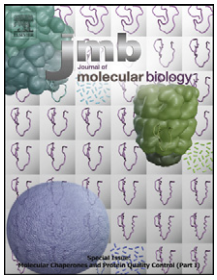

A First Line of Stress Defense: Small Heat Shock Proteins and Their Function in Protein Homeostasis

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Abstract

Small heat shock proteins (sHsps) are virtually ubiquitous molecular chaperones that can prevent the irreversible aggregation of denaturing proteins. sHsps complex with a variety of non-native proteins in an ATP-independent manner and, in the context of the stress response, form a first line of defense against protein aggregation in order to maintain protein homeostasis. In vertebrates, they act to maintain the clarity of the eye lens, and in humans, sHsp mutations are linked to myopathies and neuropathies. Although found in all domains of life, sHsps are quite diverse and have evolved independently in metazoans, plants and fungi. sHsp monomers range in size from approximately 12 to 42 kDa and are defined by a conserved β-sandwich α-crystallin domain, flanked by variable N- and C-terminal sequences. Most sHsps form large oligomeric ensembles with a broad distribution of different, sphere- or barrel-like oligomers, with the size and structure of the oligomers dictated by features of the N- and C-termini. The activity of sHsps is regulated by mechanisms that change the equilibrium distribution in tertiary features and/or quaternary structure of the sHsp ensembles. Cooperation and/or co-assembly between different sHsps in the same cellular compartment add an underexplored level of complexity to sHsp structure and function.

Evolution of sHsps

In the course of evolution, a network of proteins arose to protect cells against stress conditions (e.g., heat, cold, oxidative stress) (cf. Ref. [1]). A prominent group of these stress proteins are the molecular chaperones, which comprise several families classified according to their molecular mass and evolutionary history [2]. Small heat shock proteins (sHsps), which are present in all three domains of life, are the least conserved of the molecular chaperones [3,4]. Among the most well-studied members of the sHsp family are the two α-crystallins, αA (or HspB4) and αB (or HspB5), which share 60% amino acid identity and account for over 30% of protein in the vertebrate eye lens. In the lens, they act to maintain the solubility of other lenticular proteins, preventing aggregate-induced light scattering [5,6]. The clinical importance of αB is highlighted by its additional expression in many other tissues [7] and the fact that αB mutations are linked to myopathies [8]. Furthermore, αB accumulates in plaques of amyloid proteins/peptides that are correlated with neurological disorders such as Alzheimer's and Creutzfeldt-Jakob, as well as other diseases [8]. The link of sHsps to human disease, along with the fact that sHsps are expressed during stress and specific stages of development in other organisms, indicates the importance of this virtually ubiquitous group of molecular chaperones.

sHsp primary structure can be dissected into a non-conserved N-terminal sequence (NTS) of variable length, a conserved α-crystallin domain (ACD) and a non-conserved short C-terminal sequence (CTS) (Fig. 1A) [3,9]. The ACD (or Hsp20 domain, PF00011) represents the conserved signature motif of sHsps. Phylogenetic analyses indicate that sHsps were already present in the last common ancestor of prokaryotes and eukaryotes [3,10,11]. Prokaryotes contain usually only one or two sHsps [3,11,12]. However, a few, mostly pathogenic bacteria do not

