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Review

Chaperone function and mechanism of small heat-shock proteins

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Small heat-shock proteins (sHSPs) are ubiquitous ATP-independent molecular chaperones that play crucial roles in protein quality control in cells. They are able to prevent the aggregation and/or inactivation of various non-native substrate proteins and assist the refolding of these substrates independently or under the help of other ATP-dependent chaperones. Substrate recognition and binding by sHSPs are essential for their chaperone functions. This review focuses on what natural substrate proteins an sHSP protects and how it binds the substrates in cells under fluctuating conditions. It appears that sHSPs of prokaryotes, although being able to bind a wide range of cellular proteins, preferentially protect certain classes of functional proteins, such as translation-related proteins and metabolic enzymes, which may well explain why they could increase the resistance of host cells against various stresses. Mechanistically, the sHSPs of prokaryotes appear to possess numerous multi-type substrate-binding residues and are able to hierarchically activate these residues in a temperature-dependent manner, and thus act as temperature-regulated chaperones. The mechanism of hierarchical activation of substrate-binding residues is also discussed regarding its implication for eukaryotic sHSPs.

Keywords protein quality control; molecular chaperone; small heat-shock protein; protein aggregation; oligomer

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Introduction

Protein quality control systems are composed of molecular chaperones, folding catalysts, and proteases, which are essential for cells to produce functional proteins as well as to remove harmful misfolded/aggregated proteins. Molecular chaperones, coined in 1978 [1] and later widely investigated (as extensively reviewed by Ellis [2]), are defined as proteins that assist other proteins to acquire functionally active structures but are not components of these final structures [3,4]. According to their molecular size, they are usually classified into the following families: Hsp100s, Hsp90s, Hsp70s, Hsp60s, Hsp40s, and small heat-shock proteins (sHSPs). Each member from these families acts upon a specific spectrum (broad or narrow) of substrate proteins in assisting their folding, refolding, oligomeric assembly, translocation, and/or degradation [4].

The sHSPs, ubiquitously existing in all forms of life [5], were found to suppress protein aggregation in an ATP-independent manner [6,7] and stabilize stress-damaged cell membranes [8–10]. Under *in vitro* conditions, sHSPs can effectively interact with unfolded model substrate proteins and keep them in a folding-competent state for subsequent refolding that is facilitated by such ATP-dependent chaperones as Hsp70s and Hsp100s [11–15]. On the other hand, heterologous overexpression of sHSPs was reported to increase the tolerance of host cells against various stresses [16–23]. Physiologically, the sHSPs of animals, as exampled by fly Hsp22, mammalian αA-crystallin and αB-crystallin, mouse Hsp25, human Hsp27 and Hsp22, have been linked to cell differentiation [24], apoptosis [25], and longevity [26], and their dysfunction has been related to many diseases such as cancer development [27], cardiovascular diseases [28,29], cataracts [30], myopathy [31], and neuron diseases [32,33].

The primary structures of sHSPs are characterized by a conserved α-crystallin domain of ∼100 amino acids [5], which is flanked by a highly variable and often structurally disordered N-terminal arm [34–37] and a short flexible C-terminal extension [35,38,39]. The 3D structure determinations revealed that sHSPs are rich in β-strands (as reviewed in [40]) and existing as immunoglobin-like β-sandwiches [35]. Under *in vitro* conditions, sHSPs are often found to assemble into large oligomers of 12–40 subunits, using dimers as the building block [34,35,41–43], although a few of them were reported to exist as monomers, dimers, or tetramers [44–46].

In the past decade, we systematically investigated the chaperone activity and mechanism of representative sHSPs mostly under *in vitro* conditions [36,47–53]. Recently, we have utilized the site-specific *in vivo* photo-crosslinking to probe the chaperone function and mechanism of sHSPs in *Escherichia coli* [54,55]. These studies suggested that prokaryotic sHSPs utilize numerous multi-type substrate-binding residues and

hierarchically activate them in a temperature-dependent manner to capture a wide range of proteins. As such, sHSPs could act as robust chaperones under fluctuating conditions and enhance their chaperone activities at elevating temperatures. Importantly, accumulative evidence indicate that sHSPs also preferentially protect certain classes of functional proteins, such as translation-related proteins and metabolic enzymes [54,56–58]. In this review, I will focus on how an sHSP activates its numerous multi-type substrate-binding residues and what functional proteins it protects in cells. For readers who are interested in sHSPs with respect to their 3D structures and oligomeric assembly and relevance to human health and physiology, nice review articles are also available [40,59–64].

Functional Diversity of the Substrate Proteins of sHSPs

The sHSPs are known to non-selectively interact with nearly all types of aggregation-prone unfolded model substrate proteins under *in vitro* conditions [6,7,11,39,65–80]. Bovine eye α-crystallin is the first sHSP that was reported to exhibit chaperone activity by suppressing the *in vitro* thermal aggregations of up to 12 types of proteins [6], including its naturally associated proteins (β_H-, β_L-, γ_S-, and γ-crystallins) and unrelated model enzymes (α-glucosidase, phosphoglucose isomerase, glutathione *S*-transferase, enolase, aldolase, lactate dehydrogenase, citrate synthase, carbonic anhydrase). Later, many model substrate proteins (e.g. malate dehydrogenase, alcohol dehydrogenase, β-galactosidase, elastase, luciferase, lysozyme, rhodanese, xylose reductase, Ataxin-3, Sup35, α-synuclein, α-lactalbumin, Abrin, β-actin, β-tubulin, β-crystallin, γ-crystallin, titin) and even peptides (insulin B chain and melitin) from different organisms were found to be the substrate proteins of sHSP family [7,11,65–73].

Furthermore, the substrate functional diversity of sHSPs was nicely demonstrated by systematic identification of the substrate proteins of specific sHSP in cells. For instances, 42 and 37 proteins were associated with the bacterium *Synechocystis* Hsp16.6 [81] and *E. coli* IbpA [82] in cells, respectively. In addition, a total of 94 proteins in the cell extract of bacterium *Deinococcus radiodurans* were found to co-aggregate with Hsp20.2 during thermal treatment [56]. Importantly, we have recently identified a total of 113 cellular proteins interacting with IbpB in living *E. coli* cells by using *in vivo* photo-crosslinking [54]. Retrospectively, these natural substrate proteins of specific sHSPs appear to have a variety of cellular functions, such as metabolism (e.g. energy production, amino acid and carbohydrate metabolism), DNA replication, recombination and repair, mRNA transcription and processing, and protein translation (**Table 1**). In particular, many secretory proteins were identified as substrates of IbpB and Hsp20.2 (**Table 1**), implicating that sHSPs are involved in the quality control of secretory proteins during their biogenesis in the cytoplasm. Together, it appears that bacterial sHSPs are capable of protecting all the cellular proteins.

