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Heat shock response in ovarian nurse cells of *Anopheles stephensi*

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Abstract. Transcriptional and translational changes following temperature shock at 37, 39 or 41°C to ovarian cells of *Anopheles stephensi* were studied. Temperature shock at 39°C induced 6 puffs on polytene chromosomes in the nurse cells as revealed by [^{3}H] uridine incorporation studies. Only the 2R-19B puff was induced at 37°C and was found to be a major temperature shock locus remaining most active at all the 3 temperatures tested. Other temperature shock loci were activated only at 39°C. There was progressive inhibi tion of general chromosomal transcription with the rise of temperature. Transcription was drastically inhibited at 41°C but all the temperature shock loci still remained relatively active. Examination of [^{35}S]methionine labelled newly synthesized ovarian proteins using sodium dodecyl sulphate-polyacrylamide slab gels revealed that all the heat shock poly-peptides except the HSP 70 were synthesized in ovarian cells even at control temperature (29°C). Temperature shock induced the synthesis of HSP 70 and elevated the levels of other heat shock polypeptides (82, 30, 29, 23 and 17 KD). Present results suggest that the threshold level for induction of a complete heat shock response in mosquitos is higher (39°C) than the other dipteran insects studied and that a 41°C treatment is not lethal as in the case of *Drosophila, Chironomus* etc. These features reflect the adaptations of mosquitos to tropical climate and their dietary habit of warm blood meal.

Keywords. Temperature shock; mosquito; heat shock polypeptides; polytene chromo somes; puffs; thermotolerance.

Introduction

Studies on heat shock response in a tropical chironomid, *Chironomus striatipennis*, revealed certain intriguing features, different from the typical *Drosophila-like* response (Nath and Lakhotia, 1989). The threshold level for the induction of heat shock loci was higher (39°C) in *C. striatipennis* than in *Drosophila* and other *Chironomus* species living in temperate zones. These and other features of the heat shock response in *C. striatipennis* (Nath and Lakhotia, 1989) suggested an important role of the heat shock response in tropical adaptations of these insects. These results provided an impetus to examine the heat shock response in other tropical dipteran insects. Mosquitos are interesting in this context because not only their population density is found to be higher during summer months (Pal, 1974) but also they are adapted to warm blood meals which in other dipterans like *Drosophila* may be warm enough to induce a heat shock response.

Anopheles stephensi is widespread in its distribution and is an important malaria vector in the tropical areas (see Rao, 1981). Polytene chromosomes are found in larval salivary glands of *A. stephensi,* transiently appearing during mid to late 4th larval instar. They are also found in ovarian nurse cells of the adult females during

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Abbreviations used: SDS, Sodium dodecyl sulphate; TS, temperature shock; HSPs, heat shock polypeptides; CBB, Coomassic brilliant blue.

the gonotrophic (post-blood meal) cycle. The ovarian nurse cell polytene chromo somes have been a favourable material for studies because of the ease with which preparations can be made and also because the chromosomes are of superior morphology compared to those in larval salivary glands (Redfern, 1981; Macgregor and Verley, 1983). The present study explores the basic heat shock response at levels of transcription and translation in the ovarian nurse cells of *A. stephensi* The results were briefly presented earlier (Nath, 1987).

Materials and methods

Animal culture

Adults and larvae of *A. stephensi* (Delhi strain: stock obtained from the Malaria Research Centre, Delhi) were reared using the standard methods in an insectary maintained at 29 ± 1°C with a relative humidity of about 80%. Adults were fed on water soaked raisins.

To initiate ovarian development, 4–5 day old females were allowed a blood meal on rabbit. After the blood meal (24–30 h), ovaries were pulled out from the semi-gravid females and subjected to heat shock at different temperature in the inorganic salt constituents of Poels′ tissue culture medium (Lakhotia and Mukherjee, 1980). Ovaries were either used for [^{3}H]uridine labelling to study transcriptional pattern or for [^{35}S] methionine labelling to study protein synthesis pattern after heat shock.

