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Tissue-specific expression patterns of heat shock protein 90 transcripts in silkworm, *Bombyx mori*

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Abstract

The present study reports the survival and expression patterns of heat shock protein 90 (hsp90) in silkworm *Bombyx mori*. The ubiquitous expression of hsp90 mRNA was observed within all examined tissues such as fat body, ovary, wing disc and dorsal abdominal epidermis during larval stage, and fat body and ovary during pupal stage. Under heat stress, depending on its severity, hsp 90 expression was found to vary among different tissues. At mild and mild-to-severe heat stressed condition (39 °C and 42 °C), its expression was found to be significantly up-regulated in all examined tissues except pupal ovary. At severe-to-lethal heat stressed condition (45 °C), no significant up-regulation in hsp90 transcript level was observed in tissues except dorsal abdominal epidermis. The up-regulation of hsp90 was found to be associated with the ability of the animal to survive at the particular heat shock temperature. The spinning larvae were found to be more heat resistant than pupae.

Keywords: Heat stress, survival, hsp90, *Bombyx mori*.

1. Introduction

The insects are the most abundant animal group on earth and the scientific interest to understand their response to environmental stresses has increased considerably because of their medical, economical and ecological values. The impact of these stresses, depending on its severity, varies from increased synthesis of heat shock proteins for the survival to the physiological and developmental insults. The heat shock proteins act as molecular chaperones and protect the cells by controlling the appropriate folding and translocation of polypeptides to different cellular compartments. There are many families of heat shock proteins (Hsps) based on their molecular weight and homology, and include Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and sHsps [1-3]. In insects, the genes for many such Hsps have been isolated and characterized [4-11]. The heat shock protein of 90 kDa (Hsp90) has been found to be highly conserved and highly expressed cellular protein [12]. It is important among other Hsps because its target proteins include steroid hormone receptor, signaling kinases and various transcription factors and it was suggested that it occupies a central position in cellular networks and plays an important role in the homeostasis of cellular proteins [13-15]. The involvement of Hsps in thermotolerance and developmental processes such as diapause, spermatogenesis, oogenesis and embryogenesis were observed in insects, such as *Delia antiqua*, *Helicoverpa zea*, *Lucilia sericata* [5, 8, 9, 16-21]. In *Bombyx*, Hsp70a was found to play a role in the reception/transduction of diapause termination (5 °C) signal via gene activation [7].

Thermotolerance in an insect is dependent on the expression of heat shock proteins such as sHsps, Hsp70 and Hsp90. Recently, it was demonstrated that in *Bombyx*, two heat shock proteins, namely, sHsps and Hsp70 are associated with the acquisition of thermal tolerance [22]. The tissue specific expression of hsp70 mRNA and the changes in its expression level along with other small heat shock proteins were demonstrated in *Bombyx* and *Antheraea pernyi* upon heat stress [11, 22-23]. For Hsp90, its involvement under heat stress in silkworm was demonstrated at protein level, using the techniques of SDS-PAGE and immunoblot analysis [24-26]. At mRNA level, its differential expression was shown only during embryonic development in *Bombyx* [27], and no reports are available till date on tissue specific mRNA expression of hsp90 gene and its induction upon heat stress in *Bombyx*. The present study thus made an attempt to study the tissue specific mRNA expression pattern of hsp90 in silkworm, *B. mori*. An attempt was also made to correlate the survival of larvae under heat stress with the transcript level of hsp90.

2. Materials and methods

2.1 Experimental Insect

Disease-free layings (dflys) of silkworms breed CSR 18 of *B. mori* were obtained from Central Sericultural Germplasm Resource Centre (CSGRC), CSB, Hosur, Tamilnadu and reared with fresh mulberry leaves in laboratory under controlled conditions (temperature, 25±1 °C; humidity, 60-90%, photoperiod, 12L:12D). Only female larvae and pupae were considered for heat shock treatment to avoid any sex-specific data variations. Larvae and pupae were staged according to days after ecdysis, and the available morphological markers such as spinneret pigmentation and gut purge.

2.2 Heat shock treatment

For heat shock treatment, day 3 spinning larvae and day 2 pupae were placed individually in glass test tubes plugged with cotton and were submerged in a water bath at 39 °C, 42 °C and 45 °C for both 1 h and 2 h, as described [28]. Following heat shock, the larvae and pupae were resumed back to controlled laboratory conditions.

2.3 Study on survival and development under heat stress

Survival, successful molting to next developmental stage and developmental abnormalities, if any, were observed under normal and experimental conditions.