Nevertheless, the substrate functional diversity of eukaryotic sHSPs is largely unknown due to the lack of systematic studies. To clarify this, database searching was performed for three representative human sHSPs regarding their interacting partners. The results presented in **Table 1** indicated that αB-crystallin binds a number of cellular proteins, including

Table 1. Functional diversity of the substrate proteins of sHSPs

Protein function[a]	Hsp16.6[b]	IbpA[b]	IbpB[b]	Hsp20.2[b]	αA[b]	αB[b]	Hsp27[b]
Metabolism	6	15	66	38	2	1	6
DNA replication/modification and chromosome structure	0	2	4	2	0	0	2
mRNA transcription and processing	2	3	4	4	1	0	15
Translation	3	8	19	30	0	0	1
Protein quality control[c]	1	5	3	5	3	5	4
Secretory protein	0	1	9	5	2	5	10
Other functions	1	7	5	10	7[d]	11[d]	27[d]
Uncharacterized	0	1	3	0	0	0	3
Total	13	42	113	94	15	22	68

[a]Protein class was assigned according to the functional annotation of each protein in the UnitProt database.

[b]42 Hsp16.6-bound proteins after heat shock were co-purified, with 13 being identified [81]; IbpA-bound proteins were co-purified, with 42 being identified [82]; IbpB-bound proteins were covalently photo-crosslinked with IbpB in living cells and then co-purified, with 113 being identified [54]; Hsp20.2-bound proteins were co-purified from the thermally treated mixture of Hsp20.2 and cell extract, with 94 being identified [56]. The information for mammalian sHSPs-bound proteins was extracted from the protein–protein interaction databases (a uniform entry is provided at http://www.ebi.ac.uk/Tools/webservices/psicquic/view/main.xhtml).

[c]Protein quality controls are considered as functional partners, instead of substrates of sHSPs.

[d]Most of the substrates for mammalian sHSPs in this classification are involved in signaling transductions that are crucial for cell differentiation, proliferation, apoptosis, and development.

those participating in signaling transductions related to apoptosis, cell–cell adhesion, cytoskeleton, and so on (classified as 'other functions'), in contrast to αA-crystallin that mainly binds the crystallin proteins specifically expressed in the eyes. Notably, Hsp27 not only binds signal transduction proteins similarly with αB-crystallin, but also interacts with a number of transcriptional factors and mRNA-processing proteins. Apparently, the cellular functions for the substrate proteins of mammalian sHSPs, or eukaryotic sHSPs in general, reflect the multi-cellular complexity of eukaryotes. In line with this, the genomes of eukaryotes usually encode a larger number of sHSPs than those of prokaryotes [63], and the sHSPs of a eukaryotic organism usually have different sub-cellular compartments [83,84] and cell/tissue specificity [85].

Functional Preference of the Substrate Proteins of sHSPs

Despite their functional diversity, the substrates of both IbpB and Hsp20.2 are apparently enriched in translation-related proteins (**Table 1**), which include a number of ribosomal proteins, amino-acyl tRNA synthetases, and translation factors. In retrospect, Hsp16.3, an sHSP of *Mycobacterium tuberculosis*, was found to be associated with ribosomes, either in cells grown under oxygen-deficient conditions as reported by Tabira *et al.* [57] or in the *in vitro* transcription/translation system as reported by our laboratory [86]. It appears that bacterial sHSPs preferentially protect the protein synthesis machine under stress conditions. Similarly, the sHSPs in plants (soybean and tomato) were reported decades ago to be associated with ribosomes under heat-shock condition [58]. On the other hand, it is documented that the ribosome is easily disrupted by heat shock [87], which may inactivate the components in the protein synthesis machine [88]. Importantly, we found that translation-related proteins are prone to aggregate in IbpB-deficient *E. coli* [54]. In light of all these observations, it is conceivable that the translation-related proteins are vulnerable to heat-induced aggregation and sHSPs are able to protect them against the aggregation. The preferential protection of sHSPs on the protein synthesis machines would substantially contribute to the commonly observed increase of thermotolerance in the host cells as a result of the overexpression of sHSPs (e.g. IbpB) [21,22,89,90].

Besides the translation-related proteins, IbpB appears to have a significant substrate preference for metabolic enzymes, including those involved in carbohydrate metabolism, amino acid metabolism, lipid metabolism, and respiratory chains/energy production, as reported in our recent study [54]. Since metabolic enzymes were found to be more vulnerable to stress-induced inactivation in cells lacking IbpB by Fu *et al.* [54] and by others [91,92], the preferential protection of IbpB on each metabolic enzyme may apparently have specific biological effects, such as helping cells to resist the oxidative stress [54]. In addition, the protection of IbpB on the metabolic enzymes as a whole may be linked to protein synthesis, a cellular process whose functional integrity definitely requires both abundant amino acids and ATP. Nevertheless, it should be pointed out that mammalian sHSPs appear not to exhibit any preferences to translation-related proteins and metabolic enzymes (**Table 1**).

Structural Diversity of the Substrate Proteins of sHSPs

It is far from clear whether certain common structural features are adopted by the substrates of sHSPs, although they were long known to be aggregation-prone [39,70,74–80] and contain a considerable portion of native-like structures [70,73,78,79,93,94]. In line with their functional diversity, the substrate proteins of prokaryotic sHSPs are also structurally diversified, as reflected by the broad range of their molecular weight (**Fig. 1A**) and isoelectric point (**Fig. 1B**). For

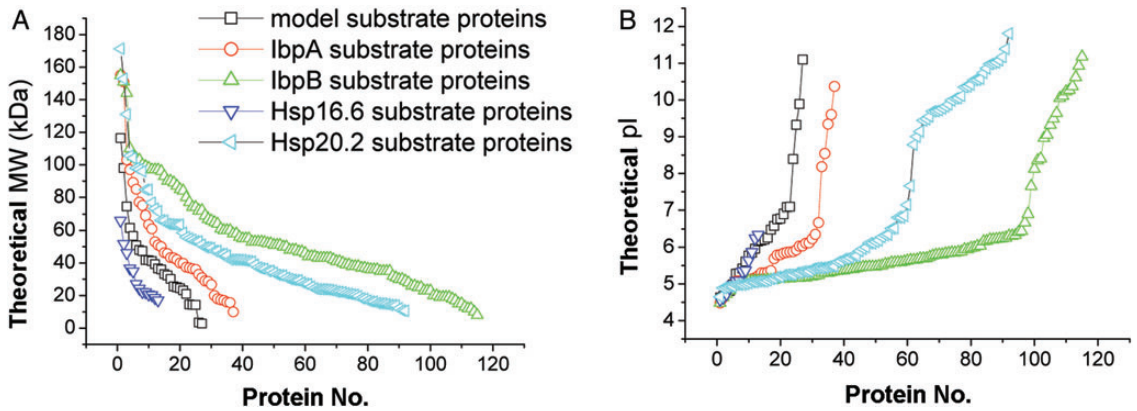

Figure 1. Broad range of molecular weights and isoelectric points of the substrates of sHSPs The model substrates of sHSPs and the *in vivo* substrates of representative sHSPs (Hsp16.6, IbpA, IbpB, and Hsp20.2) were subjected to molecular weight (A) and isoelectric point (B) analysis by online software Compute pI/Mw (http://web.expasy.org/compute_pi/), with the value of each substrate protein being shown here.

example, the molecular weights of 27 representative model substrate proteins range from 3 to 116 kDa (**Fig. 1A**). Furthermore, the molecular weights of IbpA and IbpB substrate proteins fall in the range of 10–155 kDa (**Fig. 1A**), which is comparable with those of the *E. coli* proteome [95]. These observations indicated that sHSPs are able to bind proteins of any molecular size that are naturally presented in cells. Notably, the apparent upper limit of molecular weight (i.e. 155 kDa) for IbpA and IbpB substrate proteins was much higher than that of *E. coli* GroEL substrate protein [96], presumable due to their different chaperone mechanisms. Specifically, the substrate proteins only need to be incorporated into the dynamic and plastic oligomers of sHSPs [97,98] but have to be enclosed in the cavity of GroEL through its apical end [99].

