Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Stress Response: A Molecular Biology Approach, 2006: 43-60 ISBN: 81-308-0109-4
Editors: Amere S. Sreedhar and Usha K. Srinivas

3 Chaperonins: In life and death

Surajit Sarkar, Richa Arya and Subhash C. Lakhotia
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University
Varanasi 221 005, India

Abstract

The Hsp60 family proteins, also known as "chaperonins", are a highly conserved subgroup of molecular chaperones found in all organisms with many species having multiple forms. This family includes the bacterial GroEL, mitochondrial Hsp60, plastid Rubisco subunit binding protein, archaeagroup II chaperonins and eukaryotic cytosolic TCP-1 proteins. These proteins are usually expressed constitutively but some of these are also stress inducible. Apart from being essential for correct folding of the nascent polypeptides, Hsp60 proteins have critical roles in sorting/refolding and stabilizing proteins denatured during stress. Likewise, the cytosolic Hsp60/TCP-1 complex is vital for the folding

Correspondence/Reprint request: Dr. Subhash C. Lakhotia, Cytogenetics Laboratory, Department of Zoology Banaras Hindu University, Varanasi 221 005, India. E-mail: lakhotia@bhu.ac.in

and assembly of tubulin and actin cytoskeletal components. Besides the chaperoning activities, Hsp60 family proteins have important roles in immunity, cell signaling, apoptosis and fertility. Multiple forms of Hsp60 family proteins in different species have acquired novel functions that appear to be independent of their basic chaperoning activities. Hsp60, thus, is a typical example of "moonlighting proteins" essential for life as well as death of the cell.

Introduction

Heat shock genes and heat shock proteins (Hsps) have continued to be fascinating models for studies on of transcriptional regulation, stress response, protein folding and evolution since their discovery [1,2]. In addition to being induced by heat shock and other abiotic and biotic stresses, most Hsps are present even under normal conditions [3,4]. These constitutively expressed Hsps make upto 5-10% of the total cellular proteins. The Hsps facilitate and stabilize macromolecular structures by their activities as molecular chaperones [5] that assist the folding of nascent polypeptides and refolding of denatured proteins or which function as proteases for degradation and removal of denatured/misfolded proteins [6,7,8,9]. In general, chaperones function as oligomers or complexes of chaperones, co-chaperones, and/or nucleotide exchange factors. Because of their involvement in diverse essential cellular functions like metabolism, growth, cellular signaling, fertility, differentiation and programmed cell death, Hsps are examples of moonlighting proteins. They also influence activation of enzymes and receptors [10]. Therefore, Hsps or chaperones are essential "molecules of life". The Hsps are broadly classified, on the basis of their apparent molecular weights, amino acid sequences and functions [11] into seven families, viz., Hsp110/Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, small Hsps (sHsp) and Hsp10. In the present chapter, we briefly describe the functions of the chaperonins or the Hsp60 family proteins and examine their "moonlighting" or non-chaperonic functions. Their roles as chaperones have been reviewed extensively [6,7,8,9,12].

General features of Hsp60 or chaperonins

The Hsp60 family includes bacterial GroEL, mitochondrial Hsp60, plastid Rubisco subunit binding protein, archaea group II chaperonins and eukaryotic cytosolic TCP-1 proteins which are generally constitutively expressed but some are stress inducible as well [13,14]. They are commonly called "chaperonins" [15]. The mammalian Hsp60 (also called CPN60) was first identified as a mitochondria-associated protein whose mutation conferred resistance to anti-mitotic drugs [16,17]. The Hsp60 family proteins are a well-characterized, highly conserved sequence-related subgroup of molecular

chaperones, found in all organisms from bacteria to human, with many species showing multiple forms. The only organisms known to lack Hsp60 are microsporidia and mycoplasma group parasites, which have very small genomes [18,19]. The eukaryotic Hsp60 family includes the organelle Hsp60 (GroEL homolog) and the cytosolic TCP-1 (also called TriC, CCT or c-cpn). All of them form oligomeric complexes of 50 to 65kDa Hsp60 family polypeptides [20,21,22,23,24,25,26,27,28,29,30].

The Hsp60 chaperonins assist correct folding of most proteins in the cell under both normal and stress conditions. It is estimated that under normal growth conditions in bacteria, GroEL folds 10-15% of all cytoplasmic proteins and under heat stress, this increases to ~30% [31,32]. Chaperonin mediated folding is achieved by sequestration of the misfolded protein in a secluded hydrophobic environment of oligomers of Hsp60 subunits associated with Hsp10 [6,33].

The higher order structure of Hsp60 chaperoning complexes is well studied. These ring-shaped oligomeric complexes are composed of fourteen 60kDa subunits, which are arranged in two stacked heptameric rings to form a barrel like structure with a large central cavity in which the unfolded protein substrate binds via hydrophobic interactions [34,35]. To be functional, the Hsp60 oligomers associate with chaperonin 10 oligomers. Chaperonin 10 forms single-ring heptamers that have a dome-like structure [36,37,38,39]. When ATP is bound to Hsp60, the chaperonin 10 forms a lid on top of the barrel structure [40,41] causing the central cavity to enlarge to provide appropriate conditions for protein folding. Each GroEL/Hsp60 polypeptide has three domains (Fig. 1), viz., an apical domain, which facilitates the binding of the substrate as well as the co-chaperone GroES, an equatorial domain, which contains a binding site for ATP and the contacts for ring binding, and the intermediate domain, which acts as a hinge to bring about conformational changes following ATP binding [6]. When the surface is in hydrophobic state, the protein substrate can bind to GroEL, thus preventing its misfolding or incorrect association of the substrate with other proteins. Binding of ATP with GroEL opens the hinge and alters the substrate-binding surface such that it becomes hydrophilic and the protein substrate is released [6,12,43,44].

The cytosolic TCP-1 complex is critical for maintenance of cellular architecture. The first indication of this function came from the presence of an abnormal cytoskeleton in a cold sensitive TCP-1 mutant in yeast [45]. Eight TCP-1 family genes are known in *Saccharomyces cereviseae* and at least four of these genes, TCP1, BIN2, BIN3, and ACN2 (or CCT1-CCT4), are necessary for normal functions of tubulin and actin [46,47,48,49,50]. All the *bin* (*binucleate*) mutants display defects in microtubule and actin assembly [46]. The different TCP-1 members in yeast have evolved to perform separate functions since mutant alleles of these genes do not complement each other [46].