2.4 RNA isolation and semi-quantitative RT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen) from tissues such as dorsal abdominal epidermis, wing disc, fat body and ovary during spinning larval period, and fat body and ovary during pupal stage. The quantification of RNA was performed spectrophotometrically at 260 nm. The purity of total RNA was assessed using A_{260/280} ratios. The denaturing agarose gel RNA electrophoresis was performed to ascertain the integrity of isolated RNA. Up to 5 µg of total RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen) and oligo dT primer. The cDNA was subsequently used as a template for PCR. The target DNA was generated using the gene specific forward and reverse primers and *Taq* polymerase. The primer sequences were hsp90 (forward 5'-CACAAATGGGATACATGGCTGC-3', and

reverse 5'-GATGAGCCGATTCAGGTTGAAG-3'; GenBank Accession No.: AB060275); and rp49 (forward 5'-GCATCAATCGGATCGCTATGAC-3' and reverse 5'-CAAGAAGACCCGTCATATGCT-3'; GenBank Accession No.:AB048205). Ribosomal protein (rp49) was used as internal standard. Multiplex PCR was performed with primer concentrations of 400nM for hsp90 and 200 nM for rp49. The reactions were performed at 95 °C for 10 minutes followed by 35 amplification cycles (95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s), and a final 10 minutes extension period at 72 °C. The amplified products were subjected to 3.5% agarose gel electrophoresis and molecular size was confirmed by running DNA standard. The expression level of hsp90 was calculated relative to rp49 expression for each sample by comparing the band densities of both hsp90 and rp49 using Image-J program.

2.5 Statistical analysis

Student's t-test was performed for the analysis of the data using GraphPad Prism 6 software.

3. Results

3.1 Survival and development of silkworm larvae and pupae under heat stress

The response of spinning larvae and pupae under heat stress was found to be dependent upon the degree of temperature elevation and the duration of its exposure. All spinning larvae and pupae were found to survive and almost all entered into subsequent developmental program, when heat shock was given at 39 °C for 1 h and 2 h respectively (Figs. 1A, B). The heat shock at this temperature did not bring any abnormalities to stressed larvae and pupae. At 42 °C for 1 h exposure, although all larvae and pupae survived and successfully entered into next developmental program, some of them (10-14%) showed abnormal development in the adult stage (Figs. 1A, B, 2). The heat shock exposure for 2 h at 42 °C showed pupal death (6%) as well as disturbed adult metamorphosis (14%) for spinning larvae (Figs. 1A, 2), whereas, for pupae (Figs. 1B, 2), it showed abnormal development in the adult stage (27%). The most observed striking effect of heat shock at this temperature was found to be failure of molting process or abnormalities in development before ensuring the death of affected animals (Fig. 2).

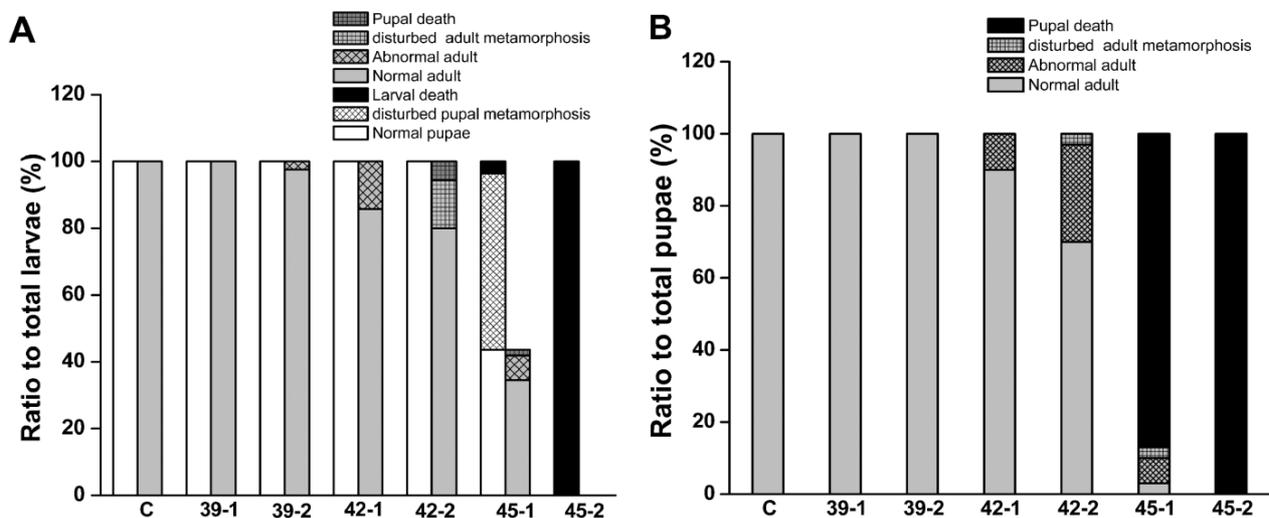


Fig 1: Effect of heat stress on survival and development of silkworm, *Bombyx mori*. (A). heat stress to day 3 spinning larvae (n=40-50). (B). Heat stress to day 2 pupae (n=30). C: control; 39-1: heat shock at 39 °C for 1 h; 39-2: heat shock at 39 °C for 2 h; 42-1: heat shock at 42 °C for 1 h; 42-2: heat shock at 42 °C for 2 h; 45-1: heat shock at 45 °C for 1 h; 45-2: heat shock at 45 °C for 2 h.

