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REVIEW

Heat shock proteins: Molecules with assorted functions

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Abstract **Heat shock proteins (Hsps) or molecular chaperones, are highly conserved protein families present in all studied organisms. Following cellular stress, the intracellular concentration of Hsps generally increases several folds. Hsps undergo ATP-driven conformational changes to stabilize unfolded proteins or unfold them for translocation across membranes or mark them for degradation. They are broadly classified in several families according to their molecular weights and functional properties. Extensive studies during the past few decades suggest that Hsps play a vital role in both normal cellular homeostasis and stress response. Hsps have been reported to interact with numerous substrates and are involved in many biological functions such as cellular communication, immune response, protein transport, apoptosis, cell cycle regulation, gametogenesis and aging. The present review attempts to provide a brief overview of various Hsps and summarizes their involvement in diverse biological activities.**

Keywords heat shock protein, chaperone, chaperonin, Hsp100, Hsp90, Hsp70, Hsp60, sHsps, fertility, apoptosis, cytoskeleton

Introduction

Although humans have long studied effects of stress/heat on themselves and on other organisms, studies of the heat-shock response began per se in 1962 with the serendipitous discovery of a new set of puffs on salivary gland chromosomes of the fruit fly, *Drosophila busckii* (Ritossa, 1962). This observation eventually led to the discovery of heat shock proteins (Hsps), whose genes were among the first eukaryotic genes to be cloned (Pauli et al., 1992). The intent of this review is to provide a brief overview of what is known about various Hsps with special emphasis on Hsp60.

Heat shock proteins (Hsps) were initially thought to stabilize macromolecular structures by binding damaged proteins during stress to prevent them from aggregating, and then release those bound proteins when suitable conditions return, to refold and regain their normal functions at the expense of ATP hydrolysis (Pelham, 1986). In addition to heat, Hsps were also reported to be induced by a wide variety of environmental or metabolic stresses, which included anoxia, ischemia, heavy metal ions, ethanol, nicotine, benzamide, surgical stress, energy depletion and viral agents etc (Lakhotia, 2001). It was later realized that Hsps, several of which are also present in unstressed cells, may function in a similar way to assist the folding of newly synthesized proteins under normal growth conditions and in this role Hsps were referred to as "molecular chaperones" (Ellis, 1987; Feder and Hofmann, 1999). Chaperones are ubiquitous and highly conserved protein families that utilize cycles of ATP-driven conformational changes to either stabilize unfolded proteins or unfold them for translocation across membranes, or mark them for degradation. Hsps selectively recognize and bind to the exposed hydrophobic surfaces of non-native proteins in a non-covalent interaction in order to inhibit irreversible aggregation.

Hsps or molecular chaperones contribute up to 5%–10% of total protein content in healthy growth conditions. Hsps function in complex with each other and recruit various smaller proteins called co-chaperones (Caplan, 2003). These co-chaperones regulate the ATPase cycle and thus the speed of Hsp-assisted folding of target polypeptides.

Hsps have been extensively studied during the past few decades, especially with regard to their cellular localization, regulation, and function. Genetic and biochemical studies in many laboratories have suggested that apart from their normal protein folding activities, members of stress protein families have also been implicated in a variety of developmental

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processes during normal conditions (Whitley et al., 1999; Lakhotia, 2001; Nollen and Morimoto, 2002). Furthermore, several Hsps have been demonstrated as signaling molecules and essential for many biological activities (Cutforth and Rubin, 1994; Ranford et al., 2000). Elevated levels of heat shock proteins are seen in certain chronic disease states including Hashimoto's thyroiditis, Graves' disease, arthritis and atherosclerosis (Heufelder et al., 1992; Slavotinek and Biesecker, 2001; Sõti et al., 2005; Kikis et al., 2010). Hsps also influence the activation of enzymes and receptors (Gething and Sambrook, 1992). Hsps play a key role in the repair of proteotoxic damage and the maintenance of cell architecture that finally regulates the aging process (Soti and Csermely, 2002). Some molecular chaperones have also been implicated in cancer development (Trepel et al., 2010). Some recent studies suggest a potential role of Hsps in miRNA processing and regulation of tumorigenesis (Iwasaki et al., 2010; Johnston et al., 2010). Table 1 attempts to classify major groups of Hsps according to their reported biological functions. It is proposed that Hsps have co-evolved as integral components of signal transduction networks with roles in maturation, activation and inactivation of signaling molecules (Nollen and Morimoto, 2002). Therefore, the uninterrupted presence of molecular chaperones is essential for viability.

The intracellular concentration of Hsps can increase two- to threefold following cellular stress resulting in either protein denaturation, protein aggregation or a flux of newly-synthesized non-native proteins. This suggests that the main purpose of increased concentrations of molecular chaperones following stress is to minimize protein aggregation and thus ensure proper protein folding and transport; in this respect, these proteins potentially play major roles in the molecular evolution of many enzymes (Csermely, 1997; Feldman and Frydman, 2000; Thirumalai and Lorimer, 2001).

Hsps are broadly classified by their molecular weight, amino acid sequence homologies and functional aspects (Nover, 1984). Conventionally, they are grouped into five major families, Hsp100 (100–104 kDa), Hsp90 (82–90 kDa), Hsp70 (68–75 kDa), Hsp60 (58–65 kDa) and small Hsp (15–30 kDa) families.

The Hsp100/Clp chaperone family

The Hsp100 or Clp family includes constitutive and stress inducible chaperones in the range of 100–110 kDa, which also have proteolytic activities (Clarke, 1996; Mayer, 2010). The Hsp100/Clp chaperone family shows strong conservation in bacteria, yeast, plants, trypanosomes and mammals and is found at various intracellular locations.

The Clp/Hsp100 protein family in prokaryotes is further classified into two major subgroups on basis of the occurrence of two highly conserved ATP binding domains. Among the five different proteins (ClpA, B, C, X and Y) present in the Hsp100/Clp protein family, the first group includes ClpA, ClpB and ClpC, with ClpA and ClpC being constitutive and ClpB being heat inducible. These three proteins possess two characteristic ATP binding domains (ATP-1 and ATP-2). Several members of this subfamily have been identified in various organisms, including Hsp104 and Hsp78 in *Saccharomyces cerevisiae* (Sanchez and Lindquist, 1990; Leonhardt et al., 1993). In *S. cerevisiae*, Hsp104 mediates the solubilization of aggregated proteins in an ATP-dependent process assisted by the Hsp70/40 system (Bösl et al., 2005). ClpB has been identified in various bacteria and is proposed to be involved in proteolysis because of its sequence similarity to the ClpA subunit (Kitagawa et al., 1991; Squires et al., 1991). The ClpC chaperone of the Hsp100 family, identified as a stress sensor molecule, is essential for the pathogenicity of *Staphylococcus aureus* (Frees et al., 2004). In plant cells, the majority of ClpC localizes in the stromal compartment of chloroplasts and is essential for leaf development and photosystem biosynthesis (Sjögren et al., 2004). The second group of Clp proteins consists of ClpX and ClpY which contain only one ATP binding domain with a greater similarity to the ATP-2 (Schirmer et al., 1996).

