Proc. Indian natn Sci Acad.(PINSA) **B67** No.5 pp 247-264 (2001)

Heat Shock Response - Regulation and Functions of Coding and Non-Coding Heat Shock Genes in *Drosophila*

S C LAKHOTIA*
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005

(Received on 28 March 2001; Accepted after revision on 25 May 2001)

Heat shock response is a homeostatic adaptive response exhibited by nearly all cells in response to thermal and a variety of other stresses. In this article, the nature, functions and regulation of the various families of heat shock proteins are presented first and this is followed by an account of studies in the author's laboratory relating to the expression and regulation of some of the heat shock protein-coding genes and the non-coding *hsrω* gene in *Drosophila*. Our studies showed that unlike the common belief, different cell types of *Drosophila* exhibit differential activation of the various heat shock genes in a developmental stage and cell type specific manner. Transcripts of the different members of the *HSP70* gene family are differentially induced and turned over in various cell types. A unique set of proteins, which includes the HSP64, is induced immediately after heat shock in larval Malpighian tubules while none of the typical heat shock proteins are induced in this tissue till at least one hour after heat shock. Interestingly, in most other tissues, HSP64 is not induced by heat shock. The heat inducible *93D* or the *hsrω* gene of *Drosophila melanogaster* does not code for any protein. This gene is developmentally active and is also singularly induced by a variety of amides. Recent studies in our laboratory showed that one of its multiple non-coding transcripts is instrumental in the organization of nuclear omega speckles, which seem to be very important for regulation of availability of the heterogeneous nuclear RNA binding proteins (hnRNPs) for RNA processing activities in unstressed and stressed conditions as per contemporary requirements of the cell. Accordingly, over- as well as non-expression of this gene under normal conditions has severe consequences. It is likely that an equivalent of the non-coding *hsrω* gene is present in all other organisms as well. Future studies are expected to reveal the physiological bases of such pronounced as well as subtle differences in the stress response mounted by different cells of an organism and their evolutionary significance.

Key Words: Stress response; HSP, Omega speckles, *hsrω*; 93D

Introduction

The heat shock response was discovered by Ritossa (1962) as the induction of a specific set of puffs in polytene chromosomes of salivary glands of *Drosophila* larvae in response to a brief exposure to elevated temperature or to chemicals that disrupt oxidative metabolism of the cell. A little more than a decade later, Tissieres et al. (1974) and Lewis et al. (1975) reported the synthesis of a novel set of polypeptides in *Drosophila* tissues exposed to elevated temperature. The discovery of heat shock (HS) induced synthesis of a novel set of polypeptides, the HS proteins or the HSPs, stimulated a remarkable flurry of research activity so that within a short time, the HS or the stress response was established as a major field of study to not only understand the survival strategies of living organisms in their every day life but also to understand the mechanistic details of gene expression and regulation (Schlesinger et al. 1982). It was obvious that the HS response evolved very early in the history of living organisms as a homeostatic mechanism to protect the cellular machinery from damages inflicted by a variety of adverse environmental factors like temperature, oxidative stress, salinity, osmolarity, heavy metals, genotoxic compounds etc. The well orchestrated changes in the transcriptional and translational activities of individual cells in response to such environmental stresses are collectively termed as "heat shock" or "stress" response (Schlesinger et al. 1982).

**Tel* (+91 542) 368145, 307136; *Telefax:* 36845; *e-mail:* lakhotia@banaras.ac.in; sclakhotia@yahoo.co.in

As remarked by Ashburner (1982), "The heat-shock response had been shown not only to be universal but also to occur under a wide variety of different stress conditions".

My own interest in the HS genes in *Drosophila* was aroused because of a serendipitous finding that one of the major HS genes of *Drosophila melanogaster*, the 93D locus, was singularly inducible with benzamide (Lakhotia & Mukherjee 1970, 1980). Later studies directed at understanding this gene's organization and functions revealed many unusual and interesting aspects, not only concerning this particular gene but also about other HS genes. In the following, the HS paradigm is described first and this is followed by an account of the studies carried out in my laboratory during the past 2-3 decades on these aspects.

Heat Shock Response

Sudden increase in the cellular temperature, beyond the normal physiological range of the species, is recognized by the cell as "heat shock", which results in transcriptional activation of a set of genes, the HS genes. The ongoing transcription of most other genes is generally inhibited under such conditions. In higher organisms, the pre-existing mRNAs are typically sequestered and not translated under the conditions of stress. The ribosomal machinery of a heat shocked cell preferentially translates the newly synthesized HS mRNAs resulting in synthesis and rapid accumulation of HSPs (Tissieres et al. 1974, Lewis et al. 1975, Lindquist 1986). Lewis et al. (1975) made an important observation that all the examined cell types of *Drosophila* displayed a remarkably similar induction of a new set of HSPs. Interestingly, the temperature at which the cells optimally respond to HS varies considerably in different organisms, e.g., 24-28°C for salmon embryos, 33-35°C for yeast, 35-38°C for *Drosophila*, 35-40°C for plants and 40-44°C for birds and mammals (Lindquist 1986). Even within a group, there are significant variations in relation to specific environmental adaptations. Thus compared to the temperate species of *Chironomus*, the tropical species show maximal response to HS at a higher temperature and also survive better at such temperatures (Nath & Lakhotia 1989). Likewise, blow flies and houseflies can withstand much higher temperature than *Drosophila* (Tiwari et al. 1995, 1997).

Apart from heat stress, other environmental stresses like amino acid analogues, transition heavy metals, uncouplers of oxidative phosphorylation, recovery from anoxia, chemotherapeutic agents, pathophysiological states like viral infection, fever, inflammation, ischemia, hypertrophy, malignancy etc. are also known to induce some or all of the HSPs (Morimoto et al. 1992, 1994, Csermely 1998). The cellular levels of HS proteins are elevated in certain chronic diseases like Hashimoto's thyroiditis, Graves' disease, arthritis and artherosclerosis (Morimoto et al. 1990). Localized tissue injury can also induce HSPs (Gower et al. 1989, Morimoto et al. 1990).

Heat Shock Protein Families

The HS genes and the mechanisms of their induction under conditions of cellular stress are conserved across the different phylogenetic groups (Schlesinger et al. 1982, Nover 1984). The HSPs are broadly classified according to the apparent molecular weight, amino acid sequence homologies and their functional aspects (Nover 1984) into 5 major families: HSP100 family (100-104 kDa), HSP90 family (82-90 kDa), HSP70 family (68-75 kDa), HSP60 family (58-65 kDa) and the small HSP family (15-30 kDa). HSP70 family proteins are the most conserved proteins among the HSP families followed by the HSP90, HSP100, HSP60 and small HSPs. Other than these, several other minor HSPs are also synthesized during HS (see later). Homologs of the HSPs are also often present under normal physiological or unstressed conditions and are termed as HS cognates (HSC). The chromosomal locations of the major HS genes in *Drosophila melanogaster* are shown in figure1.

The HSP100 Family

The *clp* or the *HSP100* gene family codes for constitutive and stress inducible proteins in the range of 100-110 kDa (Parsell et al. 1991, Squires & Squires 1992), which function as regulators of energy dependent proteolysis and as molecular chaperones (Clarke 1996). They stabilize certain polypeptides during severe thermal stress and either enable resolubilization of non-functional protein aggregates or target the irreversibly damaged polypeptides for degradation. The eukaryotic HSP100 members play an important role in thermotolerance (Sanchez & Lindquist 1990, Parsell et al. 1991). An *HSP100* homolog has not yet been identified in *Drosophila*.

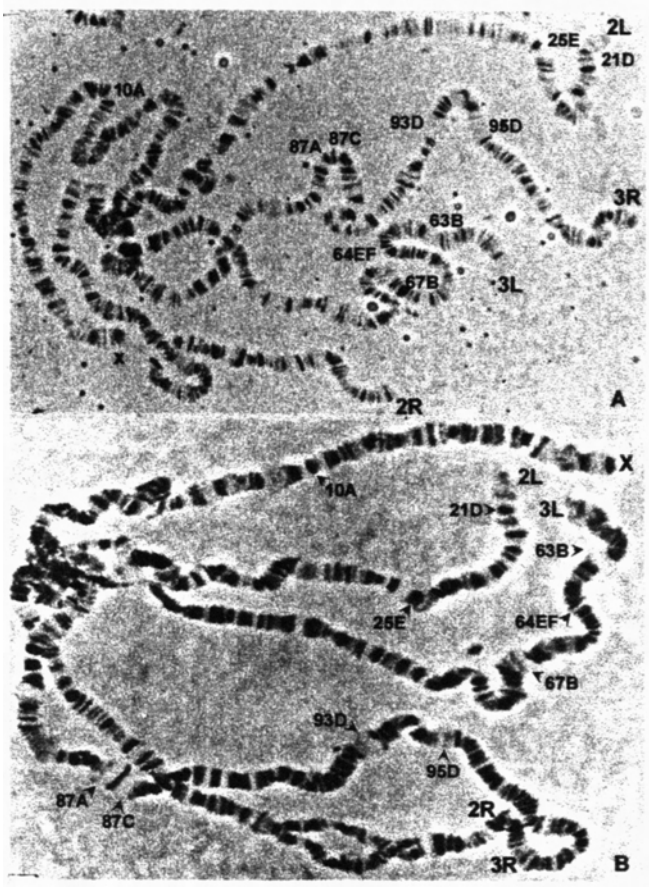

Figure 1 Polytene chromosomes of *D. melanogaster* larval salivary glands showing locations of the major heat shock genes. The chromosomes in A are from an unstressed salivary gland while those in B are from a gland that was heat shocked at 37°C for 30 min before squash preparation. Note the presence of heat shock induced major puffs at 87A, 87C, 93D, 95D sites on 3R and 70B, 67B, 64F and 63B sites on 3L in B but not in A. The three HSP64 genes, located at the sites 10A on X, 21D and 25E on 2L, respectively, are not induced by heat shock in larval salivary glands

The HSP90 Family

Members of the HSP90 molecular chaperone family are mainly cytoplasmic (Lindquist & Craig 1988). In mammals, this family includes the two major molecular chaperones of the cytosol, the HSP90 (90 kD) and the 94 kD glucose regulated protein, grp94. In most cells the HSP90 family proteins account for 1-2% of all cellular proteins (Csermely et al. 1998). HSP90 family members are apparently involved in diverse cellular functions in view of their association with a wide variety of cellular proteins like steroid receptors, histone H1, cytoskeleton, HSP56, HSP70 etc. (Jakob & Buchner 1994, Csermely et al. 1998, Caplan 1999).

The *HSP90* family member in *Drosophila* is the *HSP83* gene located at the 63BC locus (figure 1). Besides being heat inducible, the HSP83 is constitutively expressed at high levels during normal growth (Chomyn & Mitchel 1982, Zimmerman et al. 1983, Xiao & Lis 1989, Ding et al. 1993, Yue et al. 1999). *HSP83* gene is the only HS protein-coding gene in *D. melanogaster* with an intron (Hackett & Lis 1983). Like the mammalian HSP90, HSP83 in *Drosophila* is also mostly cytoplasmic in unstressed as well as heat shocked cells but Morcillo et al. (1993) showed that after heat shock, HSP83 is also present at the heat induced 93D puff. Lange et al. (2000) reported HSP83 in centrosomes in *Drosophila* and vertebrates (HSP90). Rutherford and Lindquist (1998) made an interesting observation that heterozygosity for *HSP83* recessive mutation in *Drosophila* results in revelation of a wide variety of otherwise cryptic morphological abnormalities, the nature and extents of which depend upon the specific genetic background and environmental conditions in which the heterozygosity for the *HSP83* mutation is introduced. Thus this family of proteins has been suggested to provide a simple molecular mechanism for evolvability (Rutherford 2000).

