Cell. Mol. Life Sci. 62 (2005) 2460–2476
1420-682X/05/212460-17
DOI 10.1007/s00018-005-5190-4
© Birkhäuser Verlag, Basel, 2005

Cellular and Molecular Life Sciences

Review

Small heat shock proteins: molecular structure and chaperone function

Y. Sun and T. H. MacRae*

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1 (Canada), Fax: + 1 902 494 3736,
e-mail: tmacrae@dal.ca

Received 8 May 2005; received after revision 24 June 2005; accepted 19 July 2005
Online First 7 September 2005

Abstract. Small heat shock proteins (sHSPs) associate with nuclei, cytoskeleton and membranes, and as molecular chaperones they bind partially denatured proteins, thereby preventing irreversible protein aggregation during stress. sHSP monomers consist of a conserved α-crystallin domain of approximately 90 amino acid residues, bordered by variable amino- and carboxy-terminal extensions. The sHSPs undergo dynamic assembly into mono- and poly-disperse oligomers where the rate of disassembly affects chaperoning. The α-crystallin domain contains several β-strands organized into two β-sheets responsible for dimer formation, the basic building block of most sHSPS. The amino-terminal extension modulates oligomerization, subunit dynamics and substrate binding, whereas the flexible carboxy-terminal extension promotes solubility, chaperoning and oligomerization, the latter by inter-subunit linkage. Crystallization studies have revealed sHSP structure and function. Additionally, site-directed mutagenesis, biophysical investigations, functional studies and the discovery of relationships between mutated sHSPs and diseases have illuminated the role of sHSP within cells.

Key words. Molecular chaperones; small heat shock proteins; the α-crystallin domain; amino- and carboxy-terminal extensions; oligomerization; chaperoning; stress resistance.

Introduction

Functional proteins assume discrete but flexible three-dimensional structures determined by their amino acid sequences. In cells, where RNA and protein concentrations reach 200–400 mg/ml, the thermodynamic activity of macromolecules increases and protein diffusion decreases, changes with opposite effects on biochemical reaction rates including those of protein folding [1–4]. Not only is the intracellular milieu crowded, but the greater complexity of eukaryotic as compared with prokaryotic cells correlates with expansion in protein size and domain number. This favours a shift from post-translational to cotranslational protein folding, where successive molecular domains acquire higher-order structure as they are manufactured [5, 6]. Cotranslational folding sequesters hydrophobic residues, protecting nascent proteins from aggregation during synthesis and upon release from ribosomes. However, protein domains may fold partially, forming molten globules with sufficient exposed hydrophobicity to allow aggregation. Crowding enhances chaperone function, although, along with greater protein complexity, it also leads to nonproductive polypeptide interactions.

How proteins reach and maintain stable conformations, especially in the face of physiological stress arising from adverse environmental conditions and disease, are fundamental issues with important implications for cells. Protein folding requires the assistance of molecular chap-

* Corresponding author.

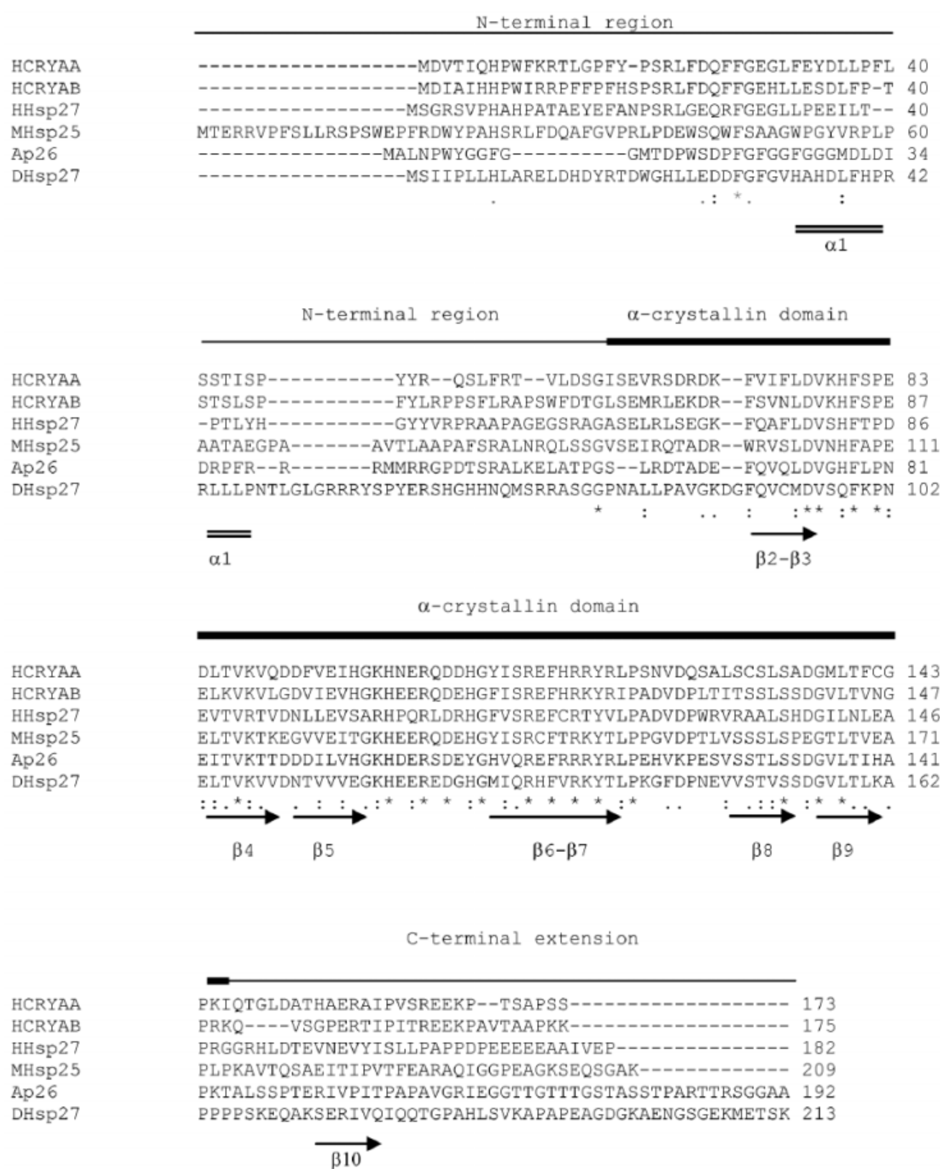

Figure 1. sHSP sequence alignment. Selected sHSP amino acid sequences were aligned by CLUSTAL W (1.82) to show the conserved α-crystallin domain bordered by variable amino- and carboxy-terminal regions and to position secondary structure elements. HCRYAA, *Homo sapiens* αA-crystallin, P04289; HCRYAB, *H. sapiens* αB-crystallin, P02511; HHsp27, *H. sapiens* Hsp27, NP_001532; MHsp25, *Mus musculus* Hsp25, JN0679; Ap26, *Artemia franciscana* p26, AAB87967; DHsp27, *Drosophila melanogaster* Hsp27, P02518. (-) no amino acid residue; (*) identical residues; (:) conserved substitution; (.) semi-conserved substitution. sHSP domains are indicated above the alignment, and the predicted secondary structure of *Artemia franciscana* p26 is depicted under the alignment. Residue number is indicated on the right.

erones, including Hsp60 (chaperonins), Hsp70, Hsp90, Hsp104/ClpB, Hsp110 and the small heat shock proteins (sHSPs) [6–14]. Chaperones actively mediate folding, some assist protein destruction through the action of an ubiquitin ligase called CHIP [12], and others protect proteins from irreversible damage upon exposure to heat, toxicants and hypoxia/anoxia, all processes characterized by transient interaction of chaperones with substrates.

Of the molecular chaperones, the sHSPs play a critical role in organismal defence during physiological stress where they protect proteins from irreversible aggregation by an energy-independent process until suitable conditions pertain for renewed cell activity, at which time protein release and refolding are mediated by ATP-dependent chaperones such as Hsp70 [14–21]. The sHSPs are phylogenetically widespread and function within several subcellular compartments where their ability to bind and protect proteins ensures a critical role in the chaperone network. To understand their mechanism of action, the sHSPs have been analyzed extensively, revealing proteins of 12–43 kDa with the ability to construct dynamic oligomers, disassembly of which is usually required for effective chaperoning. Representative sequences (fig. 1), of the many available, demonstrate sHSP monomers are composed of a conserved α-crystallin domain, enriched in β-strands organized in a β-sheet sandwich responsible for dimer formation, the basic structural unit of many sHSPs. An amino-terminal extension, variable in length and sequence and thought to influence higher-order oligomerization, subunit dynamics and chaperoning, attaches to one end of the α-crystallin domain. At the other end, the charged, highly flexible carboxy-terminal exten-

sion stabilizes oligomers while mediating sHSP solubility and chaperoning, roles that are, like those proposed for the amino-terminal extension, linked to one another. Elucidation of sHSP structure and function received a decided boost upon crystallization of *Methanococcus jannaschii* Hsp16.5 [22, 23] and *Triticum aestivum* Hsp16.9 [24], which allowed interpretation at the molecular level of data derived from site-directed mutagenesis, in vitro chaperoning assays and biophysical studies, among other approaches. These and related findings presented herein lead to a model of sHSP design and function that continues to evolve and shows promise for theoretical as well as practical exploitation [25, 26].

sHSP substrates

Proteins are major substrates of the sHSPs

sHSPs confer stability on the cell proteome by protecting diverse proteins engaged in signal transduction, metabolism, translation, transcription and other activities [27–29]. Partially denatured proteins in an unstable molten globule state bind sHSPs, suppressing non-specific, irreversible aggregation [30, 31]. sHSPs tend not to interact with native proteins, entirely unfolded proteins or stable monomeric molten globule state proteins, nor do they appear to assist with folding, although uncertainty surrounds the extent of sHSP association with folding proteins [31, 32]. Substrates range from peptides to oligomeric proteins without sequence or structure specificity, and because potentially at least one target molecule binds per subunit, the sHSPs prevent protein denaturation efficiently [33-35]. Several different proteins incorporate into individual sHSP-substrate complexes, an important functional attribute in the crowded cell because many polypeptides require protection during stress [34, 36]. To accommodate substrates there are low (transient) and high (stable) affinity binding sites, as for the α-crystallins [37, 38], regions potentially affected by ATP binding [39], although sHSP chaperoning is generally considered to be nucleotide independent. Substrates are released from sHSPs upon stress termination, probably by the action of energy-requiring chaperones such as Hsp70, and they refold with co-operation from the same chaperones, as discussed in more detail later [28, 29, 34, 40, 41]. By interacting with a wide range of proteins, the sHSPs protect cells from oxidative stress [42], heat [27, 29, 43] and apoptosis [44, 45], the latter by modulation of signalling pathways.

Cytoskeletal elements as sHSP substrates

sHSPs associate with microfilaments, intermediate filaments and microtubules, the cytoskeletal elements of eukaryotic cells [46–50]. Up-regulation of αB-crystallin stabilizes glial fibrillary acidic protein (GFAP) filaments, while Hsp27 and αB-crystallin interact with intermediate filaments, preventing network formation and regulating spatial organization [51, 52]. Additionally, αB-crystallin and desmin, a type III intermediate filament, couple specifically within cardiac muscle cells [53]. p26, a sHSP from *Artemia franciscana*, affiliates with tubulin, the major structural protein of microtubules, preventing denaturation and potentially increasing encysted embryo stress resistance [47]. As a consequence of tubulin recognition, p26 may inhibit mitosis by disrupting microtubule formation and contribute to developmental arrest in oviparously developing *Artemia* embryos [54]. αB-crystallin is reported to bind microtubules by way of microtubule-associated proteins leading to increased microtubule stability [55], and when phosphorylated at Ser-59 the protein localizes to centrosomes and midbodies [56]. The relationship between sHSPs, actin and microfilaments has been well studied, with phosphorylation receiving particular attention [50]. Monomeric, non-phosphorylated Hsp25/27 caps actin and inhibits microfilament formation, but phosphorylated monomers and non-phosphorylated multimers do not affect polymerization [57]. Peptides corresponding to residues W43-R57 and I92-N106 of murine Hsp25 strongly inhibit actin assembly, with I92-N106 effectiveness diminished by phosphorylation. The homologous αB-crystallin peptide has a similar response to phosphorylation [58]. Wild-type Hsp27 and a pseudo-phosphorylated derivative, but not a non-phosphorylatable variant, stabilize microfilaments against heat, oxidative stress and actin-reactive drugs [59-61], with comparable protection by phosphorylated Hsp25 in rat embryonic H9c2 myoblasts [62]. sHSPs recognize actin in vitro, preventing salt-induced formation of insoluble aggregates [49], and Hsp20 is dynamically associated with actinin, an actin cross-linking protein [63]. This cursory view demonstrates that sHSPs maintain and remodel the cytoskeleton while confirming these intracellular polymers as important sHSP substrates.