Clps/Hsp100 help to stabilize selected polypeptides during severe thermal stress and either enable resolubilization of non-functional protein aggregates or target the irreversibly damaged polypeptides for degradation. The eukaryotic homolog of heat inducible ClpB, the Hsp100, plays an important role in thermotolerance (Parsell et al., 1991; Parsell

Table 1 Classification of major heat shock proteins (Hsps) based on their proposed biological functions

Biological functions	Heat shock proteins
Stress response, thermotolerance, protein folding (stress induced)	Hsp100, Hsp90, Hsp70, Hsp60, sHsps
Protein folding (newly synthesized), maintenance of protein homeostasis, microfilament stabilization, maintenance of cytoskeletal components, cellular communication, apoptosis, epithelial remodeling, tumorigenesis	Hsp90, Hsc70, Hsp60, sHsps
Aging and longevity	Hsp90, Hsc70, Hsp60, sHsps
Immune response, development of autoimmune disorders	Hsp90, Hsp60, sHsps
Modifier of PolyQ induced phenotypes/neurodegeneration	Hsp70, Hsc70, Hsp60, sHsps
Fertility, gametogenesis	Hsp90, Hsp60
microRNA processing	Hsp90, Hsc70

and Lindquist, 1994). Interestingly, no Hsp100 homolog has been found in *Drosophila melanogaster*.

Hsp90/HtpG chaperone family

Hsp90 is one of the most abundant proteins of eukaryotic cells, making 1%–2% of total proteins under normal physiological conditions. Hsp90, together with several different co-chaperones, play an important role in folding of at least 200 specific proteins of various signaling pathways under normal conditions; it is also important for refolding of denatured proteins following stress (Pratt and Toft, 2003).

Hsp90 family members are the major molecular chaperones in cytosol and endoplasmic reticulum. Mammalian cells contain four distinct members of the Hsp90 molecular chaperone family (Csermely et al., 1998). The cytosolic Hsp90 has two isoforms, Hsp90α and Hsp90β, which are 76% identical at amino acid level (Csermely et al., 1998; Houlihan et al. 2009). Another isoform, the 94-kDa glucose-regulated protein Grp94, is localized primarily in the endoplasmic reticulum (ER) and shows 50% homology with Hsp90. Lastly, tumor necrosis factor receptor-associated protein-1 is primarily located in the mitochondria of mammalian cells (Song et al., 1995). *Drosophila* possesses only one Hsp90 family protein of 83 kDa size. The *Hsp83* is the only Hsp-coding gene in *D. melanogaster* with an intron (Hackett and Lis, 1983). Immunofluorescence studies showed that Hsp83 is mainly distributed in cytoplasm during unstressed as well as heat shock conditions (Lindquist, 1980; Voellmy et al., 1983). However, Tanguay and his group showed the presence of Hsp83 in polytene nuclei of salivary glands where it is localized at 93D puff of polytene chromosome following heat stress (Carbajal et al., 1990; Morcillo et al., 1993).

Hsp90 is an ATP-dependent chaperone consisting of an ATP binding domain located at the N-terminal site and exhibits auto-phosphorylation (Csermely and Kahn, 1991; Csermely et al., 1998). Like many other chaperones, Hsp90 is a hydrophobic protein and its hydrophobicity further increases after heat shock (Yamamoto et al., 1991). ATP binding facilitates conformational changes in Hsp90 allowing its interaction with other proteins (Kellermayer and Csermely, 1995). Since Hsp90 binds with histone H1 and enhances its binding to DNA, it has been suggested that Hsp90 may be a nuclear chaperone as well (Csermely et al., 1994). Various steroid receptors (glucocorticoid, estrogen, progesterone) reversibly associate with Hsp90 family proteins immediately after synthesis, in ATP dependent manner, and only the receptors that are bound to Hsp90 are recognized by the hormone ligand (Inano et al., 1994).

Hsp83 mutations were first isolated on the basis of genetic interactions with a temperature-sensitive *sevenless* allele, which codes for a receptor tyrosine kinase (Cutforth and Rubin, 1994). Later it was shown that Hsp83 is also essential for the Raf-kinase mediated signaling in *Drosophila* (van der Straten et al., 1997). Rutherford and Lindquist (1998) observed multiple phenotypic variations in flies heterozygous for the *Hsp83* mutant allele. They proposed that Hsp83 normally suppresses genetic variation by buffering the mutant allele expression, possibly at the level of protein folding. Since the frequency of phenotypic variation in *Hsp83* mutant strains was increased under stressful conditions, Rutherford and Lindquist argued that the evolutionary change could be stimulated by conditions that transiently cause a decrease in the cellular level of Hsp83. In an interesting study, using a sensitized isogenic *D. melanogaster* strain, *iso-Kr*$^{If-1}$, Sollars et al., (2003) presented evidence in support of an epigenetic mechanism for Hsp90 capacitor function, whereby reduced activity of Hsp90 induces a heritably altered chromatin state.

Inactivation/deletion of *Hsp90* gene is deleterious for eukaryotes but deletion of *HtpG* gene, the prokaryotic homolog of *Hsp90* is not lethal for bacteria (Lopatin et al., 2000). Pandey et al. (2000) have reported that Hsp90 forms a cytosolic complex with APAF-1 (apoptosis protease activating factor-1), the key adaptor molecule in the mitochondrial process of apoptosis, and thereby inhibits formation of the active complex. Immunodepletion of Hsp90 depletes APAF-1 and thereby inhibits cytochrome-c-mediated activation of caspase-9. Hsp90 thus appears to function as a negative cytosolic regulator of apoptotic cell death by interfering with the formation of the apoptosome complex (Pandey et al., 2000; Garrido et al., 2001). Hsp90 has also been shown to interact with and stabilize RIP-1 (receptor interacting protein-1) kinase, a protein that connects death receptors to NF-κB, a family of transcription factors that usually prevent cell death (Lewis et al., 2000). It is suggested that tumor cells use Hsp90 chaperone machinery to protect an array of mutated and overexpressed oncoproteins from misfolding and degradation. Therefore, Hsp90 is proposed to function as a crucial facilitator of oncogene addiction and cancer cell survival (Trepel et al., 2010). Furthermore, disruption of Hsp90 functions by its specific inhibitor, geldanamycin, has been shown to promote the degradation of the death domain kinase RIP, which results in susceptibility to TNF (tumor necrosis factor)-induced apoptosis (Lewis et al., 2000). In view of the multiple interaction of Hsp90, its regulated inhibition may emerge as a very promising therapy for various forms of cancer (Hwang et al., 2009).