The HSP70 Family

The HSP70 protein family is highly conserved with ~50% amino acid identity among all characterized species from bacteria to man (Feige & Polla 1994, Morimoto et al. 1994, Macario et al. 1999) and is the most abundantly induced protein in stressed cells. An important feature of this family is its multi-member composition, e.g., 14 in yeast and 11 in *Drosophila*, (Rassow et al. 1997). Some members of the *HSP70* gene family, the HS cognate or *HSC70* genes, are constitutively expressed while others, the *HSP70* genes, are induced by heat and other stresses.

HSP70 plays critical role in thermotolerance in bacteria (the DnaK protein), yeast, *Drosophila* and mammalian cells (Parsell & Lindquist 1994). A member of this family, the HSP73, is present in the centrosome and seems to help in its reorganization after damage by the heat stress (Perret et al. 1995, Brown et al. 1996). HSP70 also protects cells from oxidative damage with mitochondria as the targets for protection (Polla et al. 1996, Mallouk et al. 1999). The interactions between HSP70 and ATP may decide the cell survival, necrosis or apoptosis (Mallouk et al. 1999). HSP70 has varied roles in modulating the

inflammatory and immune responses (Polla et al. 1998, Basu & Srivastava 2001). There are inter-individual variations in the basal levels of HSP70/HSC70, which correlate with the level of stress-induced synthesis of the major HSPs (Boshoff et al. 2000). Such inter-individual variations may be one of the factors that make different individuals differentially susceptible/resistant to various disease conditions (Boshoff et al. 2000). Seasonal and geographical variations have also been reported in the constitutive and induced synthesis of HSPs (Nath & Lakhotia 1989, Ultimasov et al. 1992). While protective under stress conditions, over-expression of HSP70 at physiological conditions is deleterious for cells (Feder et al. 1992). It may also prevent damaged and, therefore, potentially threatening cells, from apoptosis (Samali & Cotter 1996).

HSP70 family in *D. melanogaster* includes both heat inducible (*HSP70* family) and constitutively expressed *HSC70* genes. Five different *HSC70* genes are expressed, with spatial and temporal variations, under normal growth conditions in *Drosophila* (Elefant & Palter 1999). Two different loci, 87A7 and 87C1, respectively, on the right arm of chromosome 3 (figure 1) carry clusters of genes coding for the heat inducible HSP70 in *D. melanogaster*. The 87A7 site carries two copies and the 87C1 site carries 3 copies of the heat inducible *HSP70* genes. The two clusters are separated from each other by ~500 kb of DNA. At the 87C1, the proximal two *HSP70* genes are separated from the third gene by ~38 kb of DNA, which contains many αβ repeats (see Nover 1984). The αβ repeats produce heat-inducible but non-coding RNAs of as yet unknown function. The protein coding sequences in these five *HSP70* genes show 97% identity amongst themselves; ~400bp upstream region also shows very high homology between the 5 genes (Ingolia et al. 1980; Karch et al. 1981). In contrast, the 3' untranslated regions (3'UTR), comprising ~250bp from the termination codon till the poly-A^+ site, of the two *HSP70* genes at 87A7 locus are similar, but different from those of the *HSP70* gene copies at the 87C1 locus. Interestingly, the 3'UTRs of the proximal and the middle *HSP70* genes at the 87C1 locus are similar with each other but are moderately diverged from that of distal most *HSP70* gene at this site (Torok et al. 1982). All the five *HSP70* genes have been shown to transcribe in response to HS (Ish-Horowicz & Pinchin 1980). Besides these 5 copies of *HSP70* genes, another heat inducible gene belonging to the *HSP70* family, the *HSP68* gene, is present at the 95D locus on the right arm of chromosome 3 and this produces a 68kD polypeptide (Holmgren et al. 1979).

The 2.4-2.5 kb intronless HSP70 transcripts are undetectable in unstressed cells but are induced more than 1000 fold upon HS (Velazquez et al. 1983). The half-life of the HSP70 mRNAs also increases at least 10 fold under these conditions (Petersen & Lindquist 1988). During recovery from heat shock, the *HSP70* genes are rapidly repressed and the HSP70 messages are selectively degraded (Dellavalle et al. 1994).

Although the presence of the inducible forms of HSP70 family members is regarded as a distinct indication of a cell under stress and the concerned genes are believed to be under a state of "readiness" for rapid activation (see later), our studies have revealed that regulation of *HSP70* genes in different cell types of *Drosophila* is much more complex. One of the first examples of a differential activation of the *HSP70* genes by the thermal stress was the differential inducibility of their two sets at the 87A and 87C loci, respectively (see Lakhotia & Sharma, 1996 for review). Our recent studies (Lakhotia 2001b, Lakhotia & Prasanth 2001) have revealed more remarkable differences in the HS induction and stability of the transcripts of the *HSP70* genes at the two loci in different embryonic, larval and adult cell types. For example, during later stages of embryonic development, transcripts of the *HSP70* genes at only the 87A, but not at the 87C, were detectable upon HS in the neuronal cells in embryonic central and peripheral nervous system from stage 13 onwards. Likewise, cells in the posterior part of the heat shocked larval mid gut expressed only the 87A genes and these cells continued to express 87A genes even 2 hrs after recovery from HS. These and other results (Lakhotia & Prasanth 2001) reveal that the HS inducibility of the *HSP70* genes from the two loci is differentially regulated during development and the two sets of transcripts are metabolized in a cell- and development-stage specific manner. Apparently, the multiple copies of *HSP70* genes are not just to make more Hsp70 protein during HS. We also found a constitutive expression of the HS inducible form of HSP70 in spermatogonial cells of *D. melanogaster* (Lakhotia 2001b, Lakhotia & Prasanth 2001).

The HSP60/Tcp1 Family

HSP60 family proteins are stress inducible as well as constitutively expressed, are essential for growth under all conditions and are found in the cytosol of bacteria (the GroEL protein), in the matrix of eukaryotic mitochondria and in the stroma of chloroplasts (Hartl et al. 1992, Houry et al. 1999). HSP60 family proteins, commonly also called "chaperonins" (Hemmingsen 1992), exert functions similar to those of HSP70 family proteins in assisting protein folding (Houry et al. 1999). The chaperonins require the presence of another co-chaperonin, the GroES or HSP10, for effective binding with and folding of proteins (Hartl et al. 1992, Georgopoulus et al. 1994, Bukau & Horwich 1998). Interestingly while the HSP70/DnaJ complex associates preferentially with short peptides or polypeptides in extended conformations, the HSP60/GroEL proteins show higher affinity towards partially folded structures. These two families of proteins function in sequential reactions during folding of a newly synthesized polypeptide. The cytosolic homologs of HSP60 in eukaryotic cells are classified as TCP-1 or TriC (TCP-1 ring complex, Gupta 1990), which are hetero-oligomeric chaperones involved in the folding of actin and tubulin.

The HSP60 family members in *Drosophila* have been identified only very recently. My laboratory (Lakhotia & Singh 1989) was the first to report the HS induced synthesis of HSP64 as a member of a novel set of polypeptides in Malpighian tubules of *Drosophila* larvae (figure 2A, Lakhotia & Singh 1989). This protein was later shown to be a member of the HSP60 family (figure 2B, Singh & Lakhotia 1995, Lakhotia & Singh 1996). The HSP64 is not induced by HS in any other cell type of *Drosophila*. It is interesting that 1 hr after heat shock, synthesis of the typical set of the common Hsps is induced in the larval Malpighian tubules (Krebs & Feder 1997, Priya Srivastava, Prasanth K. V. & Lakhotia, unpublished) but at this time the Hsp64 and other Malpighian tubule-specific HS induced proteins are no longer synthesized. Most intriguing, however, is our observation that the *HSP70* transcripts from both the 87A and 87C gene copies are induced immediately in the larval Malpighian tubule cells after HS but their transport to cytoplasm and translation are delayed for about an hour. The significance of the HS induced

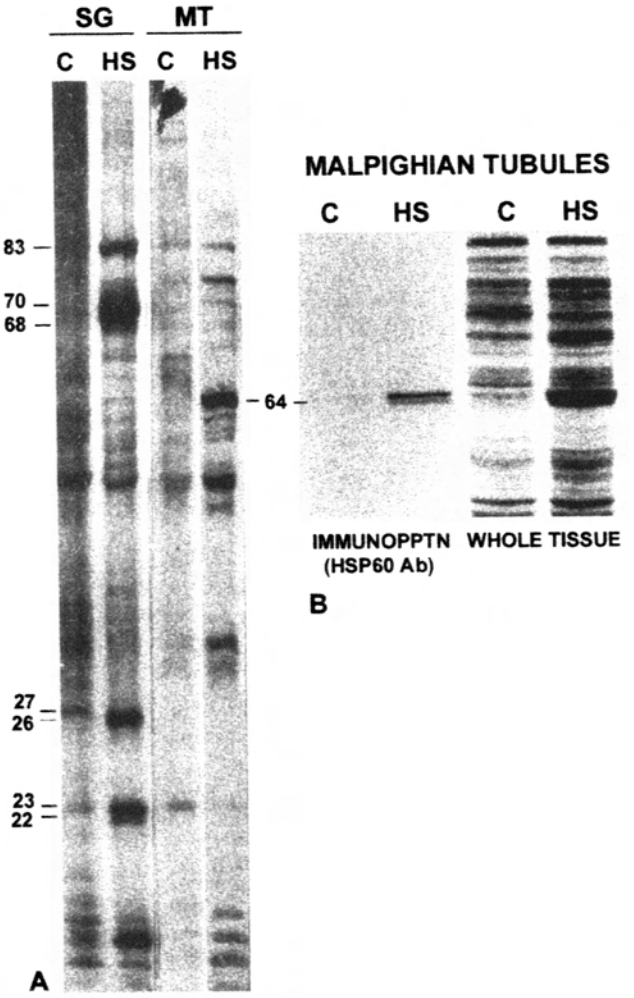

Figure 2 Heat shock response in Malpighian tubules of *Drosophila* larvae is very different from that in other tissues. A. Fluorograph of ^{35}S-methionine labelled and SDS-PAGE separated polypeptides from control (C) and heat shocked (HS) salivary glands (SG) and Malpighian tubules (MT) of *D. melanogaster* larvae. Note that unlike in salivary glands, none of the typical HSPs (83, 70, 68, 27, 26, 23 and 22 kDa) are induced by heat shock in Malpighian tubules; instead, a different set with a major polypeptide of 64 kDa is induced in this tissue. B. The 64 kDa heat shock induced polypeptide in Malpighian tubules is a member of the HSP60 family as revealed by immunoprecipitation of ^{35}S-methionine labelled polypeptides using an HSP60 antibody and fluorography of the SDS-PAGE fractionated immuno-precipitates from control (C) and heat shocked (HS) Malpighian tubules. Note the specific and stronger labeling of the 64 kDa polypeptide in the heat shock lane (lane 2). The lanes 3 and 4 show fluorograph of total proteins from ^{35}S-methionine labeled control and heat shocked Malpighian tubules. Figures adapted from Lakhotia and Singh (1989, 1996).

transcriptional activation of one of the HSP64 genes in Malpighian tubules and its negative correlation with the synthesis of HSP70 and other typical HSPs remains to be understood. It is interesting to note in this context that in several insect species, the HS response in different tissues, especially in Malpighian tubules (in some cases in testes also), shows specific deviations in terms of induction of the "HSP60", "HSP70" or a "mixed" type response (see Singh & Lakhotia 2000). It appears that tissues like Malpighian tubules have a different stress physiology and, therefore, have evolved special regulatory mechanisms. Further studies on this aspect are expected to provide exciting insights.