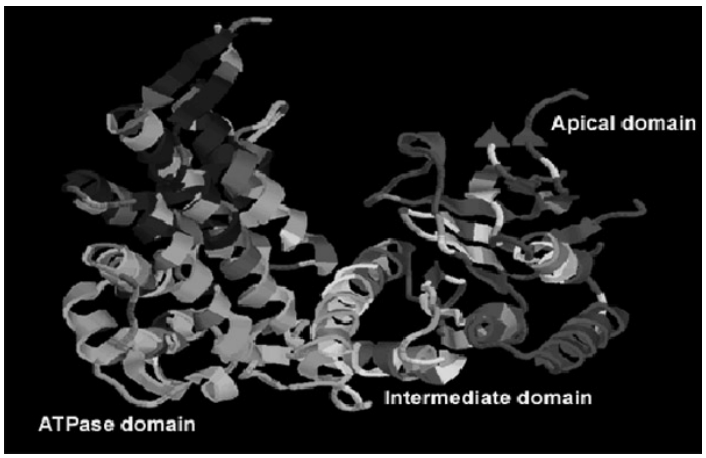

Figure 1. Predicted 3D-structure of one of the Hsp60 proteins (Hsp60C) of *Drosophila melanogaster*, generated with 3D-PSSM [42], showing the three characteristic GroEL domains.

In vitro [51] as well as *in vivo* [52] studies show that nascent tubulin and actin polypeptides enter a 900 kDa TCP-1 complex and emerge as assembly-competent forms. While additional protein cofactors are required for the folding of α and β tubulin, only TCP-1 complex can fold the actin [53,54]. Besides the α and β tubulins and actin, TCP-1 complex is also critical for folding of centrosome related proteins like γ-tubulin and centractin [27]. Continuous presence of tubulin and actin in the TCP-1 complexes suggests that they are its major substrates [51,52]. TCP-1 subunits selectively bind with F-actin at the microfilament assembly site [55,56,57]. Unlike the archetypal GroEL chaperonin, which has a rather general range of substrates [14], the main substrates *in vivo* of TCP-1 seems to be the major cytoskeletal proteins like actin and tubulin [52]. However, some other reports [58,59,60,61] have suggested a wider range substrates for eukaryotic TCP-1 proteins. 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, which may provide a region for its binding with the TCP-1 complex [62]. All the cytoskeletal components that interact with TCP-1 complex, share a high sequence homology in a carboxy-terminal peptide with that in the TCP-1 proteins. These sequences appear to explain specificity of association of the nascent substrate with the TCP-1 and the displacement of the chaperone from the substrate as its synthesis progresses [63]. However, a more complex mode of interaction of TCP-1 and actin during folding has also been suggested [64].

The TCP-1 complex is also associated with tubulin during its transport along neurites [55,65]. The CCTα component of TCP-1 complex enters neuritic 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

[55]. In *Tetrahymena*, TCP-1 subunits and tubulin are co-synthesized during cilia recovery [66]. Our studies in *Drosophila melanogaster* (Sarkar and Lakhotia, in preparation) also suggest a role for of Hsp60 in maintenance of cytoskeletal integrity (see later). The TCP-1 complex 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 [67].

Non-chaperone activities of Hsp60 family members

Initial studies on Hsp60 chaperonin in animal cells established this protein to be localized to the mitochondrial matrix [16]. However, the variety of cellular activities performed by Hsp60 family proteins is inconsistent with an exclusive mitochondrial location of Hsp60. The first indication of non-chaperone activities of Hsp60 followed the observation that cells could be activated to synthesize and secrete cytokines if Hsp60 was delivered onto their external surfaces [68,69]. It was estimated that in a variety of cells and tissues, 15-20% of Hsp60 reactivity is present at discrete extra-mitochondrial sites, including the cell surface, unidentified cytoplasmic vesicles and granules, peroxisomes and endoplasmic reticulum and in zymogen granules of pancreatic acinar cells, on the surface of human endothelial cells and in circulating serum [17,70,71,72,73,74,75]. Hsp60 is also highly expressed in germ cells of several organisms [76,77,78,79,80,81]. Further, the Hsp60 members have been shown to participate in a variety of other activities, like amino acid transport, signal transduction, peptide presentation etc [8,82,83,84,85,86]. Mutations in Hsp60 family members are also known to cause several genetic diseases [87,88]. These diverse observations suggest multiple roles of Hsp60 family proteins independent of their chaperonic activities. These are considered below.

Hsp60 in immunity and cellular signaling

Prior to the discovery of a protein-folding role for bacterial Hsp60, it was identified as a ‘common antigen’ [74,89,90]. GroEL is highly immunogenic and is capable of eliciting innate as well as acquired immune responses in the host. Stefan et al [91] showed that following infection of mice with *Mycobacterium tuberculosis*, one fifth of T-cells were reactive to the bacterial Cpn60.2. Moreover, children immunized against DPT (Diphtheria, Pertussis, Tetanus) also carry high titers of anti-Cpn60 antibody [92]. The immune response following exposure to pathogenic Hsp60 recognizes the mammalian homologue because of its sequence similarity and this has been implicated in the pathogenesis of several autoimmune diseases like type I diabetes [93], Crohn’s disease [94], atherosclerosis [95,96] and juvenile chronic arthritis [97,98]. Antibodies against *Chlamydia trachomatis* cross react with human Hsp60 and may result in female infertility and embryo or fetal loss [99].

In vitro studies have revealed binding of exogenous Cpn60 to cell through specific receptors like 'Monocyte specific lipopolysaccharide receptor', CD14 [100], and 'Toll-like receptors' (TLRs) [101]. Both human and chlamydial Cpn60 proteins activate the Toll–IL-1 receptor signaling pathway by binding with TLR2 and TLR4 [102]. On the other hand, an immunosuppressive role of Hsp60 has also been reported [103,104]. Although the role of Hsp60 in immune processes is still to be clearly elucidated, it is clear that this protein is not merely an antigen but a potent immunoreactive molecule that directly modulates the immune response.

Hsp60 family members have been found to play a critical role in epithelial remodeling and cell migration by triggering various signaling cascades. Epithelial migration is a complex and essential phenomenon for organogenesis/tissue repair and is controlled by cross-talks between cell surface receptors, extracellular matrix molecules and growth factors. It has been demonstrated that levels of Hsp60 increase in migrating epithelial cells during tissue regeneration [105,106]. Exogenous bacterial Hsp60 was shown to enhance cell proliferation by 25-75% [107]. Hsp60 induces activation of p38 MAP kinase and ERK signaling cascades implicated in cell motility [107,108,109]. p38 activates MAP kinase-activated protein kinase-2 (MAP kinase AP kinase-2), which in turn phosphorylates and activates Hsp27 [110]. The activated Hsp27 regulates actin polymerization resulting in cytoskeletal reorganization and mitogenic activity [111].

