Eisenhardt BD, Forreiter C. Insights in small heat shock protein/client interaction by combined protection analysis of two different client proteins. FEBS Lett 2012; 586: 1772–7.

19. Stromer T, Ehrnsperger M, Gaestel M, Buchner J. Analysis of the interaction of small heat shock proteins with unfolding proteins. J Biol Chem 2003; 278: 18015–21.

20. Baniwal SK, Bharti K, Chan KY, Fauth M, Ganguli A, Kotak S, Mishra SK, Nover L, Port M, Scharf KD, Tripp J, Weber C, Zielinski D, von Koskull-Döring P. Heat stress response in plants: a complex game with chaperones and more than twenty heat stress transcription factors. J Biosci 2004; 29: 471–87.

21. Nover L, Bharti K, Doring P, Mishra SK, Ganguli A, Scharf KD. Arabidopsis and the heat stress transcription factor world: how many heat stress transcription factors do we need? Cell Stress Chaperones 2001; 6: 177–89.

22. Mishra SK, Tripp J, Winkelhaus S, Tschiersch B, Theres K, Nover L, Scharf K-D. In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. Genes Dev 2002; 16: 1555–67.

23. Swindell WR, Huebner M, Weber AP. Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. Genomics 2007; 8: 125.

24. Kotak S, Vierling E, Baumlein H, von Koskull-Doring P. A novel transcriptional cascade regulating expression of heat stress proteins during seed development of Arabidopsis. Plant Cell 2007; 19: 182–95.

25. Dafny-Yelin M, Tzfira T, Vainstein A, Adam Z. Non-redundant functions of sHSP-CIs in acquired thermotolerance and their role in early seed development in Arabidopsis. Plant Mol Biol 2008; 67: 363–73.

26. Carranco R, Almoguera C, Jordano J. An imperfect heat shock element and different upstream sequences are required for the seed-specific expression of a small heat shock protein gene. Plant Physiol 1999; 121: 723–30.

27. Volkov RA, Panchuk, II, Schoffl F. Small heat shock proteins are differentially regulated during pollen development and following heat stress in tobacco. Plant Mol Biol 2005; 57: 487–502.

28. Carranco R, Almoguera C, Jordano J. A plant small heat shock protein gene expressed during zygotic embryogenesis but noninducible by heat stress. J Biol Chem 1997; 272: 27470–5.

29. Almoguera C, Prieto-Dapena P, Jordano J. Dual regulation of a heat shock promoter during embryogenesis: stage-dependent role of heat shock elements. Plant J 1998; 13: 437–46.

30. Diaz-Martin J, Almoguera C, Prieto-Dapena P, Espinosa JM, Jordano J. Functional interaction between two transcription factors involved in the developmental regulation of a small heat stress protein gene promoter. Plant Physiol 2005; 139: 1483–94.

31. Hahn A, Bublak D, Schleiff E, Scharf KD. Crosstalk between Hsp90 and Hsp70 chaperones and heat stress transcription factors in tomato. Plant Cell 2011; 23: 741–55.

32. Scharf KD, Heider H, Hohfeld I, Lyck R, Schmidt E, Nover L. The tomato Hsf system: HsfA2 needs interaction with HsfA1 for efficient nuclear import and be localized in cytoplasmic heat stress granules. Mol Cell Biol 1998; 18: 2240–51.

33. Port M, Tripp J, Zielinski D, Weber C, Heerklotz D, Winkelhaus S, Bublak D, Scharf KD. Role of Hsp17.4-CII as coregulator and cytoplasmic retention factor of tomato heat stress transcription factor HsfA2. Plant Physiol 2004; 135: 1457–70.

34. Lee JH, Schoffl F. 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 1996; 252: 11–9.

35. Kim BH, Schoffl F. Interaction between Arabidopsis heat shock transcription factor 1 and 70 kDa heat shock proteins. J Exp Bot 2002; 53: 371–5.

36. Kotak S, Larkindale J, Lee U, von Koskull-Doring P, Vierling E, Scharf KD. Complexity of the heat stress response in plants. Curr Opin Plant Biol 2007; 10: 310–6.

37. Abravaya K, Myers MP, Murphy SP, Morimoto RI. The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev 1992; 6: 1153–64.