The first *HSP60* gene in *D. melanogaster*, localized at the 10A cytogenetic region of the X chromosome (figure 1), was cloned by Kozlova et al. (1997). The Berkeley *Drosophila* Genome Project has unraveled two more *HSP60* genes in *D. melanogaster*, one at 21D locus and the other at 25E locus (figure 1). The putative protein sequences of the two *HSP60* genes located at 10A and 25E are ~80% similar with each other but the 21D gene shows only ~60% homology with the other two. Recent studies in my laboratory (Srivastava & Lakhotia 2000 and other unpublished results) have revealed very interesting functional differentiation of the three *HSP60* genes in *D. melanogaster*. The HSP64 from the 10A gene is widely expressed in nearly all cell types in a developmentally regulated manner while the HSP64 encoded by the 21D gene seems to have male germ cell specific functions (also see Timakov & Zhang 2001). On the other hand, the HSP64 gene at 25E appears to be HS inducible in larval Malpighian tubules, gut and fat bodies (Srivastava & Lakhotia 2000 and other unpublished results).

The Small HSP Family

The small HSPs, a heterogeneous group with sizes ranging from 20-30kDa, are the least conserved among the HSPs (Lindquist 1986). This group of genes also belongs to a multigene family in most organisms (3 in nematodes, at least 4 major proteins in *Drosophila*, 3 in mammals including crystallins and >20 in plants but only 1 in yeast). Cytoplasmic particles containing the small HSPs (sHSPs) complexed with small RNAs have been isolated from *Drosophila* and yeast during recovery from HS (Arrigo et al.1985). sHSPs suppress aggre- gation of denatured proteins and promote their activation but unlike HSP70 and HSP60, the chaperon activity of sHSPs is independent of ATP (Jakob et al. 1993, Arrigo & Landry 1994).

There are four well characterized sHSPs (HSP28, HSP26, HSP23 and HSP22) in *D. melanogaster* and genes for all four of them are located at the 67B region on the left arm of chromosome 3 (figure 1), within a short stretch of DNA in the order *HSP28, HSP23, HSP26, HSP22*. Three other HS inducible genes are also localized at this site but these have not been well characterized yet. Each of the sHSP genes has its own promoter, with *HSP26* being transcribed in a direction opposite to the other three (Petersen et al. 1979, Craig & McCarthy 1980, Lindquist 1986). The four *Drosophila* sHSPs have an overall homology of ~50% (Southgate et al. 1983). The sHSPs shuttle between nucleus and cytoplasm during HS and recovery (Arrigo & Landry 1994) and are found associated with cytoskeletal components also (Leicht et al. 1986). All these genes are expressed at normal temperatures with each being independently regulated in a tissue-specific manner (Southgate et al. 1983, Arrigo & Tanguay 1991, Marin et al. 1993, Michaud et al.1997), suggesting their different roles under normal (and stressed) conditions. Their HS inducibility can also be independently modulated. Thus we found (Lakhotia & Singh 1988) that when *D. melanogaster* larvae were reared at 10°C, their salivary glands showed reduced synthesis of HSP23 not only developmentally but also after heat shock, while the other sHSPs did not appear to be much affected.

Other Members of the Heat Shock Gene Family

Besides the above major HS gene families, several other genes/proteins that are induced by the heat stress have been identified in different organisms.

Several peptidyl prolyl isomerases have been identified as HSPs (Lilie et al. 1993). The DnaJ of *E.coli*, stimulates the ATPase activity and release of protein substrate from DnaK, the HSP70 homolog in prokaryotes. The eukaryotic counterpart of DnaJ, the HSP40, is also heat inducible and is believed to participate in protein folding in association with HSP70 (Wild et al. 1992). The ubiquitin too is heat inducible. Ubiquitin binds with the heat denatured proteins and targets them for degradation (Hochstrasser 1992, Craig et al. 1994). In addition,

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

Regulation of Heat Shock Response

HS affects gene activity at transcriptional, post-transcriptional as well as translational levels (table 1). The most obvious effect is the selective and rapid transcriptional induction of HS genes with a concomitant repression of most of the ongoing chromosomal transcription. RNA polymerase II is withdrawn from most chromosomal sites after HS and is redistributed at the HS loci (Bonner & Kerby 1982). To facilitate such rapid activation, the 5m ends of the HS genes (especially HSP70 genes) are maintained in an open configuration (Costlow & Lis 1984, Lu et al. 1992). The GAGA factor, TATA-binding protein (TBP) and the RNA polymerase II remain associated with the uninduced promoter of the *HSP* genes like *HSP70, HSP26, HSP27* etc with the polymerase remaining paused after transcribing about 25-30 nucleotides of these genes (Rougive & Lis 1988, Giardina et al. 1992, Li et al. 1996, Weber et al. 1997).

HS activates the HS factor (HSF), which binds to the highly conserved HS elements (HSE) in the promoter of all eukaryotic HS genes (Morimoto et al. 1994, Wu et al. 1994). HSE is typically composed of 3-6 alternatively oriented repeats of a 5bp sequence motif, 5m nGAAn-3m (Amin et al. 1988). The number of HSEs at individual genes varies (Amin et al. 1988, Fernandes et al. 1994). The monomers of HSF present in unstressed nucleus trimerize in response to HS, which binds to HSEs to transcriptionally activate the HS genes (Westwood et al. 1991, Westwood & Wu 1993). A single HSF coding gene exists in *Drosophila* as well as yeast, but multiple HSFs are present in other organisms like tomato, mouse and man (Nover & Scharf 1997, Morimoto 1998). Transcription of HS genes under stress is autoregulated (DiDomenico et al. 1982) so that inhibition of the synthesis of functional HSPs during HS causes transcription of HS gene to continue for much longer period; under such conditions the HS mRNAs are very stable.

Table 1 *Transcriptional and post-transcriptional regulation in heat shocked cells.*

Level of regulation	Heat shock genes	Other genes
Transcriptional	HS genes are rapidly transcribed due to binding of activated HSF with HSEs in HS gene ptomoters	Transcription of most other genes is inhibited and RNA polymerase II is redistributed on HS genes
Post-transcriptional	Most HS mRNAs are without introns and thus are rapidly transported to cytoplasm without the need for much processing	Splicing and polyadenylation of non-HS mRNAs are generally reduced/ inhibited and the RNA-processing factors are reorganized in the nucleus
Translational	HS mRNAs are selectively translated by ribosomes in heat shocked cells	In higher eukaryotes translation of other normal transcripts is inhibited and the mRNAs are sequestered till the cell recovers from HS

RNA splicing and polyadenylation of the pre-existing transcripts are also affected by HS (Yost et al. 1990, Shen et al. 1993). Since most of the HS genes do not contain introns, their transcripts are rapidly transported to the cytoplasm without the need of the usual post-transcriptional processing (Yost & Lindquist 1986, 1988, 1991, Bond 1988). About 40% of the *HSP70* transcripts in *D. melanogaster* lack a poly-A tail because of a rapid and selective removal of poly-A from previously adenylated transcripts (Dellavalle et al. 1994). High temperatures block the turnover of the normally short-lived *HSP70* mRNA, allowing its rapid accumulation (Petersen & Lindquist 1988). The 3m UTRs on *HSP70* transcripts destabilize them during unstressed conditions and during recovery (Petersen & Lindquist 1988). Since new transcription and processing of pre-existing transcripts of most of the non-heat shock genes is inhibited, the nuclear RNA processing proteins also get redistributed in heat shocked cells (see later)

Translational regulation during HS in *Drosophila* involves a rapid decay of pre-existing polysomes (Krugger & Benecke 1981, Duncan 1996). Ribosomes in heat shocked cells selectively translate the HS mRNAs which bear unusually long and A-rich 5m untranslated leader sequences (Klemenz et al. 1985, McGarry & Lindquist 1985, Lindquist 1986, Kozak

1988, Duncan 1996). Though synthesis of most of the proteins is rapidly inhibited following heat shock, translation of some mRNAs like those of core histones continues unaffected (Farrel-Towt & Sanders 1984).

It is obvious that the above elaborate regulatory strategies ensure a rapid production of the different HSPs to salvage the damages inflicted by the stress and to prevent further damages. Also since HSPs can have a negative effect under non-stress conditions (Feder & Hoffmann 1999), the regulatory pathways ensure their quick disposal after the stress is withdrawn. As already noted above, studies in our (Lakhotia 2001b, Lakhotia & Prasanth 2001) and other laboratories (see Feder & Hoffmann 1999) also show that specific cell types can over-ride these apparently universal regulatory circuits so that some cells do not synthesize any of the HSPs under stress while in other cell types, some or all *HSP* genes may be subject to different transcriptional and/or post-transcriptional regulations.

The 93D or the hsrω is an Unusual Heat Shock Gene in *Drosophila*

The *93D* (named after its location in the 93D cytogenetic region of polytene chromosomes of *Drosophila melanogaster*) or the *hsrω* (named after its transcription products, HS RNA omega) locus is an unusual member of the HS gene family in *Drosophila*. This gene does not code for any protein but seems to have very important cellular functions under normal as well as stressed conditions.

The Inducibility and Architecture of the hsrω Gene are Conserved in the Genus *Drosophila* but the Base Sequence is not

The *hsrω* gene is developmentally expressed in most cell types (Lakhotia et al. 2001) and is strongly induced by HS alongwith the other HS genes (Mukherjee & Lakhotia 1979, figures 1B, 3B). However, the most interesting feature of this gene which attracted our attention was its singular inducibility with benzamide (Lakhotia & Mukherjee, 1970, 1980, figure 3C). As a member of the HS gene family, the *93D* gene is induced by carbon dioxide, recovery from anoxia, 2-4 dinitrophenol, arsenic compounds etc (Ashburner & Bonner 1979, Mukherjee & Lakhotia 1979, Lakhotia & Sharma 1996, Lakhotia et al. 1999), but it is uniquely induced by chemicals like benzamide (Lakhotia & Mukherjee 1970, Lakhotia & Mukherjee 1980), colchicine or colcemid (Lakhotia & Mukherjee 1984, figure 4A) and several other amides (Tapadia & Lakhotia 1997). These chemicals also inhibit most of the chromosomal RNA synthesis (Lakhotia & Mukherjee 1970, 1980, Tapadia & Lakhotia 1997).

An equivalent of the *hsrω* gene is present in all species of *Drosophila* (figure 4B-I, Lakhotia & Singh 1982). In all species of *Drosophila* examined so far, the *hsrω* transcription unit includes a characteristic 5' end (with 2 exons and an intron spanning ~1.9kb length) followed by a long stretch (more than 5kb to ~15kb) of short tandem repeats, which are unique to this locus (Garbe et al. 1986, Hovemann et al. 1986, Lakhotia et al. 1999). An intriguing feature of the *hsrω* gene is the rapid divergence of base sequence in its transcribed region in different species of *Drosophila*. In spite of the high sequence diverg-ence, it shows comparable inducible properties and produces several transcripts of similar sizes and properties (Garbe et al. 1986, Hovemann et al. 1986, Fini et al. 1989).