Subsequently, Hsp90 family members are suggested to be involved in various cellular functions in view of their association with a wide variety of cellular proteins including steroid receptors, histone H1, cytoskeleton proteins, Apaf-1, Hsp56 and Hsp70 (Sõti et al., 2005; Zhao et al., 2005). Association of Hsp90 with actin and tubulin led to the suggestion that the Hsp90 containing hetero-complex may serve as a "transportosome" for intracellular trafficking of proteins in an inactive state to their destination along the cytoskeleton (Czar et al., 1994). Some reports also indicate possible involvement of Hsp90 in the regulation of aging

processes (Morange, 2006).

Interestingly, Hsp90 family members have been shown to buffer natural polygenic variations that affect the morphological changes and signaling pathways (Yahara, 1999; Rutherford, 2003). Furthermore, it has also been suggested that Hsp90 acts as a nodal point for various signaling pathways, and therefore, it maintains the clarity and strength of communication within and between cells (Rutherford et al., 2007; Taipale et al., 2010).

The Hsp70/DnaK chaperone family

The Hsp70 proteins constitute the most conserved family with ~50% amino acid identity among all characterized species from bacteria to humans. Their functional diversity is remarkable considering their high sequence identity within and across species. Some *Hsp70* genes, the heat shock cognate *Hsc70* genes, are constitutively expressed while others are maximally induced in stressed cells. Members of Hsp70 family exist in the cytosol of archea, eubacteria and in eukaryotic cytosol, organelles (mitochondria, ER and chloroplast) and nuclei (Lakhotia, 2001). Additionally, Hsp70 has been detected on the surface of tumor cells (Kampinga and Craig, 2010). Occurrence of multiple copies of *Hsp70* genes in most species, e.g. 14 in yeast and 13 in *Drosophila*, is a unique feature of this Hsp family (Günther and Walter, 1994; Rassow et al., 1997). The prokaryotic homologs of Hsp70 family members are referred to as DnaK proteins. Members of the Hsp70 family cooperate with cofactors of the DnaJ/Hsp40 family in an ATP-dependent manner.

The Hsp70 proteins contain a conserved N-terminal 44-kDa ATP binding domain and a less conserved 30-kDa C-terminal domain (McKay, 1991; Hightower and Seth, 1994). The C-terminal domain is responsible for peptide binding. Although Hsp70 is not itself a protease, it is now established that co-chaperone can control its activity to direct substrate proteins either to refold, or to be degraded. CHIP (C terminus of Hsc70 interacting protein) is an ubiquitin ligase that associates with Hsp70 and Hsp90 and their cognates to direct substrate proteins to the proteasome (Murata et al., 2001; McDonough and Patterson, 2003). CHIP is also involved in regulation of heat shock response (McDonough and Patterson, 2003).

Hsp70 plays critical roles in thermotolerance from bacteria to mammalian cells. Flies with a reduction in *Hsp70* gene copy number or those with no copies of heat inducible *Hsp70* are viable (Gong and Golic, 2006). However, mutations in several of the constitutively expressed homologs of *Hsp70* (*Hsc70*) in *Drosophila* cause lethality (Burmester et al., 2000), indicating that the Hsc70 family proteins have critical functions at normal temperature. It seems that presence of *Hsp70* genes are essential for *D. melanogaster* to survive a severe heat shock, but are not essential to survive a milder heat shock (Gong and Golic, 2006). This indicates that a substantial level of thermotolerance remains even in the absence of Hsp70. However, flies without Hsp70 have a prolonged heat shock response and an extended developmental delay after a non-lethal heat shock, indicating that Hsp70 has an important role in recovery from stress, even at lower temperatures. Lack of Hsp70 also confers enhanced sensitivity to a temperature-sensitive lethal mutation and to the neurodegenerative effects produced by expression of a human polyglutamine disease protein (Chan et al., 2002; Gong and Golic, 2006).

Apart from its chaperoning activities, Hsp70 family proteins have also been demonstrated to perform several other activities as well, which include protein degradation, reorganization of cytoskeletal components, translation initiation, nuclear protein import and export, ribosome assembly, chromatin structure and DNA synthesis (Kampinga and Craig, 2010). In the past few years, many studies have implicated roles for Hsp70 in the ecology and evolutionary physiology of stress tolerance and aging (Feder and Hofman, 1999). Overexpression of Hsp70 has been shown to diminish the neurodegenerative effects of human genes carrying expanded polyQ starches (Chan et al., 2002; Novoselova et al., 2005). It is also interesting to note that Hsp70 null *Drosophila* females have significantly reduced fertility, indicating that heat inducible genes have some roles at normal temperature (Gong and Golic, 2006).

The Hsp60/Tcp1 family

Hsp60 family proteins, commonly also called “chaperonins” (Hemmingsen, 1992), exert functions similar to those of Hsp70 family proteins in assisting the process of protein folding. They are primarily found in the cytosol of bacteria (GroEL), in the matrix component of mitochondria and in the stromal compartment of chloroplasts (Hartl et al., 1992; Houry et al., 1999). The nucleotide sequence of *Hsp60* is highly conserved through different species and therefore, very useful for phylogenetic studies and identification of organisms (Hill et al., 2004). Interestingly, some parasites, such as microsporidia and mycoplasma, which have extremely small genomes, completely lack Hsp60 (Glass et al., 2000; Katinka et al., 2001).

The Hsp60 family proteins (chaperonin-60 or cpn60 proteins) are stress inducible as well as constitutively expressed and are essential for growth under all conditions. Although, initial studies on Hsp60 established this protein to be localized to the mitochondrial matrix, some new findings about its diverse roles are inconsistent with an exclusive mitochondrial location of Hsp60 (Gupta et al., 2008). The eukaryotic cytosolic homolog of Hsp60 proteins discovered more recently and termed Tcp-1 (also called TriC, CCT, C-cpn), is a heteromeric assembly of several polypeptides uninducible by stress. Tcp-1 has distinct but significant sequence homology to the Hsp60 chaperonins, suggesting an

evolutionary relationship between these proteins (Gupta, 1995).

