

MINIREVIEW

Expression of heat shock protein genes in insect stress responses**L Zhao, WA Jones***Biological Control of Pests Research Unit, National Biological Control Laboratory, Agricultural Research Service, United States Department of Agriculture, P.O. Box 67, Stoneville, MS 38776, USA**Accepted May 14, 2012***Abstract**

The heat shock proteins (Hsps) that are abundantly expressed in insects are important modulators of insect survival. The expression of different Hsp genes are induced and modulated in insects in response to environmental inputs including abiotic stresses such as heat shock, ultraviolet radiation, chemical pesticides, as well as biotic stresses such as viruses, bacteria, fungi and other insects. This minireview will provide useful information related to the expression of Hsp genes in response to abiotic and biotic stressors as well as developmental regulation and modulation of Hsp genes involved with insect survival.

Key Words: heat shock protein (Hsp); gene expression; abiotic stress; biotic stress

Introduction

Insects respond to elevated temperature and to a variety of chemical and physical stresses by a rapid increase in the synthesis of a set of conserved polypeptides collectively referred to as heat shock proteins (Hsps). Hsps, named according to their molecular weight, such as Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, small Hsp (sHsp) and Hsp10, are a class of functionally related proteins involved in the folding and unfolding of other proteins. Ritossa first reported that heat and the metabolic uncoupler dinitrophenol induced a characteristic pattern of puffing in salivary gland chromosomes in the fruit fly, *Drosophila busckii* (Ritossa, 1962, 1963). This discovery eventually led to the identification of Hsps which were represented by these puffs. Increased synthesis of selected proteins in the cells of *Drosophila* following stresses such as heat shock was first reported in 1974 (Tissieres *et al.*, 1974). An enormous literature has now accumulated that describes a wide variety of events in a cell's response to a wide array of biotic and abiotic sources of stress in a variety of insects (Lindquist, 1981; Schlesinger, 1990; Wu, 1995; Garcia *et al.*, 2002, Lakhota, 2011).

Hsps are found in practically all living organisms, from bacteria to humans (Nevins, 1982; Wu *et al.*, 1985; Walter *et al.*, 1989; Marrs *et al.*, 1993; Zhang *et al.*, 1998; Kanagasabai *et al.*, 2011). More than 7,000 related Hsps papers have been published in various journals. Table 1 lists Hsp genes expressed by insects in response to environmental stresses that have been published during past three decades. This minireview is focused on the expression of Hsp genes in response to abiotic and biotic stressors as well as developmental regulation.

Abiotic stress responses

Insects respond to elevated temperatures and to chemical and other stresses by an increase in the synthesis of Hsps. Hsps appear to serve a significant role in the insect's responses to abiotic stressors such as elevated temperature (Garcia *et al.*, 2003, Huang *et al.*, 2007; Wang *et al.*, 2008; Kostal and Tollarova-Borovanska, 2009; Zhao *et al.*, 2009, 2010a), ultraviolet radiation (Rangel *et al.*, 2008, Nguyen *et al.*, 2009), drought and dehydration (Xu *et al.*, 2010, Cornette and Kikawada 2011), anhydrobiosis (Lopez-Martinez *et al.*, 2009, Gusev *et al.*, 2010, Cornette and Kikawada, 2011), chemical (Planello *et al.*, 2008, 2011), metal (Shu *et al.*, 2010; Zhao *et al.*, 2010b), nutrient (Benoit *et al.*, 2011), injury or adaptation (Colinet *et al.*, 2009; Kostal and Tollarova-Borovanska, 2009), hypoxia (Michaud *et al.*, 2011) and double stranded RNA (Benoit *et al.*, 2009, Kostal and Tollarova-Borovanska, 2009; Lu and Wan, 2011).

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Table 1 Examples of heat shock protein gene expression in insects