RNA synthesis after heat shock

The excised ovaries were heat shocked at 37, 39 or 41°C (treated) or were kept at 29°C (control) for 60 min. The treated and control ovaries were labelled with [^{3}H] uridine (activity 40μCi/ml; specific activity 75 Ci/mM; Amersham, UK) for 10 min at respective temperatures. After labelling, ovaries were fixed in 1:3 aceto-methanol for 1 min and stained in 1% aceto-orcein. Finally the ovaries were dissociated in 50% propionic acid using fine needles to separate the ovarioles. A gentle tapping of the coverglass completed the squashing.

After squashing, the slides were either frozen on dry ice or in liquid nitrogen and coverslips were removed in the usual manner. The preparations were processed for autoradiography as described by Nath and Lakhotia (1989). Identification of heat shock loci was carried out using the polytene chromosome maps of Coluzzi *et al.* (1970).

Protein synthesis after heat shock

Excised ovaries were heat shocked at 37, 39 or 41 °C or were incubated at 29°C (control) for 60 min and then labelled with [^{35}S]methionine (activity 100 μCi/ml; specific activity > 800 Ci/mM; Amersham, UK). Following labelling, ovaries were washed in radio-isotope free medium and transferred to sodium dodecyl sulphate (SDS)-sample buffer (Laemmli, 1970) containing 2 mM PMSF. Samples were then heated in a boiling water bath for 5 min after mild homogenization and were immediately cooled on ice. The samples were kept frozen at –70°C until

electrophoresis. Electrophoresis followed by fluorography was carried out on SDS polyacrylamide (10-15% gradient) slabs as described earlier (Singh and Lakhotia, 1988).

Results

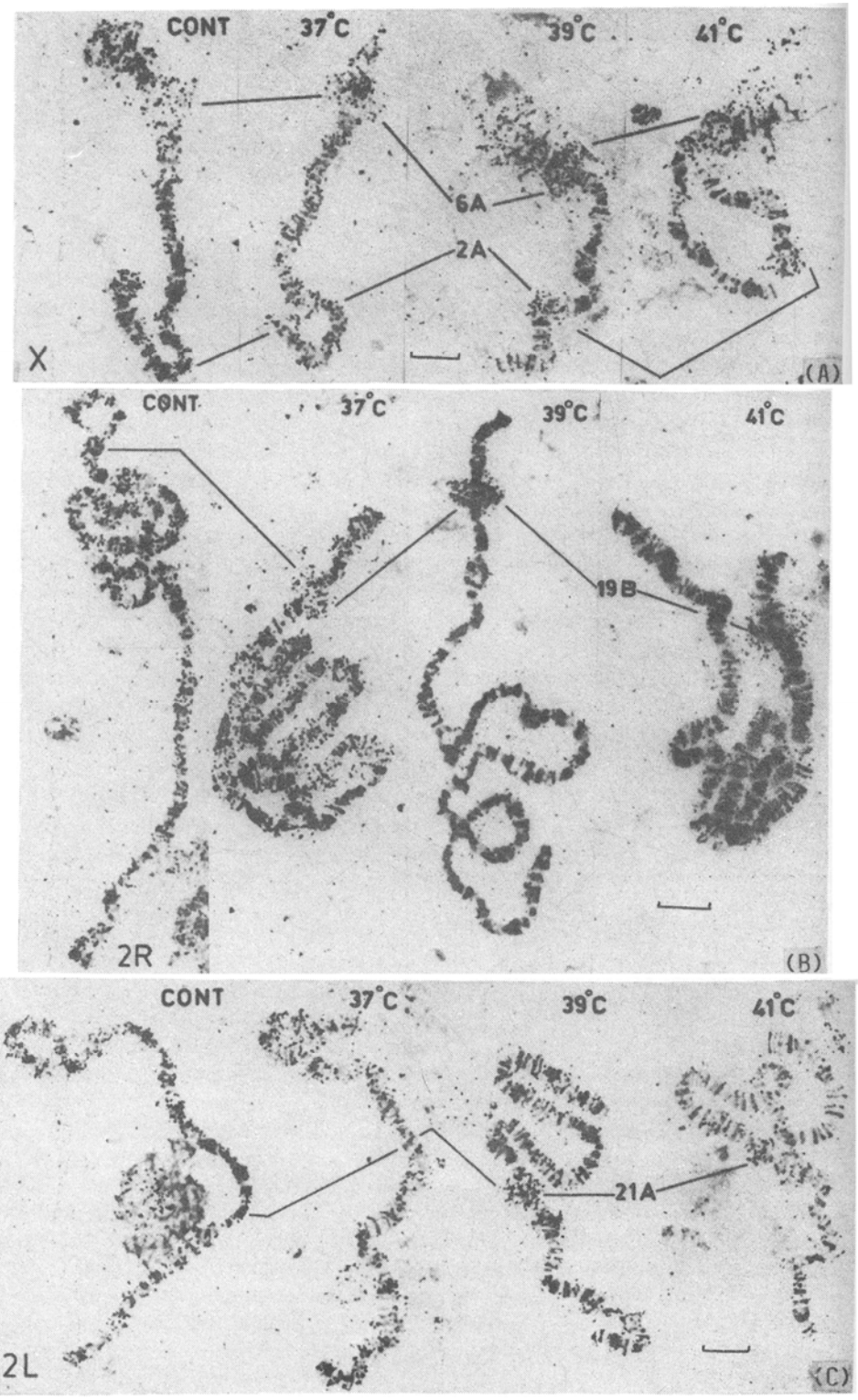
Induction of puffs and [^3H] uridine incorporation after heat shock at different temperatures in A. stephensi

