Heat Shock Response in *Drosophila*

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INTRODUCTION

Temperature is a very important environmental factor with a profound effect on biological activities. Although the range over which temperature varies in different habitats and in different seasons is very wide, a given species is normally exposed to a rather narrow range of temperature variations and usually suffers if exposed to temperatures lower or higher than this "physiological range". A living organism displays a wide variety of physiological, behavioral and other adaptations to protect itself from adverse effects of environmental temperatures beyond its physiological range. However, in addition to these organism-level adaptive responses, individual cells also cope up with sudden increase in their surrounding temperature by altering their transcriptional, translational and other activities in a characteristic fashion. All these changes in cellular activity in response to the sudden increase in surrounding temperature beyond the physiological range are collectively termed the "heat shock response". In recent years the topic of heat shock response has been reviewed many times and has also been the subject matter of several symposia. Some general reviews of this topic may be found in Lindquist (1986), Bienz & Pelham (1987), Lindquist and Craig (1988), Burdon (1988), Pelham (1989), Bond and Schlesinger (1987), Schlesinger (1990), Morimoto *et al* (1990), Nagao *et al* (1990). This article covers primarily the basic aspects of heat shock response in *Drosophila.*

F. Ritossa for the first time used polytene chromosomes of *Drosophila* larvae to examine the cellular response to heat shock in the form of newly induced puffs (Ritossa, 1962, 1964). Further interest in the heat shock response was stimulated when it was shown that the induction of new puffs by heat shock in salivary gland polytene chromosomes was associated with synthesis of a new set of polypeptides, the heat shock polypeptides. Following these seminal studies on the heat shock response using *Drosophila* cells, many workers began to examine the cellular response to heat shock in a wide variety of organisms. Techniques available in early 70's did not permit a direct study of heat shock induced gene activity in organisms that lacked polytene chromosomes, but the discovery of induced synthesis of heat shock polypeptides in *Drosophila* stimulated search for a similar response at protein synthesis level in other organisma since technically this was a rather simple approach in all kinds of living systems. These studies not only revealed that *all* organisms show a comparable heat shock response but that a variety of other cellular stress conditions also elicit a similar response.

During the past two decades, the heat shock response has been studied extensively in pro- and eukaryotes. Initially an important consideration for these studies was that the conditional switching on of the heat shock gene provided a simple but elegant model system for understanding the mechanism of gene expression and its regulation at transcriptional and translational levels. In more recent years, the emphasis has shifted to biological significance of the heat shock response and the role played by heat shock polypeptides in normal life and under conditions of cellular stress.

THE HEAT SHOCK RESPONSE IN DROSOPHILA

Heat shock induces new puffs and new species of RNA

The special structure of polytene chromosomes in salivary glands of late 3rd instar larvae of *Drosophila* permits a direct visualization of any alteration in gene

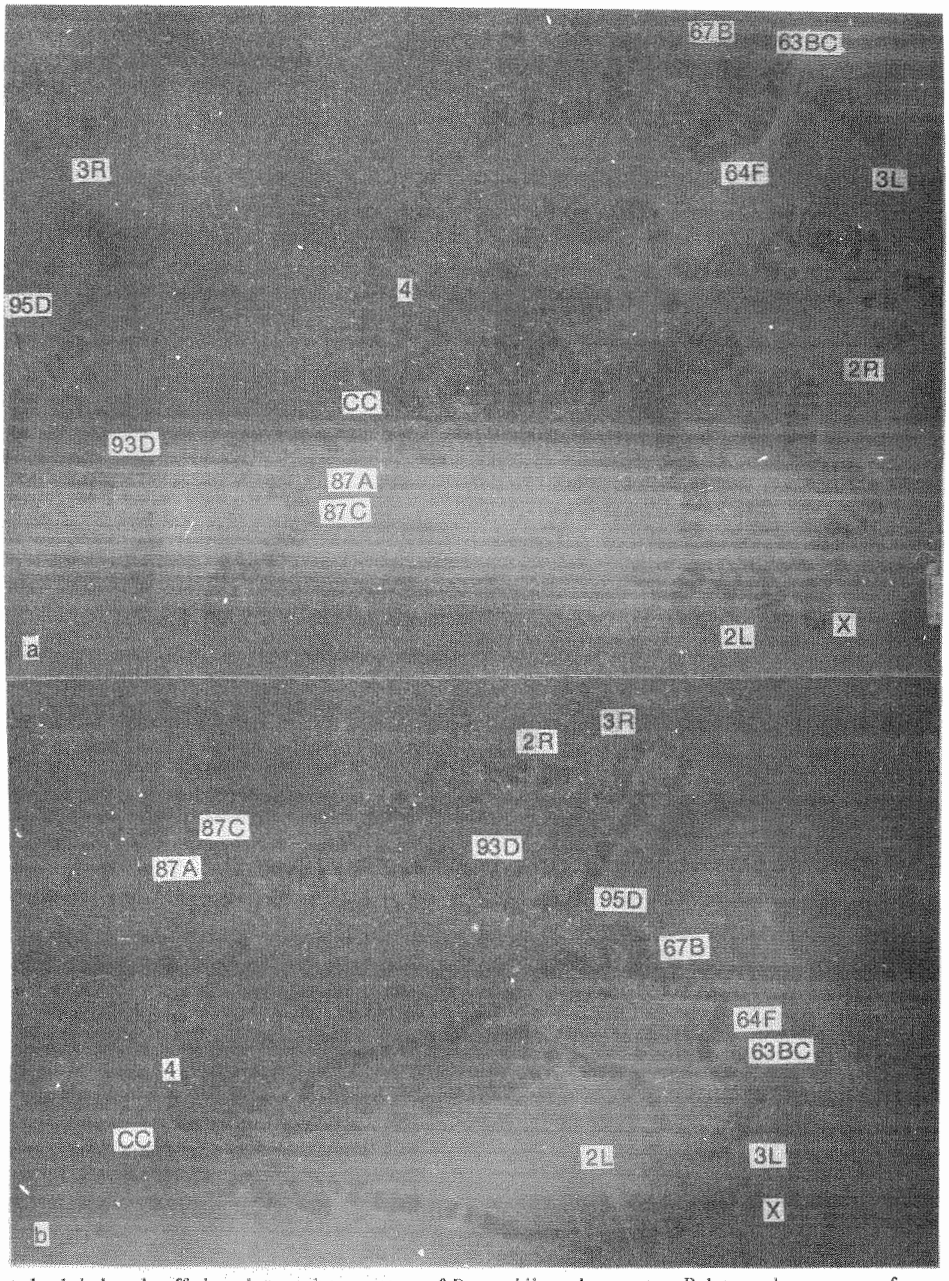

Fig. 1. Heat shock induced puffs in polytene chromosomes of *Drosophila melanogaster*. Polytene chromosomes from a non-heat shocked salivary gland are shown in **a** while those after heat shock are shown in **b**. For heat shock, salivary glands of late third instar larvae (grown at 24°C) were kept in a physiological saline solution at 37°C for 30 min prior to aceto-orcein stained squash preparation. The characteristic banding pattern (alternate dark and light stained regions) of these giant chromosomes in polytene nuclei permits easy identification of not only individual chromosomes and their arms (left and right arm in the case of a metacentric or sub-metacentric chromosome) but also of specific chromosome regions. Each dark stained band is given a number following a standard numbering system; the different heat shock puffs are designated after the number of the band that puffs out in response to heat shock. X = X-chromosome; 2L and 2R = left and right arms, respectively, of chromosome 2; 3L and 3R = left and right arms, respectively, of chromosome 3; sites of the major heat shock induced puffs on 3L and 3R are also marked : these regions appear non-puffed in **a** (Control) but as puffs in **b** (heat shocked).

activity (see the article "Polytene Chromosome Puffing and Gene Expression" in this issue) since the active regions are visible as distinct puffs under light microscope (see Fig. 1). Puffs are locally decondensed regions with an enlarged diameter and lighter staining. It was shown (see Beermann 1972) that a local decondensation of the parallely aligned multitudes of sister chromatin fibrils (arising due to repeated endoreduplication) of a polytene chromosome was necessary for active transcription at the puff site. Thus the appearance of a puff is indicative of transcriptional activity at the site. Taking advantage of the phenomenon of puffing in polytene chromosomes of *Drosophila* larvae, Ritossa (1962, 1964) examined effect of a brief exposure of larval salivary glands to 37°C or to 2-4-dinitrophenol or Na-salicylate. It was found that new transcriptionally active puffs were seen at certain specific regions of the polytene chromosomes in the treated glands while in untreated control glands, no puffs were seen at those sites.

It was remarkable that all the three treatments led to regression of pre-exitsting puffs and induction of the same set of new puffs, the heat shock puffs. Studies with polytene chromosomes of *D.melanogaster* identified the following 9 loci (named after designation of the polytene band that is involved in puffing) as the heat shock puff sites: 33B on 2L (left arm of chromosome 2), 63BC, 64EF, 67B and 70A on 3L (left arm of chromosome 3), 87A, 87C, 93D and 95D on 3R (right arm of chromosome 3). Of these, the heat shock induced puffs at 63BC, 67B, 87A, 87C and 93D are relatively large and are considered as the major heat shock puff sites (Ashburner, 1970; Ashburner and Bonner, 1979; Mukherjee and Lakhotia, 1979; see Fig. 1). In a very elegant study, Spradling et at (1977) fractionated the ^{3}H-uridine labelled poly A^{+} and poly A^{-} RNA from heat shocked salivary glands of *D. melanogaster* larvae by gel electrophoresis and hybridized individual fractions to polytene chromosomes in situ. This study confirmed that the different heat shock puffs made distinct mRNAs.

Heat shock induces synthesis of new polypeptides

To study the effect of heat shock on protein synthesis in cells, Tissieres *et al* (1974) and Lewis *et al* (1975)

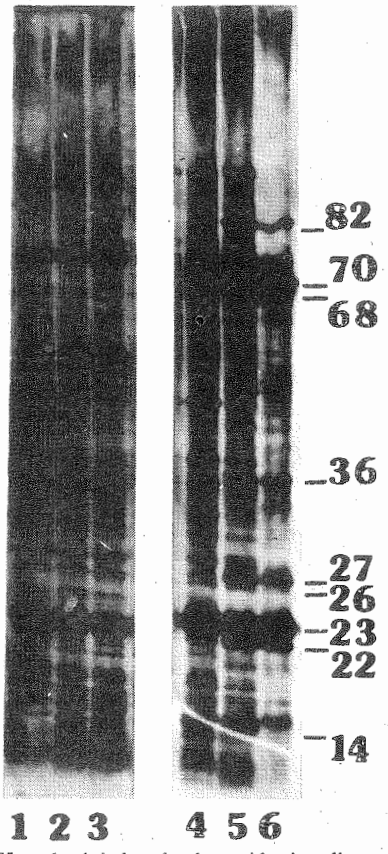

Fig. 2. Heat shock induced polypeptides in salivary glands of late 3rd instar larvae of *Drosophila melanogaster*. Newly synthesized proteins in control (lanes 1 and 4), 33°C heat shocked (lanes 2 and 5) and 37°C heat shocked (lanes 3 and 6) salivary glands were labelled with ^{35}S-methionine for 30 min by incubation in medium containing the radioactive precursor. Salivary gland proteins were dissloved and separated by eletrophoresis in polyacrylamide gels (containing a detergent, sodium-dodecyl-sulphate, to dissociate individual polypeptides). the gels were first stained with Coomassie Brilliant Blue (CBB) to reveal different polypeptides (see lanes 1, 2 and 3) and then dried and placed in close contact with X-ray film for detecting the radioactively labelled polypeptides. The autoradiograms corresponding to CBB stained lanes 1, 2 and 3 are shown in lanes 4, 5 and 6, respectively. While CBB staining does not show any appreciable difference between control and heat shocked samples, the autoradiograms reveal dramatic effect of heat shock on protein synthesis: synthesis of most of the proteins is inhibited (slightly at 33°C but more severely at 37°C) after heat shock while synthesis of a specific set of polypeptides is greatly enhanced in heat shocked glands; these heat shock polypeptides are indicated by their apparent molecular weights (in kilodaltons) on the rightmost panel.

labelled the newly synthesized proteins in control and heat shocked cells from different tissues of *Drosophila* larvae, pupae and adults with radiolabelled amino acids. Polypeptides from such control and heat shocked cells were fractionated by sodium-do-decyl sulfate polyacrylamide gel eletrophoresis (SDS-PAGE). The newly synthesized polypeptides were then identified by autoradiography of the gels (the newly synthesized polypeptides only had incorporated radio labelled amino acids; these could be detected on X-ray film placed in close contact with the gel). A comparison of the labelled polypeptide bands in samples from control and heat shocked cells of different tissues revealed that while the types of polypeptides synthesized in different cell types under control (non-heat shock) condition varied in a tissue-specific manner, the heat shocked cells of all tissues synthesized the same set of 7 different polypeptides that were generally not synthesized in control cells. The apparent molecular weights (in kilodaltons, kd) of these heat shock induced polypeptides were 82kd, 70kd, 68kd, 27kd, 26kd, 23kd and 22kd (see Fig. 2). These were termed the heat shock polypeptides or hsp.

Heat shock induced puffs, transcripts and polypeptides are correlated

Subsequent application of other molecular, genetic and recombinant DNA techniques permitted a correlation of the different hsps with puff sites and their transcripts. Location of the different heat shock genes on polytene chromosomes and the product/s made by them in *D. melanogaster* are given in Table 1.

Based on the results of a large number of studies the heat shock response in *D. melanogaster* may be summarized as follows: when the animal or its isolated tissues/cells *in vitro* are transferred from 24°C-25°C (the normal growth temperature) to temperature beyond 33°C, the cells experience heat shock. A 30 to 60 min exposure to 37° elicits optimal heat shock response while 39°C may be lethal. Within a few minutes after shift to 37°C, the heat shock genes are induced to transcribe while the ongoing chromosomal transcription is more or less inhibited (see Figs. 1 and 3) due to a redistribution of RNA polymerase II. The newly synthesized transcripts of heat shock genes are rapidly translated into the heat shock polypeptides. At the same time, the ongoing translational activity is also inhibited (Fig. 2) due to certain changes in ribosome properties which result in preferential translation of mRNAs that carry the heat shock leader sequence. Thus heat shock regulates cellular activity at transcriptional as well as translational levels. If the heat shock condition is continued, synthesis of hsps declines after some time. When the cells are returned to normal growth temperature (24°C), synthesis of the usual cell type specific polypeptides and mRNA is resumed.

Table 1. Heat Shock Genes of *Drosophila melanogaster*

Polytene Chromosome Puff Site	Gene	Product
33B	?	?
63B	hsp 83	83kd hsp
63F	Polyubiquitin	ubiquitin
64F	?	?
67B	hsp 27	27kd hsp
	hsp 26	26kd hsp
	hsp 23	23kd hsp
	hsp 22	22kd hsp
	gene 1	?
	gene 2	?
	gene 3	?
70A	?	?
87A	hsp 70	70kd hsp
87C	hsp 70	70kd hsp
	alpha-beta repeats	alpha-beta RNA
93D	hsr w	w1 RNA
		w2 RNA
		w3 RNA
95D	hsp 68	68kd hsp

Heat shock polypeptides are grouped into major families

On the basis of molecular weights and other functional properties, the different heat shock polypeptides in

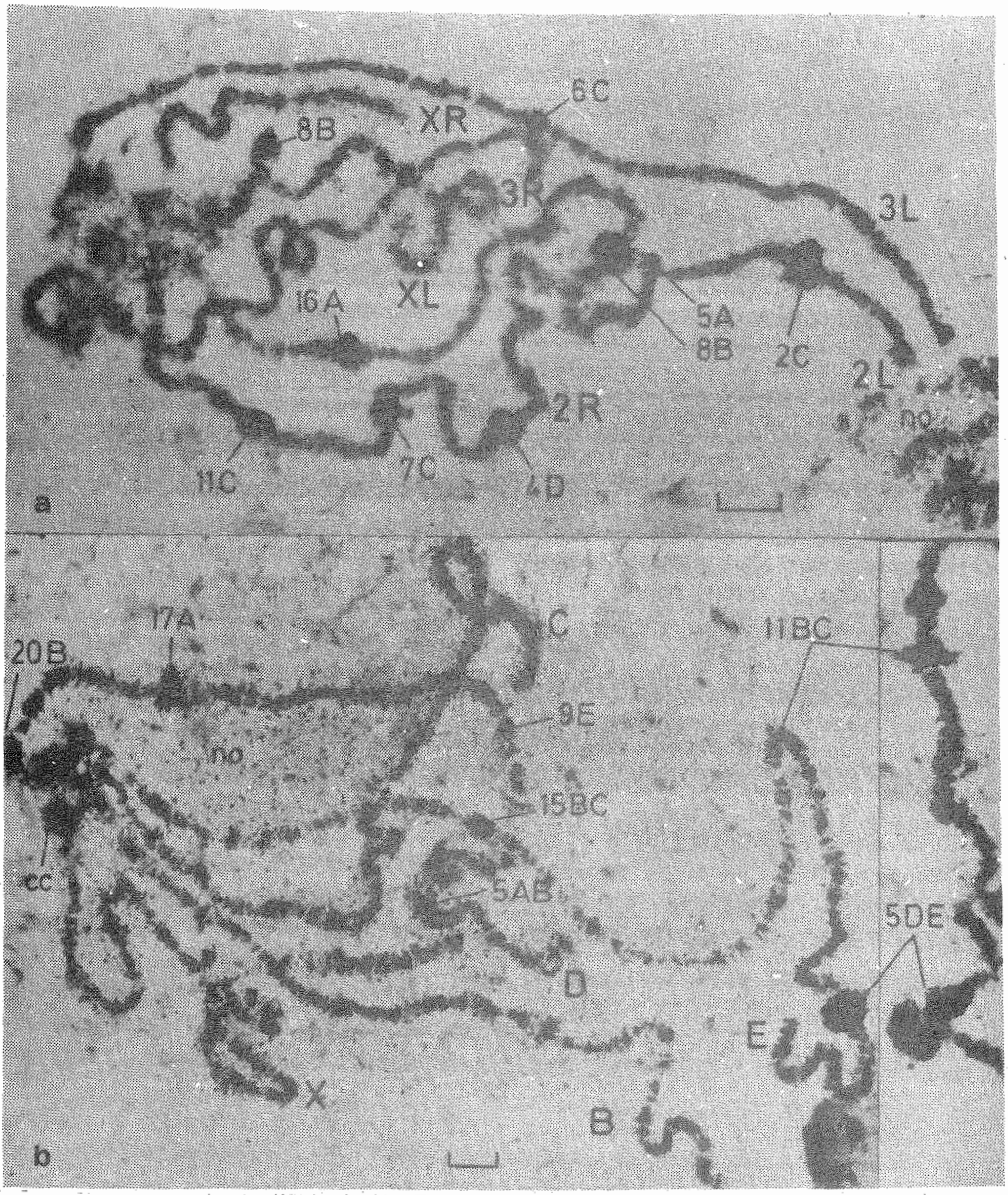

Fig. 3. Autoradiograms of ^{3}H-uridine labelled polytene chromosomes from heat shocked salivary glands of *D.nasuta* (a) and *D.hydei* (b) to show specific transcriptional activity of heat shock induced puffs : after the heat shock (37°C for 30 min), the salivary glands were labelled with ^{3}H-uridine for 10 min; their squash preparations were autoradiographed to identify (by presence of clusters of dense silver grains) the sites of ^{3}H-uridine incorporation or active transcription. The different chromosome arms and the heat shock puff sites are marked. Although the chromosomes and the puff sites are named differently in the two species, it is known from other evidences that the sites of heat shock puffs are homologous between these species. Among the different heat shock puff sites, some are more labelled (more active in RNA synthesis) than others. Heat shock inhibits most of the general chromosomal transcription so that most of the chromosomal regions (other than heat shock puff sites) are sparesely labelled. The nucleolus (no) continues to be active in RNA synthesis as evidenced by its very heavy labelling. cc= common chromocentre where all chromosomes remain attached.

diverse organisms have been grouped into 4 major families: the hsp 90 family, hsp 70 family, hsp 60 family and the low mol.wt. hsp family.

In *Drosophila,* the hsp 90 family is represented by the 83kd hsp with its gene being located at 63BC puff site. This is the only heat shock protein gene in *D. melanogaster* which has an intron and requires splicing. The 83kd hsp gene is also expressed without heat shock in a developmentally regulated manner. The hsp70 family is represented by 70kd and 68kd hsps. Hsp70 is the most abundant among induced hsps and is also the most highly conserved in all organisms. In *D. melanogaster,* there are multiple gene copies for hsp70, with at least 2 copies at 87A and three at 87C locus. In addition, there are a number of other gene loci that code for polypeptides related to hsp 70 in their sequence but which are expressed under non-heat shock condition (constitutive expression). These hsp-related proteins are termed heat shock cognates (hsc 70 for hsp 70 related polypeptides). The gene for hsp 68 is located at 95D puff site and shows some homology with the hsp 70 genes. However, the induction of hsp 68 is much less compared to that of hsp 70.

The low molecular weight hsp family in *D. melanogaster* is represented by 4 polypeptides, the 27kd, 26kd, 23kd and 22kd hsps. The genes for these four polypeptides and for three other heat inducible transcripts are clustered within 11kb of DNA sequence at the 67B puff site. All these plypeptides share some homology with each other and with the lens crystallins. The 23kd hsp appears more prominently induced by heat shock than the other three of the low mol. wt. hsp family. All the four low mol. wt. hsps are also expressed without heat shock in a developmentally regulated manner. They show a complex pattern of cell type and developmental stage-specific regulation by ecdysone.

In addition to the above well known hsps, a 14kb histone, H2b variant, is also known to be heat-inducible in *D. melanogaster* (Fig. 2). The polyubiquitin gene, coding for tandem repeats of ubiquitin mRNA and located at 63F site is also heat inducible. A 36kd polypeptide has also been reported in several studies to be heat inducible but not much is known about this polypeptide.

So far nothing is known about products of heat-inducible genes located at 33B, 64F or 70A puff sites. These sites were identified as minor heat shock puffs due to formation of small sized puffs in response to heat shock (Ashburner, 1970).

A heat shock polypeptide with molecular weight around 60kd has been seen in bacteria (the 58kd gorEL polypeptide of *E. coli*), plants and many animals. In plant and animal cells this 60kd family hsp has been localized to within mitochondria and chloroplasts (McMullin and Hallberg, 1988), although it is product of a nuclear gene. A 60kd family hsp was so far not known to exist in *Drosophila.* However, Lakhotia and A.K. Singh recently (1989) reported induction of a 58kd hsp in Malpighian tubules of *Drosophila;* more recently, using western blotting this 58kd polypeptide has been found (Lakhotia and Bhupendra. N. Singh, unpublished) to be homologous to the 60kd hsp known in other organisms.

Some heat shock genes in *Drosophila* do not make any HSP

There are two other heat shock genes, the αβ - repeat sequence and the 93D locus which are different from the rest. The so-called αβ repeat sequences are interspersed with hsp70 coding sequences at the 87C puff site and are heat inducible. Similar sequences present elsewhere in the genome, however, are not heat inducible. The heat inducible αβ repeat sequences make abundant transcripts when heat shocked but these transcripts are not translated (Lengyel and Graham, 1984). Functions of these heat inducible αβ repeat sequences are not clear. It is also intriguing that although similar αβ sequences are present at 87C locus of *D.simulans,* a sibling species of *D. melanogaster,* those are not heat-inducible.

The 93D heat shock locus of *D. melanogaster* is an unusual gene

The heat shock locus at 93D site is unique among all heat shock genes of *D. melanogaster.* This is iden-

tified as a major heat shock puff site due to its large size and a higher level of ^{3}H-uridine incorporation (Ashburner, 1970; Mukherjee and Lakhotia, 1979). This puff is also uniquely inducible with benzamide, colchicine, thiamphenicol etc without concomitant induction of any of the other heat shock puffs (see review by Lakhotia, 1987, 1989). Lakhotia and Mukherjee (1982) found that the singular transcriptional induction of the 93D puff by benzamide etc was not correlated with appearance of any novel polypeptide in such cells. In another study, Lengyel *et al* (1980) had shown certain other unusual properties of the RNA transcribed at the 93D locus. Considering these observations, Lakhotia and Mukherjee (1982) suggested that the transcripts of 93D are not translated. This was subsequently confirmed by a direct analysis of the base sequence of this locus (see Lakhotia 1987, 1989; Pardue *et al*, 1990). The 93D locus, called *hsr*Ω (heat shock RNA omega, Bendena et al 1989), makes three major tanscripts: the largest is about 12kb long while the other two are 1.9 and 1.2kb long, respectively. These are present in all cells all the time but their relative levels increase following the heat shock. The transcribed part of the *hsr*Ω gene includes a 5′ region which is unique and a 3′ region consisting of tandem repeats of a 280bp sequence extending over 10-12kb long stretch (this repeat sequence is unique to this locus). The 12kb transcript covers the entire length of *hsr*Ω while the 1.9kb transcript represents only the proximal part including an intron of ~700bp. The 1.9kb transcript is processed to yield the 1.2kb RNA. All species of *Drosophila* have a 93D-homologue identified on the basis of specific inducibility with benzamide, colchicine etc (Lakhotia and Singh, 1982). These homologous loci in different species show similar genomic organization with respect to the size and location of the two exons and the intron and in having a long stretch of repeat sequences at the 3′ end (review by Lakhotia, 1987; 1989; Pardue *et al*, 1990). As discussed later, heat shock genes are remarkable for conservation of their function and base sequence. Similar genomic organization and inducible properties of the *hsr*Ω gene in different species of *Drosophila* are in agreement with the general conservation of heat shock genes. However, it was very surprising to find that the primary base sequence of the transcribed part of *hsr*Ω gene showed very rapid sequence divergence so that even closely related species do not share much base sequence homology at this locus. It is also intriguing that in spite of its apparently non-coding transcripts and its rapid sequence divergence in related species, this gene is essential for normal survival of the fly since complete deficiency of this locus leads to considerable larval lethality and almost complete adult lethality (Lakhotia, 1987). Apparently, the *hsr*Ω gene functions without a protein product. At present it is believed that its large 12kb transcript, localized to the nucleus, has protein-binding properties for its regulatory functions while the smaller and cytoplasmic 1.2kb transcript somehow monitors "health" of the translational machinery of cell (Bendena et al, 1989; Lakhotia, 1989; Pardue *et al*, 1990).

Heat Shock Response is Universal

A most remarkable revelation of the very early studies on heat shock induced puffing changes was that all species of *Drosophila* showed heat shock puffs on chromosomally homologous regions (Fig. 3): this suggested that the gene loci responding to this cellular stress were also homologous. The initial observations on protein synthesis in various tissues of different developmental stages in several species of *Drosophila* also revealed striking similarities in the molecular weights of the new polypeptides induced by heat shock (Tissieres *et al*, 1974; Lewis et al, 1975). These initial observations suggested that the response to thermal stress was similar in different cell types of *Drosophila*, notwithstanding their otherwise highly specialized activities. Search for heat shock induced changes in protein synthesis in diverse organisms, initiated soon after the discovery of heat shock proteins in *Drosophila*, quickly confirmed universality of the heat shock response. The cellular response to thermal and related stresses was found to be remarkably similar in all organisms from bacteria to man (Schlesinger *et al*, 1982). With the advent of recombinant DNA techniques, heat shock genes from different organisms were

cloned and sequenced. The most important aspect to emerge from these studies was that the heat shock genes are highly conserved and that all cell types of all organisms generally respond in a similar fashion to thermal stress. As an example of the evolutionary conservation of heat shock genes, it may be noted that the hsp70 genes of pro- and eukaryotes show about 50 to 70% homology. In all organisms, the heat shock polypeptides can be grouped into the four major (the 90kd, 70kd, 60kd and the low mol. wt.) families mentioned earlier. Among these, as in the case of *Drosophila*,the hsp70 is always the most abundantly induced after heat shock.

It was also seen that a *Drosophila* heat shock gene when placed in mammalian cells, would be induced by heat shock at a temperature at which the host mammalian cells experience heat shock. This is interesting since while 37°C is normal for mammalian cells it is a strong heat shock condition for *Drosophila*; therefore, one may have expected that a *Drosophila* heat shock gene when placed in mammalian cells should always be under thermal stress and should remain induced. However, what was seen was that the *Drosophila* heat shock gene in mammalian cells responded to heat shock only alongwith the host's heat shock genes at 42°C-43°C. Experiments of this kind thus showed that not only the heat shock genes were conserved, their regulation was also conserved through evolution and that something else than the gene itself detects thermal stress in cell.

The heat shock response can be modulated by developmental and environmental conditions

While the heat shock response is generally universal for all cell types of all organisms, a few situations are known when the response to thermal stress may vary in relation to specific cell type, developmental stage and other conditions of development. Very early embryonic cells often are unable to mount a heat shock response. Certain stages of gametogenesis in some animal groups also have been found to be unable to show heat shock induced synthesis of hsps. It has been seen that development of *Drosophila* larvae at 10°C specifically affects synthesis of hsp23: heat shock to such larvae fails to induce synthesis of hsp23 while all other hsps are induced in the usual fashion (Lakhotia and Singh, 1988). Another interesting feature of the heat shock response is that the temperature which is assessed by the cells as strong heat shock is relative to the temperature at which the individual organism was growing: it was seen that *Chrionomus* larvae (dipteran larvae coloured red due to hemoglobin in their hemolymph) growing in water with temperature > 33°C (as happens during summer months) do not suffer a strong heat shock at 37°C while the same larvae if grown at 24°C (in laboratory or during winter months) would already experience heat shock at 33°C (Nath and Lakohtia, 1989).

In the context of global nature of the heat shock response, it is remarkable that the Malpighian tubules of *Drosophila* respond to heat shock by synthesizing a very different set of proteins: Lakhotia and Singh (1989) recently reported that while none of the usual hsps was synthesized in Malpighian tubules of *Drosophila* larvae following a heat shock, an entirely different set of polypeptides, the Malpighian tubule specific hsps, was induced by the thermal stress. This observation opens a new dimension to nature and mechanism of the heat shock response.

Mechanism of Heat Shock Response

Soon after discovery of the heat shock response in *Drosophila* cells, it was found by Sin (1975) that microinjection of mitochondrial extracts of heat shocked cells into polytene cells of non-heat shocked salivary glands of *D. melanogaster* caused induction of heat shock puffs. Thus the heat shock genes themselves were not directly sensing elevated temperature, rather the thermal stress brought about certain changes in cell which in turn stimulated the heat shock genes to transcribe. Earlier mentioned experiments on expression of *Drosophila* heat shock genes in mammalian cells also agreed with this. It is now well known that all heat-inducible genes in all organisms carry more than one copy of a 14bp consensus sequence, GAnTTCnnGAAnTC (n is any base), upstream of

their transcribed region. This 14bp sequence is termed the heat shock element (HSE) or heat shock promoter and is responsible for heat shock induced transcription of the gene. Recent studies show that the core HSE is only a 10bp sequence (nTTCnnGAAn), an inverted repeat of the 5 base pair, nGAAn (Schlesinger, 1990; Nagao et al, 1990). The transcriptional activation signal is dependent upon binding of an active heat shock transcription factor (HSTF or HSF) with one or more of these upstream HSEs. Every cell is believed to carry the HSTF protein in an inactive form so that it cannot bind with the HSEs. However, heat shock and some other stress conditions bring about certain changes in the HSTF so that it is activated and can bind with the HSEs which in turn promote active transcription of the downstream gene. Genes for Yeast and *Drosophila* HSTF have been cloned and a comparison of these shows that the HSTF gene has not been as strongly conserved as the heat shock genes and their HSEs. Nevertheless, it is apparent that the HSTF of one species is able to identify the HSE on another species' heat shock genes.

Biological significance of the heat shock response and functions

The remarkable conservation of the heat shock response in all organisms (prokaryotes, plants and animals) suggests that the heat shock genes evolved very early in the history of life and their cellular functions under conditions of thermal and other stresses are of vital importance. Soon after the discovery of heat shock response, it was shown that *Drosophila* larvae or flies briefly exposed to a milder heat shock at 33°C could survive better a severe heat shock at 39°C, which otherwise would be lethal (Lindquist, 1986). These and other similar studies in *Drosophila* and other organisms revealed that the heat shock response was involved in thermoprotection and in development of thermotolerance. It was also seen that if RNA and/or protein systhesis was inhibited during the period of milder heat shock, thermotolerance did not develop. Similarly at the cellular level, if syntheses of heat shock protein was inhibited during exposure to 37°C, the cells did not resume normal protein synthesis whe returned to 25°C. It was clear from these experiment that the heat shock proteins somehow help the ce escape deleterious effects of elevated temperature. Al though full details are still not clear, significan progress has been made in recent years in elucidatin some of the cellular activities of the various hsps (fo recent reviews, see Pelham, 1989; Lindquist, 198 Lindquist and Craig, 1988; Nagao *et al*, 199 Schesinger, 1990).

Members of the hsp70 family are involved in trans location of various proteins across eukaryotic cell o ganelle membranes, including the endoplasmi reticulum, chloroplast and lysosome: it is propose that hsp70 functions as a molecular chaperone by u folding the partially folded polypeptides so that thes can be translocated through a membrane pore. In th presence of ATP, hsp70-like proteins have been show to bind and to dissociate protein complexes.

The hsp60 family proteins also complex wit polypetides and have ATPase activity. However, contrast to the disassembly and unfolding function the hsp70, the hsp60s are involved in folding and a sembly of polypeptides in mitochondria an chloroplasts.

The hsp90 family of proteins are abundant in no mal cell cytoplasm and form complexes with a wid variety of other proteins, like glucocorticoid and oth steroid hormone receptors, several kinases tubuli actin etc. In summary, the hsp90, hsp70 and hsp6 family of proteins protect, preserve and recover th functions of various other proteins (Schlesinge 1990). Another heat shock protein, the ubiquitin ha functions in protein degradation. In the course of no mal proteolytic turnover in cells, the proteins to b degraded are flagged by ubiquitin-binding. He shock also leads to thermal denaturation of man proteins which could be toxic if not immediatel removed. It is in this process that the product of th heat-inducible polyubiquitin gene participate. I agreement with this is the observation that polyub quitinated proteins increase about 2-fold in heat shoc

ed cells which are subsequently removed by ubiquitin-dependent proteolytic system.

The low mol. wt. family hsps seem to have a structural role since they are abundant in normal cells also (Nagao et al, 1990). In *Drosophila* and other systems, it has been shown that these proteins form large aggregates during heat shock, that these aggregates concentrate in perinuclear regions and that they are tightly associated with normal cellular mRNAs. This association may help protect the normal cellular mRNAs which are not being translated during heat shock (Arrigo, 1987; Leicht *et al*, 1986; Nover *et al*, 1989).

A new dimension to studies on heat shock response has been added by recent discoveries that these are dominant antigens of infectious microorganisms and that a significant fraction of microbial-infection induced immunoglobulins and cytotoxic T- lymphocytes is directed against peptides derived from hsps. Both the invading microorganisms and host cells suffer stress during infection and thus produce hsps which stimulated the immune response. However, since uninfected host cells also contain hsps, there is a risk of autoimmune response: cases of autoimmune diseases involving hsps are known (see Lydyard and van Eden, 1990; Schlesinger, 1990).

Concluding Remarks

Initial studies on the heat shock response in *Drosophila* provided a very useful experimental approach to correlate morphological puffing of polytene chromosomes with syntheses of specific mRNAs and corresponding proteins. Eversince then, studies on heat shock genes and heat shock proteins in very diverse organisms are continuing to provide newer insight into gene regulatory and cellular adaptive processes.

In addition to the heat shock paradigm being extensively used for studies on organization, expression and regulation of genes, *Drosophila* geneticists have also made novel and a very fruitful use of the knowledge gained from studies on regulation of heat shock genes: a variety of chimeric genes have been synthesized *in vitro* where the coding sequence of a given gene is placed downstream of a heat shock promoter and the fusion gene put back in *Drosophila* genome using P-element mediated germline transformation (for details of this, see the article on "New approaches in *Drosophila* Genetics" in this issue). This strategy permits *in vivo* activation of that gene as and when required by applying heat shock to such transformed individuals (see "Demonstration of heat shock induced gene activity in transgenic *Drosophila melanogaster* with a reporter gene fused to a heat shock promoter" in this issue). Particularly interesting examples of this approach are recent studies in which some homeo-box, containing genes (for details of homeotic genes and homeo-box, see the article on "Genetics of Body Pattern Formation During Embryonic Development in *Drosophila*" in this issue) of human or mouse origin were combined with hsp70 promoter of *Drosophila* and the fusion gene inserted in *Drosophila* genome; when activated by heat shock, the human or mouse homeotic gene did the same function in *Drosophila* as was expected from their molecular homology to corresponding *Drosophila* homeotic genes (Malicki *et al*, 1990; McGinnis *et al*, 1990).

Elucidation of molecular details of the role of diverse heat shock gene products in normal cell and under conditions of stress is progressing very fast and one would expect that within the next few years the biological significance of this very ancient adaptive response would be clearly understood.

References

Arrigo, P. 1987. Cellular localization of HSP23 during *Drosophila* development and following subsequent heat shock. *Dev. Biol.* 122 : 39-48

Ashburner, M. 1970. Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. V. Responses to environmental treatments. *Chromosoma* 31 : 356-376.

Ashburner, M. and J.J. Bonner. 1979. The induction of gene activity in *Drosophila* by heat shock. *Cell* 17 : 241-254

Beermann, W. (ed) 1972. *Developemental studies in giant chromosomes.* Springer-Verlag, New York.

Bendena, W.G., J.C. Garbe, K.L. Traverse, S.C. Lakhotia and M.L. Pardue. 1989. Multiple inducers of heat shock locus 93D of *D.*

melanogaster : inducer-specific patterns of three transcripts. *J. Cell Biol.* 108 : 2017-2028.

Bienz, M. and H.R.B. Pelham. 1987. Mechanisms of heat shock gene activation in higher eukaryotes. *Adv. Genetics* 24 : 31-72.

Bond, U. and M.J. Schlesinger. 1987. Heat shock proteins and development. *Adv. Genet.* 24 : 1-29.

Burdon, R.H. 1988. The heat shock proteins. *Endeavour* 12 : 133-138.

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

Lakhotia, S.C. 1989. The 93D heat shock locus of *Drosophila melangaster* : modulation by genetic and developmental factors. *Genome* 31 : 677-683.

Lakhotia, S.C. and T. Mukherjee. 1982. Absence of novel translation products in relation to induced activity of the 93D puff of *Drosophila melanogaster. Chromosoma* 85 : 369-374.

Lakhotia, S.C. and A.K. Singh. 1982. Conservation of the 93D puff of *Drosophila melanogaster* in different species of *Drosophila. Chromosoma* 86 : 265-278.

Lakhotia, S.C. and A.K. Singh. 1988. Effect of low-temperature rearing on heat shock protein synthesis and heat sensitivity in *Drosophila melanogaster. Dev. Genet.* 9 : 193-201.

Lakhotia, S.C. and A.K. Singh. 1989. A novel set of heat shock polypeptides in malpighian tubules of *Drosophila melanogaster. J. Genet.* 68 : 129-137.

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.

Lengyel, J.A., L.J. Ransom, M.L. Graham and M.L. Pardue. 1980. Transcription and metabolism of RNA from the *D. melanogaster* puff site 93D. *Chromosoma* 80: 237-252

Lengyel, J.A. and M.L. Graham. 1984. Transcription, export and turnover of hsp70 and αβ, two *Drosophila* heat shock genes sharing a 400 nucleotide 5' upstream region. *Nucleic Acids Res.* 12: 5719-5735.

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

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

Lindquist, S. and E.A. Craig. 1988. the heat shock proteins. *Annu. Rev. Genet.* 22 : 631-677.

Lydyard, P.M. and W. van Eden. 1990. Heat shock proteins : immunity and immunopathology. *Immuno. Today* 11 : 228-229.

Malicki, J., K. Schughart and McGinnis. 1990. Mouse *hox-2.2.* specifies thoracic segmental identity in *Drosophila* embryos and larvae. *Cell* 63 : 961-967.

McGinnis, N., M.A. Kuziora, and W. McGinnis. 1990. Human *hox-4.2* and *Drosophila deformed* encode similar regulatory specificities in *Drosophila* embryos and larvae. *Cell* 63 : 969-976.

McMullin W. and R.L. Hallberg. 1988. A highly evolutionarily conserved mitochondrial protein is structurally related to the protein encoded by the *Escherichia coli* groEL gene. *Mol. Cell Biol.* 8 : 371-380.

Morimoto, R., A. Tissieres and C. Georgopolous (eds.). 1990. *Stress proteins in biology and medicine* (Cold Spr. Harb. Lab, Cold-spring Harbor, NY)

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

Nagao, R.T., Kimpel J.A. and J.L. Key. 1990. Molecular and cellular biology of the heat shock response. *Adv. Genet.* 28 : 235-274.

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

Nover, L., K.D. Schraf and D. Neumann. 1989. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. *Mol. Cell Biol.* 9 : 1298-1308.

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

Pelham. H.R.B. 1989. Heat shock and the sorting of luminal proteins. *EMBO J.* 8 : 3171-3176.

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

Ritossa, F.M. 1964. Experimental activation of specific puff loci in polytene chromosomes of *Drosophila. Exp. Cell Res.* 35 : 601-607.

Schlesinger, M.J. 1990. Heat shock proteins. *J. Biol. Chem.* 265 : 12111-12114.

Schlesinger, M.J., A. Tissieres and M. Ashburner (eds.). 1982, Heat shock proteins: from bacteria to man. (Cold Spr. Harb. Lab., Cold Spring Harbvor, NY).

Sin, Y.T. 1975. Induction of puffs in *Drosophila* salivary gland cells by mitochodrial factor (s). *Nature* 258 : 159-160.

Spradling, A., M.L. Pardue and S. Penman. 1977. Messenger RNA in heat-shocked *Drosophila* cells. *J. Mol. Biol.* 109 : 559-587.

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