38. Morimoto RI. Cells in stress: transcriptional activation of heat shock genes. Science 1993; 259: 1409–10.

39. Sarge KD, Murphy SP, Morimoto RI. Activation of heat shock gene transcription by heat shock factor 1 involves oligomer-ization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Mol Cell Biol 1993; 13: 1392–407.

40. Knauf U, Newton EM, Kyriakis J, Kingston RE. Repression of human heat shock factor 1 activity at control temperature by phosphorylation. Genes Dev 1996; 10: 2782–93.

41. Evrard A, Kumar M, Lecourieux D, Lucks J, von Koskull-Doring P, Hirt H. Regulation of the heat stress response in Arabidopsis by MPK6-targeted phosphorylation of the heat stress factor HsfA2. PeerJ 2013; 1: e59.

42. Servant P, Mazodier P. Negative regulation of the heat shock response in Streptomyces. Arch Microbiol 2001; 176: 237–42.

43. Zuber U, Schumann W. CIRCE, ael heat shock element involved in regulation of heat shock operon dnaK of Bacillus subtilis. J Bacteriol 1994; 176: 1359–63.

44. Roberts RC, Toochinda C, Avedissian M, Baldini RL, Gomes SL, Shapiro L. Identification of a Caulobacter crescentus operon encoding hrcA, involved in negatively regulating heat-inducible transcription, and the chaperone gene grpE. J Bacteriol 1996; 178: 1829–41.

45. Narberhaus F. Negative regulation of bacterial heat shock genes. Mol Microbiol 1999; 31: 1–8.

46. Babst M, Hennecke H, Fischer HM. Two different mechanisms are involved in the heat-shock regulation of chaperonin gene expression in Bradyrhizobium japonicum. Mol Microbiol 1996; 19: 827–39.

47. Mogk A, Homuth G, Scholz C, Kim L, Schmid FX, Schumann W. The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of Bacillus subtilis. EMBO J 1997 1; 16: 4579–90.

48. Nocker A, Hausherr T, Balsiger S, Krstulovic NP, Hennecke H, Narberhaus F. A mRNA-based thermosensor controls expression of rhizobial heat shock genes. Nucleic Acids Res 2001; 29: 4800–7.

49. McCarty JS, Rüdiger S, Schönfeld HJ, Schneider-Mergener J, Nakahigashi K, Yura T, Bukau B. Regulatory region C of the E. coli heat shock transcription factor, sigma32, constitutes a DnaK binding site and is conserved among eubacteria. J Mol Biol 1996; 256: 829–37.

50. Yura T, Guisbert E, Poritz M, Lu CZ, Campbell E, Gross CA. Analysis of sigma32 mutants defective in chaperone-mediated feedback control reveals unexpected complexity of the heat shock response. Proc Natl Acad Sci USA 2007; 104: 17638–43.

51. Liberek K, Galitski TP, Zylicz M, Georgopoulos C. The DnaK chaperone modulates the heat shock response of Escherichia coli by binding to the sigma 32 transcription factor. Proc Natl Acad Sci USA 1992; 89: 3516–20.

52. Dufour YS, Imam S, Koo BM, Green HA, Donohue TJ. Convergence of the transcriptional responses to heat shock and singlet oxygen stresses. PLoS Genet 2012; 8: e1002929.

53. Nuss AM, Glaeser J, Berghoff BA, Klug G. Overlapping alternative sigma factor regulons in the response to singlet oxygen in Rhodobacter sphaeroides. J Bacteriol 2010; 192: 2613–23.

54. Nuss AM, Glaeser J, Klug G. RpoH (II) activates oxidative-stress defense systems and is controlled by RpoE in the singlet oxygen-dependent response in Rhodobacter sphaeroides. J Bacteriol 2009; 191: 220–30.

55. Gamer J, Bujard H, Bukau B. Physical interaction between heat shock proteins DnaK, DnaJ, and GrpE and the bacterial heat shock transcription factor sigma 32. Cell 1992; 69: 833–42.

56. Tomoyasu T, Gamer J, Bukau B, Kanemori M, Mori H, Rutman AJ, Oppenheim AB, Yura T, Yamanaka K, Niki H. Escherichia coli FtsH is a membrane-bound, ATP-dependent protease which degrades the heat-shock transcription factor sigma 32. EMBO J 1995; 14: 2551–60.