The *hsrω* gene does not code for any protein (Lakhotia & Mukherjee, 1982, Garbe & Pardue 1986,

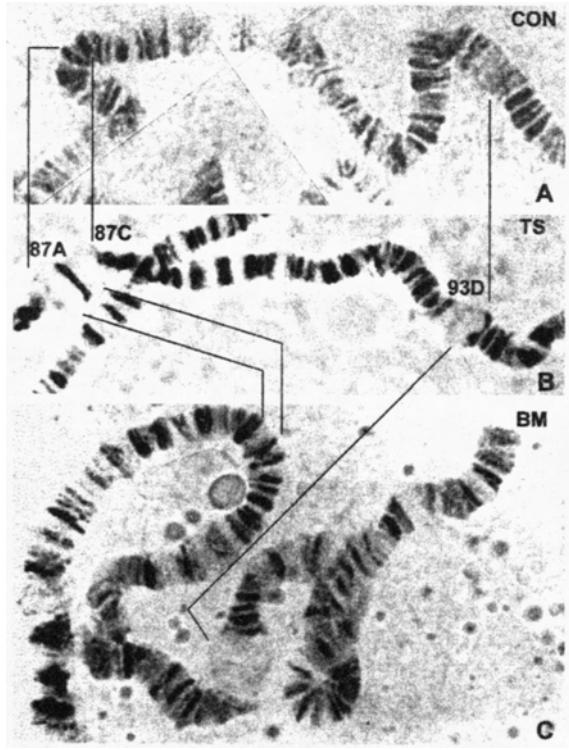

Figure 3 The 93D puff is induced by heat shock in larval salivary gland polytene chromosomes of *D. melanogaster* alongwith the other heat shock puffs (like 87A and 87C) but is singularly induced by a 10min treatment with 10mM benzamide at 24°C. Parts of the right arm of chromosome 3 (3R) are shown from control (CON, A), heat shocked (TS, B) and benzamide (BM, C) treated salivary glands. Locations of the 87A, 87C and 93D sites are indicated.

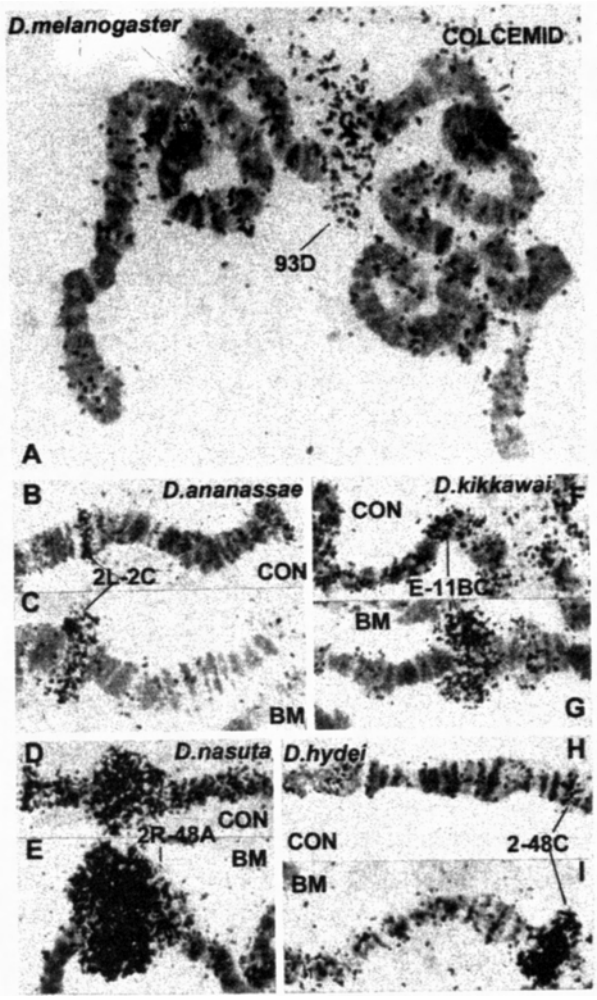

Figure 4A-I A 93D-like puff site is present in all species of *Drosophila*. ^{3}H-uridine-labeled autoradiograms of polytene chromosomes of *D. melanogaster* (A, colcemid treated), *D. ananassae* (B & C), *D.nasuta* (D & E,), *D.kikkawai* (F & G), and *D.hydei* (H & I,) *CON*, Control and *BM*, BM-treated, Note the intense labeling of a single puff site (93D in *D. melanogaster*, 2L-2C in *D. ananassae*, 2R-48A in *D.nasuta*, E-11BC in *D.kikkawai* and 2-48C in *D.hydei*) in the colcemid or BM-treated nucleus (A, *from Lakhotia & Mukherjee*, 1984 and B-I, *from Lakhotia & Singh 1982*).

Hovemann et al. 1986). *hsrω* gene in all species of *Drosophila*, that have been examined, produces two primary transcripts, viz., the hsrω-n transcript of ~10 to 15kb length (which spans the entire transcription unit and remains localized in the nucleus) and the hsrω-pre-c transcript of ~1.9kb length (spanning only the 5' region comprising of the two exons and the intron). The 1.9kb hsrω-pre-c transcript is typically spliced to give rise to the cytoplasmic 1.2kb hsrω-c transcript (Hovemann et al. 1986, Garbe et al. 1986, Bendena et al. 1989, Fini et al. 1989). The rapid sequence divergence at this locus in *Drosophila* species is perhaps related to its non-coding nature. But the production of transcripts of comparable properties suggests that the architecture of the gene and its transcripts is more important than the base sequence itself (Lakhotia et al. 1999).

Profiles of the different hsrω transcripts show inducer specific variations. Following HS, levels of all the three transcripts increase markedly. However, the benzamide and colchicine treatments lead to a significant increase of only the >10kb long nuclear hsrω-n (Bendena et al. 1989, Lakhotia & Sharma 1995). This difference seems to be related to the different effects of HS and amides on transcriptional and translational activities. As already noted, HS affects transcriptional as well as translational activities while the amides affect only chromosomal transcription without any effect on translational activity (Lakhotia & Mukherjee 1982). Turnover patterns of the hsrω-n and the ~1.2kb cytoplasmic hsrω-c transcripts are also different since while the hsrω-n is stabilized by inhibition of transcription with Actinomycin D, the hsrω-c is stabilized by protein synthesis inhibitors (Bendena et al. 1989, Bendena et al. 1991). This differential stability relates to the nucleus-limited functions of the hsrω-n transcript (see below) and to a cytoplasmic role of the hsrω-c RNA. In view of a very small but translatable ORF in the hsrω-c (Fini et al. 1989) and the stabilization of this transcript species by translational inhibitors (Bendena et al. 1989), it has been suggested that translation of the small ORF in the hsrω-c RNA serves to monitor the "health" of the cell's translational machinery (Lakhotia 1989, Lakhotia & Sharma, 1996).

The hsrω Gene is Important for Viability and Thermo-resistance

In addition to its strong induction by HS and amides, the *hsrω* gene is also developmentally expressed in a dynamically regulated manner (Bendena et al. 1991, Mutsuddi & Lakhotia 1995, Lakhotia et al. 2001). As may be expected from the production of multiple transcripts, their dynamic developmental expression in different tissues and their differential inducibility by HS and amides, our studies (Mutsuddi & Lakhotia 1995, Lakhotia & Tapadia 1998, Lakhotia et al. 2001) revealed the promoter to be complex with multiple regulatory elements in promoter region of the *hsrω* gene.

An intensive screen for mutations at the 93D locus by Mohler & Pardue (1982) did not recover any point

mutations. In hindsight this appears to be related to the non-coding nature of this gene so that base changes are well tolerated, as has actually happened at this locus during evolution. However, two small overlapping deletions, viz. *Df(3R)e*^{Gp4} and *Df(3R)GC14*, whose overlap specifically defines the HS and amide-inducible *hsrω* locus (Mohler & Pardue 1982, Burma & Lakhotia,1986) have been informative about functions of this enigmatic gene. Most of the *Df(3R)e*^{Gp4}*/Df(3R)GC14* trans-heterozygotes (*hsrω*-nullosomic) die as embryo while the few (~20% of the nullosomics) who survive to adult stage are weak flies, unable to walk or fly properly, and which die within a few days (Mohler & Pardue 1984, Lakhotia & Ray 1996, Lakhotia et al. 1999). Unlike wild type flies, *hsrω*-nullosomics are relatively poor in acquiring thermotolerance and do not survive when grown at 31°C (Lakhotia 1987, Pardue et al. 1990). This suggests that, like the HSP70, *hsrω* also plays important role in thermotolerance. This is further supported by significant differences in the levels of the nuclear and cytoplasmic hsrω transcripts between lines selected and unselected for thermo-resistance (McColl et al. 1996, McKechnie et al.1998). In addition, our recent studies on mutations due to insertion of P-transposon in the promoter region of the *hsrω* locus of *D. melanogaster* have also provided significant insight in the way this non-coding RNA functions (see below).

The hsrω-n Transcripts Organize "omega" Speckles in Nucleoplasm to Regulate the Availability of hnRNPs for RNA Processing Activities

It has been known for some time (Dangli & Bautz 1983, reviewed by Lakhotia et al. 1999) that antibodies against several nuclear non-histone proteins, mostly belonging to the heterogenous nuclear RNA-binding family of proteins (hnRNPs, Krecic & Swanson 1999), specifically bind with the 93D puff in heat shocked salivary glands of *D. melanogaster* larvae. We exploited this information to understand the functional significance of the association between the hsrω transcripts and hnRNPs under different genetic and environmental conditions (Lakhotia et al. 1999, 2001, Prasanth et al. 2000, Rajendra et al. 2001). Unlike any known RNA-polymerase II dependent eukaryotic transcripts, the hsrω-n transcripts are present in the nucleus, besides at the site of transcription, as small granules or speckles distributed in nucleoplasm in close vicinity of chromosomes and whose abundance varies in cell type specific manner (figure 5, Prasanth et al. 2000, Lakhotia et al. 2001). The hnRNPs and the related proteins remain bound either to transcriptionally active chromatin sites or to the hsrω-n RNA speckles (figure 5). Significantly, the SR-family of RNA-binding nuclear proteins, which form the well known inter-chromatin granule clusters (Spector 1993), do not localize with the hsrω-n RNA speckles (Prasanth et al. 2000, Rajendra et al. 2001). Therefore, the speckles containing the hsrω-n RNA and hnRNPs and the related proteins are novel nuclear structures and have been designated as "omega speckles" (Prasanth et al. 2000). These studies suggested that this gene's large nuclear-limited hsrω-n transcript sequesters the various hnRNPs and related proteins to regulate their availability for nuclear RNA processing activities (Lakhotia et al. 1999, Prasanth et al. 2000). As illustrated in figure 5, *all the nuclear hnRNPs, which are not associated with the transcriptionally active chromatin sites, colocalize exclusively with the hsrω-n transcripts in the form of omega speckles in all the cell types of Drosophila* (Lakhotia et al. 1999, 2001, Prasanth et al. 2000, Rajendra et al. 2001). The presence of the hsrω-n transcripts is essential for organizing the omega speckles since in the *hsrω* nullosomic (*Df(3R)e*^{Gp4}*/ Df(3R)GC14*) cells, the omega speckles are completely absent so that the hnRNPs remain distributed in a diffuse manner through the nucleoplasm (Prasanth et al. 2000).