Fourteen Hsp60 (GroEL) polypeptides form ring-shaped oligomeric complex with the subunits arranged in two stacked heptameric rings that form a barrel like structure (Bukau and Horwich, 1998; Mayer, 2010). The unfolded protein substrate binds via hydrophobic interactions in the large central cavity of this barrel (Spiess et al., 2004; Mayer, 2010). Hsp60 polypeptides work together with Hsp10 in an oligomeric assembly to facilitate correct binding and folding of native proteins, without being involved in the final protein structure themselves (Ranson et al., 1998). When ATP is bound to Hsp60, the Hsp10 forms a lid on the Hsp60 barrel structure (Chandrasekhar et al., 1986; Saibil, 1996; Mayer, 2010) that causes the central cavity to enlarge, thus providing appropriate conditions for protein folding.

Each subunit of Hsp60 has three domains with different characteristics: i) an apical domain, which facilitates the binding of the substrate and a co-chaperone Hsp10 (GroES), ii) an equatorial domain, which contains a binding site for adenosine triphosphate (ATP) and the contacts for ring binding, and iii) the intermediate domain, which connects these two domains. The intermediate domain acts as a hinge, which undergoes conformational changes upon ATP binding (Ranson et al., 1998), and thereby allowing the substrate binding surface to alternate between hydrophobic and hydrophilic states. When the surface is in the hydrophobic state, a protein substrate can bind to Hsp60, thus preventing incorrect association of the substrate with other proteins, which might lead to misfolding. When ATP binds to Hsp60, the hinge opens up, altering the substrate binding surface such that it becomes hydrophilic, and the protein substrate is then released (Ranson et al., 1998, Feltham and Gierasch, 2000). If the released substrate protein is still not correctly folded, it will go through another round of interaction with Hsp60.

It is estimated that under normal growth conditions, Hsp60/GroEL folds 10%–15% of all cytoplasmic proteins in bacterial cells and under heat stress, this increases to 30% (Ellis, 2005). Hsp60/GroEL proteins show higher affinity to partially folded structures, whereas the Hsp70/DnaJ complex associates preferentially with short peptides or polypeptides in extended conformations. These differential binding capabilities of Hsp60 and Hsp70 chaperone systems suggest that these proteins could function in a sequential reaction accompanying a newly synthesized polypeptide along its folding pathway. *In vitro* studies show that Hsp60 prevents the aggregation of proteins that denature at physiologically relevant temperatures.

The first indication of non-chaperone activities of Hsp60 was the observation that if delivered onto their external surface, it can activate cells to synthesize and secrete cytokines (Retzlaff et al., 1994). It was estimated that in a variety of cells and tissues, 15%–20% of Hsp60 reactivity is present at discrete extra-mitochondrial sites, including cell surface, unidentified cytoplasmic vesicles and granules, peroxisomes and endoplasmic reticulum, pancreatic β cells and zymogen granules in pancreatic acinar cells (Pfister et al., 2005). In this context, it is interesting to note that through atomic force microscopy Hsp60 has been demonstrated to be present on stressed human endothelial cells (Pfister et al., 2005). In addition, upregulation of Hsp60 has been detected in stem cells (Ramalho-Santos et al., 2002). Recently, cytosolic Hsp60 has been demonstrated to promote tumor necrosis factor (TNF)-α-mediated activation of the IKK/NF-κB dependent survival of cancer cells via upregulating serine phosphorylation in a chaperone-independent manner (Chun et al., 2010).

Although activation of heat shock genes and the new synthesis of heat shock proteins were originally described in *Drosophila* (Ritossa, 1962; Tissières, 1974), initial studies on Hsps in *Drosophila* did not identify any Hsp60 family member in this organism. The first report of the existence of Hsp60 in *D. melanogater* came in 1989, when heat shock induced synthesis of Hsp60 was observed as a member of a novel set of polypeptides in Malpighian tubules of *Drosophila larvae* (Lakhotia and Singh, 1989; Singh and Lakhotia, 1995). Subsequently, the Berkeley Drosophila Genome Project revealed the presence of four Hsp60-like DNA sequences at different cytogenetic positions (Adams et al., 2000, Rubin et al., 2000). These genes were named as *Hsp60A* at 10A4 polytene band, *Hsp60B* at 21D2 band, *Hsp60C* at 25F2 band and *Hsp60D* at 34C1 band (Sarkar and Lakhotia, 2005). It is suggested that multiple copies of *Hsp60* genes in *Drosophila* evolved from the X-linked *Hsp60A* gene by retrotransposition on autosomes (Betrán et al., 2002). Presence of multiple copies of Hsp60 in *D. melanogaster* provides a unique opportunity to study its role in diverse biological phenomena.

Hsp60 and cytoskeleton proteins

Molecular chaperones influence several cell components, one of which is the cytoskeleton, an assortment of filamentous and tubular polymers composed of microtubules, microfilaments and intermediate filaments (Sarkar et al., 2006). Although varied in molecular composition, the activities of cytoskeletal elements overlap with and are influenced by one another. Function of the cytoskeleton, modulated by polymerization dynamics and spatial organization, include determination of cell shape, partitioning of organelles and molecules by intracellular transport mechanisms mediated by mechano-chemical accessory proteins, division and motility. Therefore, proper synthesis and assembly of cytoskeletal elements are vital to cells survival, as disruption of these macromolecular complexes is often disastrous. In this context, it is interesting to note that existence of chaperonin-based cytoskeleton has been demonstrated in a hyperthermophilic archaeon *Sulfolobus shibatae* (Sarkar et al., 2006). It has also been suggested that in absence of actin/tubulin-based cytoskeleton and rigid

cell wall, some archaeal species have developed a chaperonin filament-based internal cytoskeleton, which is functionally similar to the eukaryotic cytoskeleton (Hixon and Searcy, 1993; Trent et al., 1997). In *S. shibatae* and other Archaea also, chaperonins are abundant and have filament-forming ability (Kagawa et al., 1995). In view of these observations, it was proposed that chaperonins are building blocks for the Archeal cytoskeleton (Trent et al., 1997).

The Hsp60 family proteins and the eukaryotic Tcp-1 are known to fold and stabilize the major cytoskeletal proteins, specially actin and tubulin in eukaryotes (Leroux and Candido, 1997; Sarkar et al., 2006). An early indication that cytoskeleton proteins are targets of the Tcp-1 complex was derived from the presence of abnormal cytoskeleton structures in a cold sensitive Tcp-1 yeast mutant (Ursic and Culbertson, 1991). In addition, continuous presence of tubulin and actin in the Tcp-1 complexe suggests that they are its major substrates (Yaffe et al., 1992; Setrnlicht, 1993). Interestingly, eight Tcp-1 family genes have been discovered in *S. cereviseae* and at least four of these genes, *Tcp-1*, *Bin-2*, *Bin-3*, and *Can-2* (or *CCT1-CCT4*), are necessary for normal functions of tubulin and actin (Ursic et al., 1994; Miklos et al., 1994; Vinh and Drubin, 1994). All the *Bin (binucleate)* mutants display defects in microtubule and actin assembly (Chen et al., 1994). It is suggested that different Tcp-1 members in yeast have evolved to perform separate functions since mutant alleles of these genes do not complement each other (Chen et al., 1994) In this context, it is interesting to note that the mammalian Hsp60 (also called CPN60) was also first identified as a tubulin-associated protein whose mutation causes resistance to anti-mitotic drugs (Soltys and Gupta, 1999).

