HEAT SHOCK PROTEINS AND TEMPERATURE TOLERANCE IN SILKWORM *Bombyx mori L.*: POSSIBILITIES AND PROSPECTS

G.K. Rajesh- 2006

Kerala State Sericulture Co operative Federation (SERIFED), Thiruvananthapuram

Insects are known to have pervasive effect of temperature on them and hence almost each and every aspect of an insect's life is influenced by temperature from direct effects on the kinetics of enzyme relations, to defining the limits of physiological function and behavior and ultimately to shaping of evolutionary pathways. It is because of this fact, insects as a group, more than any other eukaryotic organisms have a strong adoptability not only to survive but also to flourish under a wide variety of thermal environments. However, the mulberry silkworm (*Bombyx mori* L.) is one of the most thermal-sensitive organisms. Intensive and careful domestication over centuries has apparently deprived this taxon of opportunities to acquire thermo tolerance. This aspect is more pronounced in bivoltine races compared to multivoltine ones. Thus, among many factors attributed to poor performance of the bivoltine strains under tropical conditions, the major one is lack of thermo tolerance. Many quantitative characters decline sharply at higher temperatures. Therefore, one of the key factor in development of bivoltine hybrids could be need for thermo tolerant bivoltine races. This could be conventionally achieved through hybridization of polyvoltine with bivoltine races which is a prolonged and tedious procedure mainly due to the delay in fixation of economic characters. Moreover, earlier efforts in this direction using Pure Mysore did not yield expected outcome which pointed out that- economic cocoon traits were negatively correlated with

high temperate resistance (Tazima & Ohnuma, 1995). With recent advances (Kato *et al*., 1989) in identification of heat shock proteins and their introduction within the silkworm breeds has opened altogether a new domain to evolve robust productive silkworm hybrids.

The Phenomenon of Heat Shock:

Heat shock is nothing but a thermal injury caused by sudden increase in temperature in biological molecules like DNA, RNA, lipids, etc., of the cell which are vulnerable to heat stress. This leads to a number of abnormalities at cellular level. Normal pattern of protein synthesis halts. Transfer RNA and ribosomal RNA loose conformational integrity leading to degradation. DNA looses ability to function properly. There is aggregation of intermediate filamentous proteins at the nucleus instead of forming the cytoskeleton. At the same time pH of body fluid also drops. Increase of temperature leads to increase in kinetic energy of macromolecules, decrease of ionic bonds, hydrogen bonds, Van - der - Waals bonds etc., and increase its hydrophobic interactions; leading to loss of its shape. Denatured proteins also get adhered to DNA and restrict enzymatic access to DNA causing large-scale DNA damage. The heat shock thus ultimately leads to death of cells.

Another most important effect of temperature (or stress of any kind) on cellular proteins is by unfolding them. Cellular proteins are typically folded in their native conformations while functioning in cells. They unfold at the following contexts: 1) during *de novo* synthesis of polypeptides and assembly of multimetric proteins; 2) during intra cellular transport and organellar import when a protein must unfold or remain

unfolded to cross the boundary of a cellular compartment; 3) during or after exposure to a protein denaturing stress. At these times unfolded proteins may be susceptible to inappropriate interactions with one another or with other cellular components. More over once unfolded, a protein can prospectively interact with folded proteins and induce them to unfold. Such interactions can result in aggregates of unfolded protein that at best diminish the pool of functional proteins and at worst are cytotoxic (Feder, 1996).

However, a brief exposure of cells to sub-lethal high temperature was found to render protection to the organism from subsequent and more severe temperature. In a study with heat shocked *Drosophila subobscura* Digley and Smith (1968) reported continued survival and acclimatization of the experimental insects at higher temperatures.

Heat Shock Proteins:

It was Ritossa (1962) who reported that the metabolic inhibitor dinitrophenol and heat induced a characteristic pattern of puffing in the chromosomes of *Drosophila*. It was observed by the author that when Drosophila larvae were shifted from 27°C to 37°C temperature similar puffs appeared in the polytene chromosomes. This discovery eventually led to the identification of the heat-shock proteins (Hsp) or stress proteins whose expression these puffs represented. By the mid-1980's, investigators recognized that many Hsps function as *molecular chaperones*. The word chaperone (pronounced as 'sha-pə-"rōn) means *'an older person who accompanies young people at a social gathering to ensure proper behavior; broadly: one delegated to ensure proper behavior'* .Molecular chaperones work more or less in a similar fashion. They are a class of proteins that enable the cell to cope with the problem of unfolding subsequent to a stress.