Species	Heat shock proteins	References
<i>Aedes aegypti</i>	Hsp26, Hsp83, Hsp70, Hsc70	Zhao <i>et al.</i> , 2009
<i>Aedes aegypti</i>	Hsp26, Hsp83, Hsp70	Zhao <i>et al.</i> , 2010a
<i>Aedes aegypti</i> , arthropods	Hsp70	Benoit <i>et al.</i> , 2011
<i>Aedes aegypti</i> , <i>Anopheles gambiae</i> , <i>Culex pipiens</i>	Hsp70, Hsp90	Benoit <i>et al.</i> , 2009
<i>Apis mellifera</i>	Hsp70	Elekonich, 2009
<i>Belgica antarctica</i>	sHsp*, Hsp70, Hsp90	Lopez-Martinez <i>et al.</i> , 2008; Lopez-Martinez <i>et al.</i> , 2009
<i>Belgica antarctica</i>	sHsp, hsp70, Hsp90	Rinehart <i>et al.</i> , 2006
<i>Bemisia tabaci</i>	Hsps**	Mahadav <i>et al.</i> , 2009
<i>Bemisia tabaci</i>	Hsp23, Hsp70, Hsp90	Lu and Wan, 2011
<i>Bombyx mori</i>	Hsp40, Hsp70, Hsp90, Hsc70***	Hong <i>et al.</i> , 2010
<i>Bombyx mori</i>	Hsp20.4, Hsp40, Hsp70, Hsp90	Ponnuvel <i>et al.</i> , 2010
<i>Bombyx mori</i>	Hsps, Hsp83, Hsp70, Hsp 90, Hsp 84, Hsp62, Hsp60, Hsp52, Hsp33	Sosalegowda <i>et al.</i> , 2010
<i>Calanus finmarchicus</i>	Hsp21, Hsp22, p26, Hsp90, Hsp70	Aruda <i>et al.</i> , 2011
<i>Chironomus ramosus</i>	Hsp70	Datkhile <i>et al.</i> , 2010
<i>Chironomus riparius</i>	Hsp40, Hsp90	Park and Kwak, 2008
<i>Chironomus riparius</i>	Hsp70	Planello <i>et al.</i> , 2008
<i>Chironomus riparius</i>	Hsp70, Hsc70	Planello <i>et al.</i> , 2011
<i>Chortoicetes terminifera</i>	Hsp40, Hsc70, Hsp90, Hsp20.5, Hsp20.6, Hsp20.7	Chapuis <i>et al.</i> , 2011
<i>Culex quinquefasciatus</i>	Hsp70	Zhao <i>et al.</i> , 2010b
<i>Drosophila melanogaster</i>	Hsps	Lindquist, 1981
<i>Drosophila</i>	sHsps, Hsp22	Berger <i>et al.</i> , 1985
<i>Drosophila melanogaster</i>	Hsp23, Hsp27	Dubrovsky <i>et al.</i> , 1994
<i>Drosophila</i>	Hsp70	Zhang and Odenwald, 1995
<i>Drosophila melanogaster</i>	Hsp70, Hsp26	Lohe <i>et al.</i> , 1995
<i>Drosophila melanogaster</i>	Hsp23	Dubrovsky <i>et al.</i> , 1996
<i>Drosophila melanogaster</i>	Hsp22, Hsp23, Hsp26, Hsp27, Hsp40, Hsp60, Hsp67Ba, Hsp68, Hsp70Aa, Hsc70-1, Hsp83	Colinet <i>et al.</i> , 2009
<i>Drosophila</i>	Hsp90	Pflanz and Hoch, 2000
<i>Drosophila melanogaster</i>	Hsps	Takahashi <i>et al.</i> , 2011
<i>Galleria mellonella</i>	Hsp90	Wojda and Jakubowicz, 2007
<i>Lepinotus reticulatus</i> , <i>Liposcelis entomophila</i>	Hsp70, Hsp23, Hsp27	Guedes <i>et al.</i> , 2008
<i>Liriomyza huidobrensis</i>	Hsp90, Hsp70, Hsp60, Hsp40, Hsp 20	Huang <i>et al.</i> , 2007
<i>Manduca sexta</i>	Hsp70/Hsc70	Rybczynski and Gilbert, 1995
<i>Penaeus monodon</i>	Hsp21, Hsp70, Hsp90	Rungrassamee <i>et al.</i> , 2010
<i>Plodia interpunctella</i>	sHsp, Hsc70, Hsp90	Shim <i>et al.</i> , 2008
<i>Polypedilum vanderplanki</i>	Hsps	Cornette <i>et al.</i> , 2010
<i>Polypedilum vanderplanki</i>	Hsp90, Hsp70, Hsc70, Hsp60, Hsp20, Hsp23	Gusev <i>et al.</i> , 2011
<i>Pteromalus puparum</i>	Hsc70	Wang <i>et al.</i> , 2008
<i>Pyrrhocoris apterus</i>	Hsp70, Hsc70	Kostal and Tollarova-Borovanska, 2009
<i>Sarcophaga crassipalpis</i>	Hsp90, Hsp70, Hsp60, Hsp40, sHsps	Michaud <i>et al.</i> , 2011
<i>Sarcophaga crassipalpis</i>	Hsps, Hsp23, Hsp70, Hsp90	Rinehart <i>et al.</i> , 2007
<i>Sarcophaga crassipalpis</i>	Hsp23, Hsp70, Hsp90	Hayward <i>et al.</i> , 2005
<i>Spodoptera frugiperda</i>	Hsp70, Hsp70	Lyupina <i>et al.</i> , 2010
<i>Spodoptera litura</i>	Hsp70, Hsp90	Shu <i>et al.</i> , 2010
<i>Stratiomys singularior</i>	Hsp70, Hsp68	Garbuz <i>et al.</i> , 2011
<i>Steinernema carpocapsae</i>	Hsps	Hao <i>et al.</i> , 2009
<i>Tribolium castaneum</i>	Hsp83	Xu <i>et al.</i> , 2010

* Small heat shock proteins.

** Heat shock protein family.

***Heat shock protein chaperones and modulation of *Hsp* genes involved with insect survival.

Temperature

Hsps can protect cells and organisms from thermal damage. In the red flour beetle, *Tribolium castaneum*, the expression of the *Hsp83* gene could be induced with heat stress at 40 °C for 1 h in teneral and mature beetles (Xu *et al.*, 2010). High temperature can alter gene expression including Hsps and other genes in a vector mosquito population using suppression subtractive hybridization (Zhao *et al.*, 2009). *AeaHsp26* and *AeaHsp83* are important markers of stress and may function as critical proteins to protect and enhance survival of *Aedes aegypti* larvae and pupae (Zhao *et al.*, 2010a). Different sequential thermal shocks can trigger different mechanisms of cellular protection against stress in the cone-nose bug, *Panstrongylus megistus*, allowing the insect to adapt to different ecosystems (Garcia *et al.*, 2002).

Pretreating insects with a mild heat stressor can induce expression of Hsp genes and result in protection from subsequent stresses. This phenomenon has been termed "rapid heat hardening" and is apparently caused by the resolubilization of proteins that were denatured during the stressing episode (Huang *et al.*, 2007; Manwell and Heikkila, 2007; de Crecy *et al.*, 2009; Elekonich 2009; Mahadav *et al.*, 2009; Rangel *et al.*, 2010). Mild heat hardening improves thermotolerance of the pea leafminer, *Liriomyza huidobrensis*, which significantly increased the expression of mRNA levels of *Hsp70* and *Hsp20* but at the cost of impairment of fecundity (Huang *et al.*, 2007). The induced expression of mRNA may play an important role in balancing the functional tradeoff of thermal protection and reproductive impairment (Huang *et al.*, 2007). Hsps also play important roles of the recovery phase for repairing chilling injuries (Colinet *et al.*, 2009).