Effect of heat shock on nurse cell polytene chromosomes was studied at 3 different temperatures *viz.,* 37, 39 and 41°C. The autoradiographic analysis of [^{3}H]uridine incorporation revealed that heat shock caused significant changes in transcriptional activity with the activation of a number of loci on different arms of polytene chromosomes (figure 1). Transcriptional activity of these temperature shock (TS) loci was scored on the basis of grain counts (figure 2A). A chromosome segment, 28A to 28E on 2L was scored to estimate the general chromosomal transcription. This chromosomal segment was chosen because it did not exhibit any developmentally active or TS induced puff. The relative transcriptional activity (figure 2B) of a TS locus was analysed as a ratio of the grain counts on the TS puff with respect to that on the 2L-28A to 28E chromosomal segment of the same nucleus. A total of 6 heat shock loci were identified on the basis of their [^{3}H]uridine labelling at different temperatures. These were located as follows: two on X-chromosome (X-2A and X-6A); one each on right and left arms of chromosome 2 (2R-19B and 2L-21A) and two on the left arm of chromosome 3 (3L-38B and 3L-40E). Right arm of chromo some 3 did not show any heat shock induced locus (see figure 1). With increasing heat shock temperature from 37–41°C there was a progressive inhibition of general chromosomal transcription (see figure 2A).

The most obvious change after a 37°C shock was induction of the 19B locus on right arm of chromosome 2. Mean grain counts on other TS loci were comparable to those in control (figure 2A). Since the gereral chromosomal labelling was partially inhibited, the relative transcriptional activity of each TS locus showed a higher level at 37°C than at 29°C (figure 2B).

At 39°C, all the other heat shock loci, in addition to the 19B locus, displayed conspicuous incorporation of [^{3}H]uridine (figure 1). In X chromosome, 6A was transcriptionally more active than 2A at 39°C (figure 1A). Left arm of chromosome 2 showed a minor TS puff at 21A (figure 1C) with its transcriptional activity being less than the other loci induced at 39°C. Two other TS loci were induced at 39°C on left arm of chromosome 3, *viz.,* 38B and 40E (figure 1E). Among all the TS loci, the 19B on 2R showed maximal induction at 39°C.

At 41 °C, transcriptional activity of all the TS loci was drastically reduced (figure 2A) with mean grain counts on the different TS puffs generally less than those on corresponding controls. However, when relative transcriptional activity was analysed, all the TS loci were found to show higher relative activity levels than the corresponding control values (figure 2B) since the general chromosomal transcrip tion was more severely inhibited at 41°C (see figure 2A). A comparison of overall pattern of heat shock response showed that 19B was the major TS puff remaining active at all the temperatures tested and of all the TS loci it showed maximal labelling.


