Heat Shock Proteins in Insects : Their Nature and Function

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ABSTRACT

All living organisms, including insects, interact with elevated temperatures and various biotic and abiotic stresses through the production of a specific group of proteins which are commonly referred to as stress proteins and molecular chaperones. Different temperature serves as triggers for the expression of HSP genes that are essential for survival. Starvation, parasitism and desiccation tolerance also influence the survivality of the insect. Differet types of heat shock proteins are found in insect depending on their molecular size and also function as thermo tolerance, protection against oxidative stress, growth and development, recovery from stress, ddaptation to climate change, insect immunity and in insect evolution. These stress proteins can be finally utelised for pest management.

Keywords : Insect, Heat shock proteins, Factors, Types of HSP, Functions, Practical utility

Introduction

All organisms including insects respond to increased temperature and other biotic as well as abiotic stresses with the help of production of defined set of proteins known as heat shock proteins (HSPs) (Morimoto *et al.*, 1994). HSPs are highly homologous proteins and an important determinant of arthropod abundance and their distribution. They are also known as stress proteins and molecular chaperones (Basha *et al.*, 2012). HSPs have been reported from a range of insects from the orders *viz.* Lepidoptera, Hemiptera, Diptera, Hymenoptera and Psocoptera (Zhao and Jones, 2012). Heat shock proteins (HSPs) are pivotal proteins and are ubiquitous among all living organisms, including arthropods. They have multifaceted roles like protecting the cells from stress viz. temperature extremes, damage due to oxidation and from any other environmental anomalies. These proteins play a key role in maintaining cellular homeostasis and survival under temperature extremes enabling proper assemblage of proteins and preventing agglutination and aggregation. Being poikilothermic, class Insecta is very much sensitive to temperature fluctuation, hence making HSPs essential for their survival under harsh climatic conditions.

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Insects are ectothermal in nature and they exhibit plasticity in various traits w.r.t temperature extremes. They are fast in completion of their generation and thus are model organisms to study temperature plasticity. Thermal extremes in insects most often lead to injury and it can cause series of changes at the morphological, biochemical, and physiological levels. Similarly, insects inhabiting higher latitudes have evolved mechanisms to survive harsh winters. Thermal acclimation of insects is a form of adaptation that enables them to adjust after a brief or chronic exposure to stress (Angilletta, 2009). The question arises that how thermal extremes or stresses are responsible for the acclimation in insects. It has been reported that heat induces a characteristic pattern of "puffing" in the chromosomes of Drosophila. This discovery eventually led to the identification of the heat-shock proteins (HSP) or stress proteins whose expression this puffing represented. Increased synthesis of selected proteins in Drosophila cells following stresses such as heat shock was first reported in 1974 (Ritossa, 1962). These proteins (HSPs) are stress chaperones and are essential for adaptation mechanisms and are associated with large number of physiological and biochemical metabolisms (King and MacRae, 2015). As per their molecular sizes, the families of these proteins have the nomenclature of HSP40, HSP60, HSP70, HSP90, HSP100 and small proteins (sHSP). HSPs play a vital role in insects' responses to either of the extreme temperatures, poisonous substances, starvation, density, UV radiation and diapause (Tedeschi *et al.*, 2016). HSP70 and HSP90 are mostly conserved in both prokaryotes and eukaryotes and consist of two highly conserved domains: an ATP-binding N-terminal and a substrate-binding C-terminal domain (Taipale *et al.*, 2010). Along with the adaptation mechanisms, these proteins contribute to predominant self protection mechanism (Guo and Feng, 2018). HSP70 includes stress inducible genes and they participate in various mechanisms in an unstressed cell *viz.* Folding of proteins after translation, membrane translocation etc. and they may or may not be influenced by stress (Daugarrd *et al.*, 2007). HSP60 provides protection against protein de-naturation during quiescence and diapause and performs the assemblage and bending of enzymes and other proteins that are related to energy metabolism (Brackley and Grantham, 2009). It is evident that HSPs perform important roles in insects, both inter and intra specifically, under different developmental, physiological and environmental states. The titre of insect HSPs under different situations are described as follows :