It has been demonstrated that expressed levels of Hsp genes of males and females are different in the silverleaf whitefly, *Bemisia tabaci* (Lu and Wan, 2011). The survival rate of females fed dsRNA significantly decreased following exposure to 44 °C for 1 h, but male survival rate was not significantly affected (Lu and Wan, 2011). Their study also revealed that the optimum mRNA expression of Hsp genes in females promoted a higher survival rate under heat shock conditions; *Hsp23* and *Hsp70* played a key role in heat tolerance in females but not in males, and *Hsp90* showed no significant role in heat tolerance in either females or males (Lu and Wan, 2011). Antarctic flightless midge, *Belgica antarctica*, has adapted in the Antarctica's terrestrial environment, whose larvae survived the lengthy austral winter to complete their two years life cycle (Rinehart *et al.*, 2006). In survival strategies, larvae *B. antarctica* constitutively up-regulates its heat shock proteins and maintain a high inherent tolerance to temperature stress (Rinehart *et al.*, 2006). The midge larvae have adopted the unusual strategy of expressing Hsps continuously, possibly to facilitate proper protein folding in a cold habitat to enhance thermotolerance (Rinehart *et al.*, 2006).

Ultraviolet radiation

Solar radiation can be important sources of abiotic stress for herbivorous insects living in close association with plants. Greater homeostatic capabilities as revealed at the proteomic level could explain the higher tolerance of the alate morph of the aphid, *Macrosiphum euphorbiae*, to environmental stress and its more stable performance and fitness (Nguyen *et al.*, 2009). A tropical species of radiation-tolerant midge, *Chironomus ramosus*, has been shown to express elevated levels of *Hsp70* mRNA and proteins in salivary gland cells of larvae immediately after gamma radiation exposure (Datkhile *et al.*, 2011). The expressed *Hsp70* might be one of the gamma radiation-induced stress proteins required during the early stages of radiation stress management in aquatic midge larvae (Datkhile *et al.*, 2011). Ultraviolet radiation can affect cross protection. Elevated tolerance to UV radiation and heat-shock may be induced in conidia produced by fungi exposed to sublethal stresses other than heat or UV radiation during mycelial growth (Rangel *et al.*, 2008).

Drought dehydration and anhydrobiosis

Some insects are able to survive the loss of almost all their body water content, entering a latent state known as anhydrobiosis. Hsp genes were identified as important up-regulated genes for anhydrobiosis in the sleeping chironomid, *Polypedilum vanderplanki* (Cornette *et al.*, 2011). Expression of the *Hsps* mRNA in response to dehydration in the *A. aegypti*, *Anopheles gambiae* and *Culex pipiens* is different, and knock-down expressions of the transcripts using RNAi have revealed potential functions of the Hsps in maintenance of water balance in these mosquito species (Benoit *et al.*, 2009). Interestingly, it has been demonstrated that in the flesh fly, *Sarcophaga crassipalpis*, expression levels for most of the Hsp genes were significantly up-regulated during hypoxia, suggesting an important role for Hsp genes in responding to low oxygen environments (Michaud *et al.*, 2011).

The molecular responses of dehydration, rehydration and overhydration were investigated in larvae of the Antarctic midge, *B. antarctica* (Lopez-Martinez *et al.*, 2009). Using suppression subtractive hybridization, heat shock proteins (sHsp, *Hsp70*, *Hsp90*) were found the most responsive to changes in the hydration state in all genes examined (Lopez-Martinez *et al.*, 2009). The authors speculated that the midge larvae are thus responding quickly to water loss and gain by expressing genes that encode Hsps and other proteins contributing to maintenance of proper protein function, protection and overall cell homeostasis during times of osmotic flux, a challenge that is particularly acute in the Antarctic environment (Lopez-Martinez *et al.*, 2009).

Chemicals and metals

Expression of *Hsp70* and other Hsp genes was significantly induced after exposure of oriental leafworm moths, *Spodoptera litura*, to zinc. The

results also showed that the induced response of *S. litura* Hsp90 to zinc was more sensitive than that of Hsp70, whereas the inhibited response of Hsp70 was much stronger than that of Hsp90 (Shu *et al.*, 2010). Magnesium is crucial for baculovirus transmission in *Culex nigripalpus* and *Culex quinquefasciatus* larvae. Target transcripts up/downregulated by magnesium included Hsp70. Magnesium can alter gene transcription in a vector mosquito population, and understanding this process can provide insight into the mechanistic role of magnesium in baculovirus transmission (Zhao *et al.*, 2010b). Chromosomal responses to heat and heavy metal shocks were studied in the trichogen polytene chromosomes of the Australian sheep blowfly, *Lucilia cuprina* (Joshi and Tiwari, 2000). Arsenate and mercury, two of the most common toxic environmental chemical pollutants, also induced almost the same set of puffs, suggesting that a common set of gene loci encoding heat shock proteins is responsive to diverse environmental stresses (Joshi and Tiwari, 2000). The function of Hsps and other genes has been recently studied using dsRNA interference (RNAi) knock down techniques (Benoit *et al.*, 2009; Kostal and Tollarova-Borovanska, 2009; Papaconstantinou *et al.*, 2010).

In addition, heat treatment can be used as a control tactic against stored-product insects such as the psocid, *Liposcelis entomophila*, a major concern in stored grain (Guedes *et al.*, 2008).