CONT
37°C
39°C
41°C
6A
2A
X
(A)
CONT
37°C
39°C
41°C
19B
2R
(B)
CONT
37°C
39°C
41°C
21A
2L
(C)

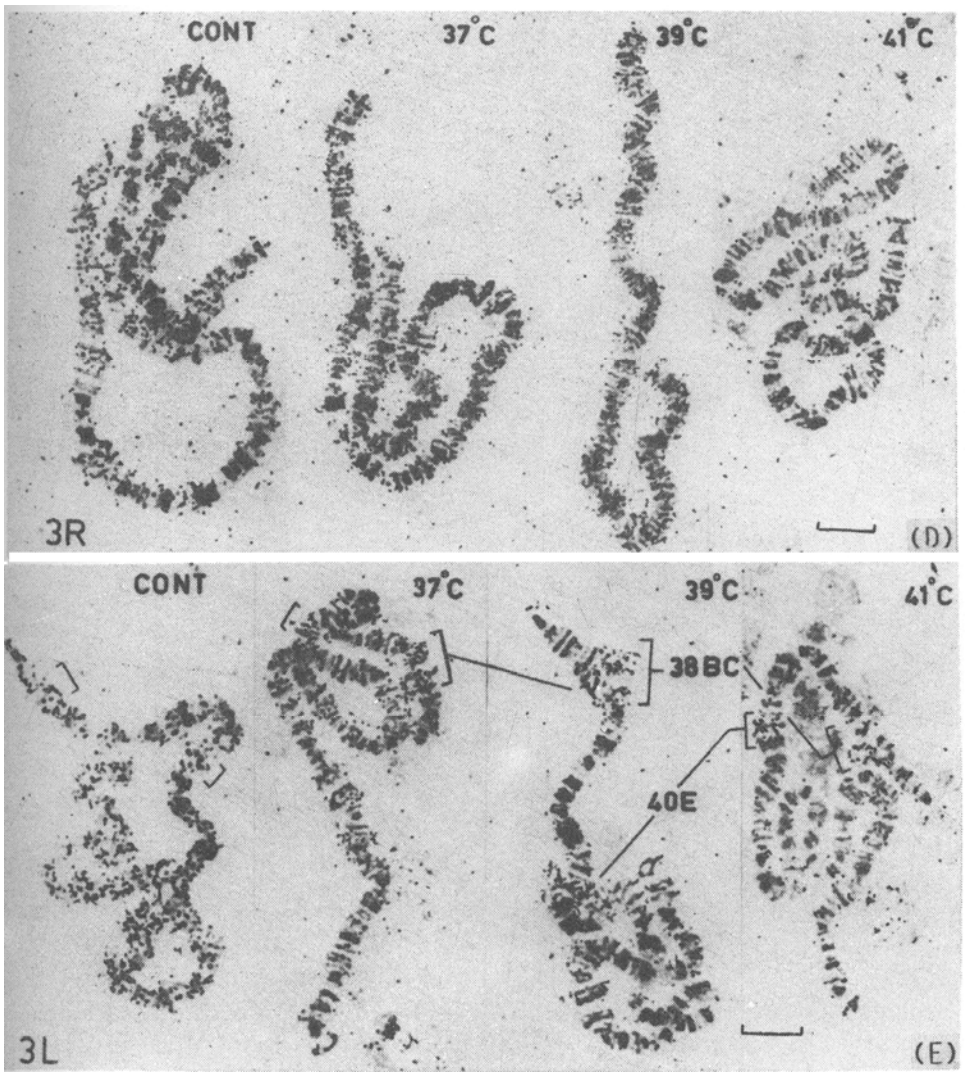


Figure 1. [^{3}H]Uridine labelled polytene chromosomes showing heat shock loci on X-chromosome (A), right arm of chromosome 2 (B), left arm of chromosome 2 (C), right arm of chromosome 3 (D), and left arm of chromosome 3 (E) from control (29°C) and heat shocked (TS 37°C, TS 39°C, TS 41°C) ovarian nurse cell polytene nuclei of *A. stephensi.* The bars represent 10 μm.