Membranes interact with sHSPs

Nonameric Hsp16.3, a major membrane protein of *Mycobacterium tuberculosis*, generates dimers upon combining with positively charged lipid layers, and is thought to protect the bacterium from reactive oxygen species of the macrophage defence system [64–66]. Reaction with the *M. tuberculosis* membrane is reversible, may involve subunit exchange with soluble Hsp16.3 and requires oligomer dissociation [64]. Oxidation of non-saturated membrane lipids, if not prevented by Hsp16.3, decreases membrane fluidity and increases permeability, leading to detrimental effects on bacterial cells [66]. A portion of lens α-crystallin resides in the plasma membrane [67], suggesting that maintenance of membranes, among the

most stress sensitive of cell components, is an important sHSP action under physiological conditions [20]. Hsp17 effects on *Synechocystis* membranes are relatively short term after stress exposure, and much of the induced sHSP interacts with thylakoids [40, 68, 69]. Hsp17 modulates the physical state of thylakoid membranes, a notion supported by increased membrane fluidity in mutants lacking the chaperone [40, 69, 70]. Hsp17 and α-crystallin, in concert with molecular chaperones such as GroEL, may regulate membrane fluidity by stabilizing the liquid crystalline state and lowering heat induced hyperfluidity.

sHSP substrates in the nucleus

Tomato Hsp16.1-CIII contains a nuclear localization signal between β-strands 5 and 6 of the α-crystallin domain and the protein resides mainly in nuclei, although by joining with other sHSPs it localizes to the cytoplasm [71]. Hsp27 migrates into transfected A549 cell nuclei during stress, but protection is not dependent on nuclear localization [72]. Rat myocardial cell Hsp20 occupies nuclei upon heat shock [73], and in normal non-stressed tissues, human testis-specific HspB9 occurs predominately in nuclei [74]; however, the consequences of nuclear placement are unknown. HspB9 lacks a nuclear localization signal, and translocation into nuclei may depend on interaction with TCTEL1, a small polypeptide associated with dynein, a microtubule-dependent motor. p26 enters nuclei early in *A. franciscana* oviparous development [75], during heat stress and upon pH reduction in vitro [76]. Thus, several sHSPs enter, and presumably function, in nuclei.

Hsp27, Hsp25 and αB-crystallin bind nucleoli and speckles, intranuclear structures populated by splicing factors, possibly regulating molecular processes or protecting proteins [77–80]. Immunofluorescent staining of cultured C2C12 myoblasts and myotubes discloses the intranuclear position of αB-crystallin and Hsp25, showing that interaction with speckles varies as cells differentiate [78]. Localization of αB-crystallin to HeLa cell nuclear speckles is affected by pseudo-phosphorylation, but exact effects are under debate [77]. Of concern for this work, the polyclonal serum K79, which recognizes the 13 carboxy-terminal residues of αB-crystallin, labels nuclear structures in lens epithelial cells from wild-type and αB (–/–) mice lacking αB-crystallin. Staining specificity is questioned and doubt cast on work employing this particular antibody [77]. Further complicating the issue, localization studies are influenced by differing cell stress responses, making extrapolation from one cell type to another difficult.

Functional relationships between sHSPs and nucleic acids are evoked in experiments where mouse α-crystallin binds αD/E/F-crystallin genes [81] and bovine lens α-crystallin recognizes single-stranded and double-stranded DNA [82, 83]. sHSPs may also protect messenger RNAs (mRNAs) during stress-induced translational arrest [84, 85], but whether RNA interaction is direct or via intermediary proteins remains uncertain.

Clearly, sHSPs interact with many essential molecules comprising different cell compartments and processes. They are, as a consequence of this promiscuity, fundamentally important to all organisms, either as a first line of defence during stress or while in pursuit of normal affairs. The remainder of the review is, therefore, dedicated to considering the structural properties of sHSPs and integrating these observations into mechanistic aspects of function.

sHSP crystallization and the α-crystallin domain

Hsp16.5 from the thermophilic archae *M. jannaschii*, the first sHSP crystallized, is a homogeneous complex of 24 subunits arranged in octahedral symmetry (fig. 2). The oligomer is a hollow sphere with eight triangular and six square windows large enough to allow entry of small molecules and extended peptides [22, 23]. Each Hsp16.5 monomer exhibits an immunoglobulin fold, although lacking sequence similarity to immunoglobulins, and contains nine α-strands in two parallel sheets, a pair of 3_{10}-helices, and an additional short β-strand. A monomer contains β-strands 1, 7, 5 and 4 in one β-sheet and 2, 3, 9 and 8 in the other, along with β-strand 6 of the adjacent subunit. Several contacts exist between Hsp16.5 monomers during dimerization with the β2-strand of one subunit hydrogen bonded to the β6-strand of a neighbouring subunit. Except for the amino-terminal 32 residues which may reside in the central cavity of the oligomer, the Hsp16.5 sequence, including the α-crystallin domain and carboxy-terminal extension, is ordered.

The second sHSP crystal structure, achieved at 2.7 Å resolution, is for wheat *T. aestivum* Hsp16.9, and although similar to Hsp16.5, interesting differences are present (figs. 2, 3) [24]. Hsp16.9, a class I cytosolic plant sHSP defined on cell localization and sequence, prevents aggregation of malate dehydrogenase upon heating and exists as a larger oligomer than class II wheat Hsp17.8 [43]. Hsp16.9 assembles a dodecameric disk arranged in two rings each of three dimers, with the oligomer:dimer ratio shifted to favour dimers at elevated temperature [24, 86]. Hydrogen deuterium exchange and mass spectrometry indicate Hsp16.9 dimers are stable and chaperone activity is enhanced by dodecamer dissociation in the absence of dimer structural change [86]. The Hsp16.9 α-crystallin domain contains a β-sandwich of two antiparallel β-sheets, constructed of β-strands 2, 3, 8, 9 and 4, 5, 7, but β-strand 1 of Hsp16.5 is absent. The extended dimerization loop comprising residues 83–105 connects β-strands 5 and 7, and there is a short β-strand termed

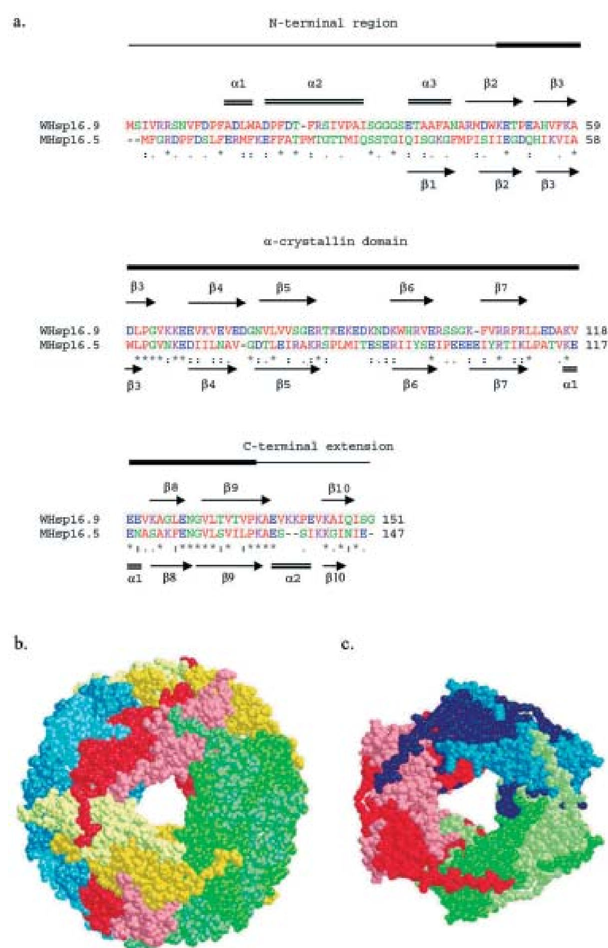

Figure 2. Structural comparison of *M. jannaschii* Hsp16.5 and *T. aestivum* Hsp16.9. *(a)* The amino acid sequences of *M. jannaschii* Hsp16.5 and *T. aestivum* Hsp16.9 were aligned by CLUSTAL W (1.82). WHsp16.9, *T. aestivum* Hsp16.9, 1GME_A; MHsp16.5, *M. jannaschii* Hsp16.5, Q57733. (-) no amino acid residue; (*) identical residues; (:) conserved substitution; (.) semi-conserved substitution. The secondary structure of *T. aestivum* Hsp16.9 is depicted above the alignment and the secondary structure of *M. jannaschii* Hsp16.5 is below the alignment. Amino acid residues in red, small and hydrophobic; blue, acidic; magenta, basic; green, contain a hydroxyl or amine group. Residue number is indicated on the right. *(b)* Quaternary structure of *M. jannaschii* Hsp16.5. The spherical 24-mer has an outer diameter of 12 nm and an inner diameter of 6.5 nm; image resolution is 0.29 nm. *(c)* Quaternary structure of *T. aestivum* Hsp16.9 arranged as a dodecameric double disk which is approximately 9.5 nm wide and 5.5 nm high with a central cavity of about 2.5 nm; image resolution is 0.27 nm. Adapted, with permission, from [23, 24].

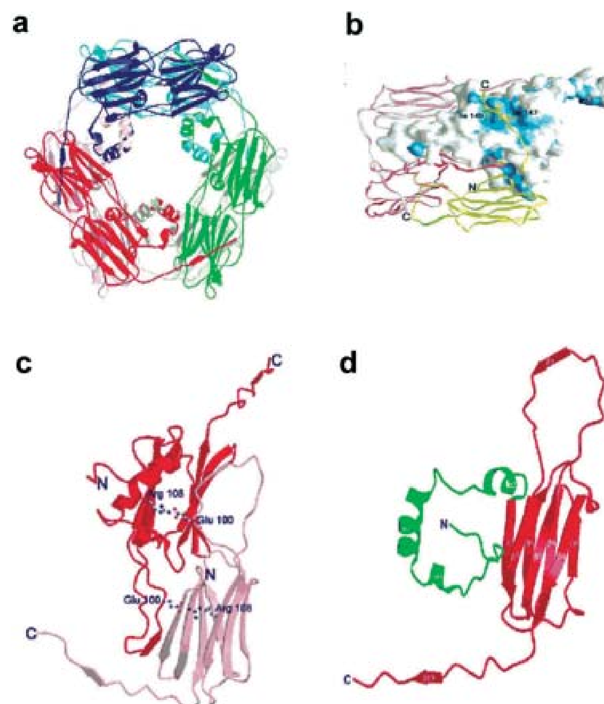

Figure 3. Structure of *T. aestivum* Hsp16.9. *(a)* An overview looking down the threefold axis of wheat Hsp16.9, which is perpendicular to three crystallographic twofold axes. Dimers in the top disk are displayed in red, green and blue, while dimers in the bottom disk are in pink, sage and turquoise. The interface that forms an eclipsed tetramer with the bottom dimers hidden when viewed down the crystallographic threefold axis is located between red-pink, blue-turquoise and green-sage dimers. *(b)* An outside view of one side of the Hsp16.9 dodecamer, showing how dimers from the top and bottom disks are related by a twofold axis to form an eclipsed tetramer. In the top dimer, the monomer with a disordered amino-terminal arm is shown as a pink ribbon, while the monomer with an ordered arm appears as a solid molecular surface, with hydrophobic patches shown in blue. Monomers of the bottom dimer are represented by yellow and red ribbons. Residues I147 and I149 of the I/VXI/V motif in the carboxy-terminal extension of the bottom monomer (yellow) extend from one dimer to another, covering hydrophobic grooves which are putative substrate binding sites in the α-crystallin domain. *(c)* An Hsp16.9 dimer composed of a complete monomer (red) and an amino-terminal arm-disordered monomer (pink). Amino (N)- and carboxy (C)-terminals are labeled, as are glutamic acid 100 and arginine 108, oppositely charged amino acid residues which form an intermolecular salt bridge in the dimerization loop. *(d)* Ribbon diagram of an Hsp16.9 monomer with the ordered amino-terminal arm shown in green, while the α-crystallin domain and the carboxy-terminal extensions are in red. Adapted, with permission, from [24].