Biotic stress responses

Biotic stress mainly refers to the stress that occurs as a result of damage to plants and animals by other living organisms such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Recently, many reports have shown that Hsp genes are induced by virus, bacteria, fungi, and insects to confer protection against stressors (Selkirk *et al.*, 1987; Wojda and Jakubowicz, 2007; Mahadav *et al.*, 2009; Hong *et al.*, 2010; Lyupina *et al.*, 2010; Rungrassamee *et al.*, 2010; Ying and Feng, 2011). High population densities are involved in resistance to other ecologically relevant types of stresses (Chapuis *et al.*, 2011).

Parasites

Stressor-induced tissue damage is involved in various diseases. Parasites are undoubtedly a biotic factor that produces stress. Parasitoid virulence and host resistance are complex interactions depending on metabolic rate and cellular activity. Among other factors, natural control of variably susceptible host populations by aphid parasitoids is more likely at moderate to high temperatures (Bensadia *et al.*, 2006). *Serratia symbiotica* is a facultative symbiont of pea aphids (*Acyrtosiphon pisum*) that provides tolerance to heat stress. Although *S. symbiotica* has a major influence on its host's metabolism and resistance to heat, it induces little change in gene expression in its host (Burke and Moran, 2011).

Envenomization by the ectoparasitoid, *Bracon hebetor*, on the expression of *sHsp*, *Hsc70* and *Hsp90* in the lepidopteran host, the Indian meal moth, *Plodia interpunctella*, suggested that upregulation of Hsp genes may produce potent factors that have important roles in the mechanism of host-parasitoid relationships (Shim *et al.*, 2008). *Steinernema carpocapsae* is an insect-parasitic nematode widely used in pest control programs. Hsp genes have been detected in the cDNA library of the parasitic phase of *S. carpocapsae*, and has provided useful information for the study of the parasitic mechanisms exhibited by this parasitoid (Hao *et al.*, 2009).

Cross-protection

Cross-protection occurred in the honeycomb moth, *Galleria mellonella*, when larvae were exposed to mild heat-shock at 38 °C, showing an enhanced humoral immune response after microbial infection in comparison to infected animals grown at 28 °C, and was correlated with the changes in Hsp90 protein and increased level of 55kDa protein, suggesting Hsp90 may play a significant role in converging pathways involved in insect immune response and heat-shock (Wojda and Jakubowicz, 2007).

The whitefly *B. tabaci* causes tremendous losses to agriculture by direct feeding on plants and by vectoring several families of plant viruses (Mahadav *et al.*, 2009). Using DNA markers and biological characteristics, the *B. tabaci* species complex was shown to have over 10 genetic biotypes, including the most dominant and damaging B and Q biotypes, which differ considerably in fecundity, host range, insecticide resistance, virus vectoring ability and in the symbiotic bacteria they harbor (Mahadav *et al.*, 2009). Exposing B biotype whiteflies to heat stress changed its gene expression, suggesting that these clear-cut differences between biotype response are due to differences in adaptation of one biotype over another and are partly responsible for observed changes in the local and global distribution of both biotypes (Mahadav *et al.*, 2009).

Pathogens

Baculovirus expression systems are broadly used for recombinant protein production in lepidopteran cells or larvae. In transgenic silkworms using its heat-shock proteins, the expression levels of the transgenes were found to be under the control of a *hsp*-promoter driven by a specific activator (Hong *et al.*, 2010). It has been shown that the His-tagged baculovirus expression system featuring the chaperone effect Hsp70 and HOP70 of transgenic silkworms increased the yield of soluble and functional foreign gene products (Hong *et al.*, 2010). Another study showed that baculoviruses serve as a stress factor that can activate both death-inducing and cytoprotective pathways in infected cells (Lyupina *et al.*, 2010). The infection potentiated the response to heat shock by boosting the Hsp/Hsc70s content in infected cells several-fold

in comparison with uninfected cells (Lyupina *et al.*, 2010). Addition of a known inhibitor of inducible Hsps decreased the rate of viral DNA synthesis in infected cells and markedly suppressed the release of budded viruses, indicating the importance of the heat shock response for baculovirus replication (Lyupina *et al.*, 2010).

There is speculation that an immune response rather than tolerance to these proteins allows, not only for an immediate protection from infection by a variety of pathogens, but also for immune surveillance, an activity of the immune system that eliminates abnormal and damaged cells (Schlesinger, 1990). A sudden increase in temperature results in heat shock stress of cultured shrimp such as the giant tiger prawn, *Penaeus monodon*. Under heat shock conditions, only *Hsp90* was induced in all tissues of *P. monodon* when compared to its untreated level (Rungrassamee *et al.*, 2010). The expression levels of *Hsp70* and *Hsp90* in *P. monodon* were significantly increased after a 3-h exposure to the marine bacterium, *Vibrio harveyi* (Johnson and Shunk) Baumann, where the *Hsp21* transcript was induced later after a 24-h exposure, suggesting putative roles and involvement of Hsp genes as a part of an immune response against *V. harveyi* (Rungrassamee *et al.*, 2010).

Developmental regulation and mutants

Development

Hsp genes are developmentally regulated in the different insects. Diapause, the dormancy common to overwintering insects, evokes a unique pattern of gene expression. Most Hsp genes are up-regulated, which appears to be common to diapause in species representing diverse insect orders including Diptera, Lepidoptera, Coleoptera and Hymenoptera as well as in diapause that occur in different developmental stages including embryos, larvae, pupae and adult stages (Rinehart *et al.*, 2007). One study of the multivoltine silkworm *B. mori* provides an overview of the differential expression levels of metabolic enzyme and Hsp genes in non-diapause and diapause-induced eggs within 48 h after oviposition, confirming the major role of in early embryogenesis (Ponnuvel *et al.*, 2010). It has been demonstrated that up-regulation of Hsp genes during diapause is a major factor contributing to cold-hardiness of overwintering insects (Rinehart *et al.*, 2007). Research on the flesh fly, *Sarcophaga crassipalpis*, has shown *Hsp23* and *Hsp70* are strongly up-regulated during pupal diapause (Rinehart and Denlinger, 2000). Expression patterns of Hsp genes and other genes associated with pupal diapause were reported in *S. crassipalpis* (Hayward *et al.*, 2005). Expression of *Hsp90* gene was downregulated two days after pupariation, while *Hsp23* and *Hsp70* transcripts were up-regulated just after the start of diapause, 5 days after pupariation (Hayward *et al.*, 2005). Although both cold and heat shock evoked elevated expression, the response of *Hsp90* to heat shock and cold shock remained intact during diapause, which indicates differential

