Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

The function of small heat-shock proteins and their implication in proteostasis

Annika Strauch[1] **and Martin Haslbeck**[1]*

[1]Department Chemie, Technische Universität München, Lichtenbergstrasse 4, 85 748 Garching, Germany

***Correspondence:** Martin Haslbeck (email martin.haslbeck@tum.de).

All organisms rely on a conserved cellular machinery supporting and controlling the life cycle of proteins: the proteostasis network. Within this network, the main players that determine the fate of proteins are molecular chaperones, the ubiquitin–proteasome and the lysosome–autophagy systems. sHsps (small heat-shock proteins) represent one family of molecular chaperones found in all domains of life. They prevent irreversible aggregation of unfolded proteins and maintain proteostasis by stabilizing promiscuously a variety of non-native proteins in an ATP-independent manner. In the cellular chaperone network, sHsps act as the first line of defence and keep their substrates in a folding-competent state until they are refolded by downstream ATP-dependent chaperone systems. Besides this interaction with unfolding substrates upon stress, sHsps show a different mode of binding for specific clients which are also recognized under physiological conditions. In vertebrates, sHsps are especially needed to maintain the refractive index of the eye lens. Additionally, sHsps are linked to a broad variety of diseases such as myopathies and neuropathies. The most striking feature of sHsps is their ability to form dynamic ensembles of higher oligomers. The activity of sHsps is regulated by changes in the composition of the ensembles.

Introduction

sHsps (small heat-shock proteins) were among the first proteins detected as part of the cellular stress response, a network of proteins which evolved to protect cells against diverse harmful conditions such as heat, cold or oxidative stress [1,2]. sHsps are the most omnipresent, but least conserved, family of molecular chaperones [3,4]. sHsps promiscuously stabilize other proteins upon severe stress and seem to be involved in several regulatory processes throughout the life cycle of cells [5,6]. The best-studied members of the sHsp family are the two α-crystallins, αA-crystallin (HspB4) and αB-crystallin (HspB5), which share 57% amino acid sequence identity. Together with β- and γ-crystallins, they account for over 90% of protein in the vertebrate eye lens [7,8]. In the lens, both α-crystallins protect proteins from irreversible aggregation and maintain lens transparency by reducing the formation of light-scattering particles as well as by balancing the interactions between lens crystallins [6–10]. The link of αB-crystallin and other sHsps to a variety of human diseases, along with the observation that sHsps are expressed during stress and specific stages of the development of many higher eukaryotes [5,11–14], highlights further the importance of this family of molecular chaperones. Additionally, enhanced expression for several sHsp family members in diverse organisms was observed upon aging, e.g. for human αB-crystallin (HSPB5) [15] and Hsp22 (heat-shock protein 22) of *Drosophila melanogaster* [16]. In correlation, the depletion of several sHsps in diverse model organisms such as *D. melanogaster* and *Caenorhabditis elegans* leads to a decrease in lifespan, whereas overexpression of the respective sHsps increases the lifespan of the organisms [16–18]. Accordingly, a higher level of sHsps generally seems to have a stabilizing effect on the proteostasis system and to enhance longevity [18,19].

Received: 14 January 2016
Revised: 13 April 2016
Accepted: 15 April 2016

Version of Record published: 15 October 2016

PORTLAND PRESS

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

Structure of sHsps

The primary structure of sHsps can be dissected into a conserved ACD (α-crystallin domain) (or Hsp20 domain, Pfam protein family PF00011) flanked by a non-conserved NTS (N-terminal sequence) of variable length, and a moderately conserved short CTS (C-terminal sequence) [3,20] (Figure 1a). The ACD, the evolutionarily conserved hallmark of the sHsp family, consists of ∼90 amino acids and adopts a compact β-sheet sandwich structure composed of two antiparallel layers of three and four β-strands connected by a short interdomain loop [21–23] (Figure 1a). Isolated ACDs commonly form dimers, but two different types of dimer interface have been described depending on the origin of the respective sHsp [4]. The first type is represented by "β7-interface' dimers where the dimer interface is formed by the interaction of the anti-parallel $\beta 6 + 7$ strands from the two protomers. This type of dimer is found in all metazoan sHsps studied so far (Figure 1) [17,24,25]. The second type of dimer are so-called 'β6-swapped" dimers where dimerization occurs via reciprocal strand swapping of the β6 strands into the β-sandwich of the neighbouring monomer. This type of dimer is common in plant, yeast, archaeal and bacterial sHsps [21,23,26,27].

A striking feature of most sHsps, which is linked to their function, is their ability to assemble into oligomers. The majority of sHsps are found as large, often polydisperse, ensembles typically ranging from 12 to 32 or even more subunits [4,22], although some dimeric sHsps, e.g. Hsp18.5 from *Arabidopsis thaliana* [28] and Hsp17.7 from *Deinococcus radiodurans* [26], have now been described as well. The oligomeric sHsps are commonly assembled from the dimers described above, with a hierarchal arrangement mediated by different sequence parts of the proteins [22,29]. Commonly, the dimers formed by the ACD represent the first level of hierarchy [22]. The second level of hierarchy is created by the assembly of, e.g., two or three dimers into a tetrameric or hexameric substructures via binding of the C-terminal I-X-I/V motif of one protomer into the hydrophobic groove formed by the β4- and β8-strands of the neighbouring monomer [17,29]. The third level of hierarchy is mediated via interactions of the NTS which are necessary for the association with higher oligomers. In line with this association mode, deletion of the NTS or parts of it commonly leads to smaller oligomers [4]. Interestingly, according to the few resolved or modelled structures of sHsp oligomers, the hierarchal oligomerization principle of dimers assembling through CTS and NTS contacts seems to be conserved among sHsps and may modulate the total number of subunits. The ability to populate a range of oligomeric states at equilibrium represents another key feature of sHsps [29–32]. The oligomers constantly exchange subunits and are thus polydisperse and dynamic ensembles [4,33,34]. The degree of structural plasticity and heterogeneity appears, however, to be variable for different members of the sHsp family [31]. Furthermore, this ability to exist in a balance between different oligomer populations is also closely correlated with the regulation of the chaperone activity of sHsps [31,32].