Effect of heat shock on ovarian protein synthesis

Heat shock to ovaries of *A. stephensi* did not result in synthesis of any novel polypeptide, except a 70 KD species. However, synthesis of several discrete species of polypeptides, already synthesized in control ovaries, was elevated after heat shock. The estimated molecular weights of these polypeptides were 82, 30, 29, 23 and 17 KD (see figure 3). Based on the criterion that the synthesis of these polypeptides was stimulated after heat shock, they are referred to as heat shock polypeptides (HSPs) (Schlesinger, 1986).

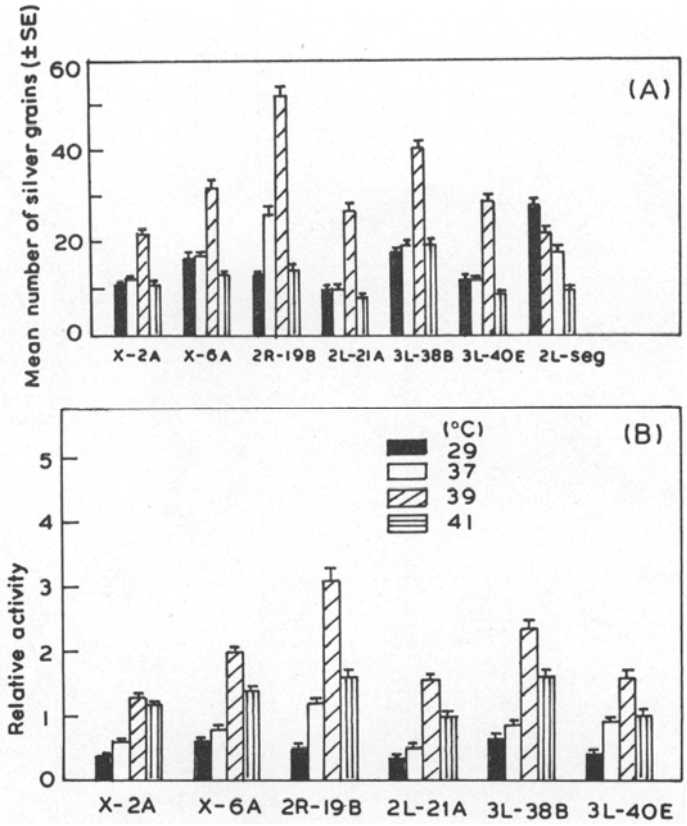


Figure 2. [^{3}H]Uridine incorporation (ordinate) on different heat shock loci and the segment of 2L (abscissa) at different temperatures. The [^{3}H] uridine uptake is shown in terms of mean numbers of silver grains ± SE in (A) and as the relative transcriptional activity of different heat shock loci in (B). For each data point 20–25 nuclei were scored.

Upon heat shock at 37°C, the synthesis of HSP 82, HSP 70, HSP 30 and HSP 29 was elevated to higher levels than in control ovaries and this persisted at 39°C as well. HSP 23 and HSP 17 were abundantly synthesized after heat shock at 37 and 39°C, although their levels seemed to be slightly lower than in control ovaries (figure 3). However, since the general protein synthesis was more inhibited at 37 and 39°C, HSP 23 and 17 also appeared prominent in fluorograms of 37 and 39°C lanes. A progressive inhibition of general protein synthesis was noted with increasing heat shock temperature: degree of inhibition was not much pronounced at 37°C but was severe at 41°C. After a 41°C shock, HSP 82, 70 and 17 were still faintly detected. Longer fluorographic exposure of the 41°C lane (see figure 3, lane D′ and E) confirmed that all the HSPs as well as other polypeptides were still being synthesized after the 41°C heat shock.

Discussion

The present study in *A. stephensi* has added another example to the growing list of organisms where the heat shock response has been found to be ubiquitous.