regulation of Hsp genes during diapause and in response to thermal injury inflicted on diapausing pupae (Rinehart and Denlinger, 2000). However, expression of most of the Hsp genes examined did not vary in response to diapause, perhaps because the diapause of *Calanus finmarchicus*, a key component of marine food webs, is not associated with extreme environmental conditions (Aruda *et al.*, 2011).

During the early stages of *Drosophila* development, the heat-shock response cannot be induced (Fang *et al.*, 2001). It is thought that the adverse effects on cell cycle and cell growth brought about by *Hsp70* induction must outweigh the beneficial aspects of *Hsp70* induction in the early embryo (Fang *et al.*, 2001). Early *Drosophila* embryos are refractory to heat shock as a result of dHSF nuclear exclusion (Fang *et al.*, 2001). However, the late embryo can respond to heat shock (Fang *et al.*, 2001).

Steroid hormone

In cultured *Drosophila* cells, Northern blot analysis showed that sHsp genes can be induced by high temperature shock and by exposure to physiological doses of the insect molting hormone ecdysterone (Berger *et al.*, 1985). The ecdysone causes dramatic changes in the genetic programs leading to the pupation of *D. melanogaster*, and regulates developmental changes in transcription and chromatin structure of four small hsp genes (Dubrovsky *et al.*, 1996). It has been shown that the ecdysone response element is necessary but not sufficient for full developmental expression of *Hsp23* in the late third instar and that there is another regulatory element (Dubrovsky *et al.*, 1996). In the tobacco hornworm *Manduca sexta*, ecdysteroids coordinate molting and metamorphosis of insects. Ecdysteroids are produced by the prothoracic glands under the acute control of the brain neuropeptide prothoracicotropic hormone, which upregulated *Hsc70* synthesis both translational and transcriptional levels (Rybczynski and Gilbert, 1995).

Mutants

Mutations can be an important means for insects to adapt to various environmental stresses and to survive in new environments. Mutants from *Drosophila* had little effect on two measures commonly used to assess heat tolerance, heat-knockdown time and heat hardening ability, suggesting that more subtle heat-related fitness components need to be examined for the effects of these mutations (Johnson *et al.*, 2011). The quantitative trait locus found in the *Drosophila melanogaster* genome encompassed Hsps and 19 heat-responsive genes, suggesting that they were strong candidates for triggering heat resistance, emphasizing the advantages of genome-wide deficiency screening using isogenic deficiency libraries (Takahashi *et al.*, 2011). During oogenesis in mutant females of the fruit fly, *Drosophila virilis*, following heat stress, there is an increase in early vitellogenic oocyte degradation and some

degradation of late-forming egg chambers (Gruntenko *et al.*, 2003). Gruntenko and his colleague showed that 20-hydroxyecdysone levels change following heat stress in mutant females of *D. virilis* (Gruntenko *et al.*, 2003). Other mutants of the *Mnn1* gene of *D. melanogaster* are hypersensitive to several stressors and display increased genome instability when subjected to conditions such as heat shock, generally regarded as non-genotoxic (Papaconstantinou *et al.*, 2010). Menin, a widespread regulator of heat shock gene expression and a critical factor in the maintenance of genome integrity, also links the stress response to the control of genome stability in *D. melanogaster* (Papaconstantinou *et al.*, 2010).

Conclusions

Hsp genes are induced and modulated in insects in response to environmental factors including abiotic and biotic stresses. *Hsp* genes are also developmentally regulated, which is important for insects to survive and adapt to their environments. The very widespread occurrence of *Hsp* activity in insects will have a significant bearing on insect adaptability as our climate changes. It may be likely that via *Hsp* activity, many pest and beneficial species will be able to adapt to global warming more than previously thought. Changes in environmental conditions can rapidly shift allele frequencies in populations of species linked to evolutionary responses to pollution, global warming and other changes (Hoffmann and Willi, 2008). New technologies such as microarray, suppression subtractive hybridization and quantitative real-time PCR advances promise to accelerate the development of genetic methods including the genomic function for monitoring insects' adaptation to environmental change in several ways. *Hsps* may play an important role in biodiversity. Many exotic invasive species displace native fauna, while others have little or no effect. The mechanisms for the differences often elude explanation. It is likely that *Hsps* could be playing a role in competition for space and resources. If *Hsps* prove to have a more widespread effect on resistance to parasites and diseases, future methods might be developed that allows increased susceptibility of pests and increased resistance to host defense by natural enemies, which would provide a great boost to biological control success while reducing pesticide use. Food security would be enhanced by similar technology development. As new studies reveal the cellular mechanisms of stress responses, new, useful applications will certainly follow.