Chaperone activity of sHsps

sHsps are ATP-independent molecular chaperones, which has been shown for several sHsps from different species [9,35,36]. Nevertheless, as explained in more detail in the next paragraph, sHsps, in most cases, are activated by different types of triggers such as phosphorylation or temperature to enable efficient recognition of unfolding proteins. As exemplified for human Hsp27 in Figure 2 (substrate pathway), sHsps bind a variety of early unfolding intermediates of aggregation-prone proteins under stress conditions and prevent them from irreversible aggregation [9,36–38]. As explained in more detail below, sHsps seem to be able to recognize and bind other proteins by different modes of interaction. Proteins which unfold upon stress are often bound tightly and with high binding capacity of ratios of up to one non-native substrate protein per sHsp dimer [37,39] and stable sHsp–substrate complexes are formed commonly [30,39]. In these sHsp–substrate complexes, the substrates are stored in a folding-competent state until the cell or organisms returns to non-stress conditions [37,40,41]. The release and refolding of the substrates then occurs with the help of ATP-dependent chaperones such as the Hsp70–Hsp40 system in plants and mammalian cells [40,42,43] or the DnaK–DnaJ–GrpE system in bacteria [26,44,45]. Under severe stress conditions, when there is an excess of substrate proteins present, sHsps are incorporated into aggregates together with the substrate proteins. This renders the aggregates easier to solubilize for the cell, but at least in *Escherichia coli* and *Saccharomyces cerevisiae* requires additional members of the Hsp100 family for refolding [44–46]. However, besides this tight binding and complexing of substrate proteins, many sHsps are able to transiently bind substrate proteins [26,39,47,48]. The substrates than are released spontaneously from the sHsps and refold on their own or with the help of ATP-dependent chaperones [26]. sHsps usually fulfil their task as molecular chaperones by stabilizing early unfolding intermediates of aggregation-prone proteins [4]. This implies that sHsps must be present during the time in which the substrate

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

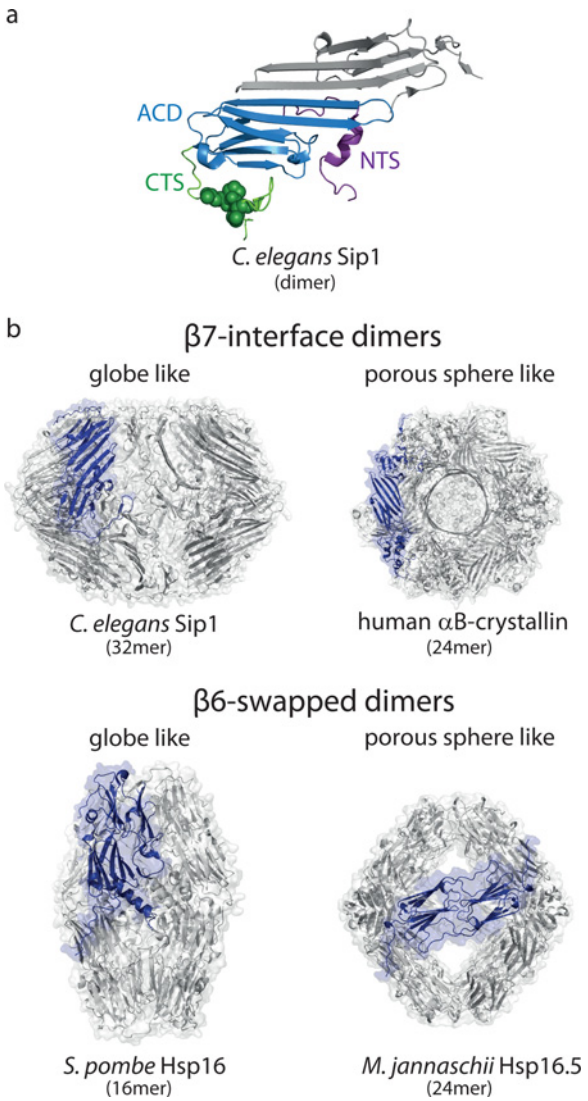

Figure 1. Structure of sHsps

(**a**) Structure of a β7-interface dimer of *C. elegans* Sip1, extracted from the oligomer structure (X-ray crystallography, PDB code 4YDZ) [17]. One monomer is coloured and one monomer is grey. Domain organization of sHsps is indicated by different colours. NTS, violet; ACD, blue; CTS, green, with the amino acids of the conserved I-X-I/V motif highlighted. (**b**) Comparison of four available oligomeric 3D structures of atomic resolution, *C. elegans* Sip1 (PDB code 4YDZ) [17], human αB-crystallin (PDB code 2WJ7) [29], *Schizosaccharomyces pombe* Hsp16 (PDB code 3W1Z) [27] and *Methanocaldococcus jannaschii* Hsp16.5 (PDB code 1HSH) [23]. Two oligomers composed of β7-interface dimers as well as two oligomers composed of β6-swapped dimers are shown. In every oligomer, one dimer is marked in blue to highlight the variable interconnections of the dimers in the respective structure. As indicated by the comparison of two 'globe-like' (one rotational axis; dimers oriented in spokes) oligomers and two 'porous sphere-like' (two rotational axis; tetrahedral symmetry) oligomers, the type of dimer interface does not define the oligomer composition.

unfolds and that they cannot rescue already aggregated substrates. The stability of the interaction seems to depend on the nature of the substrate as well as on the type of the unfolding intermediate [4]. Additionally, the specific properties of the sHsp as well determine the stability of the interaction, e.g. the dimeric sHsp Hsp17.7 from *D. radiodurans* seems to always interact only transiently with its substrate and is unable to from stable sHsp–substrate complexes [26].