In vitro as well as *in vivo* studies show that nascent tubulin and actin polypeptides enter a 900 kDa Tcp-1 complex and emerge as assembly-competent forms (Yaffe et al., 1992; Sternlicht, 1993). While additional protein cofactors are required for the folding of α- and β- tubulin, only Tcp-1 complex can fold the actin (Gao et al., 1992). Tcp-1 subunits selectively bind with F-actin at the microfilament assembly site (Roobol and Carden, 1999; Grantham et al., 2002). Besides the α- and β- tubulin and actin, Tcp-1 complex is also critical for folding of centrosome related proteins such as γ-tubulin and centractin (Melki and Cowan, 1994). The preference of Tcp-1 complex for cytoskeleton proteins seems to be related to the presence of abundant proline and other hydrophobic residues in the β-tubulin peptide, that may provide a region for its binding with the Tcp-1 complex. All the cytoskeletal components that interact with Tcp-1 complex, share a high sequence homology in a C-terminal peptide with Tcp-1 proteins, which may provide a basis for the specificity of association of the nascent substrate with Tcp-1 and the displacement of the chaperone from the substrate as its synthesis progresses (Burns and Surridge, 1994). However, other reports have suggested a wider range of substrates for eukaryotic Tcp-1 proteins.

The Tcp-1 complex is also associated with tubulin during its transport along neuritis. The CCTα component of the Tcp-1 complex enters neurite processes and co-localizes with G-actin at the leading edge of the growth cone, while CCT-β, ε and γ remain largely in the perikaryal cytoplasm (Roobol et al., 1995). In *Tetrahymena*, Tcp-1 subunits and tubulin are co-synthesized during cilia recovery (Soares et al., 1994). The Tcp-1 complex in medulla cells has been identified as chromobindin A, which associates with the chromaffin granules and thus seems to have a role in vesicle transport and/or fusion (Creutz et al., 1994). Hsp60 also exhibits association with cytoskeleton components in higher eukaryotic cells. Specific association of F-actin and Hsp60 was detected in *Drosophila* striated muscle where it was significantly localize in light bands (Fig. 1, A, B). The Z-disc in light bands also showed presence of Hsp60. In addition, a close interaction was also evident between Hsp60 and cytoskeletal components during *Drosophila* oogenesis being essential for microtubule remodeling, cell-cell adhesion and determination of oocyte polarity (Sarkar and Lakhotia, 2008). Abnormal arrangement of F-actin-based cytoskeleton in *Drosophila Hsp60C* mutant allele suggests a pivotal role of this gene in maintenance of cellular integrity (Fig. 1, C, D).

Hsp60 in immune response and cell signaling

Hsps and Hsp60 chaperonin in particular are potent immunogens. Hsp60 may affect both the innate and acquired immune system. Hsp60 proteins from *Escherichia coli*, *Mycobacterium tuberculosis* and *Chlamydia trachomatiscan* induce production of pro-inflammatory cytokines by monocytes (Kol et al., 2000). Although the *in vivo* mechanism for this activation is not resolved completely, it is suggested that they bind to a cell surface receptor, and activate cells via one or more intracellular signaling pathways (Ranford et al., 2000). It is reported that murine monocytes respond to both human and chlamydial chaperonin 60 proteins via the lipopolysaccharide (LPS) receptor CD14 (Kol et al., 2000). *In vitro*, however, it is demonstrated that exogenous Hsp60 can bind to cells via specific receptors like lipopolysaccharide receptor, CD14 and the Toll-like receptors (TLRs) and can stimulate changes in those cells (Kol et al., 2000; Ranford et al., 2000).

In humans and rodents, Hsp60 proteins have been shown to be associated with a number of autoimmune diseases like Rheumatoid arthritis, Multiple sclerosis, Kawasaki disease and Behcet's disease (reviewed by van Eden, 2006). Atherosclerosis, an inflammatory disease with hardening or ulceration of the innermost portion of the arteries, is suggested to be an outcome of the action of antibodies generated by a human host after exposure to bacterial Hsp60 (Xu and Wick, 1996). It has been hypothesized that this protein is expressed on the surface of stressed human vascular