We suggested that the omega speckles are storage sites for the unengaged hnRNPs and depending upon the cellular needs, the hnRNPs are dynamically released from or sequestered in the omega speckles (figure 6, Lakhotia et al. 1999, Prasanth et al. 2000). It is believed that such a regulated release/withdrawal of the hnRNPs is important for ensuring the desired splicing and other processings of the nascent transcripts. As already noted earlier (table 1), HS causes a drastic reduction in general transcription and RNA processing activities in the nucleus. Consequently, most of the nuclear hnRNPs in heat shocked cells have no substrates (nascent transcripts) for processing and, therefore, need to be kept in an inactive state. We have shown (Lakhotia et al. 1999, Prasanth et al. 2000) that under these conditions, the omega speckles increase in size (due to the greater amounts of the hnRNPs being sequestered) and coalesce with each other to form large aggregates and finally all of them get localized to the 93D chromosomal site itself (see figure 6). It is likely that the massive clustering of omega speckles in fully stressed cells

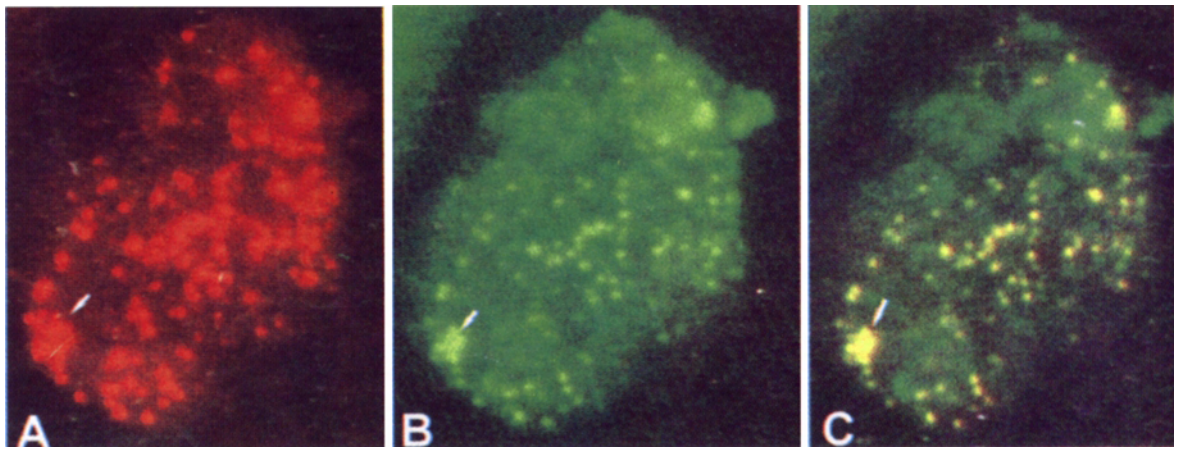

Figure 5. Omega speckles in a nucleus in cyst cell from testes of adult *D. melanogaster*. The hsrω-n RNA is present at the hsrω gene locus (arrowhead) and as numerous speckles in the nucleoplasmic space close to the chromatin area (red speckles in A, seen after RNA:RNA fluorescence in situ hybridization). The HRB87F (hnRNPA1 homolog in D. melanogaster) loacalizes diffusely over the chromatin but as speckles in the nucleoplasmic space (green speckles in B, seen after immuno-fluorescent staining with HSB87F antibody of the same cyst cell as in A). As shown in C (overlap of RNA:RNA in situ hybridization in A and immunostaining in B), nucleoplasmic speckles formed by the hsrω-n RNA and HSB87F are the same (for details, see Prasanth et al, 2000).

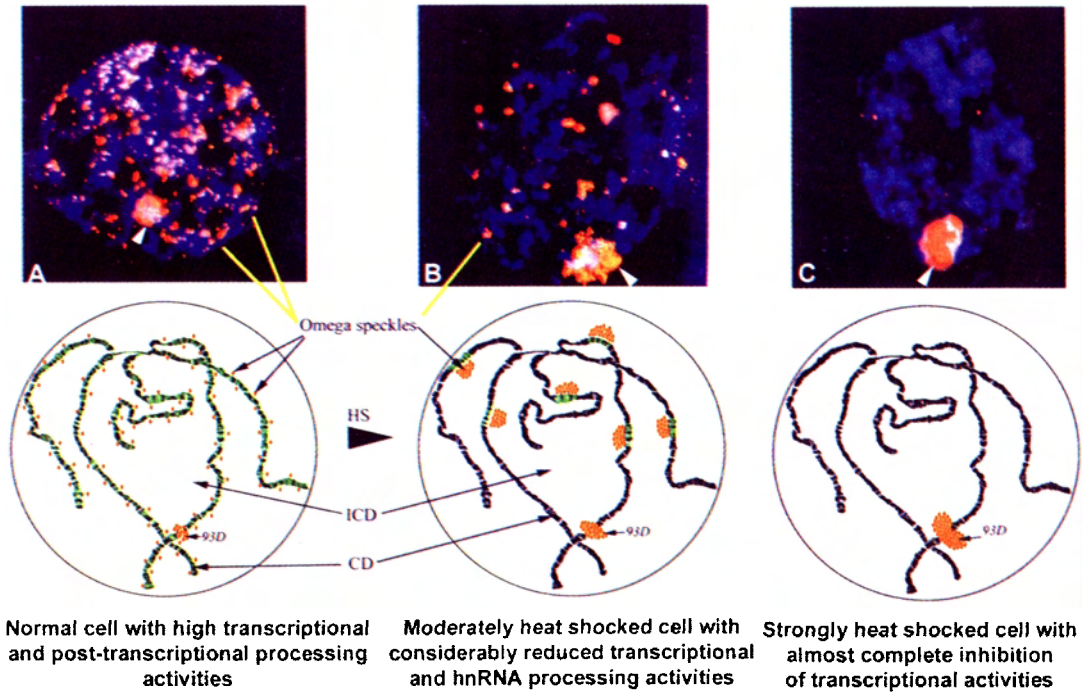

Figure 6. Dynamic re-distribution of the omega speckles in nucleus following heat shock. The upper panel shows the nuclear distribution of hsrω-n transcripts (red fluorescence following in situ RNA:RNA hybridization; chromosomal DNA shows blue DAPI fluorescence) in partially squashed cells from control (A), 30min heat shocked (B) and 40min heat shocked (C) Malpighian tubules of *D. melanogaster* larvae. The lower panel shows diagrammatic representation of the distribution of hsrω-n transcripts and the hnRNPs in these three situations. Chromosome bands are shown in black, the hnRNPs bound to transcriptionally active chromosomal sites in green and the omega speckles (containing the hsrω-n RNA and the hnRNPs) in orange. In unstressed nuclei (A), the omega speckles are present in close proximity to, but not on, the chromosomal domains (CD); the omega speckles are also absent from the inter-chromatin domain (ICD). The hsrω-n transcripts are not present anywhere on the chromosomes, except the 93D site itself (arrowheads in upper panel), where they remain complexed with the hnRNPs. Following heat shock (B), in parallel with increasing inhibition of chromosomal transcription and processing of nascent transcripts, the hnRNPs are gradually withdrawn from chromosomal locations and accumulate as clusters of omega speckles in close proximity to chromosomal domain in addition to the 93D site. As the heat shock condition continues and there is near complete inhibition of RNA processing activities, the clusters of omega speckles completely disappear from the nucleoplasmic space and get exclusively localized at the 93D site itself (C). Figure modified from Prasanth et al. (2000).

ensures against illegitimate RNA processing activities under adverse conditions (Lakhotia et al. 1999, Prasanth et al. 2000). When the cells recover from heat stress and resume their normal transcriptional and RNA processing activities, the hnRNPs are released from clusters of omega speckles and within 1hr, the hnRNPs get restored to active chromosomal sites and typical omega speckles also reappear in the nucleoplasm. Recent studies in our laboratory on over-expressing P-insertion mutant alleles of *hsrω* have provided further evidence for the importance of association between hnRNPs and hsrω transcripts. An over-expression of these transcripts due to promoter mutation would result in excessive sequestering of the hnRNPs. Since the quantity and quality of the available hnRNPs affects processing and alternative splicing of nascent transcripts (Krecic & Swanson 1999), excessive sequestering of hnRNPs by *hsrω* transcripts in the inactive compartment (omega speckles or their clusters) is expected to have trans-dominant effect on processing of several nuclear transcripts. One such P-insertion mutant allele, $hsr\omega^{05241}$, shows over-expression of hsrω-n transcripts only in cyst cells in testes, which have important role in sperm maturation and individualization. The $hsr\omega^{05241}$ mutant cyst cells display large clusters of omega speckles even without HS. The excessive sequestering of hnRNPs in the large clusters of omega speckles apparently disrupts the normal RNA processing activity in cyst cells, the consequent compromise in their function seems to be responsible for sterility of the $hsr\omega^{05241}$ homozygous male flies (Rajendra et al. 2001). In another P-insertion promoter mutant allele, the *hsrω* gene is over-expressed in a variety of 3rd instar larval tissues and this is associated with prolongation of the larval period and ultimate lethality (Sengupta & Lakhotia 2000 and other unpublished results). Other recent results (Lakhotia, Surajit Sarkar & Noopur Thakur, unpublished) show that splicing of the Ddc (dopa decarboxylase) transcripts in the central nervous system of *hsrω*-nullosomic larvae is aberrant; this seems to correlate with the absence of omega speckles in these nullosomics (Prasanth et al. 2000) and a consequent abnormal availability of the hnRNPs etc for the RNAprocessing machinery.

Although the possibility of some RNA species providing a structural role in sequestering the RNA processing factors that are not actively engaged in the post-transcriptional processing at a given time has been raised in past (Wieghardt et al. 1999), our studies (Lakhotia et al. 1999, Prasanth et al. 2000) for the first time actually identified the hsrω-n RNA as one such RNA species. The discovery of the omega speckles and the role played by the hsrω-n transcripts in organizing these speckles thus provides a new paradigm to understand the regulation of nuclear RNA processing activities. It is very likely that a regulatory system, comparable to the omega speckles of *Drosophila* cells, exists in other higher organisms, including man, since the post-transcriptional processing events in the nucleus are highly conserved (Krecic & Swanson 1999). In certain pathological conditions in man, it is known that some of the RNA processing proteins are abnormally sequestered and this trans-dominantly affects processing of several other nascent transcripts (see Singer 1998). Obviously, it is essential to fine tune the availability of RNA-processing factors in the nucleus in relation to the specific and dynamically changing cellular needs to ensure that the RNA-processing events progress smoothly in a well coordinated manner. Therefore, a non-coding RNA, similar to the hsrω-n transcript in *Drosophila*, must exist in other higher organisms as well and an active search for this would be rewarding not only from the basic cell biology point of view, but from human health point of view also.

Interaction of hsrω with Other Heat Shock Genes

Our earlier studies (for review see Lakhotia 1989, Lakhotia & Sharma 1996) revealed an intriguing relation between activities of the 93D, 87A (containing two copies of *HSP70* genes) and 87C (containing three copies of *HSP70* genes) loci. A standard HS results in high transcriptional activity at the 93D, 87A and 87C puffs with the 87A and 87C puffs being equal in size and showing similar ^{3}H-uridine incorporation (Mukherjee & Lakhotia 1979). However, when HS and another inducer of 93D (e.g., an amide or recovery from anoxia) were applied together, the 93D puff was seen not to incorporate ^{3}H-uridine and at the same time the 87A and 87C loci puffed unequally. Interestingly, depending upon the specific combination of HS and the other 93D inducer, the 87A or the 87C puff was the larger of the two in a highly reproducible manner (Lakhotia 1987, 1989, Lakhotia et al. 1990, Sharma & Lakhotia 1995, Lakhotia & Sharma 1996). We suggested that the hsrω transcripts influence RNA metabolism at the 87A and 87C sites (Lakhotia 1989, Sharma & Lakhotia 1995). Our recent observations (Lakhotia & Prasanth 2001) on the binding of hnRNPs provide a novel basis for this effect. Some of the hnRNPs have been reported to bind with the HSP70

RNA in *Drosophila* (Hamann & Stratling 1998, Reim et al. 1999), which during heat shock are normally completely sequestered by the hsωw transcipts (see above). Our studies show that under conditions when the *hsrω* locus is not transcriptionally induced by heat shock, the hnRNPs continue to remain associated with several other chromosomal sites, including the 87A and 87C puffs (Lakhotia & Prasanth 2001). It appears that in the absence of a threshold level of hsrω transcripts during HS, the removal of hnRNPs from 87A and 87C (and some other chromosomal) sites is affected. Since the hnRNPs have roles in RNA processing and transport (Krecic & Swanson 1999), their continued presence at the 87A and 87C sites in differential amounts may affect puffing of these sites differently. The functional significance and long term consequences of the unequal puffing of the two HSP70 puffs and the altered hnRNP binding at these sites needs to be examined further.