The heat stress at 45 °C-1 h to spinning larvae (Fig. 1A) showed normal development till adult stage (35%) as well as disturbed pupal metamorphosis (53%), whereas, 87% of heat stressed pupae died after survival for few days, as evident by

response to a stimulus like touch (Fig. 1B). The heat shock exposure for 2 h at 45 °C caused 100% mortality in all heat stressed larvae and pupae (Figs. 1A, B).

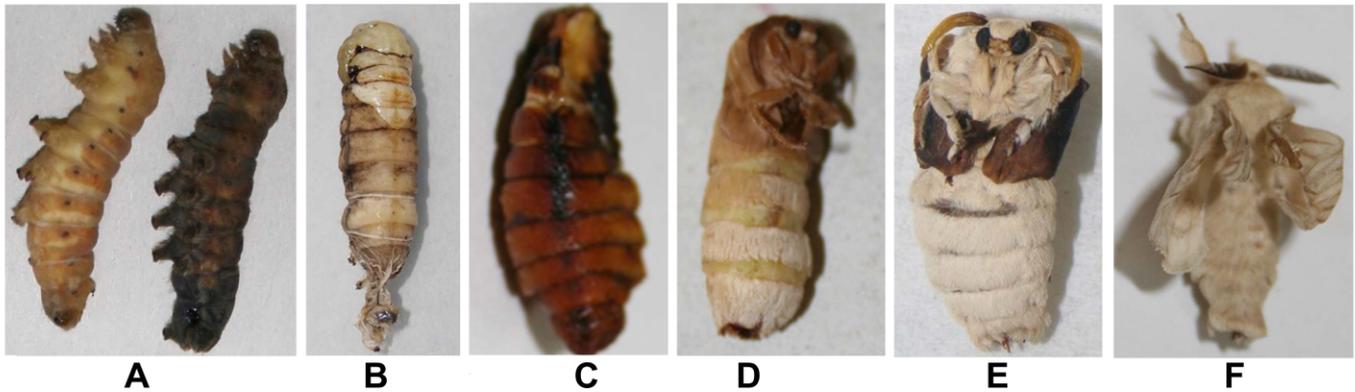


Fig 2: Developmental abnormalities of silkworm, *Bombyx mori* under heat stress. A, B: disturbed pupal metamorphosis; C: abnormal pupa; D, E: disturbed adult metamorphosis; F: abnormal adult.

3.2 Tissue specific expression of hsp90 transcript

Tissue specific expressions of hsp90 transcript were studied in fat body, ovary, dorsal abdominal epidermis and wing disc in spinning larvae, and fat body and ovary of day 2 pupae (Fig. 3). During spinning larval phase, ovary showed less expression of hsp90 transcript than other tissues examined viz., fat body,

dorsal abdominal epidermis and wing disc. During pupal stage, ovary showed more expression than fat body. Thus, ovary displayed a higher level of hsp90 expression in day 2 pupae than in spinning larvae, and fat body showed more expression in spinning larvae than pupae.