An important part of cellular signaling is autocrine activation of the EGFR cascade, which controls cell survival, proliferation, and motility [112]. Blockage of EGFR activation can completely inhibit both bacterial and human Hsp60 induced cell migration [108,109] suggesting that exogenous Hsp60 may transduce its role in epithelial remodeling through the EGFR pathway.

Hsp60 in apoptosis

Various Hsps have been implicated in cell death [113,114]. Anti-apoptotic as well as pro-apoptotic roles of Hsp60 are reported. The bacterial homologues of Hsp60 and Hsp10 were found to have a protective role in cell survival [115,116]. Hsp60 seems to have an anti-apoptotic role in neonatal cardiac myocytes presumably because in combination with Hsp10 it prevents cell death by maintaining the mitochondrial integrity and function [117]. The pro-apoptotic members of bcl-2 family, viz. Bak and Bax, are unable to trigger the apoptotic machinery when bound to Hsp60 [118]. Shan et al [119] reported that over expression of Hsp60 in normal heart leads to increased levels of the anti-apoptotic Bcl-XL, resulting in a reduced level of the pro-apoptotic Bax.

A proapoptotic role of mitochondrial Hsp60 has also been suggested [120,121]. Samali et al [122] and Xanthoudakis et al [123] independently reported evidence for a direct interaction between Hsp60 and pro-caspase-3 in

Jurkat and HeLa cell lines and thus the involvement of Hsp60 and Hsp10 in the activation of apoptosis. These studies suggested that binding of Hsp60 to pro-caspase-3 maintains it in a protease sensitive state and thus makes it more susceptible to the action of cytochrome-c and dATP; this facilitates subsequent induction of the downstream apoptotic cascade. Esophageal squamous cell carcinomas have elevated levels of Hsp60 and in agreement with Hsp60's proapoptotic role, the apoptotic index in tissues from patients of esophageal squamous cell carcinomas was high [124].

Chaperonins in fertility

The three phases of spermatogenesis, viz., mitotic proliferation of spermatogonia, meiotic development of spermatocytes and post-meiotic development/maturation of spermatids into spermatozoa [125], represent situations where dramatic transformations and cellular differentiation along with mitochondrial remodeling take place. It is, therefore, not surprising that spermatogenesis is accompanied by expression of different Hsps [126,127]. Hsp60 is dynamically expressed during germ cell development and embryogenesis [76,77,78,128]. Relationships between developmental expression of Hsp60 and fertility have been demonstrated in several organisms like *Cenorhabditis, Drosophila*, rat, mouse, monkey, human etc. In some organisms like *Heliothis viresens* (moth) and *Drosophila melanogaster* (see later), testis specific isoforms of Hsp60 have been reported [76,129]. Knockdown of *Hsp60* in *Cenorhabditis elegans* causes sterility [81]. During mouse and rat spermatogenesis, Hsp60 shows cell-type specific dynamic expression pattern [130]. The number of Hsp60 expressing spermatogonia in an infertile man parallels the spermatogenic function [78]. It seems that the early stages of spermatogenesis may be less protected when the levels of Hsp60 are low and this in turn may result in lower spermatogenic efficiency. In addition to their abundant presence during germ cell development, Hsp60 is also seen in mature sperms, on head of ejaculated spermatozoa and on the surface of oocytes [79,80]. Tyrosine phosphorylation is suggested to activate Hsp60 on the head of ejaculated spermatozoa during capacitation and the phosphorylated Hsp60 is believed to trigger conformational changes, which facilitate the formation of a functional zona pellucida receptor complex on the surface of spermatozoa; this finally brings about sperm-egg interaction [79].

Tabibzadeh et al [131] reported Hsp60 in endometrium of healthy women. It was also detectable in the decidua during the first trimestar of pregnancy [99]. Hsp60 has been demonstrated in follicular fluid of patients undergoing *in vitro* fertilization [99]. Immunity to Hsp60 epitopes is associated with a poor prognosis for reproductive outcome and impaired IVF results [99]. Hsp10, the co-chaperonin of Hsp60, is well known as an early pregnancy factor, which is required for successful establishment of pregnancy and for proliferation of

both normal and neoplastic cells [132]. Nakahara et al [133] identified *Hsp60* as one of the highly expressed genes during *Drosophila* oogenesis. Recent studies in our laboratory also show that the *Hsp60C* of *Drosophila melanogaster* is essential for spermatogenesis [134] as well as oogenesis (Sarkar and Lakhotia, in preparation).

Hsp60 in *Drosophila melanogaster*

It is interesting that although activation of heat shock genes and the new synthesis of heat shock proteins were originally described in *Drosophila* [1,2,135], initial studies on heat shock proteins in *Drosophila* [2,135,136,137,138] did not identify any Hsp60 family member in this organism. The first report of the existence of a Hsp60 family protein in *Drosophila* came from our laboratory in 1989, when it was demonstrated that heat shock elicits synthesis of Hsp60 as a major member of a novel set of induced polypeptides in Malpighian tubules of *Drosophila* larvae [139,140,141]. It is interesting to note that in several other species of insects also, induction of Hsp60 family proteins in heat shocked Malpighian tubules shows variations [142,143]. An intriguing feature of the induced synthesis of Hsp60 in Malpighian tubules of *Drosophila* is that control and heat shocked Malpighian tubule cells maintain a constant level of Hsp60 since its level is maintained whether the heat shock induced new synthesis is allowed or prevented by transcriptional or translational inhibitors [144]. This suggests a tight coupling between new synthesis and turnover of existing Hsp60 [144].

Multiple *Hsp60* genes in *Drosophila melanogaster*

The first member of the *Hsp60* gene family *(Hsp60A)* in *D. melanogaster* was localized to the 10A4 cytogenetic region of the X chromosome by Kozlova et al [145,146], who found it to be identical with the "*essential gene at 10A*" which was studied earlier by Zhimulev's group [147]. Subsequently, the Berkeley Drosophila Genome Project revealed the presence of three more Hsp60-like DNA sequences at different cytogenetic positions [148,149]. We have recently named these four genes as *Hsp60A* at 10A4 polytene band, *Hsp60B* at 21D2 band, *Hsp60C* at 25F2 band and *Hsp60D* at 34C1 band [134]. Interestingly, nucleotide sequence homologies among these four *Hsp60* genes are restricted to the protein coding sequence with little homology in their 5' and 3' UTRs [134]. This suggests differential regulation and expression of these four *Hsp60* genes. A summary of the general features of the four *Hsp60* genes in *Drosophila melanogaster* is presented in Table 1 and the predicted phylogenetic relationship between these four genes is shown in Fig. 2. Characteristic features of the four members of the Hsp60 family in *Drosophila melanogaster* are discussed below.