Chaperones can recognize and bind to the exposed side groups that charecterise unfolded proteins. In so doing molecular chaperones prevent the bound side groups from engaging in inappropriate interactions with other cellular components, as well as stabilize the bound proteins in an unfolded state. Alternatively, chaperones can target bound proteins for degradation or removal from the cell. The constitutively expressed heat shock proteins (heat shock cognates) perform these roles for nascent polypeptides or proteins that unfold during normal cellular processes, while the inducible hsps function in response to the protein denaturation due to stress.

A summary of the mechanism for expression of heat shock proteins within a cell is given by Kregel (2002) as follows. 'Heat shock factors (HSFs), present in the cytosol, are bound by heat shock proteins (HSPs) and maintained in an inactive state. A broad array of physiological stimuli ("stressors") are thought to activate HSFs, causing them to separate from HSPs. HSFs are phosphorylated by protein kinases and form trimers in the cytosol. These HSF trimer complexes enter the nucleus and bind to heat shock elements (HSE) in the promoter region of the Hsp gene. Hsp mRNA is then transcribed and leaves the nucleus for the cytosol, where new Hsp is synthesized'.

Subsequently, every form of stress is known to induce these proteins in all tested organisms from bacteria to man. Inducing stresses include ethanol, heavy metals, hypoxia, hyperoxia, changes in pH, free radicals, various poisons and toxins, ischemia, osmotic shock, ionizing radiation and many others (Feder, 1996). Thus the term "heat shock protein" is a bit of a misnomer, and it is more accurate to refer to these proteins as "stress proteins". Yet the term heat shock proteins is so deeply entrenched in the

literature and so accurate a descriptor of the response at high temperature that, it is likely to persist for years to come (Denlinger and Yocum -1998).

Heat Shock Proteins (HSP) – Types and functions:

Heat-shock proteins are classified into families on the basis of sequence homology and typical molecular weight as Hsp 110, Hsp 100, Hsp 90, Hsp 70, Hsp 40, Hsp 10 and small heat- shock protein families. In eukaryotes many families comprise multiple members that differ in inducibility, intra cellular localisation and function which are presented briefly in the following table:-

Size (kDa)	**Major Function**	**Cellular Localization**
27-28	Stabilization of microfilaments Cytokine signal transduction	Cytosol and nucleus
60	Protein Assembly	Mitochondria
70-73	Protein folding and translocation	Cytosol, nucleus, endoplasmic reticulum, mitochondria
90	Protein translocation Receptor regulation	Cytosol, nucleus, endoplasmic reticulum
100-104	Protein folding	Cytosol

(After Moseley, 1997)

The threshold temperature for Hsp induction is correlated with the typical temperature at which species live. Thermophilic species have a higher threshold than the psychrophilic species. Many species exhibit characteristic and distinctive patterns of Hsp expression (or non expression) during the various stages of development, including

gametogenesis, embryogenesis and metamorphosis. Extensive studies have been conducted on the heat- shock response by a large number of workers across the world in a variety of insect species such as *Drosophila sp.* (Tissiers *et.al.,* 1974*;* Lindquist, 1980, Gilchrist & Huey 1999; Karunanidhi *et al,* 1999), the locust *Locusta migratoria* (Whyard *et al.,* 1986) *Anopheles stephensi* (Nath and Lakhotia*,* 1989), the tobacco hornworm - *Manduca sexta* (Fittinghoff and Riddiford 1990), the fleshfly-*Sarcophaga crassipalpis,* (Joplin and Denlinger 1990), Lymantria dispar (Denlinger *et al*., 1992). Studies examining stress protein expression in the wild or in response to laboratory stimulations or of natural stress regimes are still few. Nonetheless, even these few studies are sufficient to demonstrate that patterns of stress protein expression can be correlated with specie's natural thermal environments; that is, cells and species from warm environments undergo a stress response at warmer temperatures than counterparts from cool environments (Lindquist,1986; Huey& Bennet, 1990; Sarge et al., 1995; Somero, 1995)