Morcillo et al. (1993) reported that the HSP83 protein gets quickly localized on the 93D puff in the polytene chromosomes of *D. melanogaster* after heat shock. Lakhotia and Ray (1996) showed that recessive mutation at *HSP83* acts as a dominant enhancer of the lethality associated with the nullosomy for the *hsrω* gene. As noted earlier, wild type HSP83 chaperone protein masks a variety of developmental abnormalities caused by genetic background (Rutherford & Lindquist 1998). In this context, the interaction of *hsrω* with HSP83 is interesting. The hsrω transcripts may have a role in this homeostasis by virtue of their involvement in regulation of the metabolism of hnRNPs, and thus in the pre-mRNA processing. The binding of HSP83 with hsrω transcripts may also help keep the associated hnRNPs etc properly chaperoned (Lakhotia et al. 1999). Mutations at *RasI* or *RasIII* genes of *D. melanogaster*, which by themselves have no effect on viability in heterozygous condition, also dominantly enhance the embryonic lethality due to *hsrω*-nullosomy (Ray & Lakhotia 1998, Lakhotia et al. 1999).

Fernandez-Funez et al. (2000) reported that P-transposon insertion mutant alleles of *hsrω* enhance the ataxin-1 induced neurodegeneration in *Drosophila*. In this context it is interesting that the *hsrω* gene is highly expressed in the nervous system (Lakhotia et al. 2001). Like the earlier noted altered splicing of Ddc transcripts in the *hsrω* nullosomic condition, it appears that the enhancement of ataxin-1 phenotype is also related to perturbations in nascent RNA processing in *hsrω* mutant background. Alterations in the dynamics of nuclear hnRNPs resulting from under- or over-expression of *hsrω* would have wide-ranging trans-dominant effects on other gene activities via its role in modulation of the availability of hnRNPs.

The *hsrω* gene provides a new paradigm for the roles of non-coding RNAs in cell's intricate and elaborate mechanisms for maintaining homeostasis.

Concluding Remarks

The HS or the stress response has been an extremely useful paradigm to understand the hierarchies of regulation of gene activity in a cell. A geneticist exploits mutations to understand the "normal" function of a gene. Likewise, the rapid and all pervading, but transient, perturbations in cellular activities by HS allow a deeper understanding of normal cell functions. The stress response paradigm has many applications in biotechnology and human health care (see Lakhotia 2001a). Increasing numbers of studies are revealing subtle or more apparent differences in stress response of different cell types and organisms and their possible ecological and evolutionary consequences (Feder & Hoffmann 1999). Since the cell's survival under the omnipresent stress conditions is most critical for the species' survival, the stress response is a vital component of any cell's defense mechanisms. Therefore, stress biology will continue to remain a fertile field of study (Lakhotia 2001a). Most of the studies on stress response have hitherto remained confined to a few model organisms maintained under laboratory conditions. It will be necessary to extend these studies to a variety of organisms living under naturally varied environmental conditions.

Acknowledgements

Research work on stress genes in my laboratory has been supported at various times by grants from the University Grants Commission, New Delhi, the Department of Atomic Energy, Govt. of India, Mumbai, Department of Science & Technology, Govt. of India, New Delhi, Department of Biotechnology, Govt. of India, New Delhi and the Council of Scientific & Industrial research, New Delhi. I thank all my former and present students who contributed to the studies on heat shock genes. I also thank the anonymous reviewer for the constructive critical comments on the manuscript.

References

Amin J, Ananthan J and Voellmy R 1988 Key features of heat shock regulatory elements; *Mol. Cell Biol.* 8 3761-3769

Arrigo A-P, Darlix, J-L, Khandijan E W, Simon M and Spahr P-P 1985 Characterization of prosome from *Drosophila* and its similarity with the cytoplasmic structures formed by the low molecular weight heat shock proteins; *EMBO J.* 4 399-406

_____ and Landry J 1994 Expression and function of the low-molecular weight heat shock proteins; in *The Biology of Heat Shock Proteins and Molecular Chaperones* pp335-373, eds R I Morimoto, A Tissieres and C Georgopoulos (New York: Cold Spring Harbor Lab Press)

_____ and Tanguay R M 1991 Expression of heat shock proteins during development in *Drosophila*; in *Heat shock and Development* pp106-119, eds L Hightower and L Nover (Berlin: Springer-Verlag)

Ashburner M 1982 The effects of heat shock and other stress on gene activity: an introduction; in *Heat Shock from Bacteria to Man* pp1-9, eds M J Schlesinger, M Ashburner and A Tissieres (New York: Cold Spring Harbor Lab Press)

_____ and Bonner J J 1979 The induction of gene activity in *Drosophila* by heat shock; *Cell* 17 241-254

Basu S and Srivastava P K 2001 Heat shock proteins: the fountainhead of innate and adaptive immune responses; *Cell Stress & Chaperones* 5 443-451

Beckmann R P, Lovett M and Welch W J 1992 Examining the function and regulation of hsp70 in cells subjected to metabolic stress; *J. Cell Biol.* 177 1137-1150

Bendena W J, Garbe J C, Traverse K L, Lakhotia S C and Pardue M L 1989 Multiple inducers of the *Drosophila* heat shock locus 93D *hsr omega* Inducer-specific patterns of the three transcripts; *J. Cell Biol.* 108 2017-2028

_____, Southgate A A, Garbe J C and Pardue M L 1991 Expression of heat shock locus *hsr omega* in non-stressed cells during development in *Drosophila melanogaster*; *Dev. Biol.* 144 65-77

Bond U 1988 Heat shock but not other stress inducers leads to the disruption of a sub-set of snRNPs and inhibition of *in vitro* splicing in HeLa cells; *EMBO J.* 7 3509-3518

Bonner J J and Kerby R L 1982 RNA polymerase II transcribes all of the heat shock induced genes of *Drosophila melanogaster*; *Chromosoma* 85 93-108

Boshoff T, Lombard F, Eiselen R, Bornman M, Bachler M, Polla B S and Bornman L 2000 Differential basal synthesis of Hsp70/Hsc70 contributes to interindividual variation in Hsp70/Hsc70 inducibility; *Cell Mol. Life Sci.* 57 1317-1325

Brown C R, Hong-Brown L W, Doxsey S J and Welch W J 1996 Molecular chaperones and the centrosome: a role for HSP73 in centrosomal repair following heat shock treatment; *J Biol. Chem.* 271 833-840

Bukau B and Horwich A L 1998 The hsp70 and hsp60 chaperone machines; *Cell* 92 351-366

Burma P K and Lakhotia S C 1986 Expression of 93D heat shock puff of *Drosophila melanogaster* in deficiency genotypes and its influence on the activity of 87C puff; *Chromosoma* 94 273-278

Caplan A J 1999 Hsp90's secrets unfold: New insights from structural and functional studies; *Trends Genet.* 9 262-268

Chomyn A and Mitchell H K 1982 Synthesis of the 84,000 dalton protein in normal and heat shocked *Drosophila melanogaster* cells as detected by specific antibody; *Insect Biochem.* 12 105-114

Clarke A K 1996 Variation on a theme: Combined molecular chaperone and proteolysis functions in Clp/HSP100 proteins; *J. Biosci.* 21 161-177

Costlow N and Lis J T 1984 High-resolution mapping of Dnase-I hypersensitive sites of *Drosophila* heat shock genes in *Drosophila melanogaster* and *Saccharomyces cerevisiae*; *Mol. Cell Biol.* 4 1853-1863

Craig E A and McCarthy B J 1980 Four *Drosophila* heat shock genes at 67B: Characterization of recombinant plasmids; *Nucl. Acids Res.* 8 4441-4457

_____, Baxter B K, Becker J, Halladay J and Ziegelhoffer T 1994 Cytosolic HSP70s of Saccharomyces cerevisiae Roles in protein synthesis, protein translocation, proteolysis and regulation; in *The Biology of Heat Shock Proteins and Molecular Chaperones* pp31-52 ed. R I Morimoto (New York : Cold Spring Harbor Lab Press)

Csermely P 1998 *Stress of life from molecules to man; Annals New York Acad. Sci.* 851 1-543

Csermely P, Schnaider T, Soti C, Prohaszka Z and Nadai G 1998 The 90kDa molecular chaperone family: Structure, function and clinical applications. A comprehensive review *J Pharmaco Theurap* 79 129-168

Dangli A and Bautz E K F 1983 Differential distribution of nonhistone proteins from polytene chromosomes of *Drosophila melanogaster* after heat shock; *Chromosoma* 88 201-207

Dellavalle R P, Petersen R and Lindquist S 1994 Preferential deadenylation of hsp70 mRNA plays a key role in regulating hsp70 expression in *Drosophila melanogaster; Mol. Cell Biol.* 14 3646-3659

DiDomenico B, Bugaisky G E and Lindquist S 1982 The heat shock response is self regulated at both the transcriptional and post-transcriptional levels; *Cell* 31 593-603

Ding D, Parkhurst S M, Halsell S R and Lipshitz H D 1993 Dynamic hsp83 RNA localization during *Drosophila* oogenesis and embryogenesis; *Mol. Cell Biol.* 13 3773-3781

Duncan R F 1996 Translational control during heat shock; in *Translational control* pp273-293 eds Hershey J W B, Mathews M B and Sonenberg N (New York: Cold Spring Harbor Lab Press)

Elefant F and Palter K B 1999 Tissue-specific expression of dominant negative mutant *Drosophila HSC70* causes developmental defects and lethality; *Mol. Biol. Cell* 10 2101-2117

Farrell-Towt J and Sanders M 1984 Non-coordinate histone synthesis in heat shocked *Drosophila* cells is regulated at multiple levels; *Mol. Cell Biol.* **4** 2676-2685

Feder M E and Hofmann G E 1999 Heat shock proteins, molecular chaperones, and the stress response-Evolutionary and ecological physiology; *Annu. Rev. Physiol.* **61** 243-282

_____, Cartano N V, Milos L, Krebs R A and Lindquist S 1996 Effect of engineering hsp70 copy number on hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*; *J. Expt. Biol.* **199** 1837-1844

_____, Rossi J M, Solomon J, Solomon N and Lindquist S 1992 The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures; *Genes. Dev.* **6** 1402-1413

Feige U and Polla S 1994 Heat shock proteins: The hsp70 family; *Experientia* **50** 979-986

Fernandes M, O'Brien T and Lis J T 1994 Structure and regulation of heat shock gene promoters; in *Biology of Heat Shock Proteins and Molecular Chaperones* pp375-393 eds R I Morimoto, A Tissieres and C Georgopoulos (New York : Cold Spring Harbor Lab Press)

Fernandez-Funez P, Nino-Rosales M L, de Gouyon B, She W-C et al. 2000 Identification of genes that modify ataxin-1-induced neurodegeneration; *Nature* **408** 101-106

Fini M E, Bendena W J and Pardue M L 1989 Unusual behaviour of the cytoplasmic transcript of *hsrω*. An abundant stress-inducible RNA that is translated, but which yields no detectable protein product; *J. Cell Biol.* **108** 2045-2057

Garbe J C and Pardue M L 1986 Heat shock locus 93D of *Drosophila melanogaster*, a spliced RNA most strongly conserved in the intron sequence; *Proc. Natl. Acad. Sci. A* **83** 1812-1816

_____, Bendena W H and Pardue M L 1986 A *Drosophila* heat shock gene with a rapidly diverging sequence but a conserved structure; *J. Biol. Chem.* **261** 16889-16894

Georgopoulos C, Liberek K, Zylicz M and Ang D 1994 Properties of the heat shock proteins of *Escherichia coli* and the autoregulation of the heat shock response. in *Biology of Heat Shock Proteins and Molecular Chaperones* pp209-249 eds R I Morimoto, A Tissieres and C Georgopoulos (New York : Cold Spring Harbor Lab Press)

Giardina C, Perez-Riba M and Lis J T 1992 Promoter melting and TFIID complexes on *Drosophila* genes *in vivo*; *Genes. Dev.* **6** 2190-2200

Gower D J, Hollman C, Lee K S and Tytell M T 1989 Spinal cord injury and the stress protein response; *J. Neurosurg.* **70** 605-611

Gupta R S 1990 Sequence and structural homology between a mouse t-complex protein TCP-1 and the chaperonin family of bacterial GroEL 60-65kDa heat shock antigen and eukaryotic proteins; *Biochem. Internatl.* **20** 833-841

Hackett R W and Lis J T 1983 Localization of the hsp83 transcript within a 3292 nucleotides sequence from the 63B hat shock locus of *D. melanogaster*; *Nucl. Acids. Res.* **11** 7011-7030

Hamann S and Stratling W H 1998 Specific binding of *Drosophila* protein PEP (protein on ecdysone puffs) to hsp70 DNA and RNA; *Nucleic Acids. Res.* **26** 4108-4115

Hartl F-U, Martin J and Neupert W 1992 Protein folding in the cell The role of molecular chaperones hsp70 and hsp60; *Annu. Rev. Biophys. Biomol. Struct.* **21** 293-322

Hemmingsen S M 1992 What is a chaperonin? *Nature* **357** 650

Hochstrasser M 1992 Ubiquitin and intracellular protein degradation; *Curr. Opin. Cell Biol.* **4** 1024-1031

Holmgren R, Livak K, Morimoto R I, Frennd R and Messelson M 1979 Studies of cloned sequences from four *Drosophila* heat shock loci; *Cell* **18** 1359-1370

Houry W A, Frishman D, Eckerskorn C, Lottspeich F and Hartl F-U 1999 Identification of *in vivo* substrates of the chaperonin GroEL; *Nature* **402** 147-154

Hovemann B T, Waldorf U and Ryseck R P 1986 Heat shock locus of 93D of *Drosophila melanogaster* An RNA with limiting coding capacity accumulates precursor transcripts after heat shock; *Mol. Gen. Genet.* **204** 334-340

Ingolia T D, Craig E A and McCarthy N J 1980 Sequence of three copies of the gene for the major *Drosophila* heat shock induced protein and their flanking regions; *Cell* **21** 669-679

Ish-Horowicz D and Pinchin S M 1980 Genetic organization of the 87A7 and 87C1 heat induced loci of *Drosophila melanogaster*; *J. Mol. Biol.* **142** 231-245

Jakob U and Buchner J 1994 Assisting spontaneity the role of hsp90 and small hsps as molecular chaperones; *Trends Biochem. Sci.* **19** 205-211

_____, Gaestel M, Engel K and Buchner J 1993 Small heat shock proteins are molecular chaperones; *J. Biol. Chem.* **268** 1517-1520

Karch F, Torok I and Tissieres A 1981 Extensive regions of homology in front of the two hsp70 heat shock variant genes in *Drosophila melanogaster*; *J. Mol. Biol.* **148** 219-230

Klemenz R, Hultmark D and Gehring W J 1985 Selective translation of heat shock mRNA in *Drosophila melanogaster* depends on sequence information in the leader; *EMBO J.* **4** 2053-2060

Kozak M 1988 Leader length and secondary structure modulate mRNA function under conditions of stress; *Mol. Cell Biol.* **8** 2737-2744

Kozlova T, Perezgasga L, Reynaud E and Zurita M 1997 The *Drosophila melanogaster* homologue of the hsp60 gene is encoded by the essential locus *l(1)10AC* and is differentially expressed during fly development; *Dev. Genes Evol.* **207** 253-263

Krebs R A and Feder M E 1997 Tissue specific variation in *hsp70* expression and thermal damage in *Drosophila melanogaster* larvae; *J. Expt. Biol.* **200** 2007-2015

Krecic A M and Swanson M S 1999 hnRNP complexes: composition, structure and function; *Curr. Opin. Cell Biol.* **11** 363-371

Krugger C H and Benecke B-J 1981 *In vitro* translation of *Drosophila* heat shock and non-heat-shock mRNAs in heterologous and homologous cell-free systems; *Cell* **23** 595-603

Lakhotia S C 1987 The 93D heat shock locus in *Drosophila* - A review; *J. Genet.* **66** 139-157

_____ 1989 The 93D heat shock locus of *Drosophila melanogaster* modulation by genetic and developmental factors; *Genome.* **31** 677-683

_____ (ed) 2001a Stress biology- a paradigm for integrative biology; *Biology International* **40** 1-36

_____ 2001b Stress response- ecological and developmental connections; *Biology International* **40** 24-27

_____ and Mukherjee A S 1970 Activation of a specific puff by benzamide in *Drosophila melanogaster*; *Dros. Inf. Serv.* **45** 108

_____ and Mukherjee T 1980 Specific activation of puff 93D of *Drosophila melanogaster* by benzamide and the effect of benzamide treatment on the heat shock induced puffing activity; *Chromosoma* **81** 125-136

_____ and _____ 1982 Absence of novel translational products in relation to induced activity of the 93d puff in *D. melanogaster*; *Chromosoma* **85** 369-374

_____ and _____ 1984 Specific induction of the 93D puff in polytene nuclei of *Drosophila melanogaster* by colchicine; *Ind. J. Exp. Biol.* **22** 67-70S

_____ and Prasanth K V 2001 Tissue and development specific induction and turnover of hsp70 transcripts from 87A and 87C loci after heat shock and during recovery in *Drosophila melanogaster*; submitted

_____ and Ray P 1996 *HSP83* mutation is a dominant enhancer of lethality associated with absence of the non-protein coding *hsrω* locus in *Drosophila melanogaster*; *J. Biosci.* **21** 207-219S

_____ and Sharma A 1995 RNA metabolism *in situ* at the 93D heat shock locus in polytene nuclei of *Drosophila melanogaster* after various treatments; *Chromosoma Research* **3** 151-161

_____ and _____ 1996 The 93D *hsr-omega* locus of *Drosophila* non-coding gene with house keeping function; *Genetica* **97** 339-348

_____ and Singh A K 1982 Conservation of the 93D puff of *Drosophila melanogaster* in different species of *Drosophila*; *Chromosoma* **86** 265-268

_____ and Singh A K 1988 Effect of low-temperature rearing on heat shock protein synthesis and heat sensitivity in *Drosophila melanogaster*; *Dev. Genet.* **9** 193-201

_____ and Singh A K 1989 A novel set of heat shock polypeptides in Malpighian tubules of *Drosophila melanogaster*; *J. Genet.* **68** 129-137

_____ and Singh B N 1996 Synthesis of a ubiquitously present new HSP60 family protein is enhanced by heat shock only in the Malpighian tubules of *Drosophila*; *Experientia* **52** 751-756

_____ and Tapadia M G 1998 Genetic mapping of the amide response element/s of the *hsrω* locus of *Drosophila melanogaster*; *Chromosoma* **107** 127-135

_____, Kar Chowdhuri D and Burma P 1990 Mutations affecting beta-alanine metabolism influence inducibility of the 93D puff by heat shock in *Drosophila melanogaster*; *Chromosoma* **99** 296-305

_____, Ray P, Rajendra T K and Prasanth K V 1999 The non-coding transcripts of *hsrω* gene in *Drosophila*: Do they regulate trafficking and availability of nuclear RNA-processing factors? *Curr. Sci.* **77** 553-563

_____, Rajendra T K and Prasanth K V 2001 Developmental regulation and complex organization of the promoter of the non-coding *hsrω* gene of *Drosophila melanogaster*; *J. Biosci.* **26** 25-38

Lange B M, Bachi A, Wilm M and Gonzalez C 2000 Hsp90 is a core centrosomal component and is required at different stages of the centrosome cycle in *Drosophila* and vertebrates; *EMBO J.* **19** 1252-1262

Leicht B G, Biessmann H, Palter K B and Bonner J J 1986 Small heat shock proteins of *Drosophila* associated with the cytoskeleton; *Proc. Natl. Acad. Sci., USA* **83** 90-94

Lewis M J, Helmsing P and Ashburner M 1975 Parallel changes in puffing activity and patterns of protein synthesis in salivary glands of *Drosophila*; *Proc. Natl. Acad. Sci., USA* **72** 3604-3608

Li B, Weber J A, Chen Y, Greenleaf A L and Gilmour D S 1996 Analyses of promoter-proximal pausing by RNA polymerase II on the hsp70 heat shock gene promoter in a *Drosophila* nuclear extract; *Mol. Cell Biol.* **16** 5433-5443

Lilie H, Lang K, Rudolph R and Buchner J 1993 Prolyl isomerases catalyze antibody folding in vitro; *Protein Sci.* **2** 1490-1496

Lindquist S 1986 The heat shock response; *Annu. Rev. Biochem.* **55** 1151-1191

Lindquist S and Craig E A 1988 The heat shock proteins; *Annu. Rev. Genet.* **163** 631-677

Lu Q, Wallrath L, Allan B D, Glaser R L, Lis J T and Elgin S C R 1992 Promoter sequence containing CTn GAn repeats is critical for the formation of the Dnase I hypersensitive sites in the *Drosophila* hsp26 gene; *J. Mol. Biol.* **222** 985-998

Macario A J L, Lange M, Ahring B K and De Macario E Y C 1999 Stress genes and proteins in the Archaea; *Microbiol. Mol. Biol. Rev.* **63** 923-967

Mallouk Y, Vayssier-Taussat M, Bonventre J V, Polla B S 1999 Heat shock protein 70 and ATP as partners in cell homeostasis; *Int. J. Mol. Med.* **4** 463-474

Marin R, Valet J P and Tanguay R M 1993 hsp23 and hsp26 exhibit distinct spatial and temporal patterns of constitutive expression in *Drosophila* adults; *Dev. Genet.* **14** 69-77

McColl G, Hoffmann A A and McKechnie S W 1996 Response of two heat shock genes to selection for knockdown heat resistance in *D. melanogaster*; *Genetics* **143** 1615-1627

McGarry T J and Lindquist S 1985 The preferential translation of *Drosophila* hsp70 mRNA requires sequence information in the untranslated leader; *Cell* **42** 903-911

McKechnie S W, Hafford M M, McColl G and Hoffmann A A 1998 Both allelic variation and expression of nuclear and cytoplasmic transcripts of *hsr?* are closely associated with thermal phenotype in *Drosophila*; *Proc. Natl. Acad. Sci., USA* **95** 2423-2428

Michaud S, Marin R, Westwood J T and Tanguay R M 1997 Cell-specific expression and heat-shock induction of hsps during spermatogenesis in *Drosophila melanogaster*; *J. Cell Sci.* **110** 1989-1997

Mohler J and Pardue M L 1982 Deficiency mapping of the 93D heat shock locus in *Drosophila*; *Chromosoma* **86** 457-467

_____ and Pardue M L 1984 Mutational analysis of the region surrounding the 93D heat shock locus of *Drosophila melanogaster*; *Genetics* **106** 249-265

Morcillo G, Diez J L, Carbajal M E and Tanguay R M 1993 HSP90 associates with specific heat shock puffs *hsrω* in polytene chromosomes of *Drosophila* and *Chironomus*; *Chromosoma* **102** 648-656

Morimoto R I 1998 Regulation of the heat shock transcriptional response cross talk between a family of heat shock factors, molecular chaperones, and negative regulators; *Genes. Dev.* **12** 3788-3796