β6. The dimerization loop interacts with a β-sheet in an adjacent monomer and is an important part of the interface between dimer subunits. The amino-terminal region, composed of intertwining α-helical domains resolved in 6 of 12 oligomer subunits, sustains interactions between monomers in separate disks. The final 10 residues of the carboxy-terminal extensions in monomers with disordered amino-terminal arms embrace the adjacent dimer in the partner disk, stabilizing the dimer-dimer interface. In contrast, the carboxy-terminal extensions of monomers with ordered amino-terminals link dimers in the same oligomeric disk but in adjacent tetramers. The intermolecular contacts made by carboxy-terminal extensions involve the conserved V/IXI/V motif, where isoleucines 147 and 149 of β-strand 10 deploy in a hydrophobic groove separating β-strands 4 and 8. The highly

conserved arginine 108 stabilizes dimers by formation of an intermolecular salt bridge with glutamic acid 100 in the dimerization loop. This contrasts Hsp16.5 where the equivalent residue, arginine 107, hydrogen bonds to glycine 41, reinforcing the interaction between β-strands 1 and 2. Characterization of the conserved arginine is an important outcome of crystallization studies because mutation of this residue has profound effects on sHSPs [25, 51, 53, 87–89].

The Hsp16.5 and Hsp16.9 crystal structures shed light on the spatial orientation of residues within the α-crystallin domain as well as details of monomer-monomer and higher-order interactions. The β6 loop, involved in Hsp16.5 dimerization, is shortened in some sHSPs [20, 90, 91], which may limit its function, but the immunoglobulin-like fold of the α-crystallin domain is conserved. Hsp16.5 and Hsp16.9 oligomers possess a central cavity, with dimeric building blocks similar in shape and size to those of other sHSPs [65, 86, 92]. These structural similarities explain why different sHSPs share functions even though molecular mass, spatial symmetry and quaternary structure vary. Of the two crystallized sHSPs, Hsp16.9 is more useful for modeling other eukaryotic sHSP three-dimensional structures, and models corresponding to α-crystallin domains of human αA- and αB-crystallin are based on the wheat protein [93]. The models, excluding 61 amino-terminal and 27 carboxy-terminal residues of αA-crystallin, and 65 amino-terminal and 25 carboxy-terminal residues of αB-crystallin, portray β-strand shifts in the α-crystallin domain and variations in loops connecting β-strands. The loops between β-strands 5 and 7 of the two α-crystallins, for example, experience a deletion of seven amino acid residues, thereby modifying the dimer interface. Similar variations are found by modeling human αB-crystallin against *M. jannaschii* Hsp16.5 [94]. Although modeling demonstrates differences between human α-crystallins and the crystallized sHSPs, the involvement of β-strands 6 and 2 in neighbouring monomers appears to be a common parameter of dimer formation.

Mutations of the sHSP α-crystallin domain

Protein modeling based on the crystal structure of related proteins requires caution, but it indicates how point mutations and deletions perturb function, and in the absence of crystal structures for most sHSPs remains a useful method for molecular dissection. As a case in point, mutation of phenylalanine 94 in *Bradyrhizobium japonicum* HspH disrupts dimerization, leading to monomer formation and function loss, whereas modification of the conserved glycine at position 114 eliminates chaperone function without affecting oligomerization [90]. When considered in light of three-dimensional modeling, phenylalanine 94 resides in β-strand 7 near a putative dimerization loop focused on β-strand 6, suggesting how the change perturbs monomer interactions. Glycine 114 is housed in the conserved G-X-L motif of a short loop joining β-strands 8 and 9, but knowing this fails to show how glycine modification affects chaperone activity and not oligomerization [90]. Mutation of leucine 122 in *M. tuberculosis* Hsp16.3, located in the conserved motif GVLTVTV, and the equivalent leucine 116 in *B. japonicum* HspH, undermines chaperone function in both proteins, although the effect on oligomerization is greater for HspH than Hsp16.3 [90, 95]. Leucine 122 of Hsp16.3 corresponds to leucine 129 of *M. jannaschii* Hsp16.5, and is located at the start of β-strand 9 between β-strands 4 and 5, close to a potential hydrophobic motif of IIL at positions 67–69.

Chaperone activity and oligomerization decrease modestly upon mutation of several different residues within the α-crystallin domain of human αB-crystallin [94, 96]. In contrast, replacement of phenylalanine 71 with glycine in rat αA-crystallin almost completely eliminates chaperone ability even when determined by the reduction-induced denaturation of insulin at low temperature. Molecular mass, intrinsic tryptophane fluorescence and secondary structure are unaffected by the mutation, but surface hydrophobicity as measured by 1,1'-bi(4-anilino)naphthalene-5,5'-disulfonic acid (bis-ANS) binding increases, and a very small difference in tertiary structure occurs [97]. The results indicate phenylalanine 71 and several neighbouring residues form a chaperone site of αA-crystallin composed of residues 70–88. Although mutations in the α-crystallin domain tend to have limited effect on function, autosomal dominant cataract is caused by an R116C mutation in αA-crystallin [98], and desmin-related myopathy is associated with the αB-crystallin mutation R120G [99], both highly conserved sHSP arginines [25].

The sHSP α-crystallin domain is bracketed by amino- and carboxy-terminal extensions differing in sequence from species to species and between sHSPs from the same organism (fig. 1). These regions contribute to sHSP structural and functional variation, and they have been examined extensively by deletion and site-directed mutagenesis, with emphasis on oligomerization, subunit exchange and chaperone activity. Although sHSP amino- and carboxy-terminal extensions have diverged, consensus is emerging in regard to their characteristics.

The sHSP amino-terminal domain

sHSP oligomerization and chaperoning are influenced by the amino-terminus in several [86, 100-109], but not all [110, 111] sHSPs. The amino-terminal domain is susceptible to phosphorylation and, as shown by amide

hydrogen exchange, has a loose exposed structure. This agrees with data obtained by use of chemical cross-linkers and mass spectrometry to examine bovine α-crystallin [112], but contrasts the idea that the amino-terminus is hidden internally due to its hydrophobicity [23, 92, 113, 114]. Analysis of crystal structure indicates the 40 amino-terminal residues of wheat Hsp16.9 are housed within oligomer interiors, but they experience rapid hydrogen/deuterium exchange, suggesting the region undergoes conformational change and solvent exposure [24, 86].

An effective method for scrutiny of the sHSP amino-terminus is to delete peptides of defined length, allowing comparison of truncated and full-length proteins, keeping in mind the possibility of non-specific changes to protein structure upon elimination of large peptides. sHSPs from several eukaryotes have been examined in this manner, and as one early example, deletion of the rat αA-crystallin amino-terminus yields dimers and tetramers unable to protect βL-crystallin against heat-induced denaturation, even though far-ultraviolet (UV) circular dichroism (CD) measurements of truncated and full-length proteins are similar [115, 116]. Removing 19 residues from αA-crystallin has little effect on quaternary structure or subunit exchange, whereas deleting 56 or more amino-terminal residues reduces oligomers to units of 3–4 monomers and eliminates exchange reactions, indicating important functions for residues 20–56 [108]. Bovine αB-crystallin and mouse Hsp25 truncated at the amino-terminus produce oligomers of 18–20 and 12–13 subunits, respectively, both larger than for αA-crystallin but lacking chaperone activity [115]. In other work, αB-crystallin oligomerization depended on the amino-terminus, and the truncated, dimeric form of αB-crystallin prevented heat-induced alcohol dehydrogenase aggregation in vitro [117]. Substrate protection against heat-induced denaturation by rice (*Oryza sativa*) Hsp16.9 is compromised by dispensing with 42 amino-terminal residues, but unexpectedly, the truncated derivative produces oligomers slightly larger than those of full-length protein [111]. Without an amino-terminal domain the 24 subunit complex formed by yeast Hsp26 reduces to a dimer and chaperone activity declines, indicating the importance of the region [104].

Eliminating 11 residues from the amino-terminus of *Escherichia coli* IbpB, a polydisperse sHSP, by either proteolytic digestion or recombinant technology, leads to dimer formation and loss of chaperone activity [100]. In comparison, removing the amino-terminus of *M. tuberculosis* Hsp16.3 dissociates the nonameric oligomer into trimers, the sHSP building block [118], and deleting more than 5 amino-terminal residues from either HspH or HspF of *B. japonicum* reduces multimerization and chaperone activity [106]. Oligomers of eight subunits devoid of chaperone activity arise upon moderate amino-terminal truncation of *B. japonicum* sHSPs, suggesting that a relatively intact amino-terminal domain is required for oligomerization and chaperoning, while a smaller portion of the region facilitates dimer formation.

For some sHSPs the amino-terminus influences monomer-monomer interaction and the region is required, but not sufficient, for oligomerization. As a case in point, an amino-terminal deletion mutant of *M. jannaschii* Hsp16.5 behaves like its full-length counterpart, suggesting, in contrast to examples just described, the region lacks a critical role in maintaining oligomer structure but assists in monomer organization before oligomerization [110, 118]. In this model, the hydrophobic amino-terminus holds α-crystallin domains in close proximity long enough to enhance oligomer assembly. To extrapolate, the amino-terminal regions of all sHSPs may encourage oligomerization by positioning monomers in such a way that stable interactions between other regions of the molecule are promoted, whereas the amino-terminals of most sHSPs also assume the related function of holding monomers in dynamic complexes.

Modification of individual residues and short internal peptides by site-directed mutagenesis is employed to reveal functional characteristics of the sHSP amino-terminal domain. Thermo-protective activity by Chinese hamster Hsp27, a sHSP for which the amino-terminus stabilizes oligomers [109], requires phosphorylation of serine residues 90 and 15, while phosphorylation of serine 90 is sufficient to dissociate oligomers [119]. Replacing serine 90 with alanine and eliminating phosphorylation of this site inhibits stress-induced dissociation of oligomers and eliminates chaperone activity. However, more than dissociation is required for Hsp27 function because replacement of serine 15 with alanine allows dissociation but the protein is inactive. The WD/EPF motif, located in the amino-terminus of many sHSPs, maintains Hsp27 oligomer structure and chaperone activity [119]. Three-dimensional modeling demonstrates homology between the residues around the Hsp27 WD/EPF motif and SXXFDPF of Hsp16.9, the latter thought to replace the β-strand 1 of Hsp16.5 and mediate inter- and intramolecular interactions within oligomers. Serine 90 is positioned near the centre of monomer β-sheets and adjacent to serine 90 in the neighbouring monomer, suggesting that introduction of negative charges by phosphorylation contributes to destabilization. Thus, the WD/EPF motif potentially associates with isoleucine 96, glutamine 98, tyrosine 150 and proline 153, residing at the β2/β7 strand interface in the α-crystallin domain of the same monomer. Phosphorylation of serine 90 destabilizes the interaction and facilitates oligomer dissociation; with phosphorylation of serine 15, the motif is liberated and free to chaperone substrates [119].