Though there are reports on the activity of heat shock proteins in silkworm, the body of literature available on the molecular mechanism of temperature sensitivity and heat shock response in silkworm is rather thin as compared to the enormous work done on other insects. Evegnev *et al*., (1987) studied heat shock response in *Bombyx mori* cells. Temperature elevation induced active transcription of heat shock mRNAs in infected cells. But at the level of translation heat shock treatment failed to induce Hsp synthesis and was not able to inhibit production of polyhedrin in such cells. Joy and Gopinathan in 1995 reported the appearance of 93, 70, 46 and 28 kDa protein bands consequent to high temperature exposure in *Bombyx mori*. in both bivoltine and multivoltine strains, but with varying kinetics. The isolated hemocyte of multivoltine

race exhibited the induction of 70 kDa protein. Lee *et.al*., in 2003 cloned a genomic DNA fragment containing a promoter region for the gene encoding an HSC70-4 homologue, the structure of which was deduced from the partial cDNA sequences that were registered in a *Bombyx mori* EST data base. The deduced amino acid sequence with 649 residues was 89% and 96% identical to those from *Drosphilla melanogaster* hsc-4 and *Muduca sexta* HSC-70-4 respectively. The expression analysis by reverse transcription PCR demonstrated that mRNA transcription occurred in all tissues examined and was not stimulated by heat shock. Thus HSC70-4, the molecular chaperon is ubiquitously expressed in every tissue of *Bombyx mori*.

Vasudha *et.al*., (2006) observed differential expression of hsps in silkworm strains. 90 kDa in the first, second and third instars, 84 kDa in the fourth instar and 84, 62, 60, 47 and 33 kDa heat shock proteins in fifth instar was observed in response to heat shock. Use of Heat shock proteins as molecular markers for evaluation and evolution of thermotolerant silkworm strains has been suggested by them.

Towards Heat tolerant silkworms:

Utilization of thermotolerance characteristics was the only way out to evolve tropical bivoltine silkworm hybrids. In this direction at CSR&TI, Mysore, a breeding technique was evolved to select the robustness genes along with its modifiers in high temperature conditions. Consequently in 1988 a temperature tolerant bivoltine hybrid namely CSR18 X CSR19 was developed and authorized for commercial exploitation (Sureshkumar, *et.al*., 2002). Though the introduction of CSR18xCSR19 in the field for commercial rearings during summer months was seen as a big success, the productivity level and returns realized did not match to that of other productive CSR hybrids.

Therefore, the acceptance level of this hybrid among farmers was not up to the expected level.

Considering the enormous investigations conducted on HSPs in a plethora of organisms ranging from bacteria to man, it is felt that there is an acute shortage of literature on the heat shock response of the silkworm *Bombyx mori*. However, the literature available on the heat-shock protein synthesis in other lepidopteran insects suggest that there is much scope for similar studies in silkworm HSPs in detail with the set target of harnessing the genes responsible for rendering thermo-tolerance in hardy polyvoltine and can be transferred in bivoltine breeds since high temperature resistance is recognize as heritable character in silkworm (Kato *et al*., 1989). In order to achieve greater success in this regard, there is dire necessity for (1) Understanding the molecular mechanism of temperature tolerance in silkworm; (2) Identification of the various families of HSPs synthesized and the threshold temperature, which induce their expression; (3) Understanding the differential expression pattern of various HSPs in bivoltine and polyvoltine races; and (4) To locate the genes responsible for the heat inducible HSPs and subsequent steps to introgress the same into the silkworm genome either by conventional breeding or by use of molecular techniques.

References:

Denlinger DL and Yoccum GD (1998). Physiology of Heat Sensitivity in Insects. In: *Lethal Temperatures in Integrated Pest Management.* Eds: GJ Hallman and DL Denlinger. Westview Press, Boulder Co., pp:7

Denlinger DL, Lee RE, Yocum GD and Kukal O (1992). Role of chilling in the acquisition of cold tolerance and the capacitation to express stress proteins in

diapausing pharate larvae of the gypsy moth *Lymantria dispar. Arch. Insect Biochem. Physiol.,* 21:271-80.

Dingley F, Maynard Smith J (1968). Temperature acclimatization in the absence of protein synthesis of *Drosophila subobscura. J. Insect Physiol.,* 14:1185-94.

Evgenev MB, Braude-Zolotareva TY, Titarenko EA, Levin AV, Denisenko ON, Ulmasov K and Karaev K (1987). Heat Shock response in *Bombyx mori* cells infected by Nuclear Polyhedrosis Virus. *Mol. Gen. Genetics,* 215:322-325.