Taken together within the protein homoeostasis network of the cell, sHsps are commonly viewed to act as a buffer system to bind unfolding proteins upon stress, protecting these unfolding proteins from irreversible aggregation

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

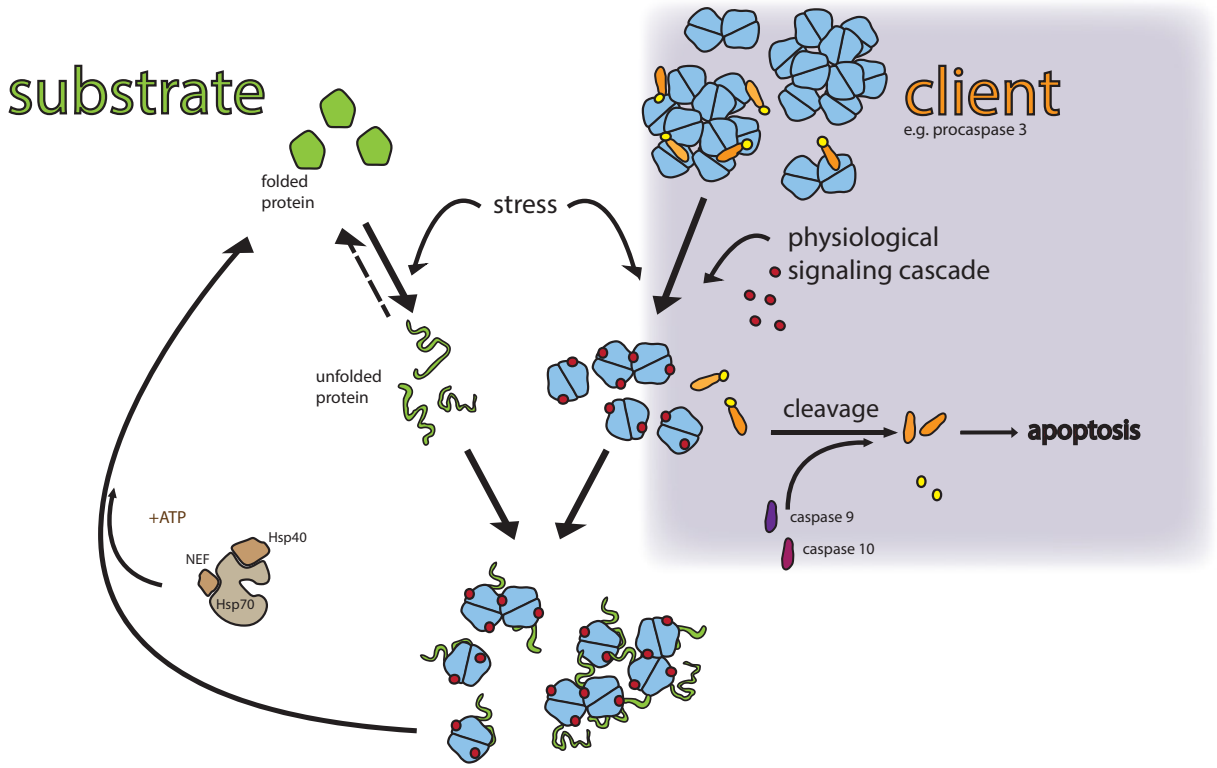

Figure 2. Simplified scheme of the chaperone function of mammalian Hsp27 (HspB1) comparing the interaction with substrates and clients

Substrate pathway (left-hand side). Under stress conditions when substrate proteins (green) are destabilized and begin to unfold, Hsp27 (blue) binds these partially unfolded proteins in an energy-independent manner and keeps them in a folding-competent state [40,69]. The physiological ensemble of Hsp27 oligomers (blue) is activated, e.g. by the temperature- and/or stress-induced phosphorylation (red circles), resulting in dissociation into smaller species [69]. The substrate is stabilized by this activated ensemble of Hsp27 and is captured in stable Hsp27–substrate complexes of still enigmatic organization. This ensemble can also include aggregates with incorporated Hsp27. Bound substrates are subsequently refolded by the ATP-dependent Hsp70–Hsp40 chaperone system [40]. Client pathway (right-hand side, coloured background). Under physiological conditions, at least part of the physiological Hsp27 molecules may also bind clients such as procaspase 3 (orange and yellow, where yellow indicates the prodomain). Upon stress-induced phosphorylation as well as upon phosphorylation via a physiological signalling cascade (highlighted by the coloured background) procaspase is released [59]. In the case of the client procaspase 3, cleavage by other caspases (e.g. caspase 9 or 10) leads to active caspase 3 and induction of apoptosis (note that the involvement of Hsp27 in the apoptosis cascade is much more complex [6,59] and only procaspase as a typical client protein is indicated for reasons of clarity).

(Figure 2; substrate pathway). This basic and highly promiscuous substrate-stabilizing mechanism as well as energy-dependent release of substrates from sHsps by other chaperone systems is conserved from bacteria to humans and allows the cell/organism to separate the prevention of aggregation from ATP-dependent refolding steps [4]. The interaction with unfolding substrates upon stress is common for all sHsps. Besides that, especially human sHps, show a different mode of binding for specific clients which are recognized also at physiological conditions which is explained further below and is exemplified for the interaction of procaspase 3 and Hsp27 in Figure 2 (client pathway).