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References

Aruda AM, Baumgartner MF, Reitzel AM, Tarrant AM. Heat shock protein expression during stress and diapause in the marine copepod

- Calanus finmarchicus*. J. Insect Physiol. 57: 665-675, 2011.
- Benoit JB, Lopez-Martinez G, Phillips ZP, Patrick KR, Denlinger DL. Heat shock proteins contribute to mosquito dehydration tolerance. J. Insect Physiol. 56: 151-156, 2009.
- Benoit JB, Lopez-Martinez G, Patrick KR, Phillips ZP, Krause TB, Denlinger DL. Drinking a hot blood meal elicits a protective heat shock response in mosquitoes. Proc. Natl. Acad. Sci. USA 108: 8026-8029, 2011.
- Bensadia F, Boudreault S, Guay JF, Michaud D, Cloutier C. Aphid clonal resistance to a parasitoid fails under heat stress. J. Insect Physiol. 52: 146-157, 2006.
- Berger EM, Vitek MP, Morganelli CM. Transcript length heterogeneity at the small heat shock protein genes of *Drosophila*. J. Mol. Biol. 186: 137-148, 1985.
- Burke GR, Moran NA. Responses of the pea aphid transcriptome to infection by facultative symbionts. Insect Mol. Biol. 20: 357-365, 2011.
- Chapuis MP, Simpson SJ, Blondin L, Sword GA. Taxa-specific heat shock proteins are over-expressed with crowding in the Australian plague locust. J. Insect Physiol. 57: 1562-1567, 2011.
- Colinet H, Lee SF, Hoffmann A. Temporal expression of heat shock genes during cold stress and recovery from chill coma in adult *Drosophila melanogaster*. FEBS J. 277: 174-185, 2009.
- Cornette R, Kikawada T. The induction of anhydrobiosis in the sleeping chironomid: current status of our knowledge. IUBMB Life 63: 419-429, 2011.
- Cornette R, Kanamori Y, Watanabe M, Nakahara Y, Gusev O, Mitsumasu K, *et al.* Identification of anhydrobiosis-related genes from an expressed sequence tag database in the cryptobiotic midge *Polypedilum vanderplanki* (Diptera; Chironomidae). J. Biol. Chem. 285: 35889-35899, 2010.
- Datkhile KD, Mukhopadhyaya R, Dongre TK, Nath BB. Hsp70 expression in *Chironomus ramosus* exposed to gamma radiation. Int. J. Radiat. Biol. 87: 213-221, 2010.
- de Crecy E, Jaronski S, Lyons B, Lyons TJ, Keyhani NO. Directed evolution of a filamentous fungus for thermotolerance. BMC Biotechnol. 9: 74, 2009.
- Dubrovsky EB, Dretzen G, Bellard M. The *Drosophila* broad-complex regulates developmental changes in transcription and chromatin structure of the 67B heat-shock gene cluster. J. Mol. Biol. 241: 353-362, 1994.
- Dubrovsky EB, Dretzen G, Berger EM. The Broad-Complex gene is a tissue-specific modulator of the ecdysone response of the *Drosophila* hsp23 gene. Mol. Cell Biol. 16: 6542-6552, 1996.
- Elekovich MM. Extreme thermotolerance and behavioral induction of 70-kDa heat shock proteins and their encoding genes in honey bees. Cell Stress Chaperones 14: 219-226, 2009.