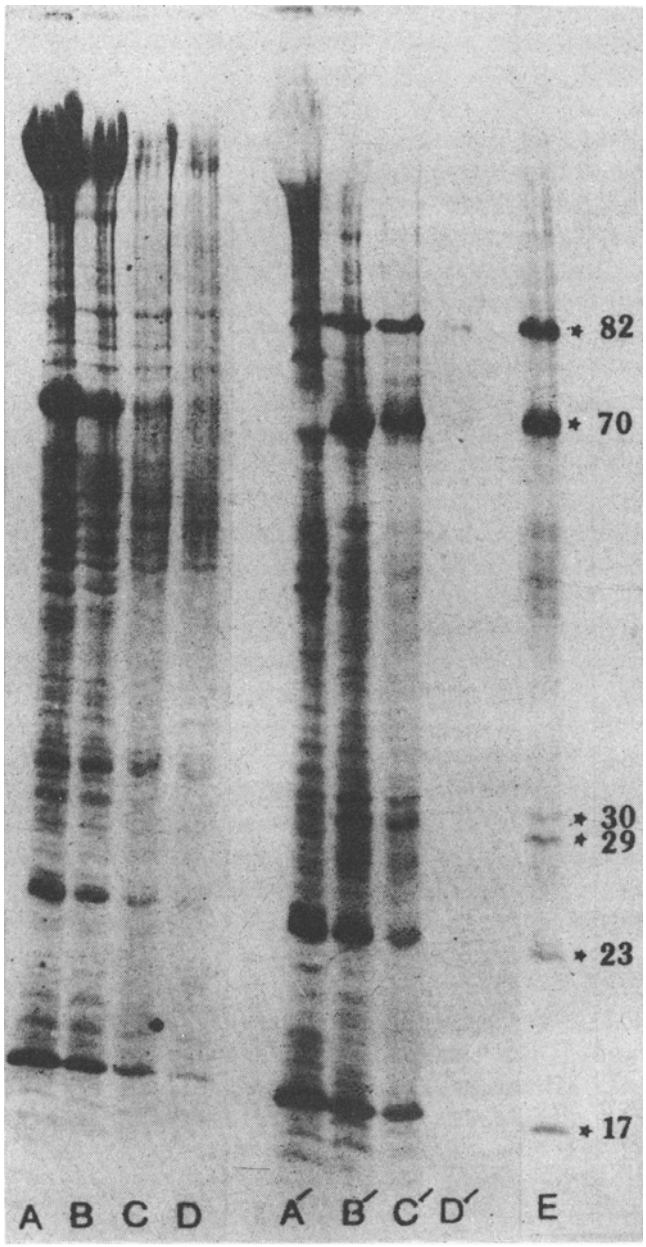


Figure 3. Patterns of normal and heat shock induced protein synthesis in ovaries of adult *Anopheles:* Lanes A-D show the CBB staining patterns in 29°C control (lane A) and in heat shocked (37°C, lane B; 39°C, lane C and 41°C, lane D) ovaries. The corresponding fluorograms are shown in lanes A′-D′ respectively after a 6 day exposure. Lane E shows a 15 day exposure of the 41°C-lane. Molecular weights (in KD) of the HSPs are marked.

Although Berger *et al.* (1985) have reported the protein synthesis pattern in heat shocked *Aedes* cell line, detailed studies on heat shock response in mosquitos at the level of transcription and translation are lacking. In *Anopheles,* heat shock response,

although analogous to other dipteran insects has some distinctive features. In *Drosophila* and in a local species of *Chironomus* (Nath and Lakhotia, 1989), the heat shock genes begin to show induction at 33°C. In contrast, in the case of *A. stephensi,* even at 37°C, only one of the TS loci showed elevated level of [^{3}H]uridine uptake; full complement of TS puffs was induced only at 39°C. The reason for only a marginal induction in *Anopheles,* even at 37°C can be attributed to its higher optimal growth temperature (29°C) than that of *Drosophila* or *Chironomus* (20–25°C) and other biological adaptations.

The two heat shock loci at 6A and 2A are notable in *Anopheles* due to their X-linkage. Since mosquitos have an XX/XY sex-chromosome system (King, 1975), X-linkage of these heat shock genes becomes of interest from the view point of dosage compensation (Muller, 1932). In the genus *Drosophila,* all heat shock genes are presumed to be originally autosomal and only in some species, a few of the heat shock genes become X-linked due to autosome-X translocation during evolution (Sturtevant and Tan, 1937; Pierce and Lucchesi, 1980). Interestingly, these neo X-linked heat shock genes have evolved a dosage compensating regulatory system (Lucchesi, 1978; Pierce and Lucchesi, 1980; Strobel *et al.,* 1978). In this context, the evolutionary origin of the X-linked heat shock genes in *Anopheles* and their dosage compensation become interesting issues for further study.