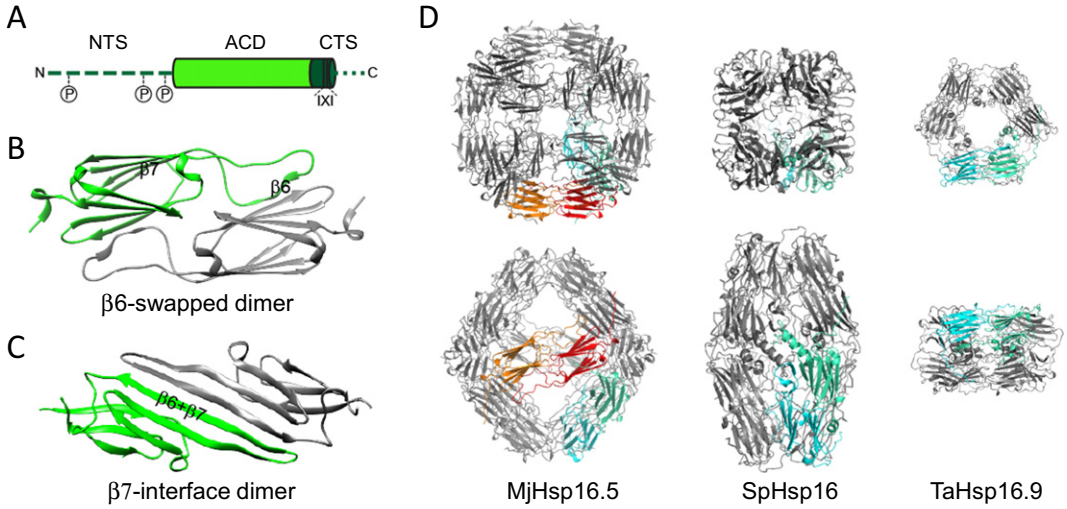

Fig. 1. (A) Domain organization of sHsps. NTS (dark green, broken line), ACD (light green) and CTS (dark green with the conserved I-X-I motive in cylinder form and the remainder as a dotted line). As indicated, up to three phosphorylation sites exist in the NTS of some sHsps as discussed in the text. (B) Structure of a β6-swapped dimer of the ACD of *M. jannaschii* Hsp16.5 (X-ray crystallography, PDB ID 1SHS; see Ref. [45]). The ACDs of individual protomers are colored green and gray. (C) Structure of a β7-interface dimer of the ACD of human αB-crystallin (solid-state NMR, PDB ID 2KLR; see Ref. [49]). (D) Scaling comparison of the three available oligomeric crystal structures of sHsps. One dimeric building block is marked in green-cyan to highlight the variable interconnections of the dimers in the respective structures. MjHsp16.5, *M. jannaschii* Hsp16.5 representing a 24mer [45]. The dimeric building block highlighted in orange-red additionally highlights the equatorial protein axis forming an octahedron. SpHsp16, *Schizosaccharomyces pombe* Hsp16 representing a 16mer ellipsoid composed of two half-spheres of four dimers [46]. TaHsp16.9, *Tritium aestivum* (wheat) Hsp16.9 representing a 12mer of two stacked rings [40].

encode sHsps, and some, often symbiotic bacteria, have as many as 12 sHsp genes [3,12,13]. In comparison, in most multicellular eukaryotes, the number of sHsps is significantly larger [3,14]. For example, in addition to αA-crystallin and αB-crystallin, there are 8 other sHsps encoded in the human genome, 16 sHsps are present in *Caenorhabditis elegans*, and at least 12 sHsp gene families are present in higher plants, with multiple family members bringing the total number of plant sHsp genes in any one species to 20 or more [3,15–17].

A number of studies have considered the evolutionary trajectory of sHsps, both as a superfamily and within individual domains of life [4,10,11,18–20]. From a comprehensive analysis of 8714 sHsp sequences, Kriehuber *et al.* were able to conclude that the sHsp signature ACD has evolved independently of the flanking NTS and CTS [3]. A phylogeny of the ACD reflects speciation events, with sHsps from different phyla clustering together on specific branches of the evolutionary tree, while analysis of the NTS and CTS shows no such relationship. In addition, bacterial sHsps are spread into several branches of the tree, even when only considering the ACD, seemingly reflecting a functional diversification of these sequences. Notably, all metazoan sHsps belong to a distinct clade and appear to have evolved from a single ancestral gene by repeated duplications. Similarly, the gene families of higher plant sHsps have arisen by duplication and divergence, which has also included acquisition of specific targeting signals to direct the encoded sHsps to intracellular organelles [17]. In this regard, higher plants are unique compared to other eukaryotes, in that sHsps are found not only in the cytosol but also in virtually every membrane-bound compartment—chloroplasts, mitochondria, endoplasmic reticulum (ER), peroxisomes and the nucleus [17,18,21–23]. *Drosophila* and *Toxoplasma gondii*, in which an sHsp is found in mitochondria, are up to now the only other eukaryotes known to have other organelle-localized sHsps [24–26]. Shuttling of cytosolic sHsps into the nucleus is observed under certain conditions in virtually all eukaryotes, but this behavior is distinct from the nuclear-targeted sHsp in plants, which possesses a canonical nuclear-targeting signal. The sessile lifestyle of plants after their movement to land may have driven the evolution of these chaperones to provide protection of proteins throughout the cell. Indeed, there is no evidence for organelle-targeted sHsps in algae [17,21]. While the presence of sHsps in mitochondria, chloroplasts and the ER may appear similar to the existence of Hsp70/DnaK homologues in the same compartments [27,28], their evolution is dramatically different. The Hsp70/DnaK homologues were present in the common ancestor of eukaryotes (or of plants in the case of