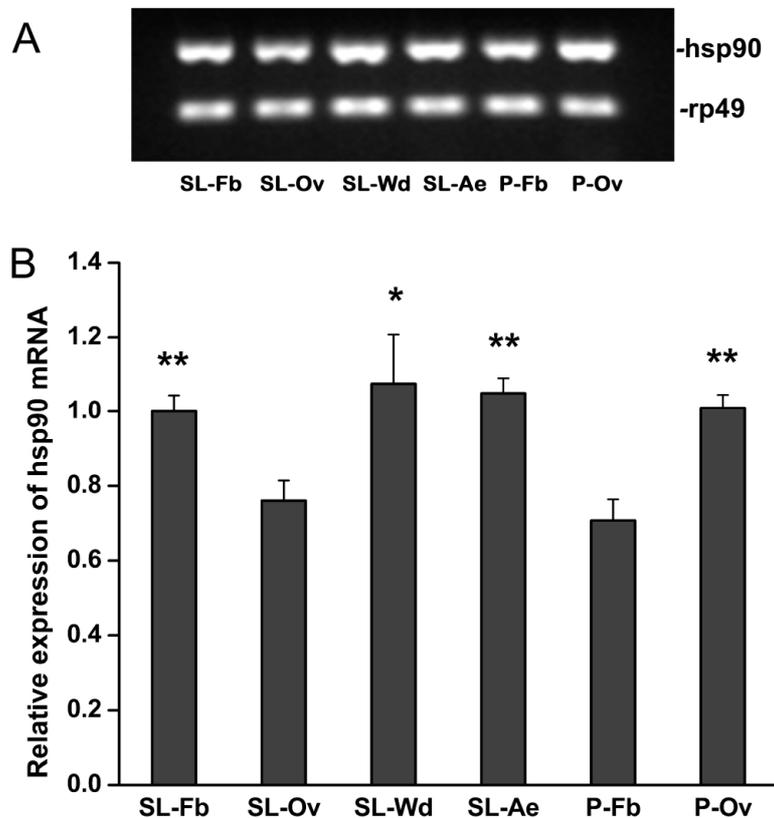


Fig 3: Tissue distribution of hsp90 mRNA in *Bombyx mori*. (A). Expression of hsp90 and rp49 as revealed by semi-quantitative RT-PCR. The expression of rp49 was used as an internal control. (B). Relative expression level of hsp90 as normalized by amount of rp49. The normalized level of hsp90 mRNA in fat body of spinning larvae was designated as 1. Each histogram bar represents the mean relative expression of hsp90 (n=7-9) and the error bar represents standard error of the mean (SEM). The asterisks (* or **) show the data which are significantly different from ovary of spinning larvae or fat body from pupae (unpaired t-test; *p<0.05, ** p<0.01- p<0.001). SL-Fb: fat body from spinning larvae; SL-Ov: ovary from spinning larvae; SL-Wd: wing disc from spinning larvae; SL-Ae: abdominal epidermis from spinning larvae; P-Ov: ovary from day 2 pupae; P-Fb: fat body from day 2 pupae.

3.3 The expression pattern of hsp90 transcript under heat stress

Depending upon the severity of heat stress, tissue- and developmental- specific alterations in the transcript level of hsp90 were found upon heat shock. The heat shock to spinning larvae at 39 °C (1 h & 2 h) and 42 °C (1 h & 2 h) caused a significant up-regulation in the expression of hsp90 transcript level in all examined tissues viz., fat body, ovary, wing disc and dorsal abdominal epidermis (Figs. 4A, B, C). However, at 45 °C (1 h & 2 h), these tissues failed to show any up-

regulation in hsp90 transcript level, except abdominal epidermis, which still maintained a significant increase in its expression level. During pupal stage (Fig. 5A), fat body showed an up-regulation in hsp90 mRNA expression at 39 °C and 42 °C (1 h & 2 h), as found during larval stage. However, pupal ovary showed a very different expression pattern of hsp90 upon heat shock. It didn't show any up-regulation in hsp90 transcript level, when heat shock was given at 39 °C and 42 °C for 1 h and 2 h, whereas, showed a down-regulation in its expression, when heat shock was given at 45°C (Fig. 5B).

