2. Review of literature
2. REVIEW OF LITERATURE

Literature on heat shock, heat shock proteins and their biological functions are reviewed here in brief from bacteria to man and silkworm in detail.

2.1 Bacteria to man

All organisms respond to heat or sudden increase in the temperature by synthesizing a group of proteins called “heat shock proteins” (hsp). This heat shock response has been highly conserved throughout evolution, not only as a physiological phenomenon but also at the level of individual proteins. Hsps comprise some of the most highly conserved known protein families, which includes constitutive as well as heat inducible members (Parsell and Lindquist, 1993). Their role in normal cellular physiology has been the subject of several excellent reviews and emphasis on how the proteins in major hsp families help organisms to survive during conditions of stress (Ellis and van der Vies, 1991; Welch, 1991; Gething and Sambrook, 1992; Hartl et al., 1992; Morimoto, 1998; Santoro, 2000; Ellen et al., 2002; Pockley, 2003).

The first discovery of hsp was from "Drosophila melanogaster" larvae and described as a set of proteins whose expression was induced by heat shock and variety of other stresses (Ritossa, 1962).
Primary function of hsps during cellular stress is to maintain translation and protein integrity. Regulation of hsp synthesis is dependent on the level of proteins exist within the cell. Hsps help cells in two general ways to cope with stress induced damage to polypeptides. First, hsps could promote degradation of abnormal proteins. Second, hsps could reactivate stress-damaged proteins. Several hsps are known to function as 'molecular chaperones', preventing the aggregation and promoting the proper refolding of denatured proteins, thus restoring protein homeostasis and promoting cell survival. This unique chaperone mechanism is conserved from Yeast to mammals (Henle and Leeper, 1982; Goff and Goldberg, 1985; Ananthan et al., 1986; Parsell and Sanchez, 1989; Rothman, 1989; Ellis and van der Vies, 1991; Parsell and Lindquist, 1993; Becker and Craig, 1994; Craig et al., 1994; Morimoto et al., 1994; Buchner, 1996; Freeman and Morimoto, 1996; Hartle, 1996; Mathew and Morimoto, 1998; Morimoto, 1998; Morimoto and Santoro, 1998; Jaattela, 1999; Jolly and Morimoto, 2000; Santoro, 2000; Kregel, 2002; Thusnelda et al., 2003; Parcellier et al., 2003).

Induction of hsp synthesis varies in different organisms when they are exposed to varied temperatures and there is a striking relationship between the induction temperature and the organism’s environment (Lindquist and Craig, 1988). Hsp synthesis was reported when thermophilic bacteria shifted from 95°C to 105°C (Phipps et al., 1993; Trent et al., 1990). In *Drosophila melanogaster*, induction of hsp occurs between 33 to 37°C. Arctic fishes growing at 0°C induce hsps when transferred from 5 to 10°C. Hsps are
induced at 122°C in mammals and in soyabean at field on hot sunny days (Kimpel and Key, 1985; Parsell and Lindquist, 1993).

The most compelling argument of the hsps is a protective function but the induction of hsps correlates well with the tolerance to extreme heat in cells and organisms (Li and Laszlo, 1985; Nagao et al., 1986; Sanchez and Lindquist, 1990; Nover, 1991; Parsell and Lindquist, 1993).

The role of individual hsps varies under common stress condition. Among, some are required for growth at temperature near upper end of the normal growth range (hsp 70 - Craig and Jacobsen, 1984); others for long term survival at moderately high temperatures (ubiquitin - Finley et al., 1987) and still other for tolerance to extreme temperatures (hsp 104 - Reading et al., 1989). On the other hand different organisms use different hsps to respond to similar levels of stress, i.e. tolerance to extreme stress depends largely upon hsp 104 in Yeast, hsp 70 in Drosophila and hsp 70, hsp 27 and hsp 110 in mammals (Li and Laszlo, 1985; Landry et al., 1989a & b; Sanchez and Lindquist, 1990; Parsell and Lindquist, 1993).