Table 1. General features of the four *Hsp60* Genes in *Drosophila melanogaster**.

Gene Name	Polytene chromosome site	Gene size (kb)	Number of exons	Predicted protein		Relative homology with the Hsp60A protein
				Number of amino acids	Molecular weight (kDa)	
Hsp60A	10A4	3.83	3	573	60.80	100
Hsp60B	21D2	2.15	1	648	68.63	~60%
Hsp60C	25F2	7.99	3	576	61.58	~80%
Hsp60D	34C1	2.15	2	558	60.21	~50%

*(*based on information at www.flybase.org)*

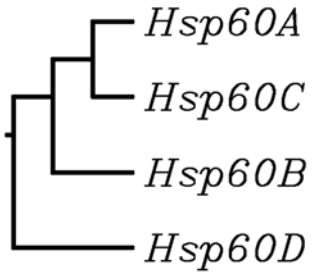

Figure 2. Dendrogram, generated by ClustalW alignment of amino acid sequences [150], showing the predicted phylogenetic relationship between the four *Hsp60* genes of *Drosophila melanogaster.*

Hsp60A

The first identified *Hsp60* gene, localized at the 10A4 polytene band [145], is ~3.8 kb long and codes for two transcripts. It is not heat inducible and a heat shock factor binding domain is also absent [145,146]. *Hsp60A* is dynamically expressed during embryogenesis with its mRNA being more abundant in 0 to 4h embryos than in later stages [145]. Expression of *Hsp60A* is significantly reduced in adult flies and the transcripts are not maternally contributed [145]. During embryogenesis, the distribution of Hsp60 protein is dynamic. Some cells, like pole cells, cells of the cephalic furrow etc, show higher amounts of Hsp60 protein [145].

Earlier studies by Zhimulev et al [147] identified a number of mutant alleles at the 10A locus; these mutations were later shown to correspond to the *Hsp60A* gene [145,146]. Pre-gastrulation embryonic lethality associated with homozygosity for the *Hsp60A* mutant alleles shows that its product is essential for embryogenesis. The Hsp60A protein seems to be the constitutively expressed chaperonin in *Drosophila* cells.

Hsp60B

Hsp60B, a second member of the *Drosophila melanogaster Hsp60* gene family, maps to the 21D2 cytogenetic position and shows ~60% sequence similarity with *Hsp60A.* It codes for a single 2.15 kb long transcript and seems to have male germ cell specific expression [129,151]. This gene is not heat inducible [151]. So far, only one P-transposon insertion allele of this gene has been reported. Male flies homozygous for this mutant allele are sterile. Although the early stages of spermatogenesis are reported to be normal in these mutant individuals, the individualization complex is abnormally assembled so that the spermatids fail to individualize and finally degenerate [129,151]. It seems that *Hsp60B* gene product is required for structural integrity of the individualization complex and/or for coordinating the movement of actin cone during individualization process. It is notable in this context that multiple caspases and caspase regulators are required for removal of much of the cytoplasmic contents of maturing bundles of spermatids and the surrounding cyst cells by a non-apoptotic process during individualization of sperm bundles [152]. In view of the earlier discussed role of Hsp60 in caspase maturation [121,122], it is possible that Hsp60B may be involved in regulating some aspect of caspase maturation/activity during sperm individualization.

Hsp60C

This is the longest (~ 8 kb) *Hsp60* gene of the family, which is located at the 25F2 cytogenetic position and which codes for three transcripts, differing in their 5'UTRs. Bioinformatic analysis suggests that *Hsp60C* shows maximum (~80%) similarity with the *Hsp60A* gene and only ~60% and ~50% homology with *Hsp60B* and *Hsp60D* genes respectively (Table 1).

Studies in our laboratory [134] showed that this gene's expression begins in late embryonic stages (stage 14 onwards), particularly in the developing tracheal system and salivary glands. In parallel with its tracheal expression, a loss of function P-transposon insertion mutant allele of this gene, $Hsp60C^{1}$, causes abnormal tracheal development and impaired liquid clearance [134]. Majority of $Hsp60C^{1}$ homozygotes die as 1^{st} instar larvae. However, ~10% survive as sterile and weak adults. The *Hsp60C* gene shares its tracheal expression and the mutant tracheal phenotype with those of genes like *DEG/Enac pickpocket*, *d-VHL* etc [153,154], and, therefore, it is likely that the Hsp60C interacts with products of these genes involved in tracheal morphogenesis. Hsp60C may be involved in signaling pathways during tracheal morphogenesis and its ATPase activity may also have a role in cation transport across the tracheal membrane [134].

Besides the strong tracheal expression, the *Hsp60C* gene is also expressed in most of the larval and adult tissues, which suggests wider functions [134].

This gene is not inducible by heat shock in any tissue except for a low induction in larval testes and accessory glands of adult male reproductive system (unpublished data).

In adult tissues, *Hsp60C* is most strongly and dynamically expressed in developing and differentiating germ cells which is in agreement with complete sterility of the rare surviving $Hsp60C^{1}$ homozygotes [134]. Compared with male sterility due to *Hsp60B* mutation, $Hsp60C^{1}$ mutation causes more severe defects prior to formation of spermatids themselves. Thus, unlike most of the "classic" male sterile mutations, $Hsp60C^{1}$ mutation affects pre-meiotic and meiotic stages in males [134].

During oogenesis also, Hsp60C transcripts show a dynamic and stage specific distribution in ovarian follicles (Sarkar and Lakhotia, in preparation). Hsp60C transcripts are present at low levels in the germarium but their abundance increases in nurse cells as they develop and beginning with stage 7, Hsp60C transcripts appear in oocyte cytoplasm, presumably through transport from nurse cells. The Hsp60C transcripts in oocyte continue to increase till stage 11, but quickly disappear following stage 12. Sterile $Hsp60C^{1}$ homozygous females show severe abnormalities in the progression of oogenesis. The first abnormality in the $Hsp60C^{1}$ mutant egg chambers is an irregular arrangement of follicle cells, particularly the posterior group of follicle cells at stage 7. In view of the close association seen between Hsp60 and actin filaments in wild type, it appears that these and other defects like abnormal cytoskeleton, premature apoptosis of nurse cells, loss of polarity of oocyte etc seen in later stages of the mutant ovarian follicles result from abnormal cytoskeleton in the absence of Hsp60C (Sarkar and Lakhotia, in preparation).