Regulation of the chaperone activity of sHsps

There is compelling evidence that the activity of sHsps is regulated by external triggers which induce transition into a state of increased chaperone activity [32] (Figure 3). Commonly the activation is achieved by shifting the equilibrium

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

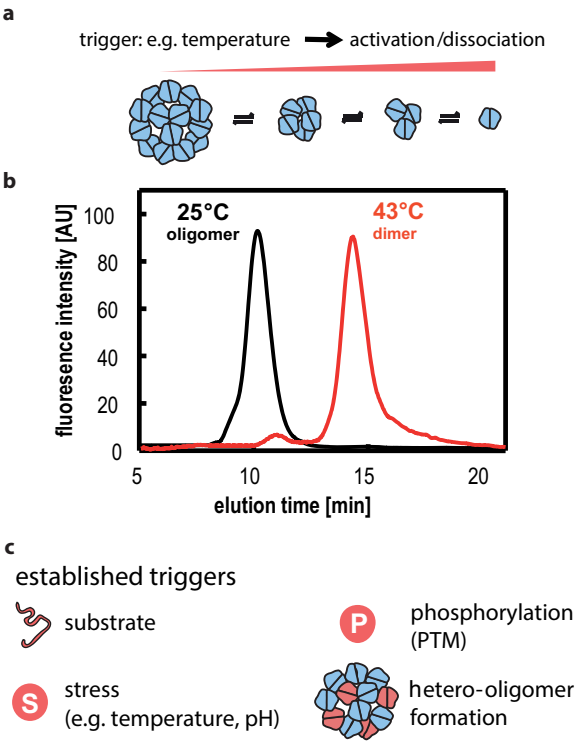

Figure 3. Regulation of sHsp activity

(**a**) sHsps populate at equilibrium a wide variety of interconverting oligomers with different substrate affinities. The transition into a state of increased chaperone activity occurs through remodelling of the ensemble composition in favour of species with enhanced substrate-binding affinity. The association/dissociation equilibrium can be modulated by different triggers such as elevated temperatures. (**b**) Analytical size-exclusion chromatography of yeast Hsp26 at physiological (25 °C; black curve) and stress (43 °C, red curve) temperatures shows that heat stress leads to dissociation. The observed dissociation correlates with an increase in chaperone activity [39]. (**c**) Besides temperature, other triggers such as the presence of substrate proteins, phosphorylation (or more generally PTMs) and hetero-oligomerization have been shown to induce activation.

of the ensemble of sHsp oligomers to a higher content of smaller species, often including dimers [4]. According to our current knowledge, at least the following triggers can affect the association/dissociation equilibria of sHsps and lead to activation [32]: (i) the presence of unfolded or partially folded substrates; (ii) the stress situation (e.g. changes in the environmental temperature, as exemplified in Figure 3b, and/or pH); (iii) PTMs (post-translational modifications), in particular phosphorylation; and (iv) hetero-oligomerization (Figure 3).

The described mechanism of shifting the ensemble of oligomers to a higher content of smaller species also implies that larger oligomers represent 'inactive' storage forms which expose through dissociation their substrate-binding sites and that smaller assemblies with modulated properties correspond to 'active' species. A paramount example for this mechanism is the activation of human αB-crystallin, which dissociates upon phosphorylation concomitant with an increase in chaperone activity [43]. A recently published molecular dynamics study using αB-crystallin as a proof-of-concept case study enabled the evaluation of different PTMs and their effects on the dynamic behaviour of the αB-crystallin oligomer [49]. The simulated phosphorylation patters of αB-crystallin again suggest the release of hexamers from the αB-crystallin oligomer when Ser^{49} and Ser^{59} are phosphorylated with the highest influence resulting from the phosphorylation of Ser^{59}. Besides several other sHsp examples, which hint in the same direction, further support for the enhanced binding affinity of smaller sHsp assemblies arises also from the observation that some sHsps exist solely as dimers which show also chaperone activity, e.g. Hsp18.5 from *A. thaliana* [28] and Hsp17.7 from *D. radiodurans* [26]. Nevertheless, it has been shown that at least Hsp18.5 is able to form large sHsps–substrate oligomers upon substrate binding [28]. The NTS, which is only fully resolved in the crystal structure of oligomeric full-length Hsp14.1 from *Sulfolobus solfataricus* where it adopts a long and straight α-helix conformation, seems to

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

be the key motif in modulating the chaperone activity. The NTS seems to act as a molecular switch facilitating the substrate recognition of Hsp14.1 by exposure of a hydrophobic surface upon changes in its conformation [50].

Substrate recognition of sHsps

Besides the wide spectrum of substrates of sHsps *in vivo* and *in vitro*, the observation that some organisms contain multiple cellular compartment-specific sHsps, which show tissue- or development-specific expression pattern, indicates a certain degree of substrate specificity. For example, p26, a sHsp from the brine shrimp *Artemia*, is only found during diapause [51,52] and Sip1 from *C. elegans* represents a sHsp which is expressed exclusively in oocytes and embryos [17]. The activity of Sip1 is optimal under the acidic conditions present in the nematode eggs [17]. Analysis of the Sip1 interactome showed that it has a specific substrate spectrum, including many proteins that are essential for embryonic development [17]. This specificity for embryonic proteins is highlighted further by studies showing that the substrate spectra of individual sHsps of one organism show substantial overlaps but are not identical [17,53]. The currently emerging picture indicates a major role of the NTS in substrate binding [4,43,50], but also the involvement of further regions of the ACD and CTS [54,55]. Thus several binding sites in sHsps seem to act together and presumably different constellations of sites facilitate the binding of different substrates. In accordance, recent studies indicate that human αB-crystallin utilizes different binding sites to capture amorphously aggregating and amyloid-forming substrates [55].