In addition, the isoelectric points of the 13 substrate proteins of Hsp16.6 have been revealed by using 2D electrophoresis with a pH ranging from 4.5 to >7.5 [81]. The theoretical isoelectric points of the 27 model substrate proteins, and IbpA, IbpB, and Hsp20.2 substrate proteins evenly fall in a broader range (i.e. from 4 to 11; **Fig. 1B**). These results indicated that sHSPs are able to capture or incorporate either negatively or positively charged substrate proteins, despite of the overall negative charge for sHSPs themselves under neutral conditions.

Multi-type Residues of sHSPs Participate in Binding Substrates in Cells

The functional and structural diversity of the substrates of sHSPs immediately raises the question as to how an sHSP binds such diversified substrates. Earlier truncation studies showed that a number of amino acids located in the three characteristic domains of sHSPs are crucial for the functional integrity of chaperone [36,50,72,100–104]. However, the truncations also significantly affect the structural integrity of sHSPs and therefore, the exact roles of the corresponding regions for substrate-binding could not be unambiguously assigned. A more reliable strategy based on chemical cross-linking coupled with mass spectrometry was successfully applied to the characterization of the substrate-binding regions of *M. tuberculosis* Hsp16.3, plant Hsp21, and mammalian αA-crystallin, αB-crystallin, and Hsp22 [105–109], revealing that the N-terminal part of the α-crystallin domain and the N-terminal arm are the major regions for substrate binding. Notably, these substrate-binding regions appear to contain both polar and non-polar amino acids. However, the exact role of each amino acid in substrate binding has not been defined.

Site-directed mutagenesis was widely adopted to characterize the substrate-binding sites of both prokaryotic and eukaryotic sHSPs, revealing that many charged residues are critical for the chaperone functions of sHSPs [104,110–114]. Notably, some point mutations of mammalian sHSPs were reported not only to significantly affect their functional integrity under *in vitro* assays [115–117], but also to cause diseases, as exampled by the mutations of R116C of αA-crystallin in autosomal-dominant cataract [30], R120G of αB-crystallin in desmin-related myopathy and cataract [31], and K141N or K141E of Hsp22 in distal hereditary motor neuropathies [32] as well as of Hsp27 in distal HMN and CMT neuropathy [33]. Again, whether these identified critical sites are directly involved in substrate binding cannot be determined, as most point mutations also affect the structural integrity similar to the truncations.

Recently, an elegant approach using unnatural amino acid-mediated photo-crosslinking, by which the side effect on the structural integrity of sHSPs is minimized and site-specific roles can be determined, has been used to characterize the substrate-binding residues of sHSPs by others [118] and by us [55]. In the first case, Bpa (an unnatural amino acid [119]) was site-specifically incorporated into pea Hsp18.1 at all the three domains, and the interactions of purified Hsp18.1 Bpa variant proteins with model substrate proteins (luciferase and malate dehydrogenase) were examined under *in vitro* conditions [118]. The authors found that both hydrophobic and charged residues of Hsp18.1 are directly involved in binding, consistent with conventional point mutation studies on αA-crystallin and αB-crystallin [120,121]. Nevertheless, the use of two model substrate proteins therein might not reflect the diversity of natural substrate proteins in cells.

In the second case, we revealed that among 48 substrate-binding residues of IbpB as identified by Bpa-mediated *in vivo* photo-crosslinking, there are nearly equivalent hydrophobic and polar amino acids (including 13 charged residues) (**Table 2**). Together, these results thus demonstrate that weak interactions, including hydrophobic interactions, electrostatic forces, and hydrogen bonds, are all involved in the interaction

Table 2. Multi-types of amino acids in IbpB are involved in substrate binding[a]

Amino acid property	Non-polar		Polar	
	Aliphatic (%)	Aromatic (%)	Uncharged (%)	Charged (%)
71 selected residues for validation	40.8	12.7	17.0	29.6
20 substrate-binding residues at 30°C	30	15	30	25
48 substrate-binding residues at 50°C	37.5	16.7	18.5	27.1

[a]Data were obtained from our recent report [55].

between sHSP and substrate proteins. Accordingly, such multi-types of interactions enable sHSPs to recognize structurally diversified substrate proteins that not only expose non-native hydrophobic surfaces, but also present certain native and/or non-native polar amino acids. Furthermore, such multi-type non-covalent interactions may also keep the substrate–sHSP complexes dynamic [97,122] and help to facilitate the substrate release and refolding under the assistance of other molecular chaperones [11–15,122].

The Mechanism of Temperature-dependent Hierarchical Activation of Multi-type Substrate-binding Residues Underlies the Temperature-regulated Chaperone Activity of sHSPs

One intriguing property for sHSPs is that their chaperone activities were enhanced at elevated temperatures, as exemplified by both prokaryotic sHSPs (e.g. *E. coli* IbpB [50], *M. tuberculosis* Hsp16.3 [49,123], *A. fulgidus* Hsp20.2 [124], *M. jannaschii* Hsp16.5 [125]), and eukaryotic sHSPs (e.g. yeast Hsp26 [68,126,127], wheat Hsp16.9 [34], pea Hsp18.1 [98,118], plant cytosolic Class I and II sHSPs [128], and mammalian α-crystallin [129]). Most probably, such heat shock-mediated enhancement in the chaperone activities of sHSPs is due to the heat shock-induced oligomeric dissociation [34,49,50,98,118,123–125,128], which, in turn, enables more substrate-binding residues to be accessible for binding substrates. Alternatively, certain heat shock-mediated structural readjustment without the oligomeric dissociation could also activate sHSPs (e.g. yeast Hsp26 [126,127] and plant cytosolic Class II sHSPs [128]). While there is no single model to sufficiently describe the activation mechanism of sHSPs [40,128], one key event is how sHSPs activate their substrate-binding residues upon temperature elevation.