57. Guisbert E, Rhodius VA, Ahuja N, Witkin E, Gross CA. Hfq modulates the sigmaE-mediated envelope stress response and the sigma32-mediated cytoplasmic stress response in Escherichia coli. J Bacteriol 2007; 189: 1963–73.

58. Vogel J, Luisi BF. Hfq and its constellation of RNA. Nat Rev Microbiol 2011; 9: 578–89.

59. Nagai H, Yuzawa H, Yura T. Interplay of two cis-acting mRNA regions in translational control of sigma 32 synthesis during the heat shock response of Escherichia coli. Proc Natl Acad Sci USA 1991; 88: 10515–9.

60. Morita MT, Tanaka Y, Kodama TS, Kyogoku Y, Yanagi H, Yura T. Translational induction of heat shock transcription factor sigma32: evidence for a built-in RNA thermosensor. Genes Dev 1999; 13: 655–65.

61. Morita M, Kanemori M, Yanagi H, Yura T. Heat-induced synthesis of sigma32 in Escherichia coli: structural and functional dissection of rpoH mRNA secondary structure. J Bacteriol 1999; 181: 401–10.

62. Kortmann J, Sczodrok S, Rinnenthal J, Schwalbe H, Narberhaus F. Translation on demand by a simple RNA-based thermosensor. Nucleic Acids Res 2010; 39: 2855–68.

63. Nocker A, Krstulovic NP, Perret X, Narberhaus F. ROSE elements occur in disparate rhizobia and are functionally interchangeable between species. Arch Microbiol 2001; 176: 44–51.

64. Narberhaus F, Kaser R, Nocker A, Hennecke H. Ael DNA element that controls bacterial heat shock gene expression. Mol Microbiol 1998; 28: 315–23.

65. Balsiger S, Ragaz C, Baron C, Narberhaus F. Replicon-specific regulation of small heat shock genes in Agrobacterium tumefaciens. J Bacteriol 2004; 186: 6824–9.

66. Franck E, Madsen O, van Rheede T, Ricard G, Huynen MA, de Jong WW. Evolutionary diversity of vertebrate small heat shock proteins. J Mol Evol 2004; 59: 792–805.

67. Bellyei S, Szigeti A, Boronkai A, Pozsgai E, Gomori E, Melegh B, Janaky T, Bognar Z, Hocsak E, Sumegi B, Gallyas F. Jr. Inhibition of cell death by ael 16.2 kD heat shock protein predominantly via Hsp90 mediated lipid rafts stabilization and Akt activation pathway. Apoptosis 2007; 12: 97–112.

68. Kourtis N, Nikoletopoulou V, Tavernarakis N. Small heat-shock proteins protect from heat-stroke-associated neurodegeneration. Nature 2012; 490: 213–8.

69. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011; 28: 2731–9.

70. Nei M. Phylogenetic analysis in molecular evolutionary genetics. Annu Rev Genet 1996; 30: 371–403.

71. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39: 783–91.

72. Sarkar NK, Kim YK, Grover A. Rice sHsp genes: genomic organization and expression profiling under stress and development. BMC Genomics 2009; 10: 393.

73. Waters ER. The molecular evolution of the small heat-shock proteins in plants. Genetics 1995; 141: 785–95.

74. Sghaier H, Le Ai TH, Horiike T, Shinozawa T. Molecular chaperones: proposal of a systematic computer-oriented nomenclature and construction of a centralized database. In Silico Biol 2004; 4: 311–22.

75. Siddique M, Port M, Tripp J, Weber C, Zielinski D, Calligaris R, Winkelhaus S, Scharf KD. Tomato heat stress protein Hsp16.1-CIII represents a member of a new class of nucleocytoplasmic small heat stress proteins in plants. Cell Stress Chaperones 2003; 8: 381–94.

76. King AM, MacRae TH. The small heat shock protein p26 aids development of encysting Artemia embryos, prevents spontaneous diapause termination and protects against stress. PLoS One 2012; 7: e43723.

77. Villeneuve TS, Ma X, Sun Y, Oulton MM, Oliver AE, MacRae TH. Inhibition of apoptosis by p26: implications for small heat shock protein function during Artemia development. Cell Stress Chaperones 2006; 11: 71–80.