At the level of protein synthesis, ovarian nurse cells exhibited enhanced synthesis of a set of polypeptides at elevated temperatures. Among the temperature induced ovarian HSPs, 82 and 70 KD were the most prominently labelled species. Except for the HSP 70, all other HSPs were also synthesized in ovaries of *A. stephensi* even at control temperature (29°C). In this context it may be noted that the constitutive synthesis of the various HSPs in ovarian cells of mosquito is unlikely to be due to heat shock that may be caused by the previous warm blood-meal since the time interval between the warm blood-meal and examination of protein synthesis in ovarian cells was long enough (24–30 h) to let the cells recover from the heat shock, if any. There is growing evidence of the presence of HSPs under non-heat shock conditions in specific developmental stages/cell types in different organisms (Lindquist, 1986; Bienz and Pelham, 1987). HSPs appear to be normal component of larval imaginal discs (Cheney and Shearn, 1983) and are also found in normal *Drosophila* embryos (Graziosi *et al,* 1980; Bergh and Arking, 1984; Zimmerman *et al.,* 1983; Mason *et al.,* 1984). Heat shock mRNAs are also reported to be con stitutively present in *Xenopus* oocytes (Bienz, 1985; Browder *et al.,* 1987). In a tropical midge *C. striatipennis* most HSPs were constitutively synthesized in ovarian cells (Nath and Lakhotia, 1989). Thus a constitutive synthesis of HSPs in ovarian cells of mosquitos may reflect a common feature seen in a wide variety of organisms. In this context, the total absence of synthesis of HSP 70 in control ovaries of *A. stephensi* is rather surprising, since HSP 70 is the most conserved and most abundant of all heat shock proteins (Schlesinger *et al.,* 1982; Schlesinger, 1986; Nover 1984; Lindquist 1986). CBB staining, however, revealed a prominently stained band in the 70 KD range in control ovaries of *A. stephensi* (see figure 3, lane A) and if this represents HSP 70, the absence of the HSP 70 synthesis in these ovaries may be understood in terms of autoregulation such that once a threshold level is attained, further synthesis is inhibited (DiDomenico *et al.,* 1982). The presence of polypeptides corresponding in molecular weight to HSP 70 in control ovaries also raises the possibility that some of them might represent HSC 70 (cognates of HSP 70), which are known in *Drosophila* as well as yeast to be essential

for growth at any temperature (Craig *et al.,* 1982; Craig and Jacobsen, 1984, 1985).

Present results showed that in *A. stephensi* ovaries, synthesis of HSP 70 alone was newly induced at 37°C while at the transcriptional level, the 19B puff on 2R alone was induced by the 37°C shock. In view of this, it is tempting to speculate that the 2R-19B locus may code for HSP 70. This correlation is further supported by the fact that the 2R-19B locus is the most active heat shock locus as would be expected for the most abundantly synthesized HSP 70. A final identification of the different HSP loci, however can only be made after further molecular studies.

A significant observation in this study was the persistence of general transcriptional and translational activities at 37°C or even at 39°C in *A. stephensi.* This contrasts with the near lethal effect of 39°C heat shock in *Drosophila.* Moreover the present results showed that *A. stephensi* cells continued some transcriptional and translational activities at 41 °C, a temperature at which even cells of a tropical species of *Chironomus* and *C. striatipennis* were found (Nath and Lakhotia, 1989) to have stopped most of their synthetic activities. This shows that in the case of mosquito, the threshold level for sensing heat shock is set to a higher temperature and their cellular activities are adapted to continue functioning even at relatively warmer temperatures. A similar finding was reported by Berger *et al.* (1985) for cultured cells of another mosquito *Aedes albopictus.* While tropical adaptation by itself would set the heat shock threshold at a higher level, the dietary habit of blood meal of warm-blooded animals would also require that mosquitos do not suffer the heat stress at 37°C. The constitutive synthesis of HSPs in ovarian tissue of mosquito as found in this study may be one of the biological adaptation of these insects to tropical climates. Further studies will show if other tissues of mosquito also synthesize HSPs constitutively.

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