Our recent report suggested that in living cells IbpB functions as a robust molecular chaperone, with its substrate-binding residues being hierarchically activated upon temperature elevation [55]. Specifically, there are three types of substrate-binding residues presented in IbpB, which are respectively activated at characteristic temperatures: Type I residues being activated at 25°C and enhanced upon temperature elevation; Type II residues being activated at 37°C and enhanced upon temperature elevation; Type III being involved in oligomerization at <37°C, but switched for substrate binding at heat-shock temperatures. Apparently, the oligomeric dissociation and/or reorganization occur in order for the Type III residues to be activated but it is not necessary for the activation of Type I and II residues. Together, Type I, II, and III residues in IbpB start to bind substrate proteins at a particular temperature (**Fig. 2**). As a result, IbpB

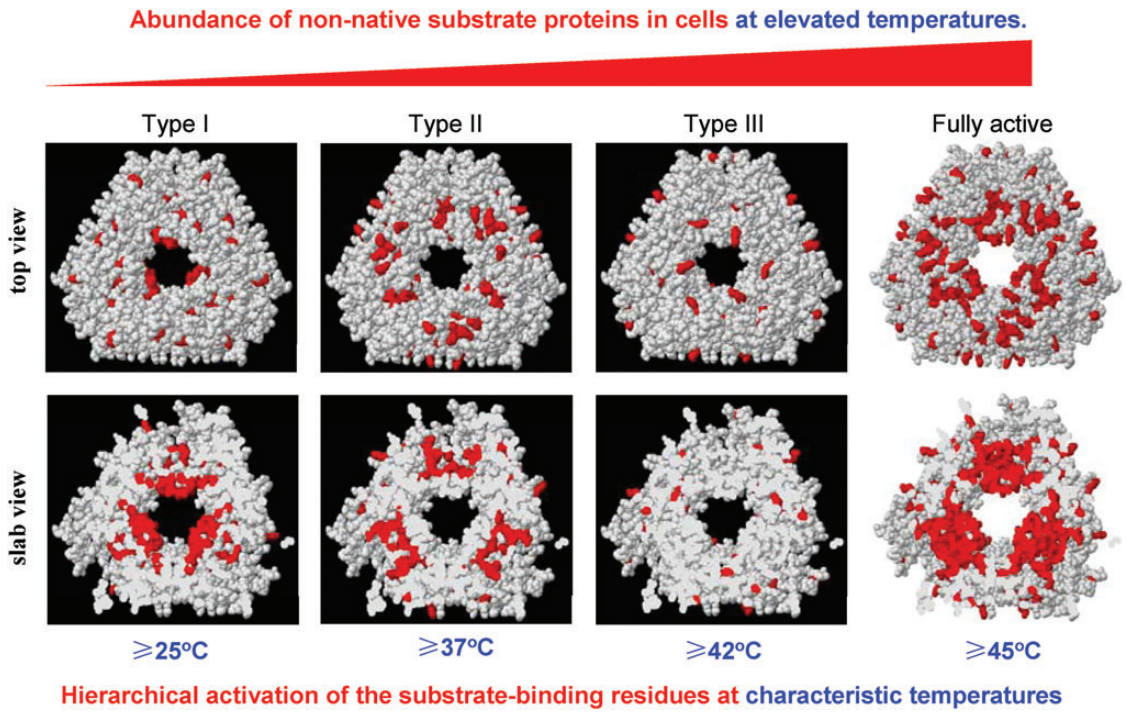

Figure 2. A mechanism of hierarchically activating multi-type substrate-binding residues of sHSPs underlies their heat shock-enhanced chaperone activities The modeled IbpB dodecameric structure was adopted from our earlier study [55] and shown here for schematically illustrating the mechanism for the substrate binding of sHSPs. At elevated temperatures, multi-type substrate-binding residues (e.g. Type I, II, and III for IbpB) of a sHSP are hierarchically activated due to temperature-regulated structural changes (e.g. oligomeric dissociation) and meanwhile, the abundance of non-native proteins in cells is also increased accordingly. As such, the substrate-binding capacity of the sHSP would be well regulated in a temperature-dependent manner to cope with the abundance of the substrates in a cell, i.e. it acts as a robust chaperone to meet the requirement for its physiological functions.

remains inactive at a temperature lower than 20°C and becomes moderately active at relatively low temperatures (between 25 and 30°C), highly active at normal/mild-heat-shock temperatures (between 37 and 42°C), and maximally active under severe heat-shock conditions (at between 45 and 50°C), as supported by our *in vitro* [50] and *in vivo* [55] studies.

Since prokaryotes, lower eukaryotes (e.g. yeast), and plants are very susceptible to fluctuating growth temperatures, the chaperone functions of sHSPs therein would be very likely regulated by temperature. It follows that the heat shock-enhanced chaperone activities, as observed for *M. tuberculosis* Hsp16.3 [49,123], *A. fulgidus* Hsp20.2 [124], *M. jannaschii* Hsp16.5 [125], yeast Hsp26 [68,126,127], wheat Hsp16.9 [34], pea Hsp18.1 [98,118], plant cytosolic Class I and II sHSPs [128], may be well explained by the temperature-dependent hierarchical activation of the multi-type substrate-binding residues, similar to the *E. coli* IbpB. Accordingly, any structural changes, including but not being limited to the oligomeric dissociation, may make the substrate-binding residues accessible to the substrates. Since the abundance of the substrates in cells is tightly dependent on the temperature and is usually increased upon temperature elevation, the hierarchical activation of the multi-type substrate residues of an sHSP at characteristic temperatures would allow it to act as a robust chaperone to smartly cope with the abundance of non-native substrate proteins occurring at fluctuating temperatures. Nonetheless, the chaperone functions of mammalian sHSPs are more likely regulated by phosphorylation in cells [28,130–134], although they may also be regulated by temperature [129]. How the phosphorylation enhances their chaperone functions is still not yet defined. Conceivably, it is of interest to investigate whether the hierarchical activation of multi-type substrate-binding residues takes place during the phosphorylation-mediated functional change for mammalian sHSPs.

Perspectives

In this review, I focus on the diversity of sHSP substrates and suggest that the temperature-dependent hierarchical activation of the multi-type substrate-binding residues may represent a mechanism underlying the heat shock-enhanced chaperone activities of prokaryotic sHSPs. Other outstanding questions include the following. First, what functional substrates are preferentially bound by eukaryotic sHSPs, particularly for mammalian sHSPs? Secondly, what common structural features are adopted by the substrates of sHSPs, despite their structural diversity? Thirdly, how sHSPs are involved in the degradation of misfolded and/or aggregated proteins in cells? Last but not least, are the substrates of secretory proteins, as captured by prokaryotic sHSPs in the cytoplasm [54,56], processed for the downstream biogenesis (e.g. the Sec-system assisted translocation across the inner membrane), or destined to degradation by cytoplasmic proteases (e.g. ClpP)?