Human αA- and αB-crystallin internal deletion mutations lacking residues SRLFDQFFG at positions 20–28 and

21–29, respectively, yield oligomers diminished in size with increased bis-ANS binding and weakened stability to urea-induced denaturation, indicating the sequence is an important determinant of quaternary structure [120]. The mutated αA-crystallin is more dynamic than the wild-type protein, and both mutated crystallins possess higher chaperone activity than unmodified proteins. Residues SRLFD within the deleted motif are analogous to wheat Hsp16.9 7-SNVFD-11, which contacts arginine 109 and phenylalanine 110 in the α-crystallin domain and contributes inter- and intra-molecular contacts. The results support the idea that oligomer dissociation is important for chaperoning, but suggest the conserved motif lacks a direct role such as substrate binding, as proposed for the WD/EPF sequence [119]. Replacing phenylalanines 24 and 27 within the conserved amino-terminal motif 22-RLFDQFF-28 of murine αB-crystallin with positively charged arginines disrupts the adjacent area and impairs chaperoning determined by heat-induced aggregation of γ-crystallin, the reduction of insulin by incubation with dithiothreitol (DTT) [121], and the decreased viability of transfected cells exposed to KCl and heat [122]. However, the equivalent mutation in human αB-crystallin fails to affect chaperone ability measured by insulin reduction at 25 °C and heat induced aggregation of α-lactalbumin at 37 °C, indicating the residue is not essential [123], a conclusion supported by mutation of phenylalanine 28 to serine in bovine αB-crystallin [124]. The modified αB-crystallin is potentially unstable at elevated temperature and this, along with using recombinant proteins from different species in these studies, may explain the variation in results [96, 123, 124].

The initial 12 amino-terminal residues, MSLIPSFFSGRR, within pea Hsp18.1, a dodecameric sHSP containing the FDPF motif at residues 16–19, bind bis-ANS, indicating surface hydrophobicity. Bis-ANS binding elevates with rising temperature and declines when Hsp18.1 associates with substrate. However, the capacity of MSLIPSFFSGRR as a substrate binding site is uncertain with this doubt reinforced, albeit indirectly, by detection of a bis-ANS binding site in the α-crystallin domain, the latter a possible chaperoning region [34].

The sHSP carboxy-terminal extension

Truncation and other modifications reveal important features of the sHSP carboxy-terminal extension, a flexible region enriched in polar and charged amino acid residues existing as a solvent-exposed random coil [35, 96, 100, 103, 113, 125–128] (fig. 1). Removal of 11 residues from the carboxy-terminus of *E. coli* IbpB leads to dimer formation and loss of chaperone activity [100]. Progressive deletion of the carboxy-terminus from rat and human αA-crystallin dramatically decreases oligomerization and alters tertiary structure once 11 or more residues are removed, with oligomer mass reduced from approximately 550 to 150 kDa [129]. Arginine 163, the 11th residue from the carboxy-end, may be particularly important because oligomerization declines once it is deleted. Truncations of less than 10 residues either slightly improve chaperoning or impose small deleterious effects, agreeing with experiments where αA-crystallin oligomerization was unaffected by loss of 10 carboxy-terminal residues [108]. Eliminating 11 residues from αA-crystallin moderately damages chaperoning, whereas truncations of greater length have severe consequences [108], as shown by the inability of bovine lens α-crystallin to protect alcohol dehydrogenase against heat-induced aggregation upon tryptic removal of 16 carboxy-terminal residues [130]. Deletion of 17 carboxy-terminal amino acid residues reduces human αA-crystallin solubility and produces larger than normal oligomers. The latter was possibly influenced by resolubilization of recombinant αA-crystallin in urea, which changes the size of oligomers formed from full-length protein and alters the near-UV CD spectrum of wild-type and truncated proteins [131]. Chaperone activity of the modified αA-crystallin, determined by inhibition of temperature-dependent aldolase aggregation and singlet oxygen-induced γD-crystallin denaturation, is reduced in comparison with normal protein. In these experiments, wild-type and truncated αA-crystallins were renatured in urea, discounting this explanation for functional differences and implicating the carboxy-terminus in chaperone activity [131]. The application of mass spectrometry shows that deletion of 5 carboxy-terminal residues from αA-crystallin reduces the average oligomer molecular mass from 540 kDa to 440 kDa, lowers poly-dispersity and severely impairs subunit exchange rate, but chaperone activity is unchanged [125]. Importantly, oligomer dissociation appears less important for poly-disperse αA-crystallin as a determinant of chaperoning activity than for mono-disperse sHSPs, and intact oligomers are the αA-crystallin chaperone units. Additionally, that the carboxy-terminus promotes polydispersity is supported by these findings.

Flexibility of the carboxy-terminal αA- and αB-crystallin extensions is maintained upon formation of substrate-sHSP complexes under reducing conditions, indicating that flexibility is important for chaperone activity [132]. Corroborating this possibility, introducing a hydrophobic residue such as tryptophan into the carboxy-terminal extension of αA-crystallin reduces protein flexibility, solubility and thermostability [96, 127]. Chaperone action measured by DTT-induced denaturation of insulin at 40 ºC and heat-induced aggregation of β-low crystallin at 55 °C is diminished, but there is little effect on oligomer assembly. However, the tryptophan containing αA-crys-

tallin mutant provides complete protection against malate dehydrogenase inactivation by glycation at 37°C [96]. The small temperature variation between insulin and malate dehydrogenase based assays is enough to change αA-crystallin secondary structure, possibly explaining the different results and providing a cautionary note when chaperone assays are done at increased temperature.

Eliminating the pair of carboxy-terminal lysines from porcine αB-crystallin, a region with reduced flexibility upon substrate binding [126], leaves a protein similar in secondary structure and surface hydrophobicity to wild type, but with smaller oligomers and superior heat stability [133]. In other experiments, doing away with the 5 carboxy-terminal residues of αB-crystallin, including the two lysines, has no effect on chaperone activity [35], whereas lysine mutagenesis to either leucine or glycine produces oligomers of normal size containing polypeptides structurally similar to wild type, but with less chaperone activity [121]. Mouse Hsp25 lacking the 18-residue carboxy-terminal extension forms oligomers similar in mass to those of wild-type protein, indicating the region is dispensable for oligomerization [132]. The truncated Hsp25 fails to protect α-lactalbumin against DTT-induced precipitation, but shields citrate synthase against heat-induced aggregation. In comparison to wild type, mutated Hsp25 has a smaller amount of ANS-available surface hydrophobicity, minimized stability to heat shown by precipitation from solution at 55 °C, and as indicated by ^{1}H nuclear magnetic resonance (NMR), added flexibility. Disruption of a β-strand which interacts with an adjoining subunit and strengthens the oligomer may explain reduced stability. The maximum oligomer size of human Hsp27 increases upon subtracting 24 carboxy-terminal residues, possibly due to modified protein solubility, but the proportion of protein as large oligomers is smaller than for wild-type Hsp27 [102].

The sHSP carboxy-termnal extension contains a relatively well conserved V/IXI/V motif. Eliminating either 5 or 15 amino acid residues from the extreme carboxy-terminus of *B. japonicum* HspH leaves the V/IXI/V motif intact, and is without effect on protection of citrate synthase against thermal denaturation in vitro, even though truncated variants are less soluble than wild-type protein [106]. Oligomerization is also indifferent to the shorter truncation; however, removing 15 residues yields oligomers larger than those obtained with full-length protein. Loss of 20 carboxy-terminal residues, including the V/IXI/V motif, completely destroys HspH chaperone activity and obstructs oligomer formation, with dimers as the maximum complex, findings duplicated by substituting alanine for either one or both isoleucines in the motif [106]. Interestingly, rat Hsp22, with amino- and carboxy-terminal regions comparable in length to most other sHSPs, lacks the V/IXI/V sequence and exists as monomers, suggesting a role in oligomerization [134]. Binding of the carboxy-terminal extension to a groove in the α-crystallin domain, as shown by crystal structure analysis, involves the V/IXI/V motif. Oligomers are stabilized by shielding hydrophobic residues from solvent and promoting interdimeric association [23, 24, 135], explaining results obtained with HspH and rat Hsp22, and upon insertion of the V143A mutation into *Synechocystis* Hsp16.6. The mutation changes valine 143, which normally associates with L66 in a hydrophobic patch of the α-crystallin domain, yielding dimeric Hsp16.6, reduced chaperone activity in vitro, and cells more susceptible to stress [135]. An L66A substitution also causes dimer formation, but suppressors of this mutation do not influence the V143A mutation, nor do suppressors of the V143A substitution affect the L66A modification. In contrast, when the V/IXI/V motifs in αA- and αB-crystallin are modified to I159G-V161G and I159G-I161G, respectively, oligomer size and chaperone activity increase [136]. Fluorescence resonance energy transfer (FRET) experiments indicate the V/IXI/V motifs of αA- and αB-crystallins occupy positions either close to regions in the α-crystallin domain, or adjacent to other V/IXI/V sequences, thus affecting sHSP oligomerization.

To summarize, the variable, highly flexible, carboxy-terminal extension maintains sHSP solubility, stability and chaperone activity, as well as protein-substrate complex solubility, but may lack a direct role in substrate binding. However, by modulating oligomerization, the carboxy-terminal extension undoubtedly contributes to chaperone activity in a critical way.

Caenorhabditis elegans sHSPs: natural deletions indicative of function

Interesting characteristics of amino- and carboxy-terminal extensions surface upon consideration of *C. elegans*, an organism boasting 16 sHSP genes [137]. Deleting the 15 amino-terminal residues of *C. elegans* Hsp16.2 (fig. 4), a stress-induced sHSP, greatly reduces oligomer size and chaperone activity [114]. Conversely, removing 16 residues from the Hsp16.2 carboxy-terminus increases oligomer size slightly with almost no effect on chaperoning even though the truncated protein precipitates upon freeze/thawing, suggesting diminished solubility. Hsp12.6, a stress-indifferent, developmentally regulated *C. elegans* sHSP, lacks a carboxy-terminal extension but possesses an amino-terminus of 25 residues, the latter corresponding in length to many artificially truncated sHSPs. Hsp12.6 exists as monomers and fails to shelter citrate synthase from heat-induced denaturation [138]. *C. elegans* Hsp12.2 and Hsp12.3, structurally similar to Hsp12.6 and essentially equivalent to the α-crystallin domains of larger sHSPs, occur as tetramers and lack chaperone action [139]. These observations suggest te-

Figure 4 Sequence alignment of multiple *C. elegans* sHSPs. The amino acid sequences of *C. elegans* sHSPs described in the text, and which mirror experimentally truncated derivatives of sHSPs from other organisms, were aligned by CLUSTAL W (1.82). CHsp12.3, *C. elegans* Hsp12.3, F38E11.1; CHsp12.6, *C. elegans* Hsp12.6, F38E11.2; CHsp12.2, *C. elegans* Hsp12.2, P34328; CHsp16-2, *C. elegans* Hsp16-2, M14334; CHsp25, *C. elegans* Hsp25, T15466. (-) no amino acid residue; (*) identical residues; (:) conserved substitution; (.) semi-conserved substitution. sHSP domains are indicated above the alignment. Residue number is indicated on the right.