78. Willsie JK, Clegg JS. Nuclear p26, a small heat shock/alpha-crystallin protein, and its relationship to stress resistance in Artemia franciscana embryos. J Exp Biol 2001; 204 (Pt 13): 2339–50.

79. Graw J. The crystallins: genes, proteins and diseases. Biol Chem 1997; 378: 1331–48.

80. Graw J. Genetics of crystallins: Cataract and beyond. Experimental Eye Research 2009; 88: 173–89.

81. Evgrafov OV, Mersiyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL, Schagina O, Verpoorten N, Van Impe K, Fedotov V, Dadali E, Auer-Grumbach M, Windpassinger C, Wagner K, Mitrovic Z, Hilton-Jones D, Talbot K, Martin JJ, Vasserman N, Tverskaya S, Polyakov A, Liem RK, Gettemans J, Robberecht W, De Jonghe P, Timmerman V. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat Genet 2004; 36: 602–6.

82. Kijima K, Numakura C, Goto T, Takahashi T, Otagiri T, Umetsu K, Hayasaka K. Small heat shock protein 27 mutation in a Japanese patient with distal hereditary motor neuropathy. J Hum Genet 2005; 50: 473–6.

83. Vicart P. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 1998; 20: 92–5.

84. Irobi J. Hot spot residue in small heat shock protein 22 causes distal motor neuropathy. Nat Genet 2004; 36: 597–601.

85. Liang P, MacRae TH. Molecular chaperones and the cytoskeleton. J Cell Sci 1997; 110 (Pt 13): 1431–40.

86. Leicht BG, Biessmann H, Palter KB, Bonner JJ. Small heat shock proteins of Drosophila associate with the cytoskeleton. Proc Natl Acad Sci USA 1986; 83: 90–4.

87. Zhu Y, O'Neill S, Saklatvala J, Tassi L, Mendelsohn ME. Phosphorylated HSP27 associates with the activation-dependent cytoskeleton in human platelets. Blood 1994; 84: 3715–23.

88. Eisenhardt SU, Habersberger J, Oliva K, Lancaster GI, Ayhan M, Woollard KJ, Bannasch H, Rice GE, Peter K. A proteomic analysis of C-reactive protein stimulated THP-1 monocytes. Proteome Sci 2011; 9: 1.

89. Tashiro M, Schafer C, Yao H, Ernst SA, Williams JA. Arginine induced acute pancreatitis alters the actin cytoskeleton and increases heat shock protein expression in rat pancreatic acinar cells. Gut 2001; 49: 241–50.

90. Smoyer WE, Ransom R, Harris RC, Welsh MJ, Lutsch G, Benndorf R. Ischemic acute renal failure induces differential expression of small heat shock proteins. J Am Soc Nephrol 2000; 11: 211–21.

91. Acarin L, Paris J, Gonzalez B, Castellano B. Glial expression of small heat shock proteins following an excitotoxic lesion in the immature rat brain. Glia 2002; 38: 1–14.

92. Kurapati R, Passananti HB, Rose MR, Tower J. Increased hsp22 RNA levels in Drosophila lines genetically selected for increased longevity. J Gerontol A Biol Sci Med Sci 2000; 55: B552–9.

93. Walker GA, White TM, McColl G, Jenkins NL, Babich S, Candido EP, Johnson TE, Lithgow GJ. Heat shock protein accumulation is upregulated in a long-lived mutant of Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci 2001; 56: B281–7.

94. Calderwood SK, Murshid A, Prince T. The shock of aging: molecular chaperones and the heat shock response in longevity and aging–a mini-review. Gerontology 2009; 55: 550–8.

95. Torok Z, Goloubinoff P, Horvath I, Tsvetkova NM, Glatz A, Balogh G, Varvasovszki V, Los DA, Vierling E, Crowe JH, Vigh L. Synechocystis HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. Proc Natl Acad Sci USA 2001; 98: 3098–103.

96. Tsvetkova NM, Horváth I, Török Z, Wolkers WF, Balogi Z, Shigapova N, Crowe LM, Tablin F, Vierling E, Crowe JH, Vigh L. Small heat-shock proteins regulate membrane lipid polymorphism. Proc Natl Acad Sci USA 2002; 99: 13504–9.