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References

1. Laskey RA, Honda BM, Mills AD and Finch JT. Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. Nature 1978, 275: 416–420.
2. Ellis RJ. Discovery of molecular chaperones. Cell Stress Chaperones 1996, 1: 155–160.
3. Ellis RJ. The general concept of molecular chaperones. Philos Trans R Soc Lond B Biol Sci 1993, 339: 257–261.
4. Hartl FU, Bracher A and Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature 2011, 475: 324–332.
5. de Jong WW, Leunissen JA and Voorter CE. Evolution of the alpha-crystallin/small heat-shock protein family. Mol Biol Evol 1993, 10: 103–126.
6. Horwitz J. Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci USA 1992, 89: 10449–10453.
7. Jakob U, Gaestel M, Engel K and Buchner J. Small heat shock proteins are molecular chaperones. J Biol Chem 1993, 268: 1517–1520.
8. Török Z, Goloubinoff P, Horváth I, Tsvetkova NM, Glatz A, Balogh G and Varvasovszki V, *et al*. 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: 3098–3103.
9. Welker S, Rudolph B, Frenzel E, Hagn F, Liebisch G, Schmitz G and Scheuring J, *et al*. Hsp12 is an intrinsically unstructured stress protein that folds upon membrane association and modulates membrane function. Mol Cell 2010, 39: 507–520.
10. Tsvetkova NM, Horváth I, Török Z, Wolkers WF, Balogi Z, Shigapova N and Crowe LM, *et al*. Small heat-shock proteins regulate membrane lipid polymorphism. Proc Natl Acad Sci USA 2002, 99: 13504–13509.
11. Veinger L, Diamant S, Buchner J and Goloubinoff P. The small heat-shock protein IbpB from *Escherichia coli* stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. J Biol Chem 1998, 273: 11032–11037.
12. Mogk A, Deuerling E, Vorderwulbecke S, Vierling E and Bukau B. Small heat shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. Mol Microbiol 2003, 50: 585–595.
13. Lee GJ, Roseman AM, Saibil HR and 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: 659–671.

14. Cashikar AG, Duennwald M and Lindquist SL. A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. J Biol Chem 2005, 280: 23869–23875.
15. Ehrnsperger M, Graber S, Gaestel M and Buchner J. Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. EMBO J 1997, 16: 221–229.
16. Balogi Z, Cheregi O, Giese KC, Juhász K, Vierling E, Vass I and Vígh L, *et al*. A mutant small heat shock protein with increased thylakoid association provides an elevated resistance against UV-B damage in synechocystis 6803. J Biol Chem 2008, 283: 22983–22991.
17. Sun L, Liu Y, Kong X, Zhang D, Pan J, Zhou Y and Wang L, *et al*. ZmHSP16.9, a cytosolic class I small heat shock protein in maize (*Zea mays*), confers heat tolerance in transgenic tobacco. Plant Cell Rep 2012, 31: 1473–1484.
18. Montero-Barrientos M, Cardoza RE, Gutierrez S, Monte E and Hermosa R. The heterologous overexpression of hsp23, a small heat-shock protein gene from *Trichoderma virens*, confers thermotolerance to *T. harzianum*. Curr Genet 2007, 52: 45–53.
19. Kim KH, Alam I, Kim YG, Sharmin SA, Lee KW, Lee SH and Lee BH. Overexpression of a chloroplast-localized small heat shock protein OsHSP26 confers enhanced tolerance against oxidative and heat stresses in tall fescue. Biotechnol Lett 2012, 34: 371–377.
20. Seo JS, Lee YM, Park HG and Lee JS. The intertidal copepod *Tigriopus japonicus* small heat shock protein 20 gene (*Hsp20*) enhances thermotolerance of transformed *Escherichia coli*. Biochem Biophys Res Commun 2006, 340: 901–908.
21. Yeh CH, Chang PF, Yeh KW, Lin WC, Chen YM and Lin CY. Expression of a gene encoding a 16.9-kDa heat-shock protein, Oshsp16.9, in *Escherichia coli* enhances thermotolerance. Proc Natl Acad Sci USA 1997, 94: 10967–10972.
22. Kitagawa M, Matsumura Y and 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: 165–171.
23. Landry J, Chretien P, Lambert H, Hickey E and Weber LA. Heat shock resistance conferred by expression of the human *HSP27* gene in rodent cells. J Cell Biol 1989, 109: 7–15.
24. Favet N, Duverger O, Loones MT, Poliard A, Kellermann O and Morange M. Overexpression of murine small heat shock protein HSP25 interferes with chondrocyte differentiation and decreases cell adhesion. Cell Death Differ 2001, 8: 603–613.
25. Salinthone S, Ba M, Hanson L, Martin JL, Halayko AJ and Gerthoffer WT. Overexpression of human Hsp27 inhibits serum-induced proliferation in airway smooth muscle myocytes and confers resistance to hydrogen peroxide cytotoxicity. Am J Physiol Lung Cell Mol Physiol 2007, 293: L1194–L1207.
26. Morrow G, Battistini S, Zhang P and Tanguay RM. Decreased lifespan in the absence of expression of the mitochondrial small heat shock protein Hsp22 in *Drosophila*. J Biol Chem 2004, 279: 43382–43385.
27. Richards EH, Hickey E, Weber L and Master JR. Effect of overexpression of the small heat shock protein HSP27 on the heat and drug sensitivities of human testis tumor cells. Cancer Res 1996, 56: 2446–2451.
28. Salinthone S, Ba M, Hanson L, Martin JL, Halayko AJ and Gerthoffer WT. Overexpression of wild-type heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. Circulation 2004, 110: 3544–3552.
29. Sanbe A, Marunouchi T, Abe T, Tezuka Y, Okada M, Aoki S and Tsumura H, *et al*. Phenotype of cardiomyopathy in cardiac-specific heat shock protein B8 K141N transgenic mouse. J Biol Chem 2013, 288: 8910–8921.
30. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW and Weleber RG. Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 1998, 7: 471–474.
31. Vicart P, Caron A, Guicheney P, Li Z, Prévost MC, Faure A and Chateau D, *et al*. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 1998, 20: 92–95.
32. Irobi J, Van Impe K, Seeman P, Jordanova A, Dierick I, Verpoorten N and Michalik A, *et al*. Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. Nat Genet 2004, 36: 597–601.
33. Evgrafov OV, Mersiyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL and Schagina O, *et al*. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat Genet 2004, 36: 602–606.
34. van Montfort RL, Basha E, Friedrich KL, Slingsby C and Vierling E. Crystal structure and assembly of a eukaryotic small heat shock protein. Nat Struct Biol 2001, 8: 1025–1030.
35. Kim KK, Kim R and Kim SH. Crystal structure of a small heat-shock protein. Nature 1998, 394: 595–599.
36. Fu X, Zhang H, Zhang X, Cao Y, Jiao W, Liu C and Song Y, *et al*. A dual role for the N-terminal region of *Mycobacterium tuberculosis* Hsp16.3 in self-oligomerization and binding denaturing substrate proteins. J Biol Chem 2005, 280: 6337–6648.
37. Cheng G, Basha E, Wysocki VH and Vierling E. Insights into small heat shock protein and substrate structure during chaperone action derived from hydrogen/deuterium exchange and mass spectrometry. J Biol Chem 2008, 283: 26634–26642.
38. Blakytny R, Carver JA, Harding JJ, Kilby GW and Sheil MM. A spectroscopic study of glycated bovine alpha-crystallin: investigation of flexibility of the C-terminal extension, chaperone activity and evidence for diglycation. Biochim Biophys Acta 1997, 1343: 299–315.
39. Carver JA and Lindner RA. NMR spectroscopy of alpha-crystallin. Insights into the structure, interactions and chaperone action of small heat-shock proteins. Int J Biol Macromol 1998, 22: 197–209.
40. Basha E, O'Neill H and Vierling E. Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. Trends Biochem Sci 2012, 37: 106–117.
41. Sobott F, Benesch JL, Vierling E and Robinson CV. Subunit exchange of multimeric protein complexes. Real-time monitoring of subunit exchange between small heat shock proteins by using electrospray mass spectrometry. J Biol Chem 2002, 277: 38921–38929.
42. Hanazono Y, Takeda K, Yohda M and Miki K. Structural studies on the oligomeric transition of a small heat shock protein, StHsp14.0. J Mol Biol 2012, 422: 100–108.
43. Hanazono Y, Takeda K, Oka T, Abe T, Tomonari T, Akiyama N and Aikawa Y, *et al*. Nonequivalence observed for the 16-meric structure of a small heat shock protein, SpHsp16.0, from *Schizosaccharomyces pombe*. Structure 2013, 21: 220–228.
44. Kokke BP, Leroux MR, Candido EP, Boelens WC and de Jong WW. *Caenorhabditis elegans* small heat-shock proteins Hsp12.2 and Hsp12.3 form tetramers and have no chaperone-like activity. FEBS Lett 1998, 433: 228–232.
45. Basha E, Jones C, Blackwell AE, Cheng G, Waters ER, Samsel KA and Siddique M, *et al*. An unusual dimeric small heat shock protein provides insight into the mechanism of this class of chaperones. J Mol Biol 2013, 425: 1683–1696.
46. Leroux MR, Ma BJ, Batelier G, Melki R and Candido EP. Unique structural features of a novel class of small heat shock proteins. J Biol Chem 1997, 272: 12847–12853.
47. Chang Z, Primm TP, Jakana J, Lee IH, Serysheva I, Chiu W and Gilbert HF, *et al*. *Mycobacterium tuberculosis* 16-kDa antigen (Hsp16.3) functions as an oligomeric structure *in vitro* to suppress thermal aggregation. J Biol Chem 1996, 271: 7218–7223.