The regulatory role of sHsps in proteostasis

Especially human sHsps have been described to interact with other proteins already at physiological conditions without severe stress [5,6]. These observations indicate that many interactions of sHsps with other proteins might not be promiscuous 'substrate' interactions, but in a perspective view seem to be more specific 'client' interactions (Figure 2, client pathway). A 'substrate' in this respect is any type of non-native protein, which is primarily recognized particularly under (severe) stress conditions when the cell tries to stabilize its proteome. A 'client' is a protein which is also bound under physiological conditions where binding and release of the client is used by the cell to regulate and/or monitor cellular processes [5,56]. It remains to be seen whether for these different modes of interactions more precise and specific terms can be found in the future, to avoid confusion as the terms substrate and client are currently often used interchangeably in the field of stress response and proteostasis.

The presence of several different substrate binding sites in sHsps would be perfectly suited to adjust the interaction of substrates or clients depending on the combinatory engagement and availability of the different sites. A prominent example for a seemingly regulatory role of sHsps in proteostasis is the client interaction with procaspase 3 which is bound and stabilized by Hsp27 (HSPB1) and αB-crystallin (HSPB5), independently of general stress conditions in the human cell [57–59]. For activation of caspase 3, an essential activator of apoptosis, the N-terminal prodomain has to be cleaved off (Figure 2). The sHsps act as negative regulators of caspase 3 activity by binding to the prodomain and inhibiting its cleavage [57,58]. The binding of procaspase 3 by Hsp27 is controlled by the phosphorylation state of Hsp27 (Figure 2). Phosphorylation triggers the release of procaspase 3 followed by its cleavage and induction of apoptosis [60]. It should also be noted that Hsp27 binds to several additional proteins of the apoptotic cascade [6,58,59]. Hence sHsps seem to represent a major regulatory tool to titrate the amount of free specific clients helping the cell in fine-tuning of proteostasis and specific cellular processes such as apoptosis [58]. Further examples for such regulatory implications of sHsps would be diapause prolongation in *Artemia* [51], as mentioned above, and modulation of the intermediate filament network [61–63]. In this context, it is also very likely that sHsps serve to monitor cell fate, since, whenever the sHsps are needed for severe stress defence, the availability of sHsps for specific client binding might decrease, resulting in a pronounced release of the former physiological stress-independently bound sHsps clients. The enhanced expression of sHsps upon stress [4] might be able to compete against client release until the stress situation becomes too severe.

Conclusion

sHsps are virtually ubiquitous molecular chaperones sharing a conserved structural domain, the ACD. Divergent NTSs and CTSs dictate differences in their dynamic oligomeric structure and are most likely to be the basis for

specificities in substrate interactions and roles within the cell. We remain far from understanding the details of substrate recognition by sHsps or the potential mechanistic differences between diverse family members. The requirement of sHsps for stress tolerance and their linkage to several human diseases all indicate the importance of further studies of these molecular chaperones. In this context, it is of special interest that a recently found small molecule interactor of human sHsps, lanosterol, which is synthesized by lanosterol synthase, is able to stabilize the eye lens α-crystallins [64]. The application of lanosterol and other sterol-based compounds was even suitable to decrease cataracts [64,65]. Besides such strategies stabilizing sHsps, a further potential strategy in disease treatment is their depletion by specific siRNAs [66]. Such a strategy seems to be especially suited for cancer therapy [19,67]. Cancer cells are usually in need of high amounts of functional chaperones (e.g. Hsp27) and their depletion might not affect normal cells, but seems to sensitize tumour cells [66,68]. Thus sHsps seem to be promising therapeutic targets. However, to be able to develop dedicated drugs to target sHsps, an in-depth analysis of their function in the context of the respective diseases and especially a detailed understanding of the interaction sites and the different modes of interaction of sHsps with substrates and/or clients is necessary. Currently we are far from understanding why seemingly specific drugs inhibit the function of some sHsps and what side reactions within the cell might be induced in parallel. sHsps, together with their clients and other molecular chaperones, seem to represent a very tightly balanced system, and any change in the balance might severely influence the fate of a cell.

Summary

- sHsps represent a first line of defence in proteostasis.
- sHsps form dynamic ensembles of different hierarchically organized oligomers.
- sHsps can be activated by several triggers such as substrate, stress situation (temperature, pH), post-translational modification (phosphorylation) or hetero-oligomerization.
- sHps seem to show different modes of binding which might be the basis for their involvement in regulatory cascades (e.g. apoptosis) within the cell.

Abbreviations

ACD, α-crystallin domain; CTS, C-terminal sequence; Hsp, heat-shock protein; NTS, N-terminal sequence; PTM, post-translational modification; sHsp, small heat-shock protein.

Acknowledgements

We apologize to our many colleagues whose work we were unable to cite due to space constraints. We thank J. Buchner for discussion and a critical reading of the article.