- Fang X, Chen T, Tran K, Parker CS. Developmental regulation of the heat shock response by nuclear transport factor karyopherin- α 3. *Development* 128: 3349-3358, 2001.
- Garbuz DG, Yushenova IA, Zatsepina OG, Przhiboro AA, Bettencourt BR, Evgen'ev M B. Organization and evolution of hsp70 clusters strikingly differ in two species of *Stratiomyidae* (Diptera) inhabiting thermally contrasting environments. *BMC Evol. Biol.* 11: 74, 2011.
- Garcia SL, Pacheco RM, Rodrigues VL, Mello ML. Effect of sequential heat and cold shocks on nuclear phenotypes of the blood-sucking insect, *Panstrongylus megistus* (Burmeister) (Hemiptera, Reduviidae). *Mem. Inst. Oswaldo Cruz* 97: 1111-1116, 2002.
- Garcia SL, Garcia NL, Oliveira LR, Rodrigues VL, Mello ML. Experimentally induced heat- and cold-shock tolerance in adult *Panstrongylus megistus* (Burmeister) (Hemiptera, Reduviidae). *Braz. J. Biol.* 63: 449-455, 2003.
- Gruntenko NE, Bownes M, Terashima J, Sukhanova M, Raushenbach IY. Heat stress affects oogenesis differently in wild-type *Drosophila virilis* and a mutant with altered juvenile hormone and 20-hydroxyecdysone levels. *Insect Mol. Biol.* 12: 393-404, 2003.
- Guedes RN, Zhu KY, Opit GP, Throne JE. Differential heat shock tolerance and expression of heat-inducible proteins in two stored-product psocids. *J. Econ. Entomol.* 101: 1974-1982, 2008.
- Gusev O, Cornette R, Kikawada T, Okuda T. Expression of heat shock protein-coding genes associated with anhydrobiosis in an African chironomid *Polypedilum vanderplanki*. *Cell Stress Chaperones* 16: 81-90, 2010.
- Hao YJ, Montiel R, Abubucker S, Mitreva M, Simoes N. Transcripts analysis of the entomopathogenic nematode *Steinernema carpocapsae* induced in vitro with insect haemolymph. *Mol. Biochem. Parasitol.* 169: 79-86, 2009.
- Hayward SA, Pavlides SC, Tammariello SP, Rinehart JP, Denlinger DL. Temporal expression patterns of diapause-associated genes in flesh fly pupae from the onset of diapause through post-diapause quiescence. *J. Insect Physiol.* 51: 631-640, 2005.
- Hoffmann AA, Willi Y. Detecting genetic responses to environmental change. *Nat. Rev. Genet.* 9: 421-432, 2008.
- Hong SM, Yamashita J, Mitsunobu H, Uchino K, Kobayashi I, Sezutsu H, *et al.* Efficient soluble protein production on transgenic silkworms expressing cytoplasmic chaperones. *Appl. Microbiol. Biotechnol.* 87: 2147-2156, 2010.
- Huang LH, Chen B, Kang L. Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in *Liriomyza huidobrensis*. *J. Insect Physiol.* 53: 1199-1205, 2007.
- Johnson TK, Cockerell FE, McKechnie SW. Transcripts from the *Drosophila* heat-shock gene hsr- ω influence rates of protein synthesis but hardly affect resistance to heat knockdown. *Mol. Genet. Genomics* 285: 313-323, 2011.
- Joshi A, Tiwari PK. Chromosomal responses of blowfly *Lucilia cuprina* to heat and heavy metal stress. *Genetica* 109: 211-218, 2000.
- Kanagasabai R, Krishnamurthy K, Druhan LJ, Ilangoan G. Forced expression of heat shock protein 27 (Hsp27) reverses P-glycoprotein (ABCB1)-mediated drug efflux and MDR1 gene expression in Adriamycin-resistant human breast cancer cells. *J. Biol. Chem.* 286: 33289-33300, 2011.
- Kostal V, Tollarova-Borovanska M. The 70 kDa heat shock protein assists during the repair of chilling injury in the insect, *Pyrrhocoris apterus*. *Plos ONE* 4: e4546, 2009.
- Lakhotia SC. Forty years of the 93D puff of *Drosophila melanogaster*. *J. Biosci.* 36: 399-423, 2011.
- Lindquist S. Regulation of protein synthesis during heat shock. *Nature* 293: 311-314, 1981.
- Lohe AR, Lidholm DA, Hartl DL. Genotypic effects, maternal effects and grand-maternal effects of immobilized derivatives of the transposable element mariner. *Genetics* 140: 183-192, 1995.
- Lopez-Martinez G, Elnitsky MA, Benoit JB, Lee RE, Denlinger DL. High resistance to oxidative damage in the Antarctic midge *Belgica antarctica*, and developmentally linked expression of genes encoding superoxide dismutase, catalase and heat shock proteins. *Insect Biochem. Mol. Biol.* 38: 796-804, 2008.
- Lopez-Martinez G, Benoit JB, Rinehart JP, Elnitsky MA, Lee RE, Denlinger DL. Dehydration, rehydration, and overhydration alter patterns of gene expression in the Antarctic midge, *Belgica antarctica*. *J. Comp. Physiol. B* 179: 481-491, 2009.
- Lu ZC, Wan FH. Using double-stranded RNA to explore the role of heat shock protein genes in heat tolerance in *Bemisia tabaci* (Gennadius). *J. Exp. Biol.* 214: 764-769, 2011.
- Lyupina YV, Dmitrieva SB, Timokhova AV, Beljelarskaya SN, Zatsepina OG, Evgen'ev MB, *et al.* An important role of the heat shock response in infected cells for replication of baculoviruses. *Virology* 406: 336-341, 2010.
- Mahadav A, Kontsedalov S, Czosnek H, Ghanim M. Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. *Insect Biochem. Mol. Biol.* 39: 668-676, 2009.
- Manwell LA, Heikkila JJ. Examination of KNK437- and quercetin-mediated inhibition of heat shock-induced heat shock protein gene expression in *Xenopus laevis* cultured cells. *Comp. Biochem. Physiol.* 148A: 521-530, 2007.
- Marrs KA, Casey ES, Capitant SA, Bouchard RA, Dietrich PS, Mettler IJ, *et al.* Characterization of two maize HSP90 heat shock protein genes: expression during heat shock, embryogenesis, and pollen development. *Dev. Genet.* 14: 27-41, 1993.