Hsp60D

Hsp60D is located at 34C1 band on the cytological map of polytene chromosomes. This gene is about 2 kb long and produces two transcripts. Initial studies in our lab (Richa Arya and Lakhotia, unpublished) on expression of the *Hsp60D* gene reveal a differential expression of this gene in various developmental stages of the fly. Transcripts of this gene accumulate in cytoplasm of all embryonic and larval cells but with a significantly high expression in the tracheal system. This gene appears to be moderately heat inducible in several larval tissues, especially in some regions of gut and the Malpighian tubules.

A mutant allele of this gene is not yet available. However, transgenic lines have been generated to either over express this gene or to ablate its transcripts in specific cell types and preliminary results (Richa Arya and Lakhotia, unpublished) with these transgenic lines suggest a pro-apoptotic role for the *Hsp60D* gene.

The multiple *Hsp60* genes in *Drosophila melanogaster* thus show functional divergence with important roles in viability, apoptosis and fertility of the fly. Betran et al [155] proposed that multiple copies of *Hsp60* genes in *Drosophila* evolved from the X-linked *Hsp60A* gene by retrotransposition on autosomes and that many such "out-of-X" retrotransposed duplicated genes often express in testis. In agreement with this proposal, it is already known that the *Hsp60B* and *Hsp60C* have essential functions in spermatogenesis, although only *Hsp60B* has a testis-specific expression. Apparently, the duplicated *Hsp60* genes acquired new UTRs and upstream regulatory sequences, which allowed them to perform novel functions in different developmental pathways.

Concluding remarks

The Hsp60 family in higher organisms is now known to comprise of multiple members with varying levels of diversification in structure and, therefore, in their functions. Typically, the Hsp60 family members are believed to be important chaperoning machines of any cell. However, it is clear that, like multiple members of the other Hsp gene families, the Hsp60 family proteins have also evolved and acquired novel functions, which may or may not depend on their basic chaperoning activities. In keeping with "moonlighting" functions of Hsps, the Hsp60 family proteins also show altered expression in cancer cells [156]. Intriguingly, however, Hsp60 and Hsp10 expression may be elevated or reduced, in concert or independently, in different types of cancer [157]. In the absence of genetic data, it is not clear in many cases if the diverse functions ascribed to Hsp60 in a given species are performed by the same protein or related products of different genes. Combined genetic, cell biological and molecular analyses in various model systems in future are expected to provide significant understanding of the diverse roles of Hsp60 family. Comparable studies in mammalian (including human) systems would help in a better understanding and management of disorders resulting from Hsp60 dysfunctions. It is clear that Hsp60 family proteins have important roles in life as well as death of cells.

Acknowledgement

Studies on the different *Hsp60* genes in *Drosophila melanogaster* in our laboratory have been supported by research grants from the Department of Biotechnology, Govt. of India (N. Delhi) to SCL. SS and RA are supported by the Council of Scientific and Industrial Research (N. Delhi).

References

1. Ritossa, F.A. 1962, Experientia, 18, 571- 573.
2. Tissieres, A., Herschel, K.M., and Tracy U.M. 1974, J. Mol. Biol., 84, 389-398.

3. Lindquist, S., and Craig, E.A. 1988, Annu. Rev. Genet., 22, 631-677.
4. Lakhotia, S.C. 2001, Proc. Indian Natl. Sci. Acad. B. Biol. Sci., 67, 247-264.
5. Laskey, R.A., Honda, B.M., Mills, A.D., and Finch, J.T. 1978, Nature, 5, 416-20.
6. Ranson, N.A., White, H.E. and Saibil, H.R. 1998, Biochem. J., 333, 233-242.
7. Ranford, J.C., Coates, A.R., and Henderson, B. 2000, Expert Rev. Mol. Med., 15, 1-17.
8. Maguire, M., Coates, A.R.M., and Henderson, B. 2002, Cell Stress Chaperones, 7, 317-329.
9. Soti, C., Nagy, E., Giricz, Z., Vigh, L., Csermely, P., and Ferdinandy, P. 2005, Br. J. Pharmacol., 146, 769-780.
10. Gething, M.J. 1997, Nature, 24, 329-331.
11. Nover, L. (Ed.). 1984, Heat Shock Response in Eukaryotic Cells. Springer-Verlag, Berlin, 78.
12. Bukau, B., and Horwich, A.L. 1998, Cell, 92, 351-366.
13. Hartl, F.U., Martin, J., and Neupert, W. 1992, Annu. Rev. Biophys. Biomol. Struct., 21, 293-322.
14. Houry, W.A., Frishman, D., Eckerskorn, C., Lottspeich, F., and Hartl, F.U. 1999, Nature, 402, 147-154.
15. Hemmingsen, S.M. 1992, Nature, 357, 650-650.
16. Gupta, R.S. 1990, Trends Biochem. Sci., 15, 415-418.
17. Soltys, B.J., and Gupta, R.S. 1999, Trends Biochem. Sci., 24, 174-177.
18. Katinka, M.D., Duprat, S., Cornillot, E., Metenier, G., Thomarat, F., Prensier, G., Barbe, V., Peyretaillade, E., Brottier, P., Wincker, P., Delbac, F., El Alaoui, H., Peyret, P., Saurin, W., Gouy, M., Weissenbach, J., and Vivares, C. P. 2001, Nature, 22, 450-453.
19. Glass, J.I., Lefkowitz, E.J., Glass, J.S., Heiner, C.R., Chen, E.Y., and Cassell, G.H. 2000, Nature, 407, 757-762.
20. Gupta, R.S. 1990, Biochem. Int., 20, 833- 841.
21. Gupta, R.S. 1995, Mol. Microbiol., 15, 1-11.
22. Trent, J.D., Nimmesgern, E., Wall, J.S., Hartl, F.-U., and Horwich, A.L. 1991, Nature, 354, 490-493.
23. Frydman, J., Nimmesgern, E., Erdjument-Bromage, H., Wall, J.S., Tempst, P., and Hartl, F.-U. 1992, EMBO J., 11, 4767-4778.
24. Lewis, V.A., Hynes, G.M., Zheng, D., Saibil, H., and Willison, K. 1992, Nature, 358, 249-252.
25. Kubota, H., Hynes, G., Carne, A., Ashworth, A., and Willison, K. 1994, Curr. Biol., 4, 89-99.
26. Willison K. R. and Kubota, H. 1994, Biology of Heat Shock Proteins and Molecular Chaperones, Morimoto, R. I., Tissieres, A., and Georgopoulos, C. (Ed.) pp. 299-312. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
27. Melki, R., and Cowan, N.J. 1994, Mol. Cell. Biol., 14, 2895-2904.
28. Melki, R., Rommelaere, H., Leguy, R., Vandekerckhove, J., and Ampe, C. 1996, Biochemistry, 35, 10422-10435.
29. Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C., and Cowan, N.J. 1996, Cell, 86, 287-296.
30. Lewis, S.A., Tian, G., Vainberg, I.E., and Cowan, N.J. 1996. J. Cell Biol., 132, 1-4.
31. Ewalt, K.L., Hendrick, J.P., Houry, W.A., and Hartl, F.U. 1997, Cell, 8, 491-500.