Funding

We thank the Deutsche Forschungsgemeinschaft [grant number SFB 1035] for financial support

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Tissieres, A., Mitchell, H.K. and Tracy, U.M. (1974) Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. J. Mol. Biol. **84**, 389–398 CrossRef PubMed
2 Richter, K., Haslbeck, M. and Buchner, J. (2010) The heat shock response: life on the verge of death. Mol. Cell **40**, 253–266 CrossRef PubMed
3 Kriehuber, T., Rattei, T., Weinmaier, T., Bepperling, A., Haslbeck, M. and Buchner, J. (2010) Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J **24**, 3633–3642 CrossRef PubMed
4 Haslbeck, M. and Vierling, E. (2015) A first line of stress defense: small heat shock proteins and their function in protein homeostasis. J. Mol. Biol. **427**, 1537–1548 CrossRef PubMed
5 Mymrikov, E.V. and Haslbeck, M. (2015) Medical implications of understanding the functions of human small heat shock proteins. Expert Rev. Proteomics **12**, 295–308 CrossRef PubMed

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

6 Bakthisaran, R., Tangirala, R. and Rao, C.M. (2014) Small heat shock proteins: role in cellular functions and pathology. Biochim. Biophys. Acta **1854**, 291–319 CrossRef PubMed
7 Haslbeck, M., Peschek, J., Buchner, J. and Weinkauf, S. (2015) Structure and function of α-crystallins: traversing from *in vitro* to *in vivo*. Biochim. Biophys. Acta **1860**, 149–166 CrossRef PubMed
8 Horwitz, J. (2003) α-Crystallin. Exp. Eye Res. **76**, 145–153 CrossRef PubMed
9 Horwitz, J. (1992) α-Crystallin can function as a molecular chaperone. Proc. Natl. Acad. Sci. U.S.A. **89**, 10449–10453 CrossRef PubMed
10 Slingsby, C., Wistow, G.J. and Clark, A.R. (2013) Evolution of crystallins for a role in the vertebrate eye lens. Protein Sci **22**, 367–380 CrossRef PubMed
11 Arrigo, A.P. (2013) Human small heat shock proteins: protein interactomes of homo- and hetero-oligomeric complexes: an update. FEBS Lett **587**, 1959–1969 CrossRef PubMed
12 Sun, Y. and MacRae, T.H. (2005) The small heat shock proteins and their role in human disease. FEBS J **272**, 2613–2627 CrossRef PubMed
13 Morrow, G. and Tanguay, R.M. (2012) Small heat shock protein expression and functions during development. Int. J. Biochem. Cell Biol. **44**, 1613–1621 CrossRef PubMed
14 Mymrikov, E.V., Seit-Nebi, A.S. and Gusev, N.B. (2011) Large potentials of small heat shock proteins. Physiol. Rev. **91**, 1123–1159 CrossRef PubMed
15 Lu, T., Pan, Y., Kao, S.Y., Li, C., Kohane, I., Chan, J. and Yanker, B.A. (2004) Gene regulation and DNA damage in the ageing human brain. Nature **429**, 8838–8891 CrossRef
16 Morrow, G. and Tanguay, R.M. (2015) *Drosophila* small heat shock proteins: an update on their features and functions. The Big Book on Small Heat Shock Proteins (Tanguay, R.M. and Hightower, L., eds), pp. 579–606, Springer International Publishing, Berlin CrossRef
17 Fleckenstein, T., Kastenmuller, A., Stein, M.L., Peters, C., Daake, M., Krause, M. et al. (2015) The chaperone activity of the developmental small heat shock protein Sip1 is regulated by pH-dependent conformational changes. Mol. Cell **58**, 1067–1078 CrossRef PubMed
18 Vos, M.J., Carra, S., Kanon, B., Bosveld, F., Klauke, K., Sibon, O.C. and Kampinga, H.H. (2015) Specific protein homeostatic functions of small heat-shock proteins increase lifespan. Aging Cell **15**, 217–226 CrossRef PubMed
19 Kakkar, V., Meister-Broekema, M., Minoia, M., Carra, S. and Kampinga, H.H. (2014) Barcoding heat shock proteins to human diseases: looking beyond the heat shock response. Dis. Model. Mech. **7**, 421–34 CrossRef PubMed