48. Yang H, Huang S, Dai H, Gong Y, Zheng C and Chang Z. The *Mycobacterium tuberculosis* small heat shock protein Hsp16.3 exposes hydrophobic surfaces at mild conditions: conformational flexibility and molecular chaperone activity. Protein Sci 1999, 8: 174–179.
49. Gu L, Abulimiti A, Li W and Chang Z. Monodisperse Hsp16.3 nonamer exhibits dynamic dissociation and reassociation, with the nonamer dissociation prerequisite for chaperone-like activity. J Mol Biol 2002, 319: 517–526.
50. Jiao W, Qian M, Li P, Zhao L and Chang Z. The essential role of the flexible termini in the temperature-responsiveness of the oligomeric state and chaperone-like activity for the polydisperse small heat shock protein IbpB from *Escherichia coli*. J Mol Biol 2005, 347: 871–884.
51. Jiao W, Hong W, Li P, Sun S, Ma J, Qian M and Hu M, *et al*. The dramatically increased chaperone activity of small heat-shock protein IbpB is retained for an extended period of time after the stress condition is removed. Biochem J 2008, 410: 63–70.
52. Shi X, Wang Z, Yan L, Ezemaduka AN, Fan G, Wang R and Fu X, *et al*. Small heat shock protein AgsA forms dynamic fibrils. FEBS Lett 2011, 585: 3396–3402.
53. Jiao W, Li P, Zhang J, Zhang H and Chang Z. Small heat-shock proteins function in the insoluble protein complex. Biochem Biophys Res Commun 2005, 335: 227–231.
54. Fu X, Shi X, Yan L, Zhang H and Chang Z. *In vivo* substrate diversity and preference of small heat shock protein IbpB as revealed by using a genetically incorporated photo-crosslinker. J Biol Chem 2013, 288: 31646–31654.
55. Fu X, Shi X, Yin L, Liu J, Joo K, Lee J and Chang Z. Small heat shock protein IbpB acts as a robust chaperone in living cells by hierarchically activating its multi-type substrate-binding residues. J Biol Chem 2013, 288: 11897–11906.
56. Bepperling A, Alte F, Kriehuber T, Braun N, Weinkauf S, Groll M and Haslbeck M, *et al*. Alternative bacterial two-component small heat shock protein systems. Proc Natl Acad Sci USA 2012, 109: 20407–20412.
57. Tabira Y, Ohara N, Ohara N, Kitaura H, Matsumoto S, Naito M and Yamada T. The 16-kDa alpha-crystallin-like protein of *Mycobacterium bovis* BCG is produced under conditions of oxygen deficiency and is associated with ribosomes. Res Microbiol 1998, 149: 255–264.
58. Mansfield MA and Key JL. Cytoplasmic distribution of heat shock proteins in soybean. Plant Physiol 1988, 86: 1240–1246.
59. Sudnitsyna MV, Mymrikov EV, Seit-Nebi AS and Gusev NB. The role of intrinsically disordered regions in the structure and functioning of small heat shock proteins. Curr Protein Pept Sci 2012, 13: 76–85.
60. Narberhaus F. Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiol Mol Biol Rev 2002, 66: 64–93.
61. Nakamoto H and Vigh L. The small heat shock proteins and their clients. Cell Mol Life Sci 2007, 64: 294–306.
62. Laskowska E, Matuszewska E and Kuczynska-Wisnik D. Small heat shock proteins and protein-misfolding diseases. Curr Pharm Biotechnol 2010, 11: 146–157.
63. Haslbeck M, Franzmann T, Weinfurtner D and Buchner J. Some like it hot: the structure and function of small heat-shock proteins. Nat Struct Mol Biol 2005, 12: 842–846.
64. Zeng L, Tan J, Lu W, Lu T and Hu Z. The potential role of small heat shock proteins in mitochondria. Cell Signal 2013, 25: 2312–2319.
65. Merck KB, Groenen PJ, Voorter CE, de Haard-Hoekman WA, Horwitz J, Bloemendal H and de Jong WW. Structural and functional similarities of bovine alpha-crystallin and mouse small heat-shock protein. A family of chaperones. J Biol Chem 1993, 268: 1046–1052.
66. Robertson AL, Headey SJ, Saunders HM, Ecroyd H, Scanlon MJ, Carver JA and Bottomley SP. Small heat-shock proteins interact with a flanking domain to suppress polyglutamine aggregation. Proc Natl Acad Sci USA 2010, 107: 10424–10429.
67. Duennwald ML, Echeverria A and Shorter J. Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans. PLoS Biol 2012, 10: e1001346.
68. Haslbeck M, Walke S, Stromer T, Ehrnsperger M, White HE, Chen S and Saibil HR, *et al*. Hsp26: a temperature-regulated chaperone. EMBO J 1999, 18: 6744–6751.
69. Tomoyasu T, Tabata A, Ishikawa Y, Whiley RA and Nagamune H. Small heat shock protein AgsA: an effective stabilizer of enzyme activities. J Biosci Bioeng 2013, 115: 15–19.
70. Das KP, Petrash JM and Surewicz WK. Conformational properties of substrate proteins bound to a molecular chaperone alpha-crystallin. J Biol Chem 1996, 271: 10449–10452.
71. Reddy GB, Narayanan S, Reddy PY and Surolia I. Suppression of DTT-induced aggregation of abrin by alphaA- and alphaB-crystallins: a model aggregation assay for alpha-crystallin chaperone activity *in vitro*. FEBS Lett 2002, 522: 59–64.
72. Leroux MR, Melki R, Gordon B, Batelier G and Candido EP. Structure-function studies on small heat shock protein oligomeric assembly and interaction with unfolded polypeptides. J Biol Chem 1997, 272: 24646–24656.
73. Rawat U and Rao M. Interactions of chaperone alpha-crystallin with the molten globule state of xylose reductase. Implications for reconstitution of the active enzyme. J Biol Chem 1998, 273: 9415–9423.
74. Carver JA, Lindner RA, Lyon C, Canet D, Hernandez H, Dobson CM and Redfield C. The interaction of the molecular chaperone alpha-crystallin with unfolding alpha-lactalbumin: a structural and kinetic spectroscopic study. J Mol Biol 2002, 318: 815–827.
75. Carver JA, Guerreiro N, Nicholls KA and Truscott RJ. On the interaction of alpha-crystallin with unfolded proteins. Biochim Biophys Acta 1995, 1252: 251–260.
76. Treweek TM, Lindner RA, Mariani M and Carver JA. The small heat-shock chaperone protein, alpha-crystallin, does not recognize stable molten globule states of cytosolic proteins. Biochim Biophys Acta 2000, 1481: 175–188.
77. Farahbakhsh ZT, Huang QL, Ding LL, Altenbach C, Steinhoff HJ, Horwitz J and Hubbell WL. Interaction of alpha-crystallin with spin-labeled peptides. Biochemistry 1995, 34: 509–516.
78. Lindner RA, Kapur A and Carver JA. The interaction of the molecular chaperone, alpha-crystallin, with molten globule states of bovine alpha-lactalbumin. J Biol Chem 1997, 272: 27722–27729.
79. Das KP, Choo-Smith LP, Petrash JM and Surewicz WK. Insight into the secondary structure of non-native proteins bound to a molecular chaperone alpha-crystallin. An isotope-edited infrared spectroscopic study. J Biol Chem 1999, 274: 33209–33212.
80. Lindner RA, Treweek TM and Carver JA. The molecular chaperone alpha-crystallin is in kinetic competition with aggregation to stabilize a monomeric molten-globule form of alpha-lactalbumin. Biochem J 2001, 354: 79–87.
81. Basha E, Lee GJ, Breci LA, Hausrath AC, Buan NR, Giese KC and 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, 279: 7566–7575.
82. Butland G, Peregrín-Alvarez JM, Li J, Yang W, Yang X, Canadien V and Starostine A, *et al*. Interaction network containing conserved and essential protein complexes in *Escherichia coli*. Nature 2005, 433: 531–537.
83. Waters ER, Nguyen SL, Eskandar R, Behan J and Sanders-Reed Z. The recent evolution of a pseudogene: diversity and divergence of a mitochondria-localized small heat shock protein in *Arabidopsis thaliana*. Genome 2008, 51: 177–186.