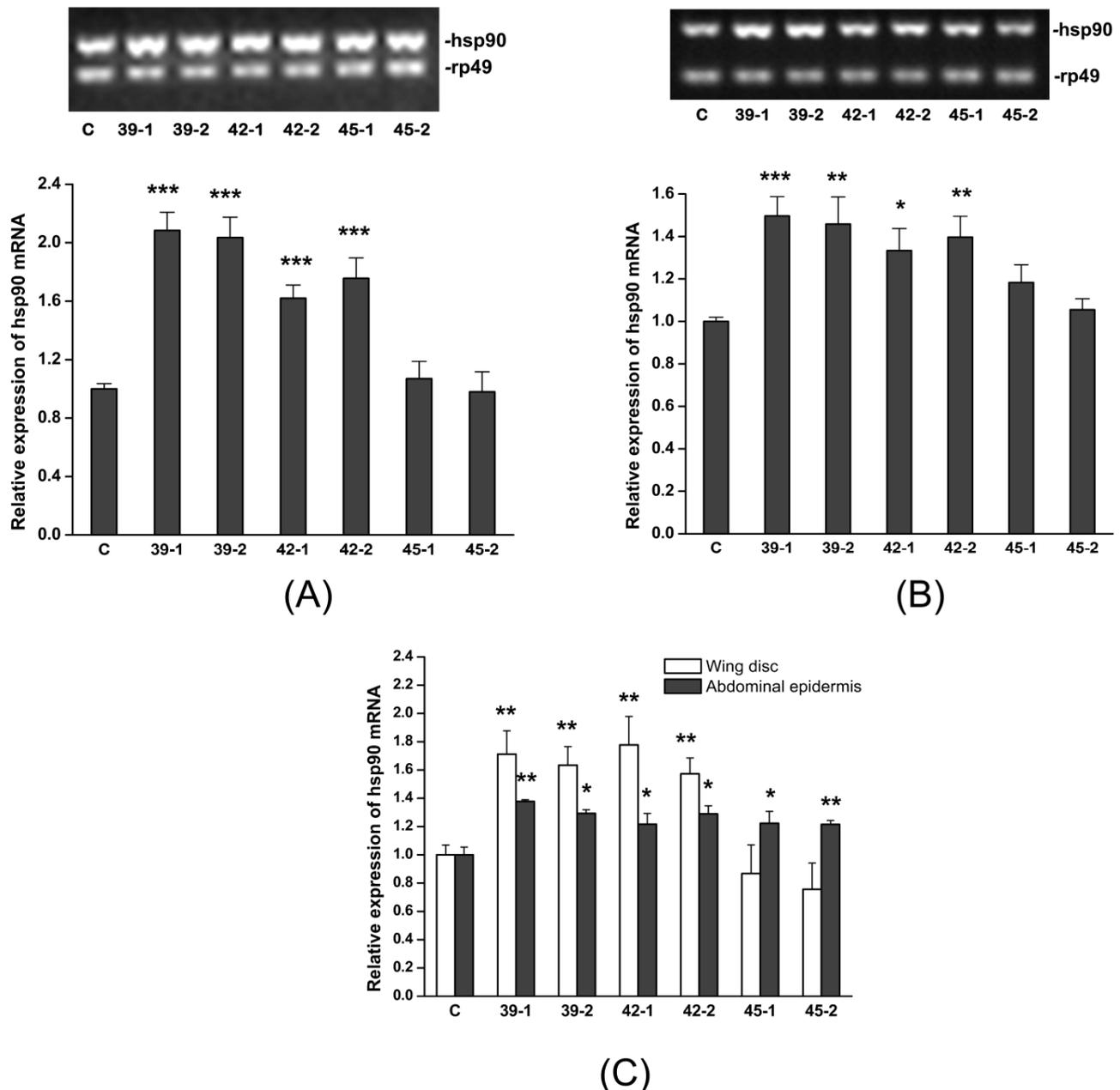


Fig 4: Tissue specific expression of hsp90 mRNA during spinning larval period upon heat shock. (A). Fat body. (B) Ovary. (C) Wing disc and abdominal epidermis. The upper panel showed expression of hsp90 and rp49 as revealed by semi-quantitative RT-PCR. The lower panel showed relative expression level of hsp90 as normalized by amount of rp49. The normalized level of hsp90 mRNA in respective control sample was designated as 1. Each histogram bar represents the mean relative expression of hsp90 (n=6-8) and the error bar represents standard error of the mean (SEM). The asterisks (* or ** or ***) show the data which are significantly different from control sample (unpaired t-test; *p<0.05, **p<0.01, ***p<0.001). The abbreviations used are as mentioned for figure 1.

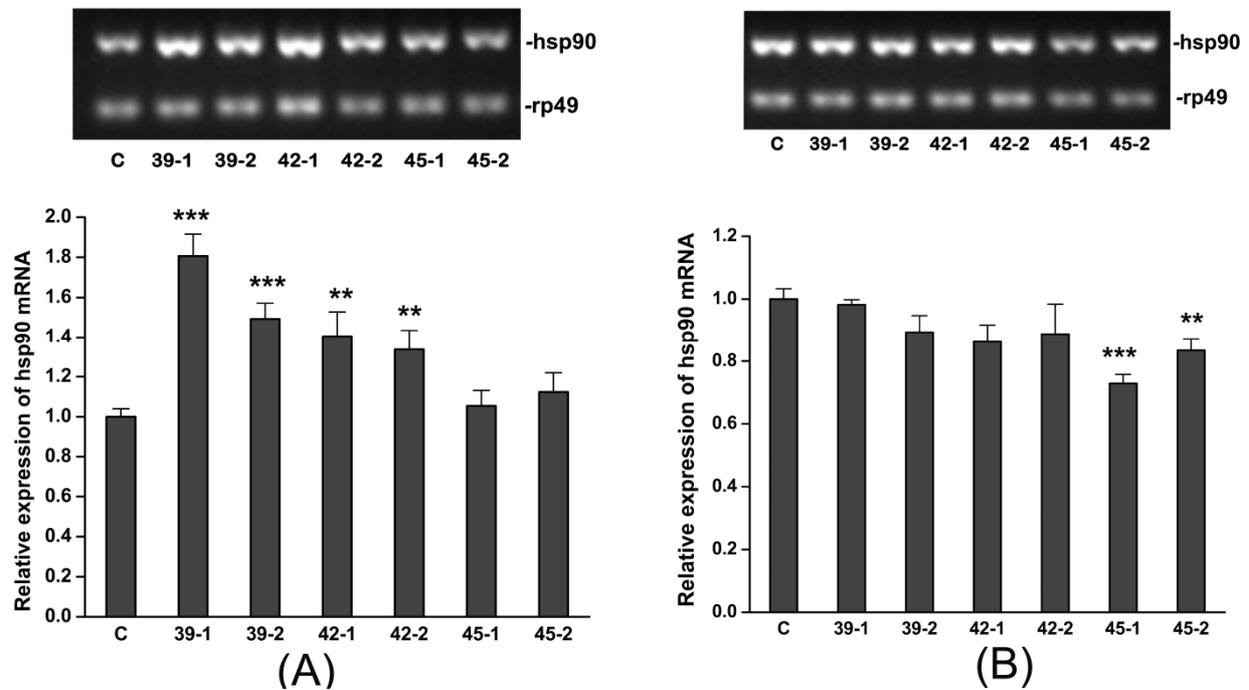


Fig 5: Tissue specific expression of hsp90 mRNA during pupal stage upon heat shock. (A) Fat body. (B) Ovary. The upper panel showed expression of hsp90 and rp49 as revealed by semi-quantitative RT-PCR. The lower panel showed relative expression level of hsp90 as normalized by amount of rp49. The normalized level of hsp90 mRNA in respective control sample was designated as 1. Each histogram bar represents the mean relative expression of hsp90 (n=6-8) and the error bar represents standard error of the mean (SEM). The asterisks (** or ***) show the data which are significantly different from control sample (unpaired t-test; ** p<0.01, ***p<0.001). The abbreviations used are as mentioned for figure 1.