The molecular mass of principal hsps ranges from ~15 to 110 kDa and are divided into groups based on size and function i.e. small hsps, hsp 40, hsp 60, hsp 70, hsp 90 and hsp100 and present in the cytosol, mitochondria, endoplasmic reticulum and nucleus. They differ in the way that contributes to proper folding, subunit assembly and cellular integrity (Table 1) (Schlesinger, 1990; Welch, 1992; Morimoto et al., 1994; Hightower and Hendershot, 1997; Moseley, 1997; Kregel, 2002; Pockley, 2003).
Physiological functions associated with the stress induced accumulation of hsp 70 was acquired thermotolerance, which is defined as the ability of a cell or organism to become resistant to heat stress after a prior sub lethal heat exposure (Landry et al., 1982; Li and Werb, 1982; Landry and Chretien, 1983; Li et al., 1983; Mizzen and Welch, 1988; Moseley, 1997; Krégel, 2002).

Table 1. Heat shock protein families and their intracellular location and function.

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<thead>
<tr>
<th>Hsp family</th>
<th>Intracellular location</th>
<th>Proposed functions</th>
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<tbody>
<tr>
<td>Small Hsps</td>
<td>Cytosol, nucleus</td>
<td>Microfilament stabilization</td>
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<tr>
<td>Hsp 40</td>
<td>Cytosol, nucleus, Endoplasmic Reticulum (ER)</td>
<td>Regulates the activity of Hsp 70 &amp; binds non-native proteins</td>
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<td>Hsp 60</td>
<td>Mitochondria</td>
<td>Refolds proteins, prevents aggregation of denatured proteins, assists correct folding</td>
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<tr>
<td>Hsp 70</td>
<td>Cytosol, nucleus, ER, Mitochondria</td>
<td>Protein folding, cytoprotection</td>
</tr>
<tr>
<td>Hsp 90</td>
<td>Cytosol, ER, Nucleus</td>
<td>Regulation of steroid hormone receptors, protein translocation</td>
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<tr>
<td>Hsp 110</td>
<td>Cytoplasm</td>
<td>Protein folding</td>
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Hsp 60, hsp 70 and hsp 90 have immunological relevance. Members of hsp 70 family bind as monomers to hydrophobic regions of polypeptide chains or denatured proteins and keep them in a folded state. Hsp 60 assists partially folded proteins in an ATP dependent manner to reach their native state inside its large central channel (Bukau and Horwich, 1998). The 90 kDa hsp interact
with substrate only in late stages of folding (Buchner, 1999). Singh et al. (2001) studied the role of heat shock proteins and their receptors in the activation of immune system and has shown that hsps act as highly stimulating agents to antigen presenting cells, especially dendritic cells.

Further the role of heat shock proteins in activation of antigen presenting cells appear to have been involved in immune responses since the emergence of phagocytes in early multicellular organisms and to have been commandeered for adaptive immune responses with the advent of specificity. These properties of hsps allow them to be used for immunotherapy of cancers and infection in novel ways (Srivastava, 2002).

Expression of hsps is also associated with certain diseases. The modulation of the heat shock or constitutive over expression of specific heat shock proteins restricts or substantially reduces the level of pathology and cell death (Mizzen and Welch, 1988; Morimoto et al., 1992; Huot et al., 1991; Jaattela et al., 1992; Parsell and Lindquist, 1994; Mestrill et al., 1994; Plumier et al., 1995; Marber et al., 1994 and 1995; Mehlen et al., 1995; Mosser et al., 1997; Morimoto, 1998; Santoro, 2000). This has led to recognition that hsps, via their chaperoning effects on protein, protect cells from many forms of stress-induced cell damage and could influence the course of disease like ischemia, fever and inflammation, metabolic disorders, cell and tissue trauma, aging, infection and cancer.