97. Schuster G, Even D, Kloppstech K, Ohad I. Evidence for protection by heat-shock proteins against photoinhibition during heat-shock. EMBO J 1988; 7: 1–6.

98. Kim DH, Xu ZY, Na YJ, Yoo YJ, Lee J, Sohn EJ, Hwang I. Small heat shock protein Hsp17.8 functions as an AKR2A cofactor in the targeting of chloroplast outer membrane proteins in Arabidopsis. Plant Physiol 2011; 157: 132–46.

99. Harndahl U, Hall RB, Osteryoung KW, Vierling E, Bornman JF, Sundby C. The chloroplast small heat shock protein undergoes oxidation-dependent conformational changes and protect plants from oxidative stress. Cell Stress Chaperones 1999; 4: 129–38.

100. Eisenberg-Domovich Y, Kloppstech K, Ohad I. Reversible membrane association of heat-shock protein 22 in Chlamydomonas reinhardtii during heat shock and recovery. Eur J Biochem 1994; 222: 1041–6.

101. Glaczinski H, Kloppstech K. Temperature-dependent binding to the thylakoid membranes of nuclear-coded chloroplast heat-shock proteins. Eur J Biochem 1988 2; 173: 579–83.

102. Balogi Z, Török Z, Balogh G, Jósvay K, Shigapova N, Vierling E, Vígh L, Horváth I. 'Heat shock lipid' in cyanobacteria during heat/light-acclimation. Arch Biochem Biophys 2005; 436: 346–54.

103. Basha E, Lee GJ, Breci LA, Hausrath AC, Buan NR, Giese KC, Vierling E. The identity of proteins associated with a small heat shock protein during heat stress in vivo indicates that these chaperones protect a wide range of cellular functions. J Biol Chem 2004 27; 279: 7566–75.

104. Wehmeyer N, Vierling E. The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance. Plant Physiol 2000; 122: 1099–108.

105. Allen SP, Polazzi JO, Gierse JK, Easton AM. Twoel heat shock genes encoding proteins produced in response to heterologous protein expression in Escherichia coli. J Bacteriol 1992; 174: 6938–47.

106. Kitagawa M, Matsumura Y, Tsuchido T. Small heat shock proteins, IbpA and IbpB, are involved in resistances to heat and superoxide stresses in Escherichia coli. FEMS Microbiol Lett 2000; 184: 165–71.

107. Kuczynska-Wisnik D, Kedzierska S, Matuszewska E, Lund P, Taylor A, Lipinska B, Laskowska E. The Escherichia coli small heat-shock proteins IbpA and IbpB prevent the aggregation of endogenous proteins denatured in vivo during extreme heat shock. Microbiology 2002; 148 (Pt 6): 1757–65.

108. Henriques AO, Beall BW, Moran CP Jr. CotM of Bacillus subtilis, a member of the α-crystallin family of stress proteins, is induced during development and participates in spore outer coat formation. J Bacteriol 1997; 179: 1887–97.

109. Reischl S, Thake S, Homuth G, Schumann W. Transcriptional analysis of three Bacillus subtilis genes coding for proteins with the α-crystallin domain characteristic of small heat shock proteins. FEMS Microbiol Lett 2001; 194: 99–103.

110. Tsai YL, Wang MH, Gao C, Klüsener S, Baron C, Narberhaus F, Lai EM. Small heat-shock protein HspL is induced by VirB protein(s) and promotes VirB/D4-mediated DNA transfer in Agrobacterium tumefaciens. Microbiology 2009; 155 (Pt 10): 3270–80.

111. Tsai YL, Chiang YR, Narberhaus F, Baron C, Lai EM. The small heat-shock protein HspL is a VirB8 chaperone promoting type IV secretion-mediated DNA transfer. J Biol Chem 2010; 285: 19757–66.

112. Tsai YL, Chiang YR, Wu CF, Narberhaus F, Lai EM. One out of four: HspL but no other small heat shock protein of Agrobacterium tumefaciens acts as efficient virulence-promoting VirB8 chaperone. PLoS One 2012; 7: e49685.