A. Temperature

Temperature is an important determinant for optimum physiological function of the insects. Both Heat and cold situations act as elicitors for HSP gene expression that is required for survival. Larvae of the apple maggot, *Rhagoletis pomonella*, respond to heat in the laboratory by up regulation of Hsp90 and Hsp70 mRNAs (Lopez-Martinez and Denlinger, 2008). Similarly, the small Hsp genes *viz.* shsp19.9, shsp20.1, shsp20.4, shsp20.8, shsp21.4, and shsp23.7 from the *Bombyx mori* are expressed in fifth instar larvae. These sHsp genes get induced in the fat body and in the reproductive organs by heat stress. The over-expression of Hsp23 genes in female silverleaf whiteflies have been found to have a important role in heat tolerance even at 44°C (Lü and Wan, 2011). In *Apolygus lucorum*, the expression of HSC70 significantly gets elevated by synthetic pyrethroids as well as by high temperature. Similarly, in Colorado potato beetle, exposure to a temperature of 43°C significantly increased the expression of HSP70 and the stressed larvae failed to respond to imidacloprid with the help of the high titre of this protein. The up-regulation of HSC70 provides a greater thermo-tolerance to Brown Plant Hopper, *Nilaparvata lugens*. Exposure to high temperature caused a proportionately higher expression of HSP90 compared to HSP70, whereas exposure to a low temperature shows exactly the reverse results (Farahani *et al.*, 2020).

B. Starvation and parasitism

In *Drosophila melanogaster*, it has been found that there was induction of HSP70 only in cold temperatures and not by starvation (Sinclair *et al.*, 2007). Contrastingly, Tian et al., 2018 reported that starvation in house fly (*Musca domestica*) induced expression of HSP27. In contrast, parasitism by Braconids in carob moth larvae increased HSP70 expression and HSP90 levels remaining unchanged. Similar observations were observed by other workers, where parasitized larvae of Indian meal moth by the Braconids enhance HSP70 and not the HSP90 levels.

C. Desiccation tolerance

Genes responsible of expressing sHSP, HSP70, and HSP90 in larvae of *Belgica Antarctica* are upregulated to react to changing levels of hydration. The HSP genes are expressed after faster rate of dehydration. Contrastingly, during slower dehydration only HSP70 enhanced, while sHSP and HSP90 remaining unaffected (Lopez-Martinez *et al.*, 2009). During quick rehydration sHSP and HSP70 levels remain on higher scale, but HSP90 declines. HSP70 gets collected in *Aedes aegypti*, *Anopheles gambiae*, and *Culex pipiens* during desiccation and dehydration (Benoit *et al.*, 2010). HSPs likely protect intracellular proteins and membranes, promoting survival under extreme conditions. However, some insects possess high levels of HSP23 and HSP70 and desiccation increases neither, suggesting these HSPs are sufficiently abundant to permit the insect to survive water loss without gene expression

D. Starvation and crowding

Various HSPs have been examined both during solitary and gregarious phases, representing lower and higher population densities respectively. In migratory locust, *Locusta migratoria,* HSP mRNA are more abundant in gregarious than solitary phase, that suggests crowding mediates HSP gene activation. The expression of HSP gene reflects increase in energy demand which further increase competitions for food and space (Wang *et al.*, 2007)

Types of HSPs in insects

Insects exhibit and express a plethora of HSPs, which can be classified based on their molecular size. Some key HSPs are discussed in the following :

1. **HSP70s :**

- These are the most rampantly studied in heat shock proteins. They function as molecular chaperones and help to fold the nascent proteins, refold the degraded proteins, and thus prevent the protein aggregation (Lu *et al.*, 2016).
- The titre of these proteins gets up-regulated during thermal stress and plays a critical role in protecting the cells from heat damage.