4. Discussion

The present study clearly illustrated the response of silkworm under heat stress, and the tissue and developmental specific expression pattern of hsp90. It was observed that the heat shock response in silkworm depends not only on the severity of heat shock but also on the developmental stages of the animal. The heat shock at 39 °C for 1 h and 2 h exposure was found to be mild, irrespective of the developmental stages, as almost all stressed larvae and pupae were able to survive and entered into successive developmental program. Depending on exposure time, the heat shock at 42 °C was found to be mild-to-severe, whereas, 45 °C proved to be severe-to-deleterious for the survival of stressed larvae and pupae. There was a decrease in thermo-tolerance both in terms of survival and successful entry to next developmental stage when the duration of heat shock exposure was increased from 1 h to 2 h at both 42 °C and 45 °C.

Although studies are available on survival of *Bombyx* under heat stress [24, 25, 29-31], but in the present study, the development of silkworm under heat stress was monitored very carefully and it was observed that at severe heat stressed conditions, although the affected larvae or pupae survived initially but afterwards they showed a failure of molting process or abnormal development before ensuring death. The data also showed that the spinning larvae are more resistant to heat stress than pupae at 42 °C and 45 °C. The heat shock exposure at 45 °C for 1 h showed the survival of almost 35% spinning larvae, whereas it was only 3% for pupae. It was reported that beyond 43°C, even a 10-20 min exposure proved lethal to all the developmental stages of the silkworm [24]. The differences in the findings might be because of the different strains used in the study. It was also shown in some studies [24, 25, 29] that the thermo-tolerance increased as larval development proceeds,

and the present data indicated that spinning larvae are more tolerant to heat stress than pupae. The fifth instar larvae were found to be even more tolerant to heat stress than adult moths [31].

The present study showed a ubiquitous expression of endogenous hsp90 mRNA in all examined tissue but with variations in its expression level between fat body and ovary during spinning larval and pupal stages. A higher hsp90 expression was observed in larval fat body, wing disc and dorsal abdominal epidermis than ovary at spinning period and pupal fat body. During pupal stage the expression was found to be more in ovary than fat body. The ubiquitous expression of hsp90 was also found in cDNA libraries representing different tissues and/or developmental stages of *Bombyx* [4]. In *H. armigera*, the variation in expression of Hsp90 was demonstrated in all developmental stages from fifth instar larvae to day 2 pupae and it was suggested that Hsp90 expression is regulated developmentally [32]. Tissue specific variations in expression of hsp90 was also observed in Chinese oak silkworm, *Antheraea pernyi*, where testis during pupal stage showed a higher expression than hemocytes, fat bodies, midgut and ovaries [33]. The finding that spinning larval fat body has higher endogenous hsp90 transcript level than pupal fat body is interesting but is difficult to explain. At the former time it is primarily taking up and storing the storage proteins for future use, whereas in the pupa it is breaking down those proteins to fuel adult development. For ovarian tissues, the endogenous expression of hsp90 was found to be more during pupal stage than spinning larval period. However it is premature to explain this data, but it might be that the ovary has not started to develop in the spinning larva whereas it is developing in the pupa. In *Bombyx* the follicular differentiation and the expression of the yolk protein start only after pupation

when ecdysteroid titer is high ^[34-35]. In *Tribolium castaneum*, it was observed that the Hsp83 is expressed in ovary of mature adults, but not in newly hatched beetles which lack mature oocytes ^[9]. It was suggested that the Hsp83 protein could be involved in oogenesis during ovarian maturation as female beetles were not able to produce mature oocytes after knock-down of the hsp83 expression ^[9]. The role of hsp90 in ovary maturation was also suggested in *Penaeus monodon* ^[36].

In the present study, at mild and mild-to severe heat stressed conditions, the up-regulation of hsp90 mRNA was demonstrated in all examined tissues except pupal ovary. This up-regulation of hsp90 in various tissues correlates with the ability of the animal to survive the particular heat shock. The heat shock exposure at a higher temperature (45 °C for 1 h and 2 h) could not elicit any significant up-regulation in hsp90 expression except dorsal abdominal epidermis, which still showed a heat shock response at the lethal temperature of 45°C. The role of hsp90 in protection against heat stress was also demonstrated in other insect species such as *Plutella xylostella*, *Liriomyza huidobrensis* ^[19, 37]. In *Bombyx* larvae, the induction of Hsp90 under heat stress was demonstrated at protein level in fat body and whole body extracts ^[24-26]. As indicated earlier, it was found that at sub-lethal temperature, pupal ovary failed to show any up-regulation in hsp90 expression. During pupal stage, ovary is at highly progressive state of ovarian development in terms of follicular differentiation and the possible induction of hsp90 expression over normal threshold level upon heat shock might have more adverse effect on ovarian development. It was suggested that though Hsps play an important role in helping insect to survive under extreme temperatures, its high level usually brings negative physiological effect on organisms ^[38].