20 De Jong, W.W., Caspers, G.J. and Leunissen, J.A. (1998) Genealogy of the α-crystallin–small heat-shock protein superfamily. Int. J. Biol. Macromol. **22**, 151–162 CrossRef PubMed
21 van Montfort, R.L., Basha, E., Friedrich, K.L., Slingsby, C. and Vierling, E. (2001) Crystal structure and assembly of a eukaryotic small heat shock protein. Nat. Struct. Biol. **8**, 1025–1030 CrossRef PubMed
22 Delbecq, S.P. and Klevit, R.E. (2013) One size does not fit all: the oligomeric states of αB crystallin. FEBS Lett **587**, 1073–1080 CrossRef PubMed
23 Kim, K.K., Kim, R. and Kim, S.H. (1998) Crystal structure of a small heat-shock protein. Nature **394**, 595–599 CrossRef PubMed
24 Jehle, S., Rajagopal, P., Bardiaux, B., Markovic, S., Kuhne, R., Stout, J.R. et al. (2010) Solid-state NMR and SAXS studies provide a structural basis for the activation of αB-crystallin oligomers. Nat. Struct. Mol. Biol. **17**, 1037–1042 CrossRef PubMed
25 Laganowsky, A., Benesch, J.L., Landau, M., Ding, L., Sawaya, M.R., Cascio, D. et al. (2010) Crystal structures of truncated αA and αB crystallins reveal structural mechanisms of polydispersity important for eye lens function. Protein Sci **19**, 1031–1043 CrossRef PubMed
26 Bepperling, A., Alte, F., Kriehuber, T., Braun, N., Weinkauf, S., Groll, M. et al. (2012) Alternative bacterial two-component small heat shock protein systems. Proc. Natl. Acad. Sci. U.S.A. **109**, 20407–20412 CrossRef PubMed
27 Hanazono, Y., Takeda, K., Oka, T., Abe, T., Tomonari, T., Akiyama, N. et al. (2013) Nonequivalence observed for the 16-meric structure of a small heat shock protein, SpHsp16.0, from *Schizosaccharomyces pombe*. Structure **21**, 220–228 CrossRef PubMed
28 Basha, E., Jones, C., Blackwell, A.E., Cheng, G., Waters, E.R., Samsel, K.A. et al. (2013) An unusual dimeric small heat shock protein provides insight into the mechanism of this class of chaperones. J. Mol. Biol. **425**, 1683–1696 CrossRef PubMed
29 Braun, N., Zacharias, M., Peschek, J., Kastenmuller, A., Zou, J., Hanzlik, M. et al. (2011) Multiple molecular architectures of the eye lens chaperone αB-crystallin elucidated by a triple hybrid approach. Proc. Natl. Acad. Sci. U.S.A. **108**, 20491–20496 CrossRef PubMed
30 Stengel, F., Baldwin, A.J., Painter, A.J., Jaya, N., Basha, E., Kay, L.E. et al. (2010) Quaternary dynamics and plasticity underlie small heat shock protein chaperone function. Proc. Natl. Acad. Sci. U.S.A. **107**, 2007–2012 CrossRef PubMed
31 Basha, E., O'Neill, H. and Vierling, E. (2012) Small heat shock proteins and α-crystallins: dynamic proteins with flexible functions. Trends Biochem. Sci. **37**, 106–117 CrossRef PubMed
32 Haslbeck, M., Weinkauf, S. and Buchner, J. (2015) Regulation of the chaperone function of small Hsps. The Big Book on Small Heat Shock Proteins (Tanguay, R.M. and Hightower, L., eds), pp. 155–176, Springer International Publishing, Berlin CrossRef
33 Bova, M.P., Ding, L.L., Horwitz, J. and Fung, B.K. (1997) Subunit exchange of αA-crystallin. J. Biol. Chem. **272**, 29511–29517 CrossRef PubMed
34 Baldwin, A.J., Lioe, H., Robinson, C.V., Kay, L.E. and Benesch, J.L. (2011) αB-Crystallin polydispersity is a consequence of unbiased quaternary dynamics. J. Mol. Biol. **413**, 297–309 CrossRef PubMed
35 Ingolia, T.D. and Craig, E.A. (1982) Four small *Drosophila* heat shock proteins are related to each other and to mammalian α-crystallin. Proc. Natl. Acad. Sci. U.S.A. **79**, 2360–2364 CrossRef PubMed
36 Jakob, U., Gaestel, M., Engel, K. and Buchner, J. (1993) Small heat shock proteins are molecular chaperones. J. Biol. Chem. **268**, 1517–1520 PubMed
37 Lee, G.J., Roseman, A.M., Saibil, H.R. and Vierling, E. (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 CrossRef PubMed