113. Hilario E, tin FJ, Bertolini MC, Fan L. Crystal structures of Xanthomonas small heat shock protein provide a structural basis for an active molecular chaperone oligomer. J Mol Biol 2011; 408: 74–86.

114. Augusteyn RC. alpha-crystallin: a review of its structure and function. Clin Exp Optom 2004; 87: 356–66.

115. Van Montfort R, Slingsby C, Vierling E. Structure and function of the small heat shock protein/α-crystallin family of molecular chaperones. Adv Protein Chem 2001; 59: 105–56.

116. Chang Z, Primm TP, Jakana J, Lee IH, Serysheva I, Chiu W, Gilbert HF, Quiocho FA. Mycobacterium tuberculosis 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation. J Biol Chem 1996; 271: 7218–23.

117. Wistow G. Possible tetramer-based quaternary structure for α-crystallins and small heat shock proteins. Exp Eye Res 1993; 56: 729–32.

118. van Montfort RL, Basha E, Friedrich KL, Slingsby C, Vierling E. Crystal structure and assembly of a eukaryotic small heat shock protein. Nat Struct Biol 2001; 8: 1025–30.

119. Basha E, O'Neill H, Vierling E. Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. Trends Biochem Sci 2011; 37: 106–17.

120. Hesterkamp T, Bukau B. Role of the DnaK and HscA homologs of Hsp70 chaperones in protein folding in E. coli. EMBO J 1998; 17: 4818–28.

121. Levy EJ, McCarty J, Bukau B, Chirico WJ. Conserved ATPase and luciferase refolding activities between bacteria and yeast Hsp70 chaperones and modulators. S Lett 1995 24; 368: 435–40.

122. Lin CH, Lee CN, Lin JW, Tsai WJ, Wang SW, Weng SF, Tseng YH. Characterization of Xanthomonas campestris pv. campestris heat shock protein A (HspA), which possesses an intrinsic ability to reactivate inactivated proteins. Appl Microbiol Biotechnol 2010; 88: 699–709.

123. Poulain P, Gelly JC, Flatters D. Detection and architecture of small heat shock protein monomers. PLoS One 2010; 5: e9990.

124. Basha E, Friedrich KL, Vierling E. The N-terminal arm of small heat shock proteins is important for both chaperone activity and substrate specificity. J Biol Chem 2006; 281: 39943–52.

125. Jaya N, Garcia V, Vierling E. Substrate binding site flexibility of the small heat shock protein molecular chaperones. Proc Natl Acad Sci USA 2009; 106: 15604–9.

126. Basha E, Jones C, Blackwell AE, Cheng G, Waters ER, Samsel KA, Siddique M, Pett V, Wysocki V, Vierling E. An unusual dimeric small heat shock protein provides insight into the mechanism of this class of chaperones. J Mol Biol 2013; 425: 1683–96.

127. Giese KC, Vierling E. Mutants in a small heat shock protein that affect the oligomeric state. Analysis and allele-specific suppression. J Biol Chem 2004; 279: 32674–83.

128. Giese KC, Vierling, E. Changes in oligomerization are essential for the chaperone activity of a small heat shock protein in vivo and in vitro. J Biol Chem 2002; 277: 46310–8.

129. Basha E, Jones C, Wysocki V, Vierling E. Mechanistic differences between two conserved classes of small heat shock proteins found in the plant cytosol. J Biol Chem 2010; 285: 11489–97.

130. Giese KC, Basha E, Catague BY, Vierling E. Evidence for an essential function of the N terminus of a small heat shock protein in vivo, independent of in vitro chaperone activity. Proc Natl Acad Sci USA 2005; 102: 18896–901.

131. de Miguel N, Braun N, Bepperling A, Kriehuber T, Kastenmüller A, Buchner J, Angel SO, Haslbeck M. Structural and functional diversity in the family of small heat shock proteins from the parasite Toxoplasma gondii. BBA-Mol Cell Res 2009; 1793: 1738–48.

132. Rinehart JP, Li A, Yocum GD, Robich RM, Hayward SA, Denlinger DL. Up-regulation of heat shock proteins is essential for cold

survival during insect diapause. Proc Natl Acad Sci USA 2007; 104: 11130–7.

133. Groenen PJ, Merck KB, de Jong WW, Bloemendal H. Structure and modifications of theior chaperone α-crystallin. From lens transparency to molecular pathology. Eur J Biochem 1994; 225: 1–19.