Over expression of either hsp 90 or hsp 70 protect neuronal cells from thermal stress (Mailhos et al., 1994). However, hsp 70 alone is sufficient to
protect the neurons against thermal or ischaemic stress (Amin et al., 1996). The major factor directing protein folding in the cytosol of eukaryotic cells is hsp 90 complexes in co-operation with hsp 70 (Bose et al., 1996). Freeman and Morimoto (1996), Jakob et al. (1995), Wiech et al. (1992) opined that hsp 90 is highly efficient in preventing protein misfolding and that the hsp 70 chaperon machinery is required to fold the hsp 90 released intermediates to the native state.

The molecular relationship between heat shock proteins, various signaling proteins and partner proteins appears to be critical for the normal functioning of signal transduction pathways. The relative level of these proteins may be important as too little or too much hsp 70 or hsp 90 can result in aberrant growth control, developmental malformations and cell death (Ellen et al., 2002).

The enhanced heat shock gene expression in response to various stimuli is regulated by heat shock transcription factors (HSFs). HSF1 has an important role in the molecular response to non-native proteins. HSF2 activity was associated with development and differentiation. HSF3 also interacts with other transcription factors and responsible for heat induced hsp expression. HSF4 was discovered in human and is expressed in tissue specific manner. It constitutively binds to DNA, but lacks the properties of transcriptional activator, and it has been suggested to be a negative regulator of the heat shock response (Morimoto, 1993; Nakai et al., 1997; Morimoto, 1998; Kawazoe et al., 1999; Santoro, 2000; Pirkkala et al., 2001).
Heat shock factor preferentially forms large complexes, probably including hexamers (Sorger and Nelson, 1989; Clos et al., 1990) and these large complexes are important for the activation of heat shock genes in vivo. Although heat shock element containing three nGAAn units bind D-HSF (Drosophila-HSF) trimers tightly in vitro, arrays of more than three units must be introduced into the Drosophila genome to stimulate high-level transcription (Xiao and Lis, 1988). These results imply that transcriptionally active complexes contain six or more heat shock factor monomers (Sorger, 1991).

The thermotolerance demonstrated by cells, tissues and animals suggest that the morbidity and mortality associated with whole body heating is due in part to the dysfunction of some critical target tissues (Weshler et al., 1984; Urano, 1986; Moseley, 1997; Hall et al., 2000a and b). However, development of thermotolerance results from the improved tolerance of the weakest organ and cell system, which are both heat sensitive and vital to the animal (Hume and Marigold, 1980). Further, cellular manipulations that either block hsp 70 accumulation or over expression of certain hsps have been shown to either increase or decrease heat sensitivity (Lewis and Pelham, 1985; Johnston and Kucey, 1988; Riabowol et al., 1988; Landry et al., 1989a & b; Kregel, 2002).

Several important cytoprotective functions that are attributed to hsps (Bakau and Horwich, 1988; Chirico et al., 1988; Deshaies et al., 1988; Mizzen and Welch, 1988; Morimoto et al., 1990; Palleros et al., 1991; Garrido et al., 2001; Kregel, 2002) are,
1. Folding of proteins in various intracellular compartments.


3. Refolding of misfolded proteins.

4. Translocation of proteins across membranes and into various cellular compartments.


6. Involvement in apoptosis-Hsp 27, Hsp 70 and Hsp 90 are predominantly antiapoptotic and Hsp 60 is proapoptotic.

Sessile marine invertebrate undergo constant direct exposure to the surrounding environmental conditions, including local and global environmental fluctuations that may lead to fatal protein damage. Induction of heat shock proteins (Hsps) constitutes an important defense mechanism that protects these organisms from deleterious stress conditions (Choresh et al., 2004).