2. **HSP90s :**

- They help in activation and stabilization of different client proteins including kinases, signal molecules and steroid receptors.
- They are essential for insect growth and development and have modulating effect on the stress responses.

3. **Small HSPs (SHSPs) :**

- They act as molecular chaperones and prevent aggregation by stabilizing the damaged proteins.
- They often accumulate in high titres and protect the cells in stress conditions.

4. **HSP60 :**

- They are found in mitochondria and help in folding of proteins within it.
- They prevent in agglutination of misfolded proteins and maintain the status quo during stress efficence.

5. **HSP40s :**

- These chaperones assist HSP70s in identifying misfolded proteins and guide them for repair.

Functions of HSPs in insects.

1. **Thermo tolerance**

- Insects, after exposure to high temperature conditions, synthesize higher titres of HSPs to stabiles cell structures and prevent the protein denaturation.

2. **Protection against oxidative stress**

- HSPs provide protection against oxidative stress by neutralizing the reactive oxygen species (RO) that are produced during high temperature stress. This helps in preserving cellular integrity and prevents damage to DNA, proteins and lipids.

3. **Growth and Development**

- During growth, development and in metamorphosis also the protein degradation is regulated during stress conditions which in turn improve the adaptability of insects to changing climatic conditions.

4. **Recovery from stress**

- HSPs help in repairment and proper folding of cellular proteins which in-turn helps in cellular homeostasis after exposure to stress conditions.

5. **Adaptation to climate change**

- Heat shock proteins are key element in insect hemolymph in deserts and high altitude areas. They provide greater resilience to heat and cold stress.\

6. **Insect immunity**

- HSPs facilitate are activate immune pathways by degrading damaged foreign proteins and maintain immune system during stress.

Role of Heat Shock Proteins in insect evolution

There can be variation in the expression of HSPs depending on the species variation, life stages of insects and variation in environment. Insects thriving in extreme ecosystems certainly harm higher level of shock proteins compared to those in stable environments. Evolution has helped the arthropods with respect to HSO responses to tackles with specific ecological niches. Insect species may even exhibit chromosomal adaptations w.r.t HSP expression. This has widely been documented in grasshoppers and fruit flies (Drosophila). In laboratory conditions, the expression of these proteins can be experimentally up-regulated which allow the researchers to study the biochemical as well as genetic mechanisms that facilitate the insects to cope up with climatic stress.

Practical utilities of Stess Proteins

1. Linking climate change and spatio-temporal distribution of insects

Steep rise in temperatures makes it mandatory for the insect's ability to over express the shock proteins for their survival. Thus the cascading change in the expression profiles with certainly dictates the species distribution over time and space. This will affect growth and development of the insects in general and adaptation to new environments in particular.

2. Role on pest management

The various pathways responsible for the synthesis of HSPs can be studied and these pathways may be targeted by physiological and biochemical means to disrupt their synthesis. In this way we can target the weak links of the insects to subside their population without application of synthetic insecticides. It has all the attributes to be integrated in the pest management schedule. Research in these areas is still in nascent stage and much of the works are yet to be done in this line.

Conclusion

We often see higher titres of HSPs during temperature extremes, starvation, hypoxia and crowding in insects. However they are produced differentially during diapause. They contribute in protecting the nascent proteins from denaturation. This sHSPs/HSP70/HSP90/HSP60 has the potential to gear up protein folding and storage. In short supply of ATP proteins usually bind stably to the aforesaid shock proteins and expand storage during diapauses. With unlimited supply of ATP the down regulation of the shock proteins may enhance protein storage. These proteins along with the co-chaperones are thought to establish the molecular networks play vital role in protein synthesis stress tolerance and diapause. Identifying the substrates that bind to the HSPs will further lead to identification of novel proteins with key functional roles during stress tolerance and diapause.

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