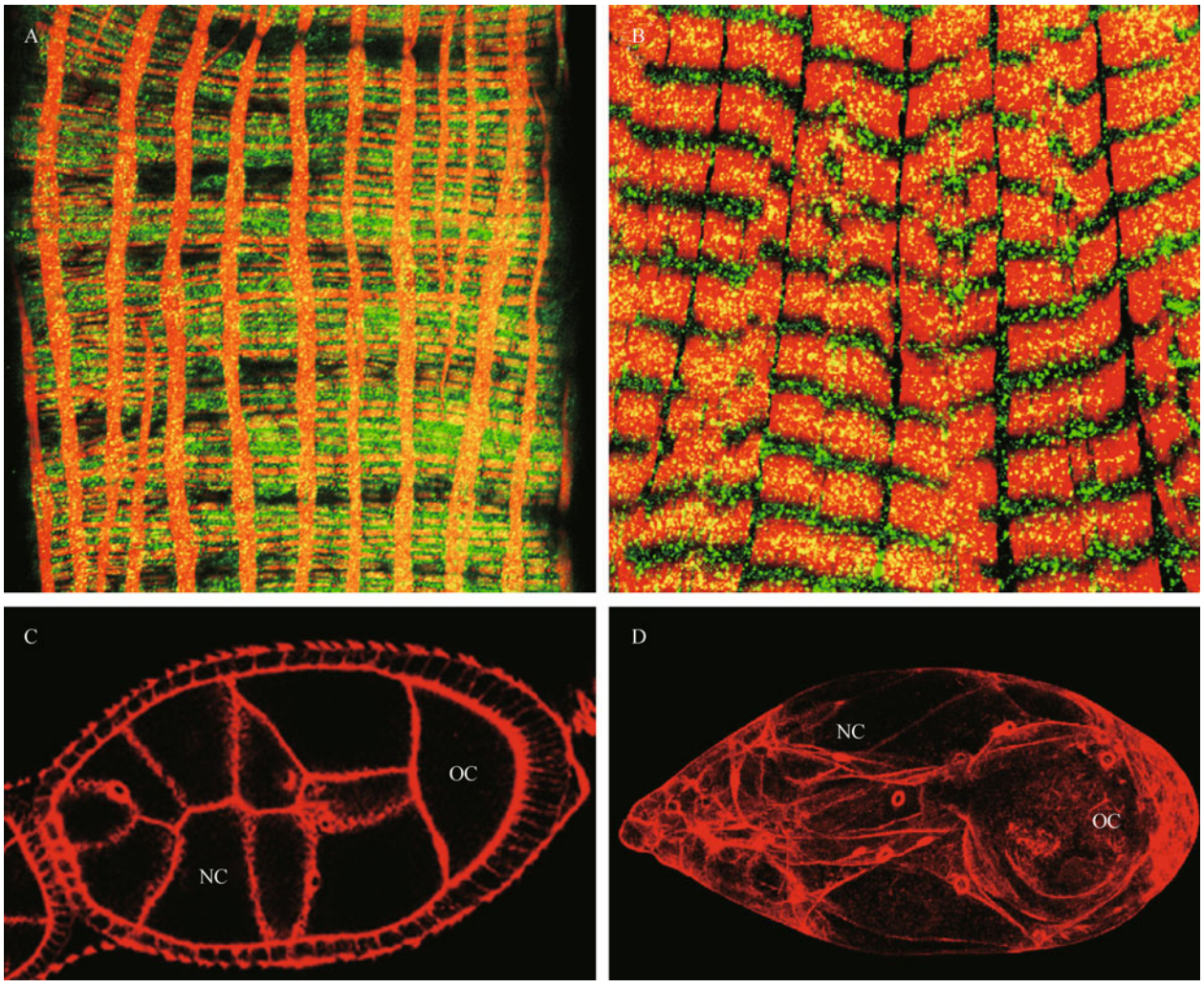

Figure 1 Confocal images showing distribution of Hsp60 (green) and F-actin (red) in *Drosophila* mid-gut muscle (A) and flight muscle (B). Compare to wild type *Drosophila* egg chamber (C), *Hsp60C*^{1} mutant egg chamber (D) exhibits abnormal arrangement of F-actin cytoskeleton. OC: Oocyte, NC: Nurse cell.

endothelial cells that cross-react with chaperonin 60. Infection is a stressful condition for the pathogen as well as the host, and therefore, results in increased production of molecular chaperones by both (Ranford et al., 2000). Although because of the high degree of sequence conservation between bacterial and mammalian molecular chaperones, the resulting immune reactivity to these bacterial proteins is expected to be minimal, however, this is not always the case with chaperonins. Binding of anti-chaperonin 60 antibodies to the surface of the vascular endothelial generates complement-mediated cytotoxicity. The denuded areas of the vasculature initiate the development of atherosclerosis (Xu and Wick, 1996). Presence of Hsp60 on the surface of stressed human endothelial cell provides strong evidence in favor of above hypothesis (Pfister et al., 2005).

In the case of rheumatoid arthritis, antibodies against Hsp60 show reactivity to the synovial tissue (de Graeff-Meeder et al., 1990). Moreover, antibodies to human Hsp60 cross-reacting with *E. coli* Hsp60 have also been demonstrated in such patients. It is still unclear why immunity to molecular chaperones is such a common characteristic of infection. The widely accepted possibility is that T cell reactivity to bacterial chaperonins may lead to the autoimmune recognition of host chaperonins (Zügel and Kaufmann, 1999). Matzinger's 'danger model' hypothesis explains immune responsiveness in terms of the recognition of host components that signal the body is under attack (Matzinger, 2002). In this model, it is the self-Hsp60 that is being recognized, rather than the Hsp60 of pathogens. This hypothesis postulates the Hsps (such as Hsp60) as good examples of a danger signal (Matzinger, 2002). The chaperonins have also been demonstrated to interact with, and activate, several different cells such as monocytes, dendritic cells, endothelial cells, and to present antigens

(Ranford et al., 2000). It has, therefore been suggested that molecular chaperonins may be classified as 'multiplex antigens'. Chaperonins and chaperonin-derived peptides can further activate antigen-presenting cells (APCs) that may enhance antigen presentation with a final outcome of a greater lymphocyte response.

In addition to the action of chaperonin 60 proteins on cytokine synthesis, several other activities have been ascribed to molecular chaperones that seem either to be independent of cytokine synthesis or to have an independent effect on cytokine synthesis. Cultured human vascular endothelial cells respond to chaperonin 60 by upregulating synthesis of adhesion molecules that are involved in controlling leukocyte trafficking in inflammation (Verdegaal et al., 1996). Hsp60 proteins from *M. tuberculosis* and *E. coli* induce the expression of intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and E-selectin in human vascular endothelial cells (Galdiero et al., 1997).

Eukaryotic cells also secrete chaperonins. For example, human neuronal cells have been reported to secrete both a chaperonin-like protein, activity-dependent neurotrophic factor (ADNF) and a chaperonin 60 protein (Gozes and Brenneman, 1996). Indeed, it has been reported that the serum of healthy human subjects contains both human chaperonin 60 and antibodies to chaperonin 60 (Pockley, 2002). Intriguingly, the serum concentrations of chaperonin 60 and antibodies to this protein are higher in women than men, regardless of their pregnancy status. However, the mechanism of secretion of Hsp60 by cells is still unclear. The finding that chaperonin 60 has a lipid-binding domain, which allows it to interact with membranes (Török et al., 1997), may provide an explanation.

Hsp60 in epithelial remodeling

Epithelial migration is a complex and essential phenomenon for organogenesis and tissue repair. This is controlled by a series of cross talks between cell surface receptors, extracellular matrix molecules and growth factors. Hsp60 proteins have been found to play a critical role in epithelial remodeling and cell migration through various signaling cascades. It has been demonstrated that the concentration of Hsp60 affects the migration rate of epithelial cells during tissue regeneration (Shinoda and Huang, 1996; Laplante et al., 1998) and exogenous bacterial Hsp60 enhances the growth of cell proliferation by 25%–75% (Zhang et al., 2001). Furthermore, Hsp60 induces activation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) cascades that have been implicated in signaling of cell motility (Zhang et al., 2001, 2004). p38 activates MAP kinase-activated protein kinase-2 (MAP kinase AP kinase-2), which in turn phosphorylates and activates Hsp27 (Larsen et al., 1997). Finally, the activated Hsp27 regulates actin polymerization that results in cytoskeletal reorganization and stimulated mitogenic activity (Gerthoffer and Gunst, 2001).