32. Ellis, R.J. 2005, Chaperomics: In Vivo GroEL Function Defined. Curr. Biol., 15, 661-663.
33. Fenton, W.A., and Horwich, A.L. 1997, Protein Sci., 6, 743-760.
34. Hutchinson, E.G., Tichelaar, W., Hofhaus, G., Weiss, H., and Leonard, K.R. 1989, EMBO J., 8, 1485-1490.
35. Braig, K., Otwinowski, Z., Hegde, R., Boisvert, D.C., Joachimiak, A., Horwich, A.L., and Sigler, P.B. 1994, Nature, 371, 578-586.
36. Langer, T., Pfeifer, G., Martin, J., Baumeister, W., and Hartl, F.U. 1992, EMBO J., 11, 4757-4765.
37. Mayhew, M., da Silva, Ana C.R., Martin, J., Erdjument-Bromage, H., Tempst, P., and Hartl, F.U. 1996, Nature, 379, 420-426.
38. Hunt, J.F., Weaver, A.J., Samuel, L.J., Gierasch, L., and Deisenhofer, J. 1996, Nature, 379, 37-45.
39. Hunt, J.F, van der Vies, S.M., Henry, L., and Deisenhofer J. 1997, Cell, 25, 361-371
40. Chandrasekhar, G.N., Tilly, K., Woolford, C., Hendrix, R., and Georgopoulos, C. 1986, J. Biol. Chem., 15, 12414-12419.
41. Saibil, H. 1996, Structure, 15, 1-4.
42. Kelley, L.A., MacCallum, R.M., and Sternberg, M.J.E. 2000, J. Mol. Biol., 299, 501-522.
43. Dobson, C.M., and Fersht, A.R. (Ed.). 1995, Protein Folding, Cambridge University Press, UK.
44. Feltham, J.L., and Gierasch, L.M. 2000, Cell, 100, 193-196.
45. Ursic, D., and Culbertson, M.R. 1991, Mol. Cell. Biol., 11, 2629-2640.
46. Chen, X., Sullivan, D.S., and Huffaker, T.C. 1994, Proc. Nat. Acad. Sci., USA, 91, 9111-9115.
47. Vinh, D. B.-N., and Drubin, D. G. 1994, Proc. Nat. Acad. Sci., USA, 91, 9116-9120.
48. Ursic, D., Sedbrook, J.C., Himmel, K.L., and Culbertson, M.R. 1994, Mol. Biol. Cell, 5, 1065-1080.
49. Miklos, D., Caplan, S., Mertens, D., Hynes, G., Pitluk, Z., Kashi, Y., Harrison-Lavoie, K., Stevenson, S., Brown, C., Barrell, B., Horwich, A.L., and Willison, K. 1994, Proc. Nat. Acad. Sci., USA, 91, 2743-2747.
50. Stoldt, V., Rademacher, F., Kehren, V., Ernst, J.F., Pearce, D.A., and Sherman, F. 1996, Yeast, 12, 523-529.
51. Yaffe, M.B., Farr, G.W., Miklos, D., Horwich, A.L., Sternlicht, M.L., and Sternlicht, H. 1992, Nature, 358, 245-248.
52. Sternlicht, H., Farr, G.W., Sternlicht, M.L., Driscoll, J.K., Willison, K., and Yaffe, M.B. 1993, Proc. Nat. Acad. Sci., USA, 90, 9422-9426.
53. Gao, Y., Thomas, J.O., Chow, R.L., Lee, G.-H., and Cowan, N.J. 1992, Cell, 69, 1043-1050.
54. Gao, Y., Vainberg, I.E., Chow, R.L., and Cowan, N.J. 1993, Mol. Cell. Biol., 13, 2478-2485.
55. Roobol, A., Holmes, F.E., Hayes, N.V.L., Baines, A.J., and Carden, M. J. 1995, J. Cell Sci., 108, 1477- 1488.
56. Roobol, A., and Carden M.J. 1999, Eur. J. Cell. Biol., 78, 21-32.