Recently, it has been established that hsps exhibit specificity to particular classe of polypeptide substrates and client proteins in vivo and that chaperons can stabilize mutations that effect the folded conformation. Likewise, over expression of chaperons has also been shown to protect cells against apoptotic cell death. The involvement of chaperons therefore in such diverse roles might suggest novel anticancer therapeutic approaches targeting hsps function for broad spectrum of tumor types (Mosser and Morimato, 2004).
Genome-wide identification of HSF target genes provides novel insights into the role of HSF in growth, development, disease and aging and in the complex metabolic reprogramming that occurs in all cells in response to stress (Hahn et al., 2004).

2.2 Insects

The heat shock protein was discovered for the first time in *Drosophila melanogaster* (Ritossa, 1962). Thereafter the first product of these genes identified and termed as "Heat shock protein" (Tissiers et al., 1974).


The studies on heat shock response in other dipteran insects viz. Chironomus tentans (Vincent and Tanguay, 1979), Sarcophaga bullata (Bultmann, 1986), Ceratitis capitata (Stephanou, 1987), Aedes albopictus (Carvalho and Rebello, 1987) and Musca domestica (Tiwari et al, 1997) suggest that the Drosophila heat shock response is typical of insects as it includes heat shock protein synthesis and the repression of non heat shock protein synthesis (Lindquist, 1980b). However, in non dipteran insects, skipper butterfly (Lepidoptera- Dean and Atkinson, 1982), the American cockroach (Dictyoptera- Ruder et al., 1989), Chironomus (Lepidoptera- Nath and Lakhotia, 1989), Locusts (Orthoptera- Whyard et al., 1986; Baldaia et al., 1987), Manduca sexta (Lepidoptera- Fittingoff and Riddiford, 1990; Lohmann and Riddiford, 1992a) and Bombyx mori (Lepidoptera; Evgen’ev et al., 1987; Lohmann and Riddiford, 1992b; Abramova et al., 1991; Hsieh et al., 1995; Joy and Gopinathan, 1995) studied revealed that there was no repression of non heat shock proteins except in Locusts and Manduca which are far less apparent than in Drosophila.
The repression of non heat shock protein synthesis has been primarily described in Diptera (Vincent and Tanguay, 1979; Lindquist, 1980b; Stephanou et al., 1983; Bultmann, 1986; Carvalho and Rebello, 1987; Stephanou, 1987) and in one lepidopteran pharate larvae of the gypsy moth, *Lymantria dispar* (Yocum et al., 1991) but was not observed in *Manduca* (Fittingoff and Riddford, 1990) and *Locusta migratoria* (Whyard et al., 1986) since both of them are tolerant to very high temperature. Whereas in *B. mori* non heat shock protein synthesis is not substantially repressed at high temperature as in *Manduca* (Lohmann and Riddiford, 1992b).

V instar larvae of *Manduca sexta* heat shocked for 1 hour (allowed to pupate at normal temperature-26°C) exhibited cent percent survivability up to 42°C and then declined substantially to only 8.0 percent at 46°C and 0.0 percent at 48°C. The animals heat shocked at 48°C and above did not recovered sufficiently to resume feeding. On the other hand 42°C heat shock induced synthesis of heat shock proteins with molecular masses of 84, 73, 71, 27, 24, 23 and 22 kDa (Fittingoff and Riddiford, 1990).

### 2.3 Heat shock response in silkworm, *Bombyx mori*

Cells of *Bombyx mori* and *Antheraea pernyi* were exceptionally resistant to the effect of high temperature and synthesis of both normal and heat shock proteins continued up to 40°C but, synthesis of all proteins ceased at temperature above 45°C. Heat shock induced polypeptides were of molecular weight 83, 80, 74, 68, 25 and 23 kDa in fat body and silk glands (Evgen'ev et al., 1987).
Broude et al. (1988) studied the action of heat shock on B. mori cells infected by nuclear polyhedrosis virus (NPV) both in vitro and in vivo. The infected cells lack the ability to synthesize heat shock proteins after temperature elevation. But northern blotting experiments revealed that the NPV infection does not interfere with the induction of transcription of hs-gene, rather inhibits hