The diverse life forms that exist today are finely adapted to live under different environmental conditions. However, since the environmental conditions do not remain constant due to diurnal and seasonal changes, the organisms too have evolved different strategies to cope up with these subtle and continuous but sometimes sudden changes. There are at least two different levels at which the living forms respond to the changing environment - i) the organismic response and ii) the cellular response. It is the cellular response to changing environmental conditions that evolved first in acellular and unicellular organisms and very interestingly, has persisted even in higher life forms that, in addition, have also acquired very intricate organism-level responses to adjust to environmental changes.

Temperature is a very important component of the changing environment which affects basic physiological processes in the cell. Therefore, it is not surprising that even the primitive organisms like bacteria have a very sophisticated cellular machinery to protect against damages caused by sudden thermal changes. The significance of this response to cells' survival is obvious from the fact that this has persisted relatively unchanged through the entire spectrum of biological diversity. Thus as diverse organisms as E.coli, yeast, Drosophila, Xenopus, mammals and higher plants, all have a conserved set of genes, the heat shock genes, which are activated whenever their
cells experience hyperthermia and certain other stressful conditions (1). When a cell's surrounding temperature suddenly increases a few degrees above the "normal" (to which the organism is adapted), the heat shock genes are triggered to actively transcribe. These heat shock transcripts are preferentially translated into heat shock proteins (HSP) which protect the cell, in an as yet poorly understood fashion, from the hyperthermic stress. This entire gamut of induced changes at transcriptional and translational levels in a cell is known as the heat shock response (recent reviews in 2-5).

In nearly all organisms, the heat shock genes generally fall into three categories depending upon the heat shock polypeptides for which they code: i) the 90kd family, ii) the 70kd family and iii) the low mol. wt. HSP family. Among these, the 70kd HSP is the most abundant under conditions of thermal stress and is also the most highly conserved HSP. Besides, the coding part, the upstream regulatory sequences that control these genes' heat inducibility are also highly conserved (2) so that a heat shock gene of Drosophila will express appropriately under conditions of stress even when placed in amphibian or mammalian cells (6). This specific regulation of heat shock genes which cuts across phylogenetic relationships is due to the fact that in all organisms, every heat shock gene shares a 14bp consensus heat shock element (HSE) or heat shock promoter sequence which specifically responds to a conserved heat shock transcription factor (HSTF) activated by the stress (2,7). It is, however, remarkable that while in Drosophila cells, the heat shock genes are activated at temperatures above 30°C, with maximal expression at 37°C, the same Drosophila gene when placed in mammalian cells, remains inactive at 37°C but is expressed only when the host cell's heat shock genes express at 40°C or higher temperature (6). Thus the threshold of temperature that acts as heat shock is relative to the normal physiological temperature of the organism.
With a view to learn something about the biological role of the heat shock response and its regulation, we have been studying the influence of developmental conditions and cell specializations on the heat shock response in Drosophila and some other diptera. The heat shock response in these studies was examined by autoradiography of \(^{3}\)H-uridine labelled polytene chromosomes to know the transcriptional status of heat shock genes and by fluorography of electrophoretically separated (in SDS-polyacrylamide slab gels) radio-labelled proteins to detect the synthesis of HSP (8-12). These studies revealed that in contrast to the notion of general universality and uniformity of the heat shock response in a given species, it can be profoundly modified not only by the temperature at which the larvae grow, but it may also dramatically vary in relation to specialization of the differentiated cells.

**EFFECT OF LOW-TEMPERATURE REARING ON HEAT SHOCK GENE ACTIVITY AND HEAT SHOCK PROTEIN SYNTHESIS IN SALIVARY GLANDS OF D. MELANOGASTER LARVAE:**

When salivary glands of late third instar larvae of *D. melanogaster* reared at 20°C-24°C, are heat shocked at 37°C, synthesis of a group of seven HSP is induced. These are identified by their apparent molecular mass in kilodaltons (kd): HSP83, HSP70, HSP68, HSP27, HSP26, HSP23 and HSP22. The last four HSP constitute the low mol. wt HSP family and their genes are clustered at the 67B locus on polytene chromosomes (13). These genes are also independently regulated by ecdysone (14,15) and during late larval stages, the HSP23 accumulates abundantly due to ecdysone-regulated expression of its gene. Singh and Lakhotia (10) found that the HSP23 was not inducible by heat shock in salivary glands of late third instar larvae that were reared at 10°C since the beginning of their larval period, although the other HSP, including the remaining low mol
wt HSP, were typically inducible by heat shock. Equally intriguing was the observation that even the ecdysone-induced constitutive accumulation of HSP23 in late third instar stage was considerably inhibited by the cold-rearing (10).

Lakhotia and Singh (8) had earlier examined the heat shock induced puffing in salivary glands of 10°C-reared larvae of D. melanogaster. While the 67B puff, which harbours the gene for HSP23 together with those for the other small HSP, appeared to puff normally, the major heat shock puff at 93D was not at all induced in these larvae. The 93D heat shock locus of D. melanogaster is unusual in many ways and also does not code for any of the known HSP; moreover, its heat shock inducibility is affected by several other conditions (reviewed in 16,17).

These specific effects on individual heat shock loci are significant from the point of view of regulation of heat shock genes. During the heat shock condition, these genes are generally co-ordinately activated by the specific interaction of their 5' HSE with the stress-activated HSTF (2,7). The heat shock genes are also subject to autoregulation (18). Our above noted observations show that the generalized regulation of heat shock genes can be over-ridden by other more specifically acting cellular physiological factors.

EFFECT OF SEASONAL VARIATIONS IN AMBIENT TEMPERATURE ON THE HEAT SHOCK RESPONSE IN CHIRONOMUS

In tropical climates, the insects which remain active throughout the year are exposed to widely varying ambient temperatures in different seasons. This raises interesting questions about their heat shock response since as mentioned earlier, the temperature at which the cells of a species experience heat shock stress is relative to its normal temperature range. The definition of a normal temperature range of a poikilothermous animal in tropics becomes difficult since
the ambient in summers and winters varies over a wide range. While some insects and other animals have evolved special mechanisms to avoid the very low or the very high ambient temperatures of winters and summers, respectively, certain insects and other animals remain active throughout the year. During the summer months these are, therefore, continuously exposed to fairly high temperatures, which would be high enough to cause heat shock stress to the same animal's cells at other times of the year. Since in insects like Drosophila, heat shock causes inhibition of developmentally regulated transcriptional and translational activities, it is intriguing that cells of these tropical insects are able to grow and maintain normal activities even when the surrounding temperature becomes rather high. To examine this aspect, the pattern of heat shock response was studied in Chironomus striatipennis, a local species which remains active throughout the year (12). One colony was maintained in the laboratory at a constant temperature of 24°C while another was kept under conditions where the temperature varied through the year in accordance with the season. The air-temperature in the ambient-reared culture varied from about 18°C to 42°C in winter and summer months, respectively. However, the temperature of the water in which larvae grew, varied between 16°C to 36°C. Salivary glands from these larvae were heat shocked, once every month of the year, for studying their heat shock response at puffing as well as protein synthesis levels and compared with that in the 24°C-reared larval salivary glands. The most significant observations of this study (12) were: 1) optimal heat shock response in the 24°C-reared larvae was seen at 39°C although heat shock puffs began to show a moderate level of induction even at 33°C; on the other hand, in the ambient-reared larvae which during the summer months were already growing at about 35°C-36°C, the heat shock puffs were inactive but were variably induced in individual polytene cells when
exposed to 39°C; 2) salivary glands of ambient-reared summer larvae constitutively synthesized the HSP at a low rate which was further stimulated by 39°C heat shock although unlike the 24°C-reared larvae the ongoing protein synthesis was not inhibited by 39°C; during other months of the year, the HSP were not synthesized constitutively; 3) while a brief exposure to 41°C caused extensive cell death in salivary glands of 24°C-reared larvae, the salivary glands of ambient-reared summer larvae continued transcriptional and translational activities even at 41°C; however, during other periods of the year, when water temperature was lower, the ambient-reared larvae behaved like the 24°C-reared larvae. Thus during summer months, the larvae growing in nature remain constantly under a mild heat shock condition and accumulation of a certain threshold level of HSP provides thermotolerance to these larvae for normal growth and development even at such high temperatures. Moreover, since the same population at other times of the year did not show constitutive expression of heat shock genes, the heat shock response in this tropical insect has remained inducible rather than becoming constitutive. Another notable observation in this study (12) was that unlike the temperate species of Chironomus which show optimal heat shock response at 37°C (19), this tropical species senses heat shock optimally at 39°C only. A further adaptation to tropical life is that, unlike the temperate species, the ongoing protein synthesis is not inhibited at 39°C in this tropical species of Chironomus. A comparable adaptation was noted in the ovarian cells of Anopheles stephensi which also responded to heat shock only at 39°C rather than at 37°C: this adaptation seems to be related to their warm blood-meal habits (11).
The pioneering study on heat shock induced protein synthesis in different cell types of Drosophila by Tissieres et al (20) revealed the heat shock response to be independent of cell differentiation since notwithstanding the cell type specific differences in ongoing protein synthesis, all cells synthesized the same set of HSP when heat shocked. This early finding of the universality of the stress-induced HSP synthesis in different cell types of a species has since been amply confirmed in a wide variety of organisms and cell types (2,21,22), although subtle differences in temperature optima and quantitative aspects of expression of specific heat shock genes are known to exist. A somewhat different situation obtains in germ-line cells and very early embryos since these cells were found to differ in their capacity to synthesize heat shock transcripts or HSP (3,22). The germ-line and somatic cells in developing egg chambers in ovaries of Drosophila show a dramatic difference in heat shock response: while the somatic components (follicle and nurse cells) synthesize the normal complement of HSP when heat shocked, the growing oocyte and early embryos (pre-blastoderm) are incapable of induced synthesis of HSP (3). The nurse and follicle cells in Drosophila ovaries also constitutively synthesize mRNA for HSP83, HSP27 and HSP26 and pass them on to the growing oocyte (22,23). In Drosophila testes also, the meiotic and post-meiotic germ cells do not synthesize HSP when heat shocked (24). In several other animal systems too the meiotic, post-meiotic and early embryonic cells have been found to be incapable of making HSP when heat shocked (3,5,22,25).

In a striking contrast to the above scenario of a generally uniform heat shock response in somatic cells of a species, we recently found (9) a uniquely different heat shock response in malpighian tubules (MT) of Drosophila. Heat
shocked MT from late third instar larvae and from freshly emerged adults did not synthesize any of the usual HSP, including the normally very abundant HSP70. Instead, synthesis of a novel set of MT-specific HSP, with a major HSP (in terms of abundant synthesis) banding at 58kd position in SDS-polyacrylamide gels, was detected. The 58kd HSP, seen abundantly in heat shocked MT, was not detectable in other heat shocked tissues of larvae or adults. In contrast to the larval MT, the MT from older flies responded to heat shock by synthesizing both the common as well as the MT-specific sets of HSP (9). The lack of induction of the usual set of HSP in larval MT was further confirmed by use of P-element mediated transformed stocks of *D. melanogaster* carrying fusion genes with HSP70 or HSP26 promoter linked to lac-Z (coding for beta-galactosidase) or ADH (coding for alcohol dehydrogenase) reporter gene (24,26,27). Neither the HSP70 nor the HSP26 promoter was active in larval MT since the reporter gene product (beta-galactosidase or alcohol dehydrogenase, as the case may be) was not detectable cytochemically in control as well as heat shocked samples.

The silencing of the common set of HSP genes in larval MT and of the MT-specific HSP genes in other tissues of *Drosophila* is most unusual and intriguing. The MT cells are polytenized and during polytene replication cycles, specific DNA sequences are known to be under-replicated (reviewed in 28). However, the possibility that genes for the common set of HSP are under-replicated in MT cells and are, therefore, not expressed is ruled out by the fact that in older flies' MT the common set of HSP genes also express. Apparently the regulation of HSP genes in MT cells is accomplished at the level of transcription. This would imply that either the larval MT cells do not contain the HSTF or the HSE on the common set of heat shock genes are blocked in some way so that the HSE-HSTF interaction does not occur as in other cell types. Whether the MT-specific HSP
genes also rely on HSE-HSTF type interaction or they follow a different mode of regulation has to remain conjectural till the genes themselves are identified and characterized.

The other very significant question raised by our observation is why the MT in Drosophila need to have an entirely different set of HSP? In all likelihood this biological adaptation is related to the specialized function of MT in osmoregulation and excretion. It is known that denatured proteins or the proteins marked for degradation in a cell can be good inducers of the heat shock response (2). As a part of their excretory function, the MT cells may accumulate degraded proteins or certain other metabolites which may act as inducer for heat shock genes. In view of these, it is tempting to speculate that the common set of HSP genes in MT cells are kept in a non-inducible state, either by modifying the HSTF or the HSE, so that these genes do not remain continuously induced due to metabolites in MT cells. To cope up with the necessity of protecting themselves against thermal and other stress-induced damages, the MT cells nevertheless need some HSP. Apparently, a different set of MT-specific HSP genes has been recruited for the purpose. Studies are in progress to resolve some of these issues.

Although considerable progress has been made in understanding the regulation of HSP genes, much less is known about their biological role. Our initial studies on the heat shock response in the context of ecological conditions and cell specializations have been encouraging from this point of view. It is expected that further studies in these directions will help to unravel the function of this cellular response in its true biological perspective.
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