An important part of the cellular signaling that controls cell survival, proliferation, and motility is the autocrine activation of epidermal growth factor (EGF) receptor cascade (Jost et al., 2000). ERK is an important signaling molecule-activated downstream of the EGFR. It has been shown that blockage of EGFR activation can completely inhibit both bacterial and human Hsp60-induced cell migration (Zhang et al., 2004). Therefore, it is suggested that exogenous Hsp60 transduces signals through the EGFR pathway that are finally responsible for enhanced epithelial remodeling.

Hsp60 in apoptosis

Any damage to cells can trigger one of two opposing responses, apoptosis, a form of programmed cell death that removes damaged cells or stress response that repairs any damage and helps maintain cell survival. Interaction between these two pathways determines the fate of a cell. However, during normal growth conditions, apoptotic cell death is a fundamental process for embryonic development, tissue homeostasis and regulation of immune system.

Apoptosis is mediated by the activity of the aspartate-specific cystein proteases, the caspases, which cleave either to inactivate or activate target substrates (Wolf and Green, 1999). Caspases initiate a cascade in which 'initiator' caspases interact with specific adaptor molecules to facilitate their own autocatalytic processing. These, in turn, cleave and activate the downstream 'executioner' caspases that orchestrate the proteolytic dismantling of cells (Thornberry and Lazebnik, 1998).

Hsps are actively involved in different aspects of apoptosis. They have a complex role to play, but primarily they are anti-apoptotic. For example, overexpression of Hsp27 and Hsp70 can protect the cells from apoptosis (Arya et al., 2007). Hsp60 is involved in both pro- and anti-apoptotic events, depending on the cell type and provoking stimulus. Functioning as an anti-apoptotic protein, cytosolic Hsp60 forms a complex with Bax and Bak (Gupta and Knowlton, 2002). Bax is a key member of Bcl-2 family proteins with pro- and anti-apoptotic functions, localized in cytosol and mitochondria. Bax is cytosolic until an apoptotic stimulus triggers its translocation into the mitochondria. Once inside the mitochondria, Bid (another Bcl-2 protein family member) triggers conformational changes in Bax, which stimulates release of cytochorome c in the cytosol, and therefore cleavage of caspase 3 followed by DNA fragmentation and finally apoptosis (Eskes et al., 2000). It has been shown that downregulation or sequestration of Bax in the cytoplasm can inhibit apoptotic processes, and conversely, overexpression of Bax may initiate apoptosis without any kind of stimulus (Arya et al., 2007). Hsp60 co-localizes with and sequesters the pro-apoptotic protein Bax, and thus, prevents apoptosis (Gupta and

Knowlton, 2002).

Interestingly, Hsp60 has also been found to enhance apoptotic processes by activating caspase 3 (Xanthoudakis et al., 1999). Hsp60 and its co-chaperonin, Hsp10, form a complex with pro-caspase-3 which is localized in the intermembrane space of mitochondria (Samali et al., 1999). It is suggested that binding of Hsp60 to pro-caspase-3 maintains it in a protease sensitive state, which makes it more susceptible to the action of cytochrome c and dATP (Samali et al., 1999). Xanthoudakis and coworkers (1999), however, also suggested a more significant cytochrome c independent role of Hsp60 in caspase activation. Subsequently, Hsp60D in *D. melanogaster* has been found to be essential for caspase-mediated induced apoptosis (Arya and Lakhotia, 2008).

Hsp60 in fertility

Germ cells provide the continuity of life between generations. Germ cells proliferate and differentiate to supply mature oocytes and spermatozoa. The process of gametogenesis is highly susceptible to stresses, such as high temperature. Relationships between normal Hsp expression and fertility have been demonstrated in several organisms like rat, mouse, *Drosophila*, monkey and human. Tabibzadeh and colleagues (1996) reported the expression of full compliment of human Hsps in endometrium of healthy women. Their expression was also detected in the decidua during the first trimester of pregnancy (Neuer et al., 1997). Maximum levels of Hsp27, Hsp60 and Hsc70 in endometrium are observed after ovulation and in the early secretory phase, which is the critical period of "endometrial receptivity" for an implanting embryo (Tabibzadeh et al., 1996).

The different stages of spermatogenesis represent situations where dramatic transformation and cellular differentiation along with mitochondrial remodeling take place. Therefore, it is not surprising that spermatogenesis is accompanied by expression of different Hsps (Dix, 1997; Eddy, 1998). Hsp60 has been demonstrated to express dynamically during germ cell development and embryogenesis in several organisms (Paranko et al., 1996; Sarge and Cullen, 1997; Werner et al., 1997). Robust accumulations of Hsp60A in *Drosophila* embryonic pole cells suggest its significant importance from the beginning of germ cell fate determination (Kozlova et al., 1997; Baena-López et al., 2008). Knockdown of *Hsp60* in *Cenorhabditis elegans* and *D. melanogaster* causes sterility. In several organisms, testis specific Hsp60 genes have been reported to be essential for progression of spermatogenesis, and therefore, male fertility (Miller et al., 1990; Timakov and Zhang, 2001). In *Drosophila*, Hsp60B expresses exclusively in testis essential for late stages of spermatogenesis (Timakov and Zhang, 2001). Interestingly, another form of Hsp60 in *Drosophila*, Hsp60C also significantly expresses during spermatogenesis (Sarkar and Lakhotia 2005; Mikhaylova et. al., 2008). In rat, high level of Hsp60 expression was detected during early stages of spermatogenesis, when most of the cell divisions occur (Meinhardt et al., 1995). Hsp60 has been suggested to have a protective role so that a low level of Hsp60 expression during spermatogenesis may decrease the level of protection (especially during early stages), which in turn could result in low spermatogenic efficiency (Werner et al., 1996, 1997; Neuer et al., 2000).

Apart from their abundance during germ cell development, Hsp60 is present in mature sperms and on the surface of oocytes (Boilard et al., 2004; Asquith et al., 2004; Naaby-Hansen and Herr, 2010). Hsp60s presence in the midpiece of fully mature ejaculated spermatozoa has been suggested to be a critical factor for sperm functioning (Boilard et al., 2004). Asquith et al. (2004) reported Hsp60 to be present at a specific head region of mature ejaculated spermatozoa with the possible role of remodeling the sperm surface during sperm-egg interaction: tyrosine phosphorylation during capacitation activates the Hsp60 on the head of ejaculated spermatozoa, which facilitates the formation of a functional zona pellucida receptor complex on the head surface of spermatozoa that finally allows sperm-egg interactions (Asquith et al., 2004).