57. Grantham, J., Ruddock, L.W., Roobol, A., and Carden, M.J. 2002, Cell Stress Chaperone, 7, 235-242.
58. Melki, R., Batelier, G. Soulie, S., and Williams R.C.Jr. 1997, Biochemistry, 36, 5817-5826.
59. Thulasiraman, V., Yang, C.F., and Frydman, J. 1999, EMBO J., 18, 85-95.
60. Leroux, M.R., and Hartl, F. 2000, Curr. Biol., 10, 260-264.
61. McCallum, C.D., Do, H., Johnson, A.E., and Frydman, J. 2000, J. Cell Biol., 149, 591-602.
62. Dobrzynski, J.K., Sternlicht, M.L., Farr, G.W., and Sternlicht, H. 1996, Biochemistry, 35, 15870-15882.
63. Burns, R.G., and Surridge, C.D. 1994, FEBS Lett., 347, 105-111.
64. Hynes, G.M., and Willison, K.R. 2000, J. Biol. Chem., 275, 18985-18994.
65. Carden, M.J., and Roobol, A. 1995, Biochem. Soc. Trans., 23, 70-76.
66. Soares, H., Penque, D., Mouta, C., and Rodrigues-Pousada, C. 1994, J. Biol. Chem., 269, 29299- 29307.
67. Creutz, C.E., Liou, A., Snyder, S.L., Brownawell, A., and Willison, K. 1994, J. Biol. Chem., 269, 32035-32038.
68. Friedland, J.S., Shattock, R., Remick, D.G., and Griffin, G.E. 1993, Clin. Exp. Immunol., 91, 58-62.
69. Retzlaff, C., Yamamoto, Y., Hoffman P.S., Friedman, H., and Klein, T.W. 1994, Infec. Immun., 62, 5689-5693.
70. Brudzynski, K. 1993, Diabetes, 42, 908-913.
71. Velez-Granell, C.S., Arias, A.E., Torres-Ruiz, J.A., and Bendayan, M. 1994, J. Cell Sci., 107, 539-549.
72. Soltys, B.J., and Gupta, R.S. 1996, Exp. Cell Res., 10, 16-27.
73. Khan, I. U., Wallin, R., Gupta, R.S., and Kammer G.M. 1998, Proc. Natl. Acad. Sci., USA, 95, 10425-10430.
74. Zugel, U., and Kaufmann, S.H. 1999, Immunobiology, 201, 22-35.
75. Pfister, G., Stroh, C.M., Perschinka, H., Kind, M., Knoflach, M., Hinterdorfer, P., and Wick, G. 2005, J. Cell Sci., 15, 1587-1594.
76. Miller, S.G., Leclerc, R.E., and Erdos, G.W. 1990, J. Mol. Bio., 214, 407-422.
77. Sarge, K.D., and Cullen, K.E. 1997, Cell Mol. Life Sci., 53, 191-197.
78. Werner, A., Meinhardt, A., Seitz, J., and Bergmann, M. 1997, Cell. Tissue. Res., 288, 539-544.
79. Asquith, K.L. Baleato, R.M. McLaughlin, E.A. Nixon, B., and Aitken, R. J. 2004, J. Cell Sci., 117, 3645-3657.
80. Boilord, M., Reyes-Moreno, C., Lachance, C., Massicotte, L., Bailey, J.L., Sirard, M.A., and Leclerc, P. 2004, Biol. Reprod., 71, 1879-1889.
81. Rual, J.F., Ceron, J., Koreth, J., Hao, T., Nicot, A.S., Hirozane-Kishikawa T., Vandenhaute, J., Orkin, S. H., Hill, D. E., van den Heuvel, S., and Vidal, M. 2004, Genome Res., 14, 2162-2168.
82. Ikawa, S., and Weinberg, R. A. 1992, Proc. Natl. Acad. Sci., USA. 89, 2012-2016.
83. Kaur, I., Voss, S.D., Gupta, R.S., Schell, K., Fisch, P., and Sondel, P.M. 1993, J. Immunol., 150, 2046-2055.
84. Jones, M., Gupta, R.S., and Englesberg, E. 1994, Proc. Natl. Acad. Sci., USA, 91, 858-862.

85. Woodlock, T.J., Chen, X., Young, D.A., Bethlendy, G., Lichtman, M.A., and Segel, G.B. 1997, Arch. Biochem. Biophy., 338, 50-56.
86. Wells, A.D., Rai, S.K, Salvato, M.S., Band, H., and Malkovsky, M. 1997, Scand. J. Immunol., 45, 605-612.
87. Slavotinek, A.M., and Biesecker, L.G. 2001, Trends Genet., 17, 528-535.
88. Hansen, J.J., Durr, A., Cournu-Rebeix, I., Georgopoulos, C., Ang, D., Nielsen, M.N., Davoine, C.S., Brice, A., Fontaine, B., Gregersen, N., and Bross, P. 2002, Am. J. Hum. Genet., 70, 1328-1332.
89. Kaufmann, S.H. 1990, Heat Immunol. Today, 11, 129-136
90. Yong, D.B. 1992, Curr. Opin. Immunol., 4, 396-400.
91. Stefan, H.E., Kaufmann, U.V., Jelle, E.R., Thole, J.D.A., Van, E., and Frank, E. 1987, Eur. J. Immunol., 17, 351-357
92. Giudice, G.D., Gervaix, A., Costantino, P., Wyler, C.A., Tougne, C., de Graeff-Meeder, E.R., van Embden, J., van der Zee, R., Nencioni, L., and Rappuoli, R. 1993, J. Immunol., 150, 2025-2032
93. Elias, D., Markovits, D., Reshef, T., van der Zee, R., and Cohen, I.R. 1990, Proc. Natl. Acad. Sci., USA, 87, 1576-1580.
94. Szewezuk, M.R., and Depew, W.T. 1992, Clin. Invest. Med., 15, 494-505,
95. Pockley, A.G. 2002, Circulation, 105, 1012-1017.
96. Xu, Q. 2002, Arterioscler Thromb. Vasc. Biol., 22, 1547-1559.
97. Pope, R.M., Lovis, R.M. and Gupta, R.S. 1992, Arthritis Rheum, 35, 43-48.
98. Res, P.C., Schear, C.G., Breedveld, F.C., van Eden, W., Van Embden, J.D., Cohen, I.R., and De Vries, R.R. 1988, Lancet, 2, 478-480.
99. Neuer, A., Spandorfer, S.D., Giraldo, P., Dieterle, S., Rosenwaks, Z., and Witkin, S. S. 2000, Hum. Repro. Updt., 6, 149-159.
100. Kol, A., Lichtman, A.H., Finberg, R.W., Libby, P. and Kurt-Jones, E.A. 2000, J. Immunol, 164, 13-17.
101. Ohashi, K., Burkart, V., Flohé, S., and Kolb, H. 2000, J. Immunol., 164, 558-561.
102. Vabulas, R.M., Ahmad-Nejad, P., da Costa, C., Miethke, T., Kirschning, C.J., Hacker, H., and Wagner, H. 2001, J. Biol. Chem., 276, 31332-31339.
103. Elias, D., and Cohen, I.R. 1996, Diabetes, 45, 1168-1172.
104. van Eden, W., Wendling, U., Paul, L., Prakken, B., van Kooten, P., van der Zee, R. 2000, Cell Stress Chaperones, 5, 452-457.
105. Shinoda, H., and Huang, C.C. 1996, Otolaryngol. Head Neck Surg., 114, 77-83.
106. Laplante, A.F., Moulin, V., Auger, F.A., Landry, J., Li, H., Morrow G., Tanguay, R.M., and Germain, L. 1998, J. Histochem. Cytochem., 46, 1291-1301.
107. Zhang, L., Pelech, S.L., Mayrand, D., Grenier, D., Heino, J., and Uitto, V.J. 2001, Exp. Cell Res., 15, 11-20.
108. Zhang, L., Koivisto, L., Heino, J., and Uitto, V.J. 2004a, Biochem. Biophys. Res. Comm., 319, 1088-1095.
109. Zhang, L., Pelech, S., and Uitto, V.J. 2004b, Infect. Immun., 72, 38-45.
110. Larsen, J.K., Yamboliev, I.A., Weber, L.A., and Gerthoffer, W.T. 1997, Am. J. Physiol., 273, 930-940.
111. Gerthoffer, W.T., and Gunst, S.J. 2001, J. Appl. Physiol., 91, 963-972.
112. Jost, M., Kari, C., and Rodeck, U. 2000, Eur. J. Dermatol., 10, 505-510.
113. Samali, A., and Orrenius S. 1998, Cell Stress Chaperones, 3, 228-236.
114. Beere, H.M. 2004, J. Cell Sci., 117, 2641-2651.