84. Waters ER and Vierling E. Chloroplast small heat shock proteins: evidence for atypical evolution of an organelle-localized protein. Proc Natl Acad Sci USA 1999, 96: 14394–14399.
85. Morrow G and Tanguay RM. Small heat shock protein expression and functions during development. Int J Biochem Cell Biol 2012, 44: 1613–1621.
86. Abulimiti A, Fu X, Gu L, Feng X and Chang Z. *Mycobacterium tuberculosis* Hsp16.3 nonamers are assembled and re-assembled via trimer and hexamer intermediates. J Mol Biol 2003, 326: 1013–1023.
87. Richter K, Haslbeck M and Buchner J. The heat shock response: life on the verge of death. Mol Cell 2010, 40: 253–266.
88. Voit EO and Radivoyevitch T. Biochemical systems analysis of genome-wide expression data. Bioinformatics 2000, 16: 1023–1037.
89. Muchowski PJ and Clark JI. ATP-enhanced molecular chaperone functions of the small heat shock protein human alpha-crystallin. Proc Natl Acad Sci USA 1998, 95: 1004–1009.
90. Soto A, Allona I, Collada C, Guevara MA, Casado R, Rodriguez-Cerezo E and Aragoncillo C, *et al*. Heterologous expression of a plant small heat-shock protein enhances *Escherichia coli* viability under heat and cold stress. Plant Physiol 1999, 120: 521–528.
91. Matuszewska E, Kwiatkowska J, Ratajczak E, Kuczynska-Wisnik D and Laskowska E. Role of *Escherichia coli* heat shock proteins IbpA and IbpB in protection of alcohol dehydrogenase AdhE against heat inactivation in the presence of oxygen. Acta Biochim Pol 2009, 56: 55–61.
92. Kuczyńska-Wiśnik D, Kedzierska S, Matuszewska E, Lund P, Taylor A, Lipińska B and 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: 1757–1765.
93. Sathish HA, Stein RA, Yang G and McHaourab HS. Mechanism of chaperone function in small heat-shock proteins. Fluorescence studies of the conformations of T4 lysozyme bound to alphaB-crystallin. J Biol Chem 2003, 278: 44214–44221.
94. Stengel F, Baldwin AJ, Bush MF, Hilton GR, Lioe H, Basha E and Jaya N, *et al*. Dissecting heterogeneous molecular chaperone complexes using a mass spectrum deconvolution approach. Chem Biol 2012, 19: 599–607.
95. Lee SY. EcoProDB databases. 2013 (http://eecoli.kaist.ac.kr/main.html).
96. Houry WA, Frishman D, Eckerskorn C, Lottspeich F and Hartl FU. Identification of *in vivo* substrates of the chaperonin GroEL. Nature 1999, 402: 147–154.
97. Friedrich KL, Giese KC, Buan NR and Vierling E. Interactions between small heat shock protein subunits and substrate in small heat shock protein-substrate complexes. J Biol Chem 2004, 279: 1080–1089.
98. Stengel F, Baldwin AJ, Painter AJ, Jaya N, Basha E, Kay LE and Vierling E, *et al*. Quaternary dynamics and plasticity underlie small heat shock protein chaperone function. Proc Natl Acad Sci USA 2010, 107: 2007–2012.
99. Xu Z, Horwich AL and Sigler PB. The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex. Nature 1997, 388: 741–750.
100. Merck KB, Horwitz J, Kersten M, Overkamp P, Gaestel M, Bloemendal H and de Jong WW. Comparison of the homologous carboxy-terminal domain and tail of alpha-crystallin and small heat shock protein. Mol Biol Rep 1993, 18: 209–215.
101. Basha E, Friedrich KL and 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: 39943–39952.
102. Ghosh JG, Shenoy AK and Clark JI. N- and C-terminal motifs in human alpha B crystallin play an important role in the recognition, selection, and solubilization of substrates. Biochemistry 2006, 45: 13847–13854.
103. Strózecka J, Chrusciel E, Górna E, Szymanska A, Ziętkiewicz S and Liberek K. Importance of N- and C-terminal regions of IbpA, *Escherichia coli* small heat shock protein, for chaperone function and oligomerization. J Biol Chem 2012, 287: 2843–2853.
104. Studer S, Obrist M, Lentze N and Narberhaus F. A critical motif for oligomerization and chaperone activity of bacterial alpha-heat shock proteins. Eur J Biochem 2002, 269: 3578–3586.
105. Fu X and Chang Z. Identification of bis-ANS binding sites in *Mycobacterium tuberculosis* small heat shock protein Hsp16.3: evidences for a two-step substrate-binding mechanism. Biochem Biophys Res Commun 2006, 349: 167–171.
106. Lambert W, Rutsdottir G, Hussein R, Bernfur K, Kjellström S and Emanuelsson C. Probing the transient interaction between the small heat-shock protein Hsp21 and a model substrate protein using crosslinking mass spectrometry. Cell Stress Chaperones 2013, 18: 75–85.
107. Shemetov AA and Gusev NB. Biochemical characterization of small heat shock protein HspB8 (Hsp22)-Bag3 interaction. Arch Biochem Biophys 2011, 513: 1–9.
108. Sharma KK, Kaur H and Kester K. Functional elements in molecular chaperone alpha-crystallin: identification of binding sites in alpha B-crystallin. Biochem Biophys Res Commun 1997, 239: 217–222.
109. Sharma KK, Kumar GS, Murphy AS and Kester K. Identification of 1,1′-bi(4-anilino)naphthalene-5,5′-disulfonic acid binding sequences in alpha-crystallin. J Biol Chem 1998, 273: 15474–15478.
110. Fu X and Chang Z. Identification of a highly conserved pro-gly doublet in non-animal small heat shock proteins and characterization of its structural and functional roles in Mycobacterium tuberculosis Hsp16.3. Biochemistry (Mosc) 2006, 71: S83–S90.
111. Giese KC and Vierling E. Mutants in a small heat shock protein that affect the oligomeric state. Analysis and allele-specific suppression. J Biol Chem 2004, 279: 32674–32683.
112. Lentze N, Studer S and Narberhaus F. Structural and functional defects caused by point mutations in the alpha-crystallin domain of a bacterial alpha-heat shock protein. J Mol Biol 2003, 328: 927–937.
113. Saji H, Iizuka R, Yoshida T, Abe T, Kidokoro S, Ishii N and Yohda M. Role of the IXI/V motif in oligomer assembly and function of StHsp14.0, a small heat shock protein from the acidothermophilic archaeon, *Sulfolobus tokodaii* strain 7. Proteins 2008, 71: 771–782.
114. Treweek TM, Ecroyd H, Williams DM, Meehan S, Carver JA and Walker MJ. Site-directed mutations in the C-terminal extension of human alphaB-crystallin affect chaperone function and block amyloid fibril formation. PLoS ONE 2007, 2: e1046.
115. Andley UP, Patel HC and Xi JH. The R116C mutation in alpha A-crystallin diminishes its protective ability against stress-induced lens epithelial cell apoptosis. J Biol Chem 2002, 277: 10178–10186.
116. Kumar LV, Ramakrishna T and Rao CM. Structural and functional consequences of the mutation of a conserved arginine residue in alphaA and alphaB crystallins. J Biol Chem 1999, 274: 24137–24141.
117. Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kayed R, Maloyan A and Robbins J. Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. Proc Natl Acad Sci USA 2004, 101: 10132–10136.
118. Jaya N, Garcia V and Vierling E. Substrate binding site flexibility of the small heat shock protein molecular chaperones. Proc Natl Acad Sci USA 2009, 106: 15604–15609.
119. Chin JW, Martin AB, King DS, Wang L and Schultz PG. Addition of a photocrosslinking amino acid to the genetic code of *Escherichia coli*. Proc Natl Acad Sci USA 2002, 99: 11020–11024.
120. Plater ML, Goode D and Crabbe MJ. Effects of site-directed mutations on the chaperone-like activity of alphaB-crystallin. J Biol Chem 1996, 271: 28558–62856.
121. Smulders RH, Merck KB, Aendekerk J, Horwitz J, Takemoto L, Slingsby C and Bloemendal H, *et al*. The mutation Asp69–>Ser affects the chaperone-like activity of alpha A-crystallin. Eur J Biochem 1995, 232: 834–838.
122. Mogk A, Schlieker C, Friedrich KL, Schönfeld HJ, Vierling E and Bukau B. Refolding of substrates bound to small Hsps relies on a disaggregation