Thus, our study on the up-regulation of hsp90 expression at mRNA level along with earlier studies on Hsp90 expression at protein level in *Bombyx* suggests that Hsp90 play a protective role on the survivability of silkworm larvae and pupae under heat stress. Beside Hsp90, other heat shock proteins have also been evaluated on their role for increased thermotolerance in *Bombyx* ^[22-23]. Further studies will provide us more understanding on the role of Hsp90 in *Bombyx* development with particular reference to its tissue- and developmental-specific expression as many of the heat shock protein genes are found to be developmentally regulated and important for insect adaptability with climate changes ^[39]. The expression of hsp90 can also be used as a biomarker for stress in sericulture industry, as its induction was found to be more rapid than traditional marker such as growth inhibition and death.

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6. References

1. Feder ME, Hofmann GE. Heat shock proteins, molecular chaperones, and the stress response: evolutionary and

ecological physiology. Annual Review of Physiology 1999; 61:243–282.

2. Sorensen JG, Kristensen TN, Loeschcke V. The evolutionary and ecological role of heat shock proteins. Ecological Letters 2003; 6:1025–1037.
3. Huang LH, Wang HS, Kang L. Different evolutionary lineages of large and small heat shock proteins in eukaryotes. Cell Research 2008; 18:1074–1076.
4. Landais I, Pommata JM, Mitab K, Nohatab J, Gimenez S, Fourniera P *et al.* Characterization of the cDNA encoding the 90 kDa heat-shock protein in the Lepidoptera *Bombyx mori* and *Spodoptera frugiperda*. Gene 2001; 271:223-231.
5. Chen B, Kayukawa T, Monteiro A, Ishikawa Y. The expression of the *HSP90* gene in response to winter and summer diapause and thermal-stress in the onion maggot, *Delia antiqua*. Insect Molecular Biology 2005; 14:697-702.
6. Sakano D, Li B, Xia Q, Yamamoto K, Fujii H, Aso Y. Genes encoding small heat shock proteins of the silkworm, *Bombyx mori*. Bioscience Biotechnology and Biochemistry 2006; 70:2443–2450.
7. Moribe Y, Oka K, Niimi T, Yamashita O, Yaginuma T. Expression of heat shock protein 70a mRNA in *Bombyx mori* diapause eggs. Journal of Insect Physiology 2010; 56:1246–1252.
8. Zhang Q, Denlinger DL. Molecular characterization of heat shock protein 90, 70 and 70 cognate cDNAs and their expression patterns during thermal stress and pupal diapause in the corn earworm. Journal of Insect Physiology 2010; 56:138–150.
9. Xu J, Shu J, Zhang Q. Expression of the *Tribolium castaneum* (Coleoptera: Tenebrionidae) hsp83 gene and its relation to oogenesis during ovarian maturation. Journal of Genetics and Genomics 2010; 37:513-522.
10. Xu Q, Zou Q, Zheng H, Zhang F, Tang B, Wang S. Three heat shock proteins from *Spodoptera exigua*: gene cloning, characterization and comparative stress response during heat and cold shocks. Comparative Biochemistry and Physiology Part B 2011; 159:92–102.
11. Liu QN, Zhu BJ, Dai LS, Fu WW, Lin KZ, Liu CL. Overexpression of small heat shock protein 21 protects the Chinese oak silkworm *Antheraea pernyi* against thermal stress. Journal of Insect Physiology 2013; 59:848-854.
12. Chen B, Zhong D, Monteiro A. Comparative genomics and evolution of the *HSP90* family of genes across all kingdoms of organisms. BMC Genomics 2006; 7:156.
13. Young JC, Moarefi I, Hartl FU. *Hsp90*: a specialized but essential protein-folding tool. Journal of Cell Biology 2001; 154:267-273.
14. Salathia N, Queitsch C. Molecular mechanisms of canalization: *Hsp90* and beyond. Journal of Biosciences 2007; 32:457-463.
15. Sawarkar R, Paro R. Hsp90@chromatin.nucleus: an emerging hub of a networker. Trends in Cell Biology 2013; 23:193-201.
16. Ding D, Parkhurst SM, Halsell SR, Lipshitz HD. Dynamic Hsp83 RNA localization during *Drosophila* oogenesis and embryogenesis. Molecular and Cellular Biology 1993; 13:3773–3781.
17. Yue L, Karr TL, Nathan DF, Swift H, Srinivasan S, Lindquist S. Genetic analysis of viable *Hsp90* alleles reveals a critical role in *Drosophila* spermatogenesis. Genetics 1999; 151:1065–1079.