_____, Sarges K D and Abravaya K 1992 Transcriptional regulation of heat shock genes; *J. Biol. Chem.* **267** 21987-2199

_____, Tissieres A and Georgopoulos C eds 1994 *The Biology of Heat Shock Proteins and Molecular Chaperones* (New York : Cold Spring Harbor Lab Press)

_____, Tissieres A, and Georgopoulous C eds 1990 Stress proteins in biology and Medicine. (New York : Cold Spring Harbor Lab Press)

Mukherjee T and Lakhotia S C 1979 3H-uridine incorporation in the puff 93D and in chromocentric heterochromatin of heat shocked salivary glands of *Drosophila melanogaster*; *Chromosoma* **74** 75-82

Mutsuddi M and Lakhotia S C 1995 Spatial expression of the *hsr-omega 93D* gene in different tissues of *Drosophila melanogaster* and identification of promoter elements controlling its developmental expression; *Dev.Genet.* **17** 303-311

Nath B B and Lakhotia S C 1989 Heat shock response in a tropical *Chironomus*: seasonal variation in response and the effect of developmental stage and tissue type on heat shock protein synthesis; *Genome* **32** 676-686

Nover L and Scharf K D 1997 Heat shock proteins and transcription factors; *Cell Mol. Life Sci.* **53** 80-103S

Nover L ed 1984 *Heat Shock Response of Eukaryotic Cells* pp1-78 (Berlin : Springer-Verlag,)

Pardue M L, Bendena W G, Fini M E, Garbe J C, Hogan N C and Traverse K L 1990 *hsrω*, a novel gene encoded by a *Drosophila* heat shock puff; *Biol. Bull.* **179** 77-86

Parsell D A and Lindquist S 1994 Heat shock proteins and stress tolerance; in *Biology of Heat Shock Proteins and Molecular Chaperones* pp.457-493 eds R I Morimoto, A Tissieres and C Georgopoulos (New York : Cold Spring Harbor Lab Press)

Parsell D A, Sanchez Y, Stizel J D and Lindquist S 1991 Hsp104 is a highly conserved protein with two essential nucleotide binding sites; *Nature* **353** 270-273

Perret E, Moudjou M, Geraud M L, Derancourt J, Soyer-Gobillard M O and Bornens M 1995 Identification of an Hsp70-related protein associated with the centrosome from dinoflagellates to human cells; *J. Cell Sci.* **108** 711-725

Petersen N S, Moller G and Mitchell H K 1979 Genetic mapping of the coding regions for three heat shock proteins in *Drosophila melanogaster*; *Genetics* **92** 891-902

Petersen R and Lindquist S 1988 The *Drosophila* hsp70 message is rapidly degraded at normal temperatures and stabilized by heat shock; *Gene.* **72** 161-168

Polla B S, Bachelet M, Elia G, Santoro M G 1998 Stress proteins in inflammation; *Ann. N Y Acad. Sci.* **851** 75-85

_____, Kantengwa S, Francois D, Salvioli S, Franceschi C, Marsac C, Cossarizza A 1996 Mitochondria are selective targets for the protective effects of heat shock against oxidative injury *Proc. Natl. Acad. Sci., USA* **93** 6458-63

Prasanth K V, Rajendra T K, Lal A K and Lakhotia S C 2000 Omega speckles - a novel class of nuclear speckles containing hnRNPs associated with non-coding hsr-omega RNA in *Drosophila*; *J. Cell Sci.* **113** 3485-3497

Rajendra T K, Prasanth K V and Lakhotia S C 2001 Male sterility associated with over-expression of the non-coding *hsrω* gene in cyst cells of testis of *Drosophila melanogaster*; *J. Genetics* accepted

Rassow J, Ahsen O V, Bomer U and Pfanner N 1997 Molecular chaperones: Towards a characterization of the heat-shock protein70 family; *Trends Genet.* **7** 129-133

Ray P and Lakhotia S C 1998 Interaction of the non-protein-coding developmental and stress-inducible *hsrω* gene with *Ras* of *Drosophila melanogaster*; *J. Biosci.* **23** 377-386

Reim I, Marttow J and Saumweber S 1999 The RRM protein NoA from *Drosophila* forms a complex with the RRM proteins Hrb87F and S5 and the Zn finger protein PEP on hnRNA; *Exp. Cell Res.* **253** 573-586

Ritossa F M 1962 A new puffing pattern induced by heat shock and DNP in *Drosophila*; *Experientia* **18** 571-573

Rougvie A E and Lis J T 1988 The RNA polymerase II molecular at the 5′ end of the uninduced hsp70 gene of *D. melanogaster* is transcriptionally engaged; *Cell* **54** 795-804

Rutherford S L 2000 From genotype to phenotype: buffering mechanisms and the storage of genetic information; *Bioessays* **22** 1095-105

Rutherford S L and Lindquist S 1998 HSP90 as a capacitor for morphological evolution; *Nature* **396** 336-342

Samali A and Cotter T G 1996 Heat shock proteins increase resistance to apoptosis; *Exp. Cell Res.* **223** 163-70

Sanchez Y and Lindquist S 1990 HSP104 required for induced thermotolerance; *Science* **248** 1112-1115

Schlesinger M J, Ashburner M and Tissieres A eds 1982 *Heat Shock Proteins from Bacteria to Man* pp1-419 (New York: Cold Spring Harbor Lab Press)

Sengupta S and Lakhotia S C 2000 Larval abnormalities and lethality due to overexpression of *hsrω* - a noncoding, developmentally regulated and stress inducible gene in *Drosophila melanogaster*; *XXIV All India Cell Biol Conf* p.111 (Abstr)

Sharma A and Lakhotia S C 1995 *In situ* quantification of hsp70 and alpha-beta transcripts at 87A and 87C loci in relation to *hsr-omega* gene activity in polytene cells of *D. melanogaster*; *Chromosome Res.* 3386-393

Shen J, Beall C J and Hirsh J 1993 Tissue-specific alternative splicing of the *Drosophila dopa decarboxylase* gene is affected by heat shock; *Mol. Cell Biol.* 13 4549-4555

Singer R H 1998 Triplet-repeat transcripts. A role for RNA in disease; *Science* 280 696-697

Singh A K and Lakhotia S C 2000 Tissue-specific variations in induction of Hsp70 and Hsp64 by heat shock in insects; *Cell Stress Chaperones* 5 90-97

Singh B N and Lakhotia S C 1995 The non-induction of hsp70 in heat shocked Malpighian tubules of *Drosophila* larvae is not due to constitutive presence of hsp70 or hsc70; *Curr. Sci.* 69 178-182

Southgate R, Ayme A and Voellmy R 1983 Nucleotide sequence analysis of the *Drosophila* small heat shock gene cluster at locus 67B; *J. Mol. Biol.* 165 35-57

Spector D L 1993 Macromolecular domains within the cell nucleus; *Ann. Rev. Cill Biol.* 9 265-315

Squires C and Squires C L 1992 The Clp proteins: proteolysis regulators or molecular chaperones? *J. Bacteriol.* **174** 1081-1085

Srinivas U K, Revathi C J and Das M R 1987 Heat induced expression of albumin during early stages of rat embryo development; *Mol. Cell Biol.* 7 4599-4602

Srivastava P and Lakhotia 2000 Multiple hsp60 genes in *Drosophila melanogaster* and their differential expression; *XXIV All India Cell Biol. Conf* p.112 (Abstr)

Tapadia M G and Lakhotia S C 1997 Specific-induction of the *hsrω* locus of *Drosophila melanogaster* by amides; *Chromosome Res.* 5 1-4

Timakov B and Zhang P 2001 The hsp60B gene of *Drosophila melanogaster* is essential for the spermatid individualization process. *Cell Stress & Chaperones* 6 71-77

Tissieres A, Mitchell H K and Tracy V 1974 Protein synthesis in salivary glands of *D. melanogaster*: relation to chromosome puffs; *J. Mol. Biol.* 84 389-398

Tiwari P K, Joshi A, Mohan D R K 1995 Developmental study of thermotolerance and the heat shock response in *Lucilia cuprina* (Weidemann); *J. Biosci.* 20 341-54

_____, Mohan D R K, Joshi A 1997 Thermotolerance and the heat shock response in *Musca domestica*; *Curr. Sci.* 72 501-506

Torok I, Mason P J, Karch F, Kiss I and Udvardy A 1982 Extensive regions of homology associated with heat-induced genes at loci 87A7 and 87C1 in *D. melanogaster*; in *Heat Shock from Bacteria to Man* pp19-25 eds M J Schlesinger, M Ashburner and A Tissieres (New York: Cold Spring Harbor Lab Press)

Ultimasov K A, Shammakov S, Karaev K, Evgen'ev M B 1992 Heat shock proteins and thermoresistance in lizards; *Proc. Natl. Acad. Sci., USA* 89 1666-70

Velazquez, J.M. Sonoda, S. Bugaisky, G. and Lindquist, S. 1983 Is the major *Drosophila* heat shock protein preset in cells that have not been heat shocked ? *J. Cell Biol.* 96 286-290

Weber J A, Taxman D J, Lu Q and Gilmour D S 1997 Molecular architecture of the hsp70 promoter after deletion of the TATA box or the upstream regulation region; *Mol. Cell Biol.* 17 3799-3808

Westwood J T and Wu C 1993 Activation of *Drosophila* heat shock factor: conformational change associated with monomer-to-trimer transition; *Mol. Cell Biol.* 13 3481-3486

Westwood J T , Clos J and Wu C 1991 Stress-induced oligomerization and chromosomal relocalization of heat shock protein; *Nature* 353 822-827

Wieghardt F, Cobianchi F, Cartegni L, Chiodi L, Villa A, Riva S and Biamonti G 1999 A novel hnRNP protein (HAP/SAF-B) enters a subset of hnRNP complexes and relocates in nuclear granules in response to heat shock; *J. Cell Sci.* 112 1465-1476

Wild J, Altman E, Yura T and Gross C A 1992 DnaK and DnaJ heat shock proteins participate in protein export in *Escherichia coli*; *Genes Dev.* 6 1165-1172S

Wu C, Clos J, Giorgi G, Haroun R I, Kim S J, Rabindran S K, Westwood J T, Wisniewski J and Yim G 1994 Structure and regulation of heat shock transcription factor; in *Biology of Heat Shock Proteins and Molecular Chaperones* pp395-416 eds R I Morimoto, A Tissieres and C Georgopoulos (New York : Cold Spring Harbor Lab Press)

Xiao H and Lis J T 1989 Heat shock and developmental regulation of the *Drosophila melanogaster hsp83* gene; *Mol. Cell Biol.* 9 1746-1753

Yost H J and Lindquist S 1986 RNA splicing is innterrupted by heat shock and is rescued by heat shock protein synthesis; *Cell* 45 185-193

_____ and Lindquist S 1988 Translation of unspliced transcripts after heat shock; *Science* 242 1544-1548

_____ and Lindquist S 1991 Heat shock proteins affect RNA processing during the heat shock response of *Saccharomyces cerevisiae*; *Mol. Cell Biol.* 11 1062-1068

_____, Petersen R B and Lindquist S 1990 RNA metabolism: strategies for regulation in the heat shock response; *Trends Genetics* 6 223-227

Yue L, Karr T L, Nathan D F, Swift H, Srinivasan S and Lindquist S 1999 Genetic analysis of viable hsp90 alleles reveals a critical role in *Drosophila* spermatogenesis; *Genetics* 151 1065-1079

Zimmerman J L, Petri W and Meselson M 1983 Accumulation of a specific subset of *D. melanogaster* heat shock mRNAs in normal development without heat shock; *Cell* 32 1161-1170