38 Mchaourab, H.S., Dodson, E.K. and Koteiche, H.A. (2002) Mechanism of chaperone function in small heat shock proteins: two-mode binding of the excited states of T4 lysozyme mutants by αA-crystallin. J. Biol. Chem. **277**, 40557–40566 CrossRef PubMed
39 Haslbeck, M., Walke, S., Stromer, T., Ehrnsperger, M., White, H.E., Chen, S. et al. (1999) Hsp26: a temperature-regulated chaperone. EMBO J **18**, 6744–67451 CrossRef PubMed
40 Ehrnsperger, M., Graber, S., Gaestel, M. and Buchner, J. (1997) Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. EMBO J **16**, 221–229 CrossRef PubMed
41 Kampinga, H.H., Brunsting, J.F., Stege, G.J., Konings, A.W. and Landry, J. (1994) Cells overexpressing Hsp27 show accelerated recovery from heat-induced nuclear protein aggregation. Biochem. Biophys. Res. Commun. **204**, 1170–1177 CrossRef PubMed
42 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 CrossRef PubMed
43 Peschek, J., Braun, N., Rohrberg, J., Back, K.C., Kriehuber, T., Kastenmuller, A. et al. (2013) Regulated structural transitions unleash the chaperone activity of αB-crystallin. Proc. Natl. Acad. Sci. U.S.A. **110**, E3780–E3789 CrossRef PubMed
44 Mogk, A., Deuerling, E., Vorderwulbecke, S., Vierling, E. and Bukau, B. (2003) Small heat shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. Mol. Microbiol. **50**, 585–595 CrossRef PubMed
45 Liberek, K., Lewandowska, A. and Zietkiewicz, S. (2008) Chaperones in control of protein disaggregation. EMBO J. **27**, 328–335 CrossRef PubMed
46 Haslbeck, M., Miess, A., Stromer, T., Walter, S. and Buchner, J. (2005) Disassembling protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and Hsp104. J. Biol. Chem. **280**, 23861–23868
47 Treweek, T.M., Meehan, S., Ecroyd, H. and Carver, J.A. (2015) Small heat-shock proteins: important players in regulating cellular proteostasis. Cell. Mol. Life Sci. **72**, 429–451 CrossRef PubMed
48 Shi, J., Koteiche, H.A., McDonald, E.T., Fox, T.L., Stewart, P.L. and McHaourab, H,S. (2013) Cryoelectron microscopy analysis of small heat shock protein 16.5 (Hsp16.5) complexes with T4 lysozyme reveals the structural basis of multimode binding. J. Biol. Chem. **288**, 4819–4830 CrossRef PubMed
49 Chiappori, F., Mattiazzi, L., Milanesi, L. and Merelli, I. (2016) A novel molecular dynamics approach to evaluate the effect of phosphorylation on multimeric protein interface: the αB-crystallin case study. BMC Bioinformatics **17** (Suppl. 4), 57 CrossRef PubMed
50 Liu, L., Chen, J.Y., Yang, B., Wang, F.H., Wang, Y.H. and Yun, C.H. (2015) Active-state structures of a small heat-shock protein revealed a molecular switch for chaperone function. Structure **23**, 2066–2075 CrossRef PubMed
51 MacRae, T.H. (2016) Stress tolerance during diapause and quiescence of the brine shrimp, *Artemia*. Cell Stress Chaperones **21**, 9–18 CrossRef PubMed
52 King, A.M. and MacRae, T.H. (2012) The small heat shock protein p26 aids development of encysting *Artemia* embryos, prevents spontaneous diapause termination and protects against stress. PLoS One **7**, e43723 CrossRef PubMed
53 Haslbeck, M., Braun, N., Stromer, T., Richter, B., Model, N., Weinkauf, S. and Buchner, J. (2004) Hsp42 is the general small heat shock protein in the cytosol of *Saccharomyces cerevisiae*. EMBO J. **23**, 638–649 CrossRef PubMed
54 Hochberg, G.K., Ecroyd, H., Liu, C., Cox, D., Cascio, D., Sawaya, M.R. et al. (2014) The structured core domain of αB-crystallin can prevent amyloid fibrillation and associated toxicity. Proc. Natl. Acad. Sci. U.S.A. **111**, E1562–E1570 CrossRef PubMed
55 Mainz, A., Peschek, J., Stavropoulou, M., Back, K.C., Bardiaux, B., Asami, S. et al. (2015) The chaperone αB-crystallin uses different interfaces to capture an amorphous and an amyloid client. Nat. Struct. Mol. Biol. **22**, 898–905 PubMed
56 Arrigo, A.P. and Gibert, B. (2013) Protein interactomes of three stress inducible small heat shock proteins: HspB1, HspB5 and HspB8. Int. J. Hyperthermia **29**, 409–422 CrossRef PubMed
57 Pandey, P., Farber, R., Nakazawa, A., Kumar, S., Bharti, A., Nalin, C. et al. (2000) Hsp27 functions as a negative regulator of cytochrome *c*-dependent activation of procaspase-3. Oncogene **19**, 1975–1981 CrossRef PubMed
58 Acunzo, J., Katsogiannou, M. and Rocchi, P. (2012) Small heat shock proteins HSP27 (HspB1), αB-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. Int. J. Biochem. Cell Biol. **44**, 1622–1631 CrossRef PubMed
59 Voss, O.H., Batra, S., Kolattukudy, S.J., Gonzalez-Mejia, M.E., Smith, J.B. and Doseff, A.I. (2007) Binding of caspase-3 prodomain to heat shock protein 27 regulates monocyte apoptosis by inhibiting caspase-3 proteolytic activation. J. Biol. Chem. **282**, 25088–25099 CrossRef PubMed
60 Gonzalez-Mejia, M.E., Voss, O.H., Murnan, E.J. and Doseff, A.I. (2010) Apigenin-induced apoptosis of leukemia cells is mediated by a bimodal and differentially regulated residue-specific phosphorylation of heat-shock protein-27. Cell Death Dis **1**, e64 CrossRef PubMed
61 Kayser, J., Haslbeck, M., Dempfle, L., Krause, M., Grashoff, C., Buchner, J. et al. (2013) The small heat shock protein Hsp27 affects assembly dynamics and structure of keratin intermediate filament networks. Biophys. J. **105**, 1778–1785 CrossRef PubMed
62 Quinlan, R.A. and Ellis, R.J. (2013) Chaperones: needed for both the good times and the bad times. Philos. Trans. R. Soc. Lond. B Biol. Sci. **36**, 20130091 CrossRef
63 Elliott, J.L., Der Perng, M., Prescott, A.R., Jansen, K.A., Koenderink, G.H. and Quinlan, R.A. (2013) The specificity of the interaction between αB-crystallin and desmin filaments and its impact on filament aggregation and cell viability. Philos. Trans. R. Soc. Lond. B Biol. Sci. **368**, 20120375 CrossRef PubMed
64 Zhao, L., Chen, X.J., Zhu, J., Xi, Y.B., Yang, X., Hu, L.D. et al. (2015) Lanosterol reverses protein aggregation in cataracts. Nature **523**, 607–611 CrossRef PubMed
65 Makley, L.N., McMenimen, K.A., DeVree, B.T., Goldman, J.W., McGlasson, B.N., Rajagopal, P. et al. (2015) Pharmacological chaperone for α-crystallin partially restores transparency in cataract models. Science **350**, 674–677 CrossRef PubMed
66 Zoubeidi, A. and Gleave, M. (2012) Small heat shock proteins in cancer therapy and prognosis. Int. J. Biochem. Cell Biol. **44**, 1646–1656 CrossRef PubMed

Essays in Biochemistry (2016) **60** 163–172
DOI: 10.1042/EBC20160010

67 Arrigo, A.P. and Gibert, B. (2014) HspB1, HspB5 and HspB4 in human cancers: potent oncogenic role of some of their client proteins. Cancers **6**, 333–365 CrossRef PubMed

68 Arrigo, A.P. (2001) Hsp27: novel regulator of intracellular redox state. IUBMB Life **52**, 303–307 CrossRef PubMed

69 Jovcevski, B., Kelly, M.A., Rote, A.P., Berg, T., Gastall, H.Y., Benesch, J.L. et al. (2015) Phosphomimics destabilize Hsp27 oligomeric assemblies and enhance chaperone activity. Chem. Biol. **22**, 186–195 CrossRef PubMed