18. Tachibana SI, Numata H, Goto SG. Gene expression of heat-shock proteins (*Hsp23*, *Hsp70* and *Hsp90*) during and after larval diapause in the blow fly *Lucilia sericata*. *Journal of Insect Physiology* 2005; 51:641–647.
19. Sonoda S, Ashfaq M, Tsumuki H. Cloning and nucleotide sequencing of three heat shock protein genes (*hsp90*, *hsc70*, and *hsp19.5*) from the diamondback moth, *Plutella xylostella* (L.) and their expression in relation to developmental stage and temperature. *Archives of Insect Biochemistry and Physiology* 2006; 62:80–90.
20. Song Y, Fee L, Lee TH, Wharton RP. The molecular chaperone *Hsp90* is required for mRNA localization in *Drosophila melanogaster* embryos. *Genetics* 2007; 176:2213–2222.
21. Astakhova LN, Zatssepina OG, Przhiboro AA, Evgen'ev MB, Garbuz DG. Novel arrangement and comparative analysis of *hsp90* family genes in three thermotolerant species of Stratiomyidae (Diptera). *Journal of Insect Physiology* 2013; 22:284–296.
22. Li J, Moghaddam SH, Du X, Zhong BX, Chen YY. Comparative analysis on the expression of inducible HSPs in the silkworm, *Bombyx mori*. *Molecular Biology Reports* 2012; 39:3915–3923.
23. Velu D, Ponnuvel KM, Qadri SMH. Expression of heat shock protein genes in response to thermal stress in silkworm *Bombyx mori*. *International Journal of Industrial Entomology* 2008; 16:21–27.
24. Joy O, Gopinathan KP. Heat shock response in mulberry silkworm races with different thermotolerances. *Journal of Biosciences* 1995; 20:499–513.
25. Vasudha BC, Aparna HS, Manjunatha HB. Impact of heat shock on heat shock proteins expression, biological and commercial traits of *Bombyx mori*. *Insect Science* 2006; 13:243–250.
26. Aparna HS, Rajesh RK, Manjunatha HB. Molecular characterization of heat shock proteins 90 (HSP83?) and 70 in tropical strains of *Bombyx mori*. *Proteomics* 2010; 10:2734–2745.
27. Ponnuvel KM, Murthy GN, Awasthi AK, Rao G, Vijayaprakash NB. Differential gene expression during early embryonic development in diapause and non-diapause eggs of multivoltine silkworm *Bombyx mori*. *Indian Journal of Experimental Biology* 2010; 48:1143–1151.
28. Fittinghoff CM, Riddiford LM. Heat sensitivity and protein synthesis during heat shock in the tobacco hornworm, *Manduca sexta*. *Journal of comparative Physiology B* 1990; 160:349–356.
29. Manjunatha HB, Rajesh RK, Aparna HS. Silkworm thermal biology: A review of heat shock response, heat shock proteins and heat acclimation in the domesticated silkworm, *Bombyx mori*. *Journal of Insect Science* 2010; 10:204.
30. Howrelia JH, Patnaik BB, Selvanayagam M, Rajkumar S. Impact of temperature on heat shock protein expression of *Bombyx mori* cross-breed and effect on commercial traits. *Journal of Environmental Biology* 2011; 32:99–103.
31. Sinha S, Sanyal S. Acclimatization to heat stress in Nistari race of *Bombyx mori*. *Journal of Entomology and Zoology Studies* 2013; 1:61–65.
32. Liu W, Zhang FX, Cai MJ, Zhao WL, Li XR, Wang JX *et al.* The hormone-dependent function of *Hsp90* in the crosstalk between 20-hydroxyecdysone and juvenile hormone signaling pathways in insects is determined by differential phosphorylation and protein interactions. *Biochimica et Biophysica Acta* 2013; 1830:5184–5192.
33. Chen L, Zhu B, Wei G, Yu L, Xu Y, Liu C *et al.* Identification of HSP90 gene from the Chinese oak silkworm, *Antheraea pernyi*. *African Journal of Biotechnology* 2012; 11:1512–11519.
34. Hanaoka K, Ohnishi E. Changes in ecdysone titre during pupal-adult development in the silkworm, *Bombyx mori*. *Journal of Insect Physiology* 1974; 20:2375–2384.
35. Tsuchida K, Nagata M, Suzuki A. Hormonal control of ovarian development in the silkworm, *Bombyx mori*. *Archives of Insect Biochemistry and Physiology* 1987; 5:167–177.
36. Jiang S, Qiu L, Zhou F, Huang J, Guo Y, Yang K. Molecular cloning and expression analysis of a heat shock protein (*Hsp90*) gene from black tiger shrimp (*Penaeus monodon*). *Molecular Biology Reports* 2009; 36:127–134.
37. Huang LH, Kang L. Cloning and interspecific altered expression of heat shock protein genes in two leafminer species in response to thermal stress. *Journal of Insect Physiology* 2007; 16:491–500.
38. Shen Y, Gong YJ, Gu J, Huang LH, Feng QL. Physiological effect of mild thermal stress and its induction of gene expression in the common cutworm, *Spodoptera litura*. *Journal of Insect Physiology* 2014; 61:34–41.
39. Zhao L, Jones WA. Expression of heat shock protein genes in insect stress responses. *Invertebrate Survival Journal* 2012; 9:93–101.