tramers are the fundamental building blocks of Hsp12.6, and oligomerization, mediated by the amino-terminal domain, is required for chaperone activity. As demonstrated by swapping amino-terminal regions, α-crystallin domains and carboxy-terminal extensions between *C. elegans* Hsp12.2 and human αB-crystallin, the αB-crystallin amino-terminus promotes multimerization [140]. Moreover, the absence of Hsp12.2 chaperone activity in vitro depends on α-crystallin domain characteristics rather than the inability to form oligomers, per se. In these experiments, the carboxy-terminus of αB-crystallin modulates hybrid protein chaperone activity for specific substrates, but oligomer size is unaffected [140]. Hsp25, the second largest *C. elegans* sHSP, is synthesized independent of heat stress and appears at all developmental stages [141]. Hsp25 forms mostly dimers and tetramers, but chaperones citrate synthase in vitro under increased temperature. Specific binding to vinculin and α-actinin suggests influence on focal adhesions. Examination of sHSP variants, including those naturally occurring in *C. elegans*, or others produced artificially by deletion and site-directed mutagenesis, demonstrates that amino- and carboxy-terminal regions have similar effects on quaternary structure from species to species, and their influence depends, at least partly, on spatial relationships with the α-crystallin domain.

sHSP oligomerization

Structural diversity of sHSPs

sHSP family members exhibit similar functions but constitute a morphological continuum of dynamic oligomers, ranging from poly-disperse to mono-disperse, and with differing symmetry [27, 92, 110, 142, 143]. Many sHSPs present as globular or ring-like structures, revealing central cavities when observed by microscopic procedures, including cryoelectron microscopy. Other arrangements exist, with Hsp16.3 from *Mycobacterium tuberculosis* composed of nonameric triangles [144] and plant sHSPs arranged as dodecameric discs [24]. Oligomerization is likely to depend on the successive union of subunits driven by the interaction of oligomerization determinants, leading to increasingly larger assembly units such as dimers, trimers or tetramers. As an example, site-directed spin labeling and electron paramagnetic resonance indicate that oligomerization determinants of *M. jannaschii* Hsp16.5 exist in the α-crystallin domain [107], dimers of which represent building blocks of higher-order structures [22, 91, 145]. Results obtained by application of the yeast two-hybrid system support the assembly of stable mammalian sHSP dimers by way of α-crystallin domain interactions [109, 146, 147]. Assembly of these building blocks then depends on the amino- and carboxy-terminal

extensions, as described earlier, yielding oligomers ranging in structure from the homogeneous mono-disperse microbial and plant sHSPs to the poly-disperse mammalian sHSPs.

Structural dynamics of sHSP oligomers

Oligomerization is thought to be required for substrate binding and chaperone function, although oligomer roles vary from one sHSP to another. In agreement with these ideas, surface plasmon resonance studies show individual α-crystallin subunits fail to interact with unfolded proteins [148], and phosphorylated Hsp27 exhibits significantly decreased oligomeric size and chaperone activity [142]. In contrast, human Hsp22 [134, 149] and Hsp20 [150] apparently never form homo-oligomers, yet exhibit chaperone activity in vitro. Tapeworm sHSPs have chaperone activity but exist as dimers and tetramers, including up to eight α-crystallin domains due to duplication of this region [151]. Adding another dimension, sHSP oligomers are dynamic, with assembly/disassembly differing in response to tissue age, temperature, pH, ionic strength, phosphorylation and protein concentration [86, 90, 102, 108, 152–158]. Subunit exchange occurs between oligomers of either the same or different sHSPs, and the exchange rate varies with differing consequences for chaperone function [24, 27, 28, 90, 155, 157–159]. In particular, subunit exchange is less critical for αA-crystallin than the plant and bacterial sHSPs [125]. As well, the 24 subunit yeast Hsp26 separates reversibly into dimers upon heat shock, enhancing chaperoning efficiency [33, 104], but yeast Hsp42 is an effective chaperone even though it escapes heat induced dissociation [27].

HspH from *B. japonicum* assembles oligomers of 400–500 kDa, a prerequisite for chaperone activity, and the subunits exchange readily with homologous and heterologous complexes [90, 160]. *M. tuberculosis* Hsp16.3 is a trimer of trimers, an inert storage oligomer that undergoes subunit exchange, even at 4 °C, and for which dissociation greatly enhances chaperone activity, presumably by exposing α-crystallin domains [93, 107, 156, 161]. Hsp16.3 chaperone capability is modified by adjusting oligomer dissociation rate in response to heat shock or urea, without change in oligomer size [152, 162], suggesting that tertiary structure modification increases chaperoning as temperature rises [95]. H_2O_2 induced methionine sulfoxidation of *M. tuberculosis* Hsp16.3 promotes oligomer dissociation and decreases chaperone action, results seemingly opposed to those just presented [66]. To explain the paradox, methionine side chains may convert from hydrophobic to hydrophilic in a region that binds substrate and/or provides inter-subunit attachment, thereby reducing chaperone activity while promoting oligomer disassembly. Wheat Hsp16.9 also undergoes heat-induced dissociation into dimers with a corresponding increase in chaperone activity [24, 86]. Moreover, concentration-dependent heterogeneous subunit exchange between pea Hsp18.1 and wheat Hsp16.9, principally as dimers, is limited by dissociation kinetics and lacks preference for specific stoichiometries, suggesting subunits interact similarly to form dodecameric oligomers [24, 155].

Human Hsp27 and Hsp20, the latter normally a dimer, undergo temperature-influenced mixing to yield oligomers with approximately equal amounts of both subunits, indicating hetero-complex formation in vivo [150]. αA-crystallin forms a reversible hetero-oligomeric complex with Hsp27 and αB-crystallin, a temperature-dependent activity modulated by amino-terminal sequences [108]. Hsp27 binding stabilizes αB-crystallin, as happens when αA- and αB-crystallins associate [163, 164]. Exchange between different sHSP oligomers signifies structural similarities between subunits, a conclusion agreeing with sequence comparisons, analysis of crystallized sHSPs and structural modeling. Contrary to the sHSPs just described, exchange of *M. jannaschii* Hsp16.5 subunits is specific, and other sHSP monomers do not associate with Hsp16.5. This implies sufficient differences within some sHSPs to preclude stable subunit interaction, examples being disparate β-strand compositions of α-crystallin domains and amino-terminal dissimilarity [107, 157]. Limitations imposed by the high temperatures at which *M. jannaschi* Hsp16.5 functions may explain the lack of sHSP subunit exchange.

The interaction of large denatured proteins with αA-crystallin markedly reduces subunit exchange rates, presumably due to substrate association with several subunits [158]. However, some subunits remain dynamic, while association between substrates and sHSPs is relatively static. Moreover, pre-formed sHSP-substrate complexes are constant in size even upon addition of more sHSPs to mixtures [28], implying a relatively stable interaction between substrate and chaperone. These comments support the proposal that ATP-dependent chaperones recognize sHSP-bound substrates and are required for refolding because they facilitate removal of proteins from oligomeric complexes [28, 29].

Combining these observations shows how the conceptual relationship between sHSP oligomerization and chaperoning has been altered by incorporating the idea of environmentally sensitive, adjustable subunit exchange rates as determinants of chaperone activity. Oligomer destabilization increases hydrophobic surface area available for chaperoning, either by subunit rearrangement within oligomers of stable size, by modifying complex size or by changing the conformation of individual monomers [34, 110, 113, 165–167]. Subunit exchange also increases hydrophobic surface area availability, and the liberated subunits react individually with substrates before reforming complexes. Some combination of these

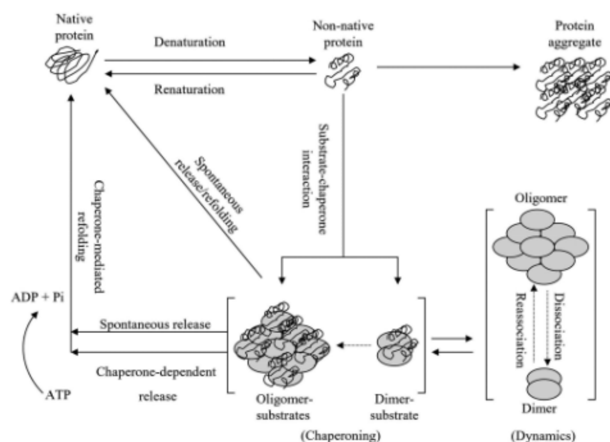

Figure 5. sHSP oligomerization and chaperoning. Exposure to stress generates non-native proteins which form protein aggregates. Partially unfolded, non-native proteins may bind large sHSP oligomers and smaller complexes such as dimers, thereby preventing irreversible aggregation. Upon return to favourable conditions, non-native proteins associated with sHSPs are released and refolded spontaneously, or with the assistance of ATP-dependent chaperones, including Hsp70 and Hsp60. sHSPs exist as oligomers in a functionally important dynamic equilibrium with dimers or other small complexes (bracket on right side of figure).

events is possible, indeed likely, during exposure of cells to adverse conditions, as structural changes occasioned by stress affect subunit packing in oligomers, leading to enhanced monomer exchange. Moreover, subunit exchange at normal temperature potentially prepares sHSPs for activities such as regulation of the cytoskeleton and protection from apoptosis, as well as providing rapid response to changing conditions in cells upon stress exposure [86] (fig. 5).

Perspectives

The sHSPs are increasingly well characterized, but much remains to be learned. Continued efforts at crystallization, either of intact sHSPs or of α-crystallin domains, will comment upon conclusions made by comparison of poly-disperse sHSPs to the crystal structures of Hsp16.9 and Hsp16.5. The role of subunit exchange in chaperone activity will benefit from further study done in light of the environmental conditions normally experienced by the experimental organisms under consideration. Identification of individual protein substrates must be extended in order to encompass sHSP roles in development, apoptosis and other vital cell activities, an objective that requires the application of proteomics. Coupled with investigation of these and other lingering questions is the continued molecular dissection of sHSP monomers by site-directed mutagenesis and related procedures in order to understand oligomerization and chaperone function. As the molecular details of sHSP design and function are resolved, the possibility for practical exploitation increases. Already, sHSPs are implicated in several diseases, either as causative agents through mutation and posttranslational changes, as protective molecules or as biochemical prognostigators of survival, all with therapeutic potential. sHSPs influence aging and they mediate diapause, a state of developmental and metabolic arrest in many animals, including insects and the crustacean, *A. franciscana*. The implications of understanding diapause are enormous when considered in the context of agriculture and aquaculture, and sHSPs may be central to this issue and to several other practical applications that will follow from continued study of these molecular chaperones.

Acknowledgements. The work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant, a Nova Scotia Health Research Foundation/Canadian Institutes of Health Research Regional Partnership Plan Grant, and a Heart and Stroke Foundation of Nova Scotia Grant to T.H.M. and a NSHRF Student Fellowship to Y.S.