134. Karmakar S, Das KP. Identification of histidine residues involved in Zn (2+) binding to αA- and αB-crystallin by chemical modification and MALDI TOF mass spectrometry. Protein J 2012; 31: 623–40.

135. Biswas A, Das KP. Zn2+ enhances the molecular chaperone function and stability of α-crystallin. Biochemistry 2008; 47: 804–16.

136. Kantorow M, Piatigorsky J. Alpha-crystallin/small heat shock protein has autokinase activity. Proc Natl Acad Sci USA 1994 12; 91: 3112–6.

137. Sredy J, Spector A. The phosphorylation of bovine and human lens polypeptides. Exp Eye Res 1984; 39: 653–64.

138. Spector A, Chiesa R, Sredy J, Garner W. cAMP-dependent phosphorylation of bovine lens alpha-crystallin. Proc Natl Acad Sci USA 1985; 82: 4712–6.

139. Benndorf R, Hayess K, Ryazantsev S, Wieske M, Behlke J, Lutsch G. Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. J Biol Chem 1994; 269: 20780–4.

140. Gustavsson N, Harndahl U, Emanuelsson A, Roepstorff P, Sundby C. Methionine sulfoxidation of the chloroplast small heat shock protein and conformational changes in the oligomer. Protein Sci 1999; 8: 2506–12.

141. Chen Q, Vierling E. Analysis of conserved domains identifies a unique structural feature of a chloroplast heat shock protein. Mol Gen Genet 1991; 226: 425–31.

142. Harndahl U, Kokke BP, Gustavsson N, Linse S, Berggren K, Tjerneld F, Boelens WC, Sundby C. The chaperone-like activity of a small heat shock protein is lost after sulfoxidation of conserved methionines in a surface-exposed amphipathic α-helix. Biochim Biophys Acta 2001 9; 1545: 227–37.

143. Suzuki TC, Krawitz DC, Vierling E. The chloroplast small heat-shock protein oligomer is not phosphorylated and does not dissociate during heat stress in vivo. Plant Physiol 1998; 116: 1151–61.

144. Gustavsson N, Kokke BP, Anzelius B, Boelens WC, Sundby C. Substitution of conserved methionines by leucines in chloroplast small heat shock protein results in loss of redox-response but retained chaperone-like activity. Protein Sci 2001; 10: 1785–93.

145. Nover L, Scharf KD, Neumann D. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol Cell Biol 1989; 9: 1298–308.

146. Kirschner M, Winkelhaus S, Thierfelder JM, Nover L. Transient expression and heat-stress-induced co-aggregation of endogenous and heterologous small heat-stress proteins in tobacco protoplasts. Plant J 2000; 24: 397–411.

147. Smykal P, Hrdy I, Pechan PM. High-molecular-mass complexes formed in vivo contain smHSPs and HSP70 and display chaperone-like activity. Eur J Biochem 2000; 267: 2195–207.

148. Weber C, Nover L, Fauth M. Plant stress granules and mRNA processing bodies are distinct from heat stress granules. Plant J 2008; 56: 517–30.

149. Stuger R, Ranostaj S, Materna T, Forreiter C. Messenger RNA-binding properties of nonpolysomal ribonucleoproteins from heat-stressed tomato cells. Plant Physiol 1999; 120: 23–32.

150. Maham A, Tang Z, Wu H, Wang J, Lin Y. Protein-based nanomedicine platforms for drug delivery. Small 2009; 5: 1706–21.

151. Sao K, Murata M, Umezaki K, Fujisaki Y, Mori T, Niidome T, Katayama Y, Hashizume M. Molecular design of protein-based nanocapsules for stimulus-responsive characteristics. Bioorganic Medicinal Chemistry 2009; 17: 85–93.

Benjamin Eisenhardt received his diploma in Biology 2009 from the Justus Liebig University Gießen. He obtained his PhD in Plant Physiology (2009–2012), studying the structure and function of cytosolic plant sHSPs. In fall of the same year he accepted a post-doctoral position at the Institute of Micro and Molecular Biology in Gießen in the group of Gabriele Klug. Currently he is interested in the heat stress response of microorganisms.