- Michaud MR, Teets NM, Peyton JT, Blobner BM, Denlinger DL. Heat shock response to hypoxia and its attenuation during recovery in the flesh fly, *Sarcophaga crassipalpis*. *J. Insect Physiol.* 57: 203-210, 2011.
- Nevins JR. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. *Cell* 29: 913-919, 1982.
- Nguyen TT, Michaud D, Cloutier C. A proteomic analysis of the aphid *Macrosiphum euphorbiae* under heat and radiation stress. *Insect Biochem. Mol. Biol.* 39: 20-30, 2009.
- Papaconstantinou M, Pepper AN, Wu Y, Kasimer D, Westwood T, Campos AR, et al. Menin links the stress response to genome stability in *Drosophila melanogaster*. *Plos ONE* 5: e14049, 2010.
- Park K, Kwak IS. Characterization of heat shock protein 40 and 90 in *Chironomus riparius* larvae: effects of di(2-ethylhexyl) phthalate exposure on gene expressions and mouthpart deformities. *Chemosphere* 74: 89-95, 2008.
- Pflanz R, Hoch M. dtrap-1 encodes a novel member of the heat shock super family of proteins and is expressed in derivatives of all three germ layers during *Drosophila* embryogenesis. *Mech. Dev.* 96: 219-222, 2000.
- Planello R, Martinez-Guitarte JL, Morcillo G. The endocrine disruptor bisphenol A increases the expression of HSP70 and ecdysone receptor genes in the aquatic larvae of *Chironomus riparius*. *Chemosphere* 71: 1870-1876, 2008.
- Planello R, Herrero O, Martinez-Guitarte JL, Morcillo G. Comparative effects of butyl benzyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP) on the aquatic larvae of *Chironomus riparius* based on gene expression assays related to the endocrine system, the stress response and ribosomes. *Aquat. Toxicol.* 105: 62-70, 2011.
- 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 J. Exp. Biol.* 48: 1143-1151, 2010.
- Rangel DE, Anderson AJ, Roberts DW. Evaluating physical and nutritional stress during mycelial growth as inducers of tolerance to heat and UV-B radiation in *Metarhizium anisopliae* conidia. *Mycol. Res.* 112: 1362-1372, 2008.
- Rangel DE, Fernandes EK, Dettenmaier SJ, Roberts DW. Thermotolerance of germlings and mycelium of the insect-pathogenic fungus *Metarhizium* spp. and mycelial recovery after heat stress. *J. Basic Microbiol.* 50: 344-350, 2010.
- Rinehart JP, Denlinger DL. Heat-shock protein 90 is down-regulated during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*, but remains responsive to thermal stress. *Insect Mol. Biol.* 9: 641-645, 2000.
- Rinehart JP, Hayward SA, Elnitsky MA, Sandro LH, Lee RE, Denlinger DL. Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. *Proc. Natl. Acad. Sci. USA* 103: 14223-14227, 2006.
- Rinehart JP, Li A, Yocum GD, Robich RM, Hayward SA, Denlinger DL. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *Proc. Natl. Acad. Sci. USA* 104: 11130-11137, 2007.
- Ritossa F. A new puffing pattern induced by temperature shock and DNP in *Drosophila*. *Experientia* 18: 571-573, 1962.
- Ritossa F. New puffs induced by temperature shock, DNP and salicylate in salivary chromosomes of *D. melanogaster*. *Drosophila Information Service* 37: 122-123, 1963.
- Runggrasamee W, Leelatanawit R, Jiravanichpaisal P, Klinbunga S, Karoonuthaisiri N. Expression and distribution of three heat shock protein genes under heat shock stress and under exposure to *Vibrio harveyi* in *Penaeus monodon*. *Dev. Comp. Immunol.* 34: 1082-1089, 2010.
- Rybczynski R, Gilbert LI. Prothoracicotropic hormone-regulated expression of a hsp 70 cognate protein in the insect prothoracic gland. *Mol. Cell. Endocrinol.* 115: 73-85, 1995.
- Schlesinger MJ. Heat shock proteins. *J. Biol. Chem.* 265: 12111-12114, 1990.
- Selkirk ME, Rutherford PJ, Denham D, Partono F, Maizels RM. Cloned antigen genes of *Brugia* filarial parasites. *Biochem. Soc. Symp.* 53: 91-102, 1987.
- Shim JK, Ha DM, Nho SK, Song KS, Lee KY. Upregulation of heat shock protein genes by envenomation of ectoparasitoid *Bracon hebetor* in larval host of Indian meal moth *Plodia interpunctella*. *J. Invertebr. Pathol.* 97: 306-309, 2008.
- Shu Y, Du Y, Wang J. Molecular characterization and expression patterns of *Spodoptera litura* heat shock protein 70/90, and their response to zinc stress. *Comp. Biochem. Physiol.* 158A: 102-110, 2010.
- Sosalegowda AH, Kundapur RR, Boregowda MH. Molecular characterization of heat shock proteins 90 (HSP83?) and 70 in tropical strains of *Bombyx mori*. *Proteomics* 10: 2734-2745, 2010.
- Takahashi KH, Okada Y, Teramura K. Genome-wide deficiency screen for the genomic regions responsible for heat resistance in *Drosophila melanogaster*. *BMC Genet.* 12: 57, 2011.
- Tissieres A, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J. Mol. Biol.* 84: 389-398, 1974.
- Walter T, Drabent B, Krebs H, Tomalak M, Heiss S, Benecke BJ. Cloning and analysis of a human 86-kDa heat-shock-protein-encoding gene. *Gene* 83: 105-115, 1989.
- Wang H, Dong SZ, Li K, Hu C, Ye GY. A heat shock cognate 70 gene in the endoparasitoid, *Pteromalus puparum*, and its expression in relation to thermal stress. *BMB Rep.* 41: 388-393, 2008.

- Wojda I, Jakubowicz T. Humoral immune response upon mild heat-shock conditions in *Galleria mellonella* larvae. *J. Insect Physiol.* 53: 1134-1144, 2007.
- Wu B, Hunt C, Morimoto R. Structure and expression of the human gene encoding major heat shock protein HSP70. *Mol. Cell. Biol.* 5: 330-341, 1985.
- Wu C. Heat shock transcription factors: structure and regulation. *Annu. Rev. Cell Dev. Biol.* 11: 441-469, 1995.
- Xu J, Shu J, Zhang Q. Expression of the *Tribolium castaneum* (Coleoptera: Tenebrionidae) hsp83 gene and its relation to oogenesis during ovarian maturation. *J. Genet. Genomics* 37: 513-522, 2010.
- Ying SH, Feng MG. A conidial protein (CP15) of *Beauveria bassiana* contributes to the conidial tolerance of the entomopathogenic fungus to thermal and oxidative stresses. *Appl. Microbiol. Biotechnol.* 90: 1711-1720, 2011.
- Zhang SD, Odenwald WF. Misexpression of the white (w) gene triggers male-male courtship in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 92: 5525-5529, 1995.
- Zhang Y, Ohashi N, Rikihisa Y. Cloning of the heat shock protein 70 (HSP70) gene of *Ehrlichia sennetsu* and differential expression of HSP70 and HSP60 mRNA after temperature upshift. *Infect. Immun.* 66: 3106-3112, 1998.
- Zhao L, Becnel JJ, Clark GG, Linthicum KJ. Expression of AeaHsp26 and AeaHsp83 in *Aedes aegypti* (Diptera: Culicidae) larvae and pupae in response to heat shock stress. *J. Med. Entomol.* 47: 367-375, 2010a.
- Zhao L, Pridgeon J, Becnel JJ, Clark GG, Linthicum KL. Identification of genes differentially expressed during heat shock treatment in *Aedes aegypti*. *J. Med. Entomol.* 46: 490-495, 2009.
- Zhao L, Becnel JJ, Clark GG, Linthicum KJ, Chen J, Jin X. Identification and expression profile of multiple genes in response to magnesium exposure in *Culex quinquefasciatus* larvae. *J. Med. Entomol.* 47: 1053-1061, 2010b.