115. Volker, U., Mach, H., Schmid, R., and Hecker, M. 1992, J. Gen. Microbiol., 138, 2125-2135.
116. Lund, P.A. 1995, Essays Biochem., 29, 113-123.
117. Lin, K.M., Lin, B., Lian, I.Y., Mestril, R., Scheffler, I., and Dillmann, W.H. 2001, Circulation, 103, 1787-1792.
118. Kirchhoff, S.R., Gupta, S., and Knowlton, A.A. 2002, Circulation, 105, 2899-2904.
119. Shan, Y.X., Liu, T.J., Su, H.F., Samsamshariat, A., Mestril, R., and Wang, P.H. 2003, J. Mol.Cell. Cardiol., 35, 1135-1143.
120. Mancini, M., Nicholson, D.W., Roy, S., Thornberry, N.A., Peterson, E.P., Casciola-Rosen, L.A., and Rosen, A. 1998, J. Cell Biol., 140, 1485-1495.
121. Samali, A., Zhivotovsky, B., Jones, D.P., and Orrenius, S., 1998, FEBS Lett., 431, 167-170.
122. Samali, A., Cai, J., Zhivotovsky, B., Jones, D.P., and Orrenius, S. 1999, EMBO J., 18, 2040-2048.
123. Xanthoudakis, S., Roy, S., Rasper, D., Hennessey, T., Cassady, R., Tawa, P., Ruel, R., Rosen, A., and Nicholson, D.W. 1999, EMBO J., 18, 2049-2056.
124. Faried A., Sohda M., Nakajima M., Miyazaki T., Kato H., Kuwano H. 2004, Eur. J. Cancer, 40, 2804-2811.
125. Eddy, E.M., O'Brien, D.A., and Welch, J.E. (Ed.) 1991, Mammalian Sperm Development in vivo and in vitro. In Wassarman, P.M. Element of Mammalian fertilization, CRC Press, Boca Raton, FL, USA. Pp. 1-28.
126. Dix, D.J. 1997, Cell Stress Chaperones, 2, 73-77.
127. Meinhardt, A., Wilhelm, B., and Seitz, J. 1999, Hum. Reprod. Update, 5, 108-119.
128. Paranko, J., Jürgen, S., and Andreas, M. 1996, Differentiation, 60, 159-167.
129. Timakov, B., and Zhang, P. 2001, Cell Stress Chaperones, 6, 71-77.
130. Meinhardt, A., Parvinen, M., Bacher, M., Aumuller, G., Hakovirta, H., Yagi, A., and Seitz, J. 1995, Biol. Reprod., 52, 798-807
131. Tabibzadeh, S., Kong, Q.F., Satyaswaroop, P. G., and Bakaknia, A. 1996, Hum. Reprod., 11, 633-640.
132. Cavanagh, A. C. 1996, Rev. Reprod., 1, 28-32.
133. Nakahara, K., Kim, K., Sciulli, C., Dowd, S.R., Minden, J.S., and Carthew R.W. 2005, Proc. Natl. Acad. Sci., USA, 102, 12023-12028.
134. Sarkar, S., and Lakhotia, S.C. 2005, J. Genet., 84, 265-281.
135. Lewis, M., Helmsing, P.J. and Ashburner M. 1975, Proc. Natl. Acad. Sci., USA, 72, 3604-3608.
136. McKenzie, S.I., Henikoff, S. and Meselson, M. 1975, Proc. Natl. Acad. Sci., USA, 72, 1117-1121.
137. Lindquist, S. 1980, Dev. Biol., 77, 463-479.
138. DiDomenico, B.J., Bugaisky, G.E., and Lindquist, S. 1982, Cell, 31, 593-603.
139. Lakhotia, S.C., and Singh, A.K. 1989, J. Genet., 68, 129-137.
140. Singh, B.N., and Lakhotia, S.C. 1995, Curr. Sci., 69, 178-182.
141. Lakhotia, S.C., and Singh, B.N. 1996, J. Genet., 68, 129-137.
142. Tiwari, P.K., Mohan, D.R.K., and Archana, J. 1995, J. Biosci., 20, 341-354.
143. Singh, A.K., and Lakhotia, S.C. 2000, Cell Stress Chaperones, 5, 90-97.
144. Lakhotia, S.C., Srivastava, P., and Prasanth, K.V. 2002, Cell Stress Chaperones, 7, 347-356.

145. Kozlova, T., Perezgasga, L., Reynaud, E., and Zurita, M. 1997, Dev. Genes. Evol., 207, 253-263.
146. Perezgasga, L., Segovia, L., and Zurita, M. 1999, FEBS. Lett., 456, 269-273.
147. Zhimulev, I.F., Pokholkova, G.V., Bgatov, A.B., Umbetova, G.H., Solov'eva IV., Khudyakov, Y.E., and Belyaeva, E.S. 1987, Biol. Zentbl., 106, 699-720.
148. Adams, M.D., Celniker, S.E., Holt, R.A., et al. 2000, Science, 287, 2185-2179.
149. Rubin, G.M., Yandell, M.D, Wortman, J.R. et al. 2000, Science, 287, 2204-2215.
150. Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G., and Thompson, J.D. 2003, Nucleic Acids Res., 31, 3497-3500.
151. Srivastava, P. 2004, Ph.D. Thesis, Banaras Hindu University, Varanasi, India.
152. Huh, J.R., Vernooy, S.Y., Yu, H., Yan, N., Shi, Y., Guo, M., and Hay, B.A. 2004, PLoS Biol., 2, E15.
153. Liu, L., Johnson, W.A., and Welsh, M.J. 2002, Proc. Natl. Acad. Sci., USA. 100, 2128-2133.
154. Adryan B., Decker H-J.H., Papas, T.S. and Hsu, T. 2000, Oncogene, 19, 2803-2811.
155. Betran, E., Thornton, K., and Long, M. 2002, Genome Res., 12, 1854-1859.
156. Ciocca, D.R., and Calderwood, S.K. 2005, Cell Stress Chaperones,10, 86-103.
157. Cappello, F., and Zummo, G. 2005, Cell Stress Chaperones, 10, 263.