Similar to spermatogenesis, Hsp expression is an integral process during oogenesis in a number of species of insects, fish, amphibians and mammals (Ambrosio and Schedl, 1984; Sarkar et al., 2006; Heikkila, 2010). Several Hsps have been reported to express dynamically in ovarian nurse cells of *Drosophila* and are subsequently transported to the oocyte (Zimmerman et al., 1983; Kurtz et al., 1986, Sarkar and Lakhotia, 2008). Carthew and coworkers identified Hsp60 and some other chaperones as highly expressed genes during *Drosophila* oogenesis (Nakahara et al., 2005). Thereafter, this gene was identified as Hsp60C, which expresses significantly during oogenesis and is essential for normal egg formation (Sarkar and Lakhotia, 2008). Presence of Hsp60 has been demonstrated on the surface of immortalized Chinese hamster ovary cells (Soltys and Gupta, 1996). Presence of Hsp60 has also been detected on the surface of bovine oviduct epithelial cells where they strongly associate with spermatozoa (Boilard et al., 2004). It is suggested that Hsp60 present in oviduct epithelium is involved in maintenance of sperm viability and integrity within the oviduct sperm reservoir, or involved in the capacitation process which makes spermatozoa competent for fertilizing the egg (Boilard et al., 2004).

Hsp60 has been demonstrated in follicular fluid of patients undergoing in vitro fertilization (Neuer et al., 1997; Jakus et al., 2008). It has been also found that immunity to Hsp60 epitopes is associated with poor prognosis for reproductive outcome (Neuer et al., 2000). Interestingly, co-chaperonin of Hsp60, Hsp10 is better known as "early pregnancy factor," and is required for successful establishment of pregnancy and for proliferation of both normal and neoplastic cells (Cavanagh, 1996).

Small Hsps

Small Hsps (sHsps) belong to a family of 12- to 43-kDa proteins that are ubiquitous and show conserved amino acid sequence in different organisms. Like the *Hsp70* genes, this group of genes also belongs to a multigene family in most organisms. The number ranges from two in yeast, 19 in *Arabidopsis* and 16 in *Caenorhabditis elegans* (Candido, 2002). In humans, nine α-crystallin-related sHsps have been recognized so far (Kappéet al., 2003). They are characterized by a conserved C-terminal region, known as α-crystallin domain; a more variable N-terminal sequence, and a short and variable C-terminal tail. sHsps occur as a homo-/heteromeric complex, comprised of 2 to 40 subunits (Kappéet al., 2003).

sHsps prevent aggregation of unfolded proteins in vitro (Jinn et al., 1995). Bound proteins are transferred to ATP-dependent chaperones, such as Hsp70, and refolded (Richter et al., 2010). The expression of most sHsps is developmentally regulated and can be upregulated by various stresses. The sHsps confer protection to a variety of cellular stressors. Under various neuropathologic conditions, αB-crystallin accumulates in reactive astrocytes and degenerating neurons (Togo and Dickson, 2002). Hsp27, Hsp25 and αB-crystallin are suggested to bind nucleoli and speckles and regulate some molecular processes or perform protective functions (Sun and MacRae, 2005). In addition, sHsps are also demonstrated to interact with cytoskeletal elements and membrane proteins and probably have great influence on aging. Modulation of their expression during aging in *Drosophila* suggests their involvement in pathways of longevity determination (Morrow and Tanguay, 2003). Interestingly, a correlation was observed between reduced levels of sHsps and neurodegenerative diseases (Ito et al., 2003). Interestingly, earlier studies indicate a correlation between reduced level of sHsps and neurodegenerative disorders (Ito et al., 2003); however, a recent report shows overexpression of HSPB7 (a member within the sHsps family) to potentially suppresses polyQ mediated neurodegeneration (Vos et al., 2010).

The *D. melanogaster* genome contains 12 open reading frames for proteins having the characteristic of sHsps (Michaud et al., 2002). However, only four of these sHsps have been examined in detail: Hsp22, Hsp23, Hsp26, and Hsp27. These four *sHsp* genes are located at 67B locus on the left arm of the third chromosome, within a 10kb stretch of DNA in the order *Hsp28, Hsp23, Hsp26, Hsp22*, and exhibit an overall ~50% amino acid sequence homology in their protein products (Southgate et al., 1983; Morrow et al., 2006). Each gene has its own promoter, with *Hsp26* transcribed in a direction opposite to that of the other three (Lindquist, 1986). Besides their distinct developmental expression pattern and intracellular localization, the four small Hsps are also induced following heat stress and in response to ecdysone (Ireland and Berger, 1982; Southgate et al., 1983; Michaud et al., 2002). Similar to *Hsp83*, s*Hsp*s are also maximally expressed at 33°C, a temperature that is lower than that needed for maximal induction of other *Drosophila* Hsps. sHsps shuttle between nucleus and cytoplasm during heat shock and recovery and are also found associated with cytoskeletal components (Leicht et al., 1986). The developmental expression of each of the sHsps is independently regulated (Arrigo and Tanguay, 1991; Richter et al., 2010).

Other members of the heat shock gene family

Besides the major Hsp families described above, several other proteins that are induced by heat have been identified in different organisms. Several peptidyl prolyl isomerases (PPIs) have been identified as Hsps. *In vitro*, the PPIs increase the yield of folded protein by reducing aggregation (Lilie et al., 1993). Heat inducible Hsp56, also known as FKB P52/P59 and possessing a peptidyl prolyl isomerase activity, directly binds to Hsp90 and is involved in the steroid receptor activation and trafficking pathway (Tai and Faber, 1985; Tai et al., 1992; Pratt et al., 1993). Several Hsps are components of the protease system. In mammalian cells, heat shock induces a burst of degradation of normally long-lived proteins mainly by an ATP-dependent proteolysis carried out by the ubiquitin system, which is also induced by heat shock. Ubiquitin binds with heat denatured proteins and targets them for degradation (Bond and Schlesinger, 1985; Hochstrasser, 1992).

Other than these proteins, several other eukaryotic proteins like γ-interferon in mammalian cells, albumin in rat liver, histone H2b in *Drosophila*, enolase and glyceraldehyde-3-phosphate dehydrogenase in yeast are induced by heat (Lindquist, 1986; Srinivas et al., 1987). The ATP-dependent lon protease, lysU, one of the *E. coli* lysyl-tRNA synthetases and rpoD, sigma^{70} subunit of RNA polymerase in prokaryotes, are also heat inducible (Lindquist, 1986).

Concluding remarks

Although much remains to be learned, it is obvious that Hsps possess multiple roles depending on their cellular location and developmental timing. Many questions await further study. Identification of interacting candidates must be extended in order to encompass Hsps roles in development, therapeutics and other vital cellular activities. The powerful modern approaches of genetics, cell biology, biochemistry and biophysics will be needed to answer these questions.

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