Fernando P and Heikkila JJ (2000). Functional charecterisation of *Xenopus* small heat shock protein, Hsp 30 c: the carboxyl end is required for stability and chaperone activity. *Cell Stress and Chaperons,* 5:148-159.

Fittinghoff CM and Riddiford LM (1990). Heat Sensitivity and Protein synthesis during heat shock in the tobacco horn worm, *Manduca Sexta*. *J. Comp. Physiol.,* B160: 349-356.

Gilchrist GW, Raymond B. Huey (1999). The direct response of *Drosophila melanogaster* to selection on knock-down temperature. *Heredity,* 83:15-29.

Huey, R.B. & Bennet, A. F. (1990) Physiological adjustments to fluctuating thermal environments: an ecological and evolutionary perspective. In Stress Proteins in Biology and Medicine, ed. R.I. Morimoto, A. Tissieres &C. Georgopoulus, pp.37-59. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press.

Joplin KH, Yocum GD and Denlinger DL (1990) Cold shock elicits expression of heat shock proteins in the flesh fly *Sarcophaga crassipalpis*. *J. Insect Physiol.,* 36:825-834.

Joy O, Gopinathan KP (1995). Heat-shock response in mulberry silkworm races with

different thermo tolerances. *J. Biosci.,* 20:499-513.

Karunanidhi S, Barclay JW, Robertson RM, Brown IR and Atwood HL (1999). Neuro protection at *Drosophilla synapsis* conferred by prior heat shock. *The Journal of Neuro Science,* 19:4360-4369.

Kato M, Nagayasu K, Ninagi O, Hara W and Watanabe A (1989). Study on resistance of the Silkworm *Bombyx mori* to high temperature. *Proceedings of the 6th International Congress of SABRAO (II)*, pp:953

KREGEL KEVIN C. (2002) Molecular Biology of Thermoregulation Invited Review: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Physiol* 92: 2177–2186

Lee JM, Kusakave TE, Kawaguchi Y, Yasunaga-Aoki C, Nho S, Nakajima Y and Koga K (2003). Molecular characterisation of heat shock cognate 70-4 promoter from the silkworm *Bombyx mori. J. Insect Biotech*. *Sericology,* 72: 33-39.

Lindquist S (1980). Variying patterns of protein synthesis in *Drosophilla* during heat shock; implications for regulation. *Developmental Biology*, 77:463-479.

Lindquist, S. (1986) The heat shock response. *Annual Review of Biochemistry* 55, 1151-91

Moseley PL (1997). Heat shock proteins and heat adaptation of the whole organism. *J. Appl. Physiol.,* 83:1413

Nath BB, Lakhotia SC. (1989). Heat shock response in ovarian nurse cells of *Anopheles stephensi. J. Biosci.,* 14:143-52.

Ritossa FA (1962) A new puffing pattern induced by temperature shock and DNP in

Drosophila. Experientia, 18: 571

Sarge, K. D., Bray, A.E. & Goodson, M. L. (1995) Altered Stress response in insects. *Nature* 374, 126

Somero, G. N(1995) Proteins and temperature. *Annual Review of Physiology* 57, 43-68.

Suresh Kumar N, Basavaraja HK, Kishore Kumar CM, Malreddy N and Datta RK (2002). On the Breeding of "CSR18 x CSR19" – A Robust Bivoltine Hybrid of Silkworm *Bombyx mori* L. for the Tropics. *Int. J. Indust. Entomol.,* 5: 153

Tazima Y and Ohnuma A (1995). Preliminary Experiments on the Breeding Procedure for Synthesising a High Temperature Resistant Commercial Strain of the Silkworm, *Bombyx mori* L. *Silk. Sci. Res. Inst. Jpn.,* 43: 1

Tissiers A,Mitchell HK and Tracy UM (1974) Protein synthesis in salivary glands of *Drosophila melanogaster* : relation to chromosome puffs. *J. Mol. Biol.,* 84:389-398.

Vasudha B. Chavadi, Aparna H. Sosalegowda and Manjunatha H Boregowda (2006) Impact of heat shock on heat shock proteins expression, biological and commercial traits of *Bombyx mori* : *Insect Science* .,13 : 243

Whyard S, Wyatt GR and Walker VK (1986). The heat-shock response in *Locusta migratoria. J. Comp. Physiol.,* 156B:813-817.