1 Rivas G., Ferrone F. and Herzfeld J. (2004) Life in a crowded world. EMBO Rep. **5:** 23–27
2 Ellis R. J. and Minton A. P. (2003) Cell biology: join the crowd. Nature **425:** 27–28
3 Ellis R. J. (2001) Macromolecular crowding: obvious but underappreciated. Trends iochem. Sci. **26:** 597–604
4 Minton A. P. (2001) The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. J. Biol. Chem. **276:** 10577–10580
5 Dobson C. M. (2003) Protein folding and misfolding. Nature **426:** 884–890
6 Frydman J. (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. Annu. Rev. Biochem. **70:** 603–647
7 Mayer M. P. and Bukau B. (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell. Mol. Life Sci. **62:** 670–6840
8 Shorter J. and Linquist S. (2005) Navigating the ClpB channel to solution. Nat. Struct. Mol. Biol. **12:** 4–6
9 Jackson S. E., Queitsch C. and Toft D. (2004) Hsp90: from structure to phenotype. Nat. Struct. Mol. Biol. **11:** 1152–1155
10 Young J. C., Agashe V. R., Siegers K. and Hartl F. U. (2004) Pathways of chaperone-mediated protein folding in the cytosol. Nat. Rev. Mol. Cell Biol. **5:** 781–791
11 Mogk A. and Bukau B. (2004) Molecular chaperones: structure of a protein disaggregase. Curr. Biol. **14:** R78–R80
12 Petrucelli L., Dickson D., Kehoe K., Taylor J., Snyder H., Grove A. et al. (2004) CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum. Mol. Genet. **13:** 703–714
13 Haslbeck M. (2002) sHSPs and their role in the chaperone network. Cell. Mol. Life Sci. **59:** 1649–1657
14 MacRae T. H. (2000) Structure and function of small heat shock/α-crystallin proteins: established concepts and emerging ideas. Cell. Mol. Life Sci. **57** 899–913
15 Matuszewska M., Kuczy ska-Wi nik D., Laskowska E. and Liberek K. (2005) The small heat shock protein IbpA of *Escherichia coli* cooperates with IbpB in stabilization of thermally aggregated proteins in a disaggregation competent state. J. Biol. Chem. **280:** 12292–12298
16 Taylor R. P. and Benjamin I. V. (2005) Small heat shock proteins: a new classification scheme in mammals. J. Mol. Cell Cardiol. **38:** 433–444

17 Frank E., Madsen O., van Rheede T., Ricard G., Huynen M. A. and de Jong W. W. (2004) Evolutionary diversity of vertebrate small heat shock proteins. J. Mol. Evol. **59:** 792–805
18 Augusteyn R. C. (2004) α-crystallin: a review of its structure and function. Clin. Exp. Optom. **87:** 356–366
19 Laksanalamai P. and Robb F. T. (2004) Small heat shock proteins from extremophiles: a review. Extremophiles **8:** 1–11
20 Narberhaus F. (2002) α-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiol. Mol. Biol. Rev. **66:** 64–93
21 Sun W., Van Montagu M. and Verbruggen N. (2002) Small heat shock proteins and stress tolerance in plants. Biochim. Biophys. Acta **1577:** 1–9
22 Kim R., Kim K. K., Yokota H. and Kim S.-H. (1998) Small heat shock protein of *Methanococcus jannaschii*, a hyperthermophile. Proc. Natl. Acad. Sci. USA **95:** 9129–9133
23 Kim K. K., Kim R. and Kim S.-H. (1998) Crystal structure of a small heat-shock protein. Nature **394:** 595–599
24 van Montfort R. L. M., 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
25 Sun Y. and MacRae T. H. (2005) The small heat shock proteins and their role in human disease. FEBS J. **272:** 2613–2627
26 MacRae T. H. (2005) Diapause: diverse states of developmental and metabolic arrest. J. Biol. Res. **3:** 3–14
27 Haslbeck M., Braun N., Stromer T., Richter B., Model N., Weinkauf S. et al. (2004) Hsp42 is the general small heat shock protein in the cytosol of *Saccharomyces cerevisiae.* EMBO J. **23:** 1–12
28 Friedrich K. L., Giese K. C., Buan N. R. and Vierling E. (2004) Interactions between small heat shock protein subunits and substrate in small heat shock protein-substrate complexes. J. Biol. Chem. **279:** 1080–1089
29 Basha E., Lee G. J., Breci L. A., Hausrath A. C., Buan N. R., Giese K. C. et al. (2004) 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. **279:** 7566–7575
30 Carver J. A., Lindner R. A., Lyon C., Canet D., Hernandez H. Dobson C. M. et al. (2002) The interaction of the molecular chaperone α-crystallin with unfolding α-lactalbumin: a structural and kinetic spectroscopic study. J. Mol. Biol. **318:** 815–827
31 Lindner R.A., Treweek T.M. and Carver J.A. (2001) The molecular chaperone α-crystallin is in kinetic competition with aggregation to stabilize a monomeric molten-globule form of α-lactalbumin. Biochem. J. **354:** 79–87
32 Treweek T. M., Lindner R. A., Mariani M. and Carver J. A. (2000) The small heat-shock chaperone protein, α-crystallin, does not recognize stable molten globule states of cytosolic proteins. Biochim. Biophys. Acta **1481:** 175–188
33 Haslbeck M., Walke S., Stromer T., Ehrnsperger M., White H. E., Chen S. et al. (1999) Hsp26: a temperature-regulated chaperone. EMBO J. **18:** 6744–6751
34 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
35 Carver J. A., Guerreiro N., Nicholls K. A. and Truscott R. J. W. (1995) On the interaction of α-crystallin with unfolded proteins. Biochim. Biophys. Acta **1252:** 251–260
36 Stromer T., Ehrnsperher M., Gaestel M. and Buchner J. (2003) Analysis of the interaction of small heat shock proteins with unfolding proteins. J. Biol. Chem. **278:** 18015–18021
37 Sathish H. A., Stein R. A., Yang G. and Mchaourab H. S. (2003) Mechanism of chaperone function in small heat-shock proteins. Fluorescence studies of the conformations of T4 lysozyme bound to αB-crystallin. J. Biol. Chem. **278:** 44214–44221
38 Rajaraman K., Raman B., Ramakrishna T. and Rao Ch. M. (2001) Interaction of human recombinant αA- and αB-crystallins with early and late unfolding intermediates of citrate synthase on its thermal denaturation. FEBS Lett. **497:** 118–123
39 Biswas A. and Das K. P. (2004) Role of ATP on the interaction of α-crystallin with its substrates and its implications for the molecular chaperone function. J. Biol. Chem. **279:** 42648–42657
40 Török Z., Goloubinoff P., Horváth I., Tsvetkova N. M., Glatz A., Balogh G. et al. (2001) *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 **98:** 3098–3103
41 Ehrnsperger M., Gräber 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
42 Derham B. K., Ellory J. C., Bron A. J. and Harding, J.J. (2003) The molecular chaperone α-crystallin incorporated into red cell ghosts protects membrane Na/K-ATPase against glycation and oxidative stress. Eur. J. Biochem. **270:** 2605–2611
43 Basha E., Lee G. J., Demeler B. and Vierling E. (2004) Chaperone activity of cytosolic small heat shock proteins from wheat. Eur. J. Biochem. **271:** 1426–1436
44 Arrigo A.-P., Firdaus W. J. J., Mellier G., Moulin M., Paul C., Diaz-latoud C. et al. (2005) Cytoxic effects induced by oxidative stress in cultured mammalian cells and protection provided by Hsp27 expression. Methods **35:** 126–138
45 Kamradt M. C., Lu M., Werner M. E., Kwan T., Chen F., Strohecker A. et al. (2005) The small heat shock protein αB-crystallin is a novel inhibitor of TRAIL-induced apoptosis that suppresses the activation of caspase-3. J. Biol. Chem. **280:** 11059–11066
46 Duverger O., Paslaru L. and Morange M. (2004) HSP25 is involved in two steps of the differentiation of PAM212 keratinocytes. J. Biol. Chem. **279:** 10252–10260
47 Day R. M., Gupta J. S. and MacRae T. H. (2003) A small heat shock/α-crystallin protein from encysted *Artemia* embryos suppresses tubulin denaturation. Cell Stress Chaperones **8:** 183–193
48 Wang X., Klevitsky R., Huang W., Glasford J., Li F. and Robbins J. (2003) αB-crystallin modulates protein aggregation of abnormal desmin. Circ. Res. **93:** 998–1005
49 Panasenko O. O., Kim M. V., Marston S. B. and Gusev N. B. (2003) Interaction of the small heat shock protein with molecular mass 25 kDa (hsp25) with actin. Eur. J. Biochem. **270:** 892–901
50 Mounier N. and Arrigo A.-P. (2002) Actin cytoskeleton and small heat shock proteins: how do they interact? Cell Stress Chaperones **7:** 167–176
51 Perng M. D., Muchowski P. J., van den IJssel P., Wu G. J. S, Hutcheson A. M., Clark J. I. et al. (1999) The cardiomyopathy and lens cataract mutation in αB-crystallin alters its protein structure, chaperone activity, and interaction with intermediate filaments in vitro. J. Biol. Chem. **274:** 33235–33243
52 Perng M. D., Cairns L., van den IJssel P., Prescott A., Hutcheson A. M. and Quinlan R. A. (1999) Intermediate filament interactions can be altered by HSP27 and αB-crystallin. J. Cell Sci. **112:** 2099–2112
53 Bova M. P., Yaron O., Huang Q., Ding L., Haley D. A., Stewart P. L. et al. (1999) Mutation R120G in αB-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. Proc. Natl. Acad. Sci. USA **96:** 6137–6142
54 MacRae T. H. (2003) Molecular chaperones, stress resistance and development in *Artemia franciscana.* Semin. Cell Dev. Biol. **14:** 251–258
55 Fujita Y., Ohto E., Katayama E. and Atomi, Y. (2004) αB-crystallin-coated MAP microtubule resists nocodazole and calcium-induced disassembly. J. Cell Sci. **117:** 1719–1726