reaction mediated most efficiently by ClpB/DnaK. J Biol Chem 2003, 278: 31033–31042.
123. Fu X and Chang Z. Temperature-dependent subunit exchange and chaperone-like activities of Hsp16.3, a small heat shock protein from *Mycobacterium tuberculosis*. Biochem Biophys Res Commun 2004, 316: 291–299.
124. Haslbeck M, Kastenmuller A, Buchner J, Weinkauf S and Braun N. Structural dynamics of archaeal small heat shock proteins. J Mol Biol 2008, 378: 362–374.
125. Bova MP, Huang Q, Ding L and Horwitz J. Subunit exchange, conformational stability, and chaperone-like function of the small heat shock protein 16.5 from *Methanococcus jannaschii*. J Biol Chem 2002, 277: 38468–38475.
126. Franzmann TM, Wuhr M, Richter K, Walter S and Buchner J. The activation mechanism of Hsp26 does not require dissociation of the oligomer. J Mol Biol 2005, 350: 1083–1093.
127. Franzmann TM, Menhorn P, Walter S and Buchner J. Activation of the chaperone Hsp26 is controlled by the rearrangement of its thermosensor domain. Mol Cell 2008, 29: 207–216.
128. Basha E, Jones C, Wysocki V and Vierling E. Mechanistic differences between two conserved classes of small heat shock proteins found in the plant cytosol. J Biol Chem 2010, 285: 11489–11497.
129. Raman B and Rao CM. Chaperone-like activity and temperature-induced structural changes of alpha-crystallin. J Biol Chem 1997, 272: 23559–23564.
130. Ecroyd H, Meehan S, Horwitz J, Aquilina JA, Benesch JL, Robinson CV and Macphee CE, *et al*. Mimicking phosphorylation of alphaB-crystallin affects its chaperone activity. Biochem J 2007, 401: 129–141.
131. Bukach OV, Seit-Nebi AS, Marston SB and Gusev NB. Some properties of human small heat shock protein Hsp20 (HspB6). Eur J Biochem 2004, 271: 291–302.
132. Shashidharamurthy R, Koteiche HA, Dong J and McHaourab HS. Mechanism of chaperone function in small heat shock proteins: dissociation of the HSP27 oligomer is required for recognition and binding of destabilized T4 lysozyme. J Biol Chem 2005, 280: 5281–5289.
133. Qiu H, Lizano P, Laure L, Sui X, Rashed E, Park JY and Hong C, *et al*. H11 kinase/heat shock protein 22 deletion impairs both nuclear and mitochondrial functions of STAT3 and accelerates the transition into heart failure on cardiac overload. Circulation 2011, 124: 406–415.
134. Peschek J, Braun N, Rohrberg J, Back KC, Kriehuber T, Kastenmüller A and Weinkauf S, *et al*. Regulated structural transitions unleash the chaperone activity of alphaB-crystallin. Proc Natl Acad Sci USA 2013, 110: E3780–E3789.