56 Inaguma Y., Ito H., Iwamoto I., Saga S. and Kato K. (2001) αB-crystallin phosphorylated at Ser-59 is localized in centrosomes and midbodies during mitosis. Eur. J. Cell Biol. **80:** 741–748
57 Benndorf R., Hayeß K., Ryazantsev S., Wieske M., Behlke J. and Lutsch G. (1994) Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. J. Biol. Chem. **269:** 20780–20784
58 Wieske M., Benndorf R., Behlke J., Dölling R., Grelle G., Bielka H. et al. (2001) Defined sequence segments of the small heat shock proteins HSP25 and αB-crystallin inhibit actin polymerization. Eur. J. Biochem. **268:** 2083–2090
59 Schäfer C., Clapp P., Welsh M. J., Benndorf R. and Williams J. A. (1999) HSP27 expression regulates CCK-induced changes of the actin cytoskeleton in CHO-CCK-A cells. Am. J. Physiol. Cell Physiol. **277:** C1032–C1043
60 Guay J., Lambert H., Gingras-Breton G., Lavoie J. N., Huot J. and Landry J. (1997) Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. J. Cell Sci. **110:** 357–368
61 Huot J., Houle F., Spitz D. R. and Landry J. (1996) HSP27 phosphorylation-mediated resistance against actin fragmentation and cell death induced by oxidative stress. Can. Res. **56:** 273–279
62 Bryantsev A. L., Loktionova S. A., Ilyinskaya O. P., Tararak E. M., Kampinga H. H. and Kabakov A. E. (2002) Distribution, phosphorylation and activities of Hsp25 in heat-stressed H9c2 myoblasts: a functinal link to cytoprotection. Cell Stress Chaperones **7:** 146–155
63 Tessier D. J., Komalavilas P., Panitch A., Joshi L. and Brophy C. M. (2003) The small heat shock protein (HSP) 20 is dynamically associated with the actin cross-linking protein actinin. J. Surg. Res. **111:** 152–157
64 Zhang H., Fu X., Jiao W., Zhang X., Liu C. and Chang Z. (2005) The association of small heat shock protein 16.3 with the plasma membrane of *Mycobacterium tuberculosis*: Dissociation of oligomers is a prerequisite. Biochem. Biophys. Res. Commun. **330:** 1055–1061
65 Chen Y., Lu Y.-J., Wang H.-W., Quan S., Chang Z. and Sui S.-F. (2003) Two-dimensional crystallization of a small heat shock protein HSP16.3 on lipid layer. Biochem. Biophys. Res. Commun. **310:** 360–366
66 Abulimiti A., Qiu X., Chen J., Liu Y. and Chang Z. (2003) Reversible methionine sulfoxidation of *Mycobacterium tuberculosis* small heat shock protein Hsp16.3 and its possible role in scavenging oxidants. Biochem. Biophys. Res. Commun. **305:** 87–93
67 Cobb B. A. and Petrash J. M. (2000) Characterization of α-crystallin-plasma membrane binding. J. Biol. Chem. **275:** 6664–6672
68 Tsvetkova N. M., Horváth I., Török Z., Wolkers W. F., Balogi Z., Shigapova N. et al. (2002) Small heat-shock proteins regulate membrane lipid polymorphism. Proc. Natl. Acad. Sci. USA **99:** 13504–13509
69 Lee S., Owen H. A., Prochaska D. J. and Barnum S. R. (2000) HSP16.6 is involved in the development of thermotolerance and thylakoid stability in the unicellular Cyanobacterium, *Synechocystis sp.* PCC 6803. Curr. Microbiol. **40:** 283–287
70 Horváth I., Glatz A., Varvasovszki V., Török Z., Páli T., Balogh G. et al. (1998) Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: identification of *hsp17* as a fluidity gene. Proc. Natl. Acad. Sci. USA **95:** 3513–3518
71 Siddique M., Port M., Tripp J., Weber C., Zielinski D., Calligaris R. et al. (2003) Tomato heat stress protein Hsp16.1-CIII represents a member of a new class of nucleocytoplasmic small heat stress proteins in plants. Cell Stress Chaperones **8:** 381–394
72 Borrelli M. J., Bernock L. J., Landry J., Spitz D. R., Weber L. A., Hickey E. et al. (2002) Stress protection by a fluorescent Hsp27 chimera that is independent of nuclear translocation or multimeric dissociation. Cell Stress Chaperones **7:** 281–296
73 van de Klundert F. A. J. M. and de Jong W. W. (1999) The small heat shock proteins Hsp20 and αB-crystallin in cultured cardiac myocytes: differences in cellular localization and solubilization after heat stress. Eur. J. Cell Biol. **78:** 567–572
74 de Wit N. J. W., Verschuure P., Kappé G., King S. M., de Jong W. W., van Muijen G. N. P. et al. (2004) Testis-specific human small heat shock protein HSPB9 is a cancer/testis antigen, and potentially interacts with the dynein subunit TCTEL1. Eur. J. Cell Biol. **83:** 337–345
75 Liang P. and MacRae T.H. (1999) The synthesis of a small heat shock/α-crystallin protein in *Artemia* and its relationship to stress tolerance during development. Dev. Biol. **207:** 445–456
76 Willsie J. K. and Clegg J. S. (2002) Small heat shock protein p26 associates with nuclear matrix and HSP70 in nuclei and nuclear matrix fractions form stressed cells. J. Cell. Biochem. **84:** 601–614
77 den Engelsman J., Bennink E. J., Doerwald L., Onnekink C., Wunderink L., Andley U. P. et al. (2004) Mimicking phosphorylation of the small heat-shock protein αB-crystallin recruits the F-box protein FBX4 to nuclear SC35 speckles. Eur. J. Biochem. **271:** 4195–4203
78 Adhikari A. S., Rao K. S. Rangaraj N., Parnaik, V. K. and Rao Ch. M. (2004) Heat stress-induced localization of small heat shock proteins in mouse myoblasts: intranuclear lamin A/C speckles as target for αB-crystallin and Hsp25. Exp. Cell Res. **299:** 393–403
79 van den IJssel P., Wheelock R., Prescott A., Russell P. and Quinlan R. A. (2003) Nuclear speckle localisation of the small heat shock protein αB-crystallin and its inhibition by the R120G cardiomyopathy-linked mutation. Exp. Cell Res. **287:** 249–261
80 van Rijk A. F., Stege G. J. J., Bennink E. J., May A. and Bloemendal H. (2003) Nuclear staining for the small heat shock protein αB-crystallin colocalizes with splicing factor SC35. Eur. J. Cell Biol. **82:** 361–368
81 Pietrowski D., Durante M. J., Liebstein A., Schmitt-John T., Werner T. and Graw J. (1994) α-crystallins are involved in specific interactions with the murine γD/E/F-crystallin-encoding gene. Gene **144:** 171–178
82 Bloemendal M., Toumadje A. and Johnson W. C. Jr (1999) Bovine lens crystallins do contain helical structure: a circular dichroism study. Biochim. Biophys. Acta **1432:** 234–238
83 Singh K., Groth-Vasselli B. and Farnsworth P. N. (1998) Interaction of DNA with bovine lens α-crystallin: its functional implications. Int. J. Biol. Macromol. **22:** 315–320
84 Kedersha N .L., Gupta M., Li W., Miller I. and Anderson P. (1999) RNA-binding proteins TIA-1 and TIAR link the phophorylation of eIF-2α to the assembly of mammalian stress granules. J. Cell Biol. **147:** 1431–1442
85 Scharf K.-D., Heider H., Höhfeld I., Lyck R., Schmidt E. and Nover L. (1998) The tomato Hsf system: HsfA2 needs interaction with HsfA1 for efficient nuclear import and may be localized in cytoplasmic heat stress granules. Mol. Cell. Biol. **18:** 2240–2251
86 Wintrode P. L., Friedrich K. L., Vierling E., Smith J. B. and Smith D. L. (2003) Solution structure and dynamics of a heat shock protein assembly probed by hydrogen exchange and mass spectrometry. Biochemistry **42:** 10667–10673
87 Bera S. and Abraham E. C. (2002) The αA-crystallin R116C mutant has a higher affinity for forming heteroaggregates with αB-crystallin. Biochemistry **41:** 297–305
88 Andley, U. P., Patel H. C. and Xi J.-H. (2002) The R116C mutation in αA-crystallin diminishes its protective ability against stress-induced lens epithelial cell apoptosis. J. Biol. Chem. **277:** 10178–10186
89 Cobb B. A. and Petrash J. M. (2000) Structural and functional changes in the αA-crystallin R116C mutant in hereditary cataracts. Biochemistry **39:** 15791–15798
90 Lentze N., Studer S. and Narberhaus F. (2003) Structural and functional defects caused by point mutations in the a-crystallin

domain of a bacterial a-heat shock protein. J. Mol. Biol. 328: 927–937
91 Koteiche H. A. and Mchaourab H. S. (1999) Folding pattern of the α-crystallin domain in αA-crystallin determined by site-directed spin labeling. J. Mol. Biol. **294:** 561–577
92 Haley D. A., Bova M. P., Huang Q. L., Mchaourab H. S. and Stewart P. L. (2000) Small heat-shock protein structures reveal a continum from symmetric to variable assemblies. J. Mol. Biol. **298:** 261–272
93 Guruprasad K. and Kumari K. (2003) Three-dimensional models corresponding to the C-terminal domain of human αA- and αB-crystallins based on the crystal structure of the small heat-shock protein HSP16.9 from wheat. Int. J. Biol. Macromol. **33:** 107–112
94 Muchowski P. J., Wu G. J. S., Liang J. J. N., Adman E. T. and Clark J. I. (1999) Site-directed mutations within the core α-crystallin domain of the small heat-shock protein, human αB-crystallin, decrease molecular chaperone functions. J. Mol. Biol. **289:** 397–411
95 Mao Q., Ke D., Feng X. and Chang Z. (2001) Preheat treatment for *Mycobacterium tuberculosis* Hsp16.3: correlation between a structural phase change at 60ºC and a dramatic increase in chaperone-like activity. Biochem. Biophys. Res. Commun. **284:** 942–947
96 Derham B. K., van Boekel M. A. M., Muchowski P. J., Clark J. I., Horwitz J., Hepburne-Scott H. W. et al. (2001) Chaperone function of mutant versions of αA- and αB-crystallin prepared to pinpoint chaperone binding sites. Eur. J. Biochem. **268:** 713–721
97 Santhoshkumar P. and Sharma K. K. (2001) Phe^{71} is essential for chaperone-like function in αA-crystallin. J. Biol. Chem. **276:** 47094–47099
98 Litt M., Kramer P., LaMorticella D. M., Murphey W., Lovrien E. W. and Weleber R. G. (1998) Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene *CRYAA*. Hum. Mol. Genet. **7:** 471–474
99 Vicart P., Caron A., Guicheney P., Li Z., Prévost M.-C., Faure A. et al. (1998) A missense mutation in the αB-crystallin chaperone gene causes a desmin-related myopathy. Nat. Genet. **20:** 92–95
100 Jiao W., Qian M., Li P., Zhao L. and Chang, Z. (2005) 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. **347:** 871–884
101 Eifert C., Burgio M. R., Bennett P. M., Salerno J. C. and Koretz J. F. (2005) N-terminal control of small heat shock protein oligomerization: Changes in aggregate size and chaperone-like function. Biochim. Biophys. Acta **1748:** 146–156
102 Lelj-Garolla B. and Mauk A. G. (2004) Self-association of a small heat shock protein. J. Mol. Biol. **345:** 631–642104
103 Sun Y., Mansour M., Crack J. A., Gass G. L. and MacRae T. H. (2004) Oligomerization, chaperone activity and nuclear localization of p26, a small heat shock protein from *Artemia franciscana*. J. Biol. Chem. **279:** 39999–40006
104 Stromer T., Fischer E., Richter K., Haslbeck M. and Buchner J. (2004) Analysis of the regulation of the molecular chaperone Hsp26 by temperature-induced dissociation. The N-terminal domain is important for oligomer assembly and the binding of unfolding proteins. J. Biol. Chem. **279:** 11222–11228
105 Salerno J. C., Eifert C. L., Salerno K. M. and Koretz J. F. (2003) Structural diversity in the small heat shock protein superfamily: control of aggregation by the N-terminal region. Prot. Eng. **16:** 847–851
106 Studer S., Obrist M., Lentre N. and Narberhaus F. (2002) A critical motif for oligomerization and chaperone activity of bacterial α-heat shock proteins. Eur. J. Biochem. **269:** 3578–3586
107 Koteiche H. A. and Mchaourab H. S. (2002) The determinants of the oligomeric structure in Hsp16.5 are encoded in the α-crystallin domain. FEBS Lett. **519:** 16–22
108 Bova M. P., Mchaourab H. S., Han Y. and Fung B. K.-K. (2000) Subunit exchange of small heat shock proteins. Analysis of oligomer formation of αA-crystallin and Hsp27 by fluorescence resonance energy transfer and site-directed truncations. J. Biol. Chem. **275:** 1035–1042
109 Lambert H., Charette S. J., Bernier A. F., Guimond A. and Landry J. (1999) HSP27 multimerization mediated by phosphorylation-sensitive intermolecular interactions at the amino terminus. J. Biol. Chem. **274:** 9378–9385
110 Kim R., Lai L., Lee H.-H., Cheong G.-W., Kim K. K., Wu Z. et al. (2003) On the mechanism of chaperone activity of the small heat-shock protein of *Methanococcus jannaschii*. Proc. Natl. Acad. Sci. USA **100:** 8151–8155
111 Young L.-S., Yeh C.-H., Chen Y.-M. and Lin C.-Y. (1999) Molecular characterization of *Oryza sativa* 16.9 kDa heat shock protein. Biochem. J. **344:** 31–38161
112 Peterson J. J., Young M. M. and Takemoto L. J. (2004) Probing α-crystallin structure using chemical cross-linkers and mass spectrometry. Mol. Vis. **10:** 857–866
113 Hasan A., Yu J., Smith D. L. and Smith J. B. (2004) Thermal stability of human α-crystallins sensed by amide hydrogen exchange. Prot. Sci. **13:** 332–341
114 Leroux M. R., Melki R., Gordon B., Batelier G. and Candido E. P. M. (1997) Structure-function studies on small heat shock protein oligomeric assembly and interaction with unfolded polypeptides. J. Biol. Chem. **272:** 24646–24656
115 Merck KB., Horwitz J., Kersten M., Overkamp P., Gaestel M., Bloemendal H. et al. (1993) Comparison of the homologous carboxy-terminal domain and tail of α-crystallin and small heat shock protein. Mol. Biol. Rep. **18:** 209–215
116 Merck K. B., De Haard-Hoekman W. A., Essink B. B. O., Bloemendal H. and De Jong W. W. (1992) Expression and aggregation of recombinant αA-crystallin and its two domains. Biochim. Biophys. Acta **1130:** 267–276
117 Feil I. K., Malfois M., Hendle J., van der Zandt, H. and Svergun D. I. (2001) A novel quaternary structure of the dimeric α-crystallin domain with chaperone-like activity. J. Biol. Chem. **276:** 12024–12029
118 Koteiche H. A. and Mchaourab H. S. (2003) Mechanism of chaperone function in small heat-shock proteins. Phosphorylation-induced activation of two-mode binding in αB-crystallin. J. Biol. Chem. **278:** 10361–10367
119 Thériault J. R., Lambert H., Chávez-Zobel A. T., Charest G., Lavigne P. and Landry J. (2004) Essential role of the NH_2-terminal WD/EPF motif in the phosphorylation-activated protective function of mammalian Hsp27. J. Biol. Chem. **279:** 23463–23471
120 Pasta S. Y., Raman B., Ramakrishna T. and Rao, Ch. M. (2003) Role of the conserved SRLFDQFFG region of α-crystallin, a small heat shock protein. Effect on oligomeric size, subunit exchange, and chaperone-like activity. J. Biol. Chem. **278:** 51159–51166
121 Plater M. L., Goode D. and Crabbe M. J. C. (1996) Effects of site-directed mutations on the chaperone-like activity of αB-crystallin. J. Biol. Chem. **271:** 28558–28566
122 Wiesmann K. E. H., Coop A., Goode D., Hepburne-Scott H. W. and Crabbe M. J. C. (1998) Effect of mutations of murine lens αB crystallin on transfected neural cell viability and cellular translocation in response to stress. FEBS Lett. **438:** 25–31
123 Horwitz J., Bova M., Huang Q.-L., Ding L., Yaron O. and Lowman S. (1998) Mutation of αB-crystallin: effects on chaperone-like activity. Int. J. Biol. Macromol. **22:** 263–269
124 Kelley P. B. and Abraham E. C. (2003) Thermally induced disintegration of the oligomeric structure of αB-crystallin mutant F28S is associated with diminished chaperone activity. Mol. Cell. Biochem. **252:** 273–278
125 Aquilina J. A., Benesch J. L. P., Ding L. L., Yaron O., Horwitz J. and Robinson C. V. (2005) Subunit exchange of polydisperse proteins. Mass spectrometry reveals consequences of αA-crystallin truncation. J. Biol. Chem. **280:** 14485–14491

126 Lindner R. A., Kapur A., Mariana M., Titmuss S. J. and Carver J. A. (1998) Structural alterations of α-crystallin during its chaperone activity. Eur. J. Biochem. **258:** 170–183

127 Smulders R. H. P. H., Carver J. A., Lindner R. A., van Boekel M. A. M., Bloemendal H. and de Jong W. W. (1996) Immobilization of the C-terminal extension of bovine αA-crystallin reduces chaperone-like activity. J. Biol. Chem. **271:** 29060–29066

128 Carver J. A, Esposito G., Schwedersky G. and Gaestel M. (1995) ^{1}H NMR spectroscopy reveals that mouse Hsp25 has a flexible C-terminal extension of 18 amino acids. FEBS Lett. **369:** 305–310

129 Thampi P. and Abraham E. C. (2003) Influence of the C-terminal residues on oligomerization of αA-crystallin. Biochemistry **42:** 11857–11863

130 Takemoto L., Emmons T. and Horwitz J. (1993) The C-terminal region of α-crystallin: involvement in protection against heat-induced denaturation. Biochem. J. **294:** 435–438

131 Andley U. P., Mathur S., Griest T. A. and Petrash J. M. (1996) Cloning, expression and chaperone-like activity of human αA-crystallin. J. Biol. Chem. **271:** 31973–31980

132 Lindner R.A., Carver J.A., Ehrnsperger M., Buchner J., Esposito G., Behlke J. et al. (2000) Mouse Hsp25, a small heat shock protein: the role of its C-terminal extension in oligomerization and chaperone action. Eur. J. Biochem. **267:** 1923–1932

133 Liao J.-H., Lee J.-S. and Chiou, S.-H. (2002) C-terminal lysine truncation increases thermostability and enhances chaperone-like function of porcine αB-crystallin. Biochem. Biophys. Res. Commun. **297:** 309–316

134 Chowdary T. K., Raman B., Ramakrishna T. and Rao, C. H. (2004) Mammalian Hsp22 is a heat-inducible small heat-shock protein with chaperone-like activity. Biochem. J. **381:** 379–387

135 Giese K.C. and Vierling E. (2004) Mutants in a small heat shock protein that affect the oligomeric state: Analysis and allele-specific suppression. J. Biol. Chem. **279:** 32674–32683

136 Pasta S. Y., Raman B., Ramakrishna T. and Rao Ch. M. (2004) The IXI/V motif in the C-terminal extension of α-crystallins: alternative interactions and oligomeric assemblies. Mol. Vis. **10:** 655–662

137 Candido E. P. M. (2002) The small heat shock proteins of the nematode *Caenorhabditis elegans*: structure, regulation and biology. In. Arrigo, A.-P. and Müller, W. E. G. (eds), Small Stress Proteins, pp. 61–67, Springer, Berlin

138 Leroux M. R., Ma B.J., Batelier G., Melki R. and Candido E. P. M. (1997) Unique structural features of a novel class of small heat shock proteins. J. Biol. Chem. **272:** 12847–12853

139 Kokke B. P. A., Leroux M. R., Candido E. P. M., Boelens W. C. and de Jong W. W. (1998) *Caenorhabditis elegans* small heat-shock proteins Hsp12.2 and Hsp12.3 form tetramers and have no chaperone-like activity. FEBS Lett. **433:** 228–232

140 Kokke B. P. A., Boelens W. C. and de Jong W. W. (2001) The lack of chaperonelike activity of *Caenorhabditis elegans* Hsp12.2 cannot be restored by domain swapping with human αB-crystallin. Cell Stress Chaperones **6:** 360–367

141 Ding L. and Candido E. P. M. (2000) HSP25, a small heat shock protein associated with dense bodies and M-lines of body wall muscle in *Caenorhabditis elegans*. J. Biol. Chem. **275:** 9510–9517

142 Rogalla T., Ehrnsperger M., Preville X., Kotlyarov A., Lutsch G., Ducasse C. et al. (1999) Regulation of HSP27 oligomerization, chaperone function and protective activity against oxidative stress/tumor necrosis factor α by phosphorylation. J. Biol. Chem. **274:** 18947–18956

143 Ehrnsperger M., Lilie H., Gaestel M. and Buchner J. (1999) The dynamics of Hsp25 quaternary structure. Structure and function of different oligomeric species. J. Biol. Chem. **274:** 14867–14874

144 Chang Z., Primm T. P., Jakana J., Lee I. H., Serysheva I., Chiu W. et al. (1996) *Mycobacterium tuberculosis* 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation. J. Biol. Chem. **271:** 7218–7223

145 Berengian A. R., Parfenova M. and Mchaourab H. S. (1999) Site-directed spin labeling study of subunit interactions in the α-crystallin domain of small heat-shock proteins. Comparison of the oligomer symmetry in αA-crystallin, HSP 27 and HSP 16.3. J. Biol. Chem. **274:** 6305– 6314

146 Liu C. and Welsh M. J. (1999) Identification of a site of Hsp27 binding with Hsp27 and αB-crystallin as indicated by the yeast two-hybrid system. Biochem. Biophys. Res. Commun. **255:** 256–261

147 Boelens W. C., Croes Y., de Ruwe M., de Reu L. and de Jong W.W. (1998) Negative charges in the C-terminal domain stabilize the αB-crystallin complex. J. Biol. Chem. **273:** 28085–28090

148 Avilov S. V. Aleksandrova N. A. and Demchenko A. P. (2004) Quaternary structure of α-crystallin is necessary for the binding of unfolded proteins: a surface plasmon resonance study. Prot. Pept. Lett. **11:** 41–48

149 Kim M. V., Seit-Nebi A. S., Marston S. B. and Gusev N. B. (2004) Some properties of human small heat shock protein Hsp22 (H11 or HspB8). Biochem. Biophys, Res. Commun. **315:** 796–801

150 Bukach O. V., Seit-Nebi A. S., Marston S. B. and Gusev N. B. (2004) Some properties of human small heat shock protein Hsp20 (HspB6). Eur. J. Biochem. **271:** 291–302

151 Kappé G., Aquilina J. A., Wunderink L., Kamps B., Robinson C. V., Garate T. et al. (2004). Tsp36, a tapeworm small heat-shock protein with a duplicated α-crystallin domain, forms dimers and tetramers with good chaperone-like activity. Proteins Struct. Funct. Bioinform. **57:** 109–117

152 Fu X. and Chang Z. (2004) Temperature-dependent subunit exchange and chaperone-like activities of Hsp16.3, a small heat shock protein from *Mycobacterium tuberculosis*. Biochem. Biophys. Res. Commun. **316:** 291–299

153 Regini J. W., Grossmann J. G., Burgio M. R., Malik N. S., Koretz J. F., Hodson S. A. et al. (2004) Structural changes in α-crystallin and whole eye lens during heating, observed by low-angle X-ray diffraction. J. Mol. Biol. **336:** 1185–1194

154 Benesch J. L. P., Sobott F. and Robinson C. V. (2003) Thermal dissociation of multimeric protein complexes by using nanoelectrospray mass spectrometry. Anal. Chem. **75:** 2208–2214

155 Sobott F., Benesch J. L. P., Vierling E. and Robinson C. V. (2002) 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. **277:** 38921–38929

156 Gu L., Abulimiti A., Li W. and Chang Z. (2002) Monodisperse HSP16.3 nonamer exhibits dynamic dissociation and reassociation, with the nonamer dissociation prerequisite for chaperone-like activity. J. Mol. Biol. **319:** 517–526

157 Bova M. P., Huang Q., Ding L. and Horwitz J. (2002) Subunit exchange, conformational stability and chaperone-like function of the small heat shock protein 16.5 from *Methanococcus jannaschii*. J. Biol. Chem. **277:** 38468–38475

158 Bova M. P., Ding L.-L., Horwitz J. and Fung, B. K.-K. (1997) Subunit exchange of αA-crystallin. J. Biol. Chem. **272:** 29511–29517

159 Vanhoudt J., Abgar S., Aerts T. and Clauwaert, J. (2000) Native quaternary structure of bovine α-crystallin. Biochemistry **39:** 4483–4492

160 Studer S. and Narberhaus F. (2000) Chaperone activity and homo- and hetero-oligomer formation of bacterial small heat shock proteins. J. Biol. Chem. **275:** 37212–37218

161 Abulimiti A., Fu X., Gu L., Feng X. and Chang Z. (2003) *Mycobacterium tuberculosis* Hsp16.3 nonamers are assembled and re-assembled via trimer and hexamer intermediates. J. Mol. Biol. **326:** 1013–1023

162 Fu X., Liu C., Liu Y., Feng X., Gu L., Chen X. et al. (2003) Small heat shock protein Hsp16.3 modulates its chaperone activity by adjusting the rate of oligomeric dissociation. Biochem. Biophys. Res. Commun. **310:** 412–420

163 Fu L. and Liang J. J.-N. (2003) Enhanced stability of αB-crystallin in the presence of small heat shock protein Hsp27. Biochem. Biophys. Res. Commun. **302:** 710–714

164 Sun T.-X. and Liang, J. J.-N. (1998) Intermolecular exchange and stabilization of recombinant human αA- and αB-crystallin. J. Biol. Chem. **273:** 286–290

165 Reddy G. B., Das K. P., Petrash J. M. and Surewicz W. K. (2000) Temperature-dependent chaperone activity and structural properties of human αA- and αB-crystallins. J. Biol. Chem. **275:** 4565–4570

166 Datta S. A. and Rao Ch. M. (1999) Differential temperature-dependent chaperone-like activity of αA- and αB-crystallin homoaggregates. J. Biol. Chem. **274:** 34773–34778

167 Rao Ch. M., Raman B., Ramakrishna T., Rajaraman K., Ghosh D., Datta S. et al. (1998) Structural perturbation of α-crystallin and its chaperone-like activity. Int. J. Biol. Macromol. **22:** 271–281

To access this journal online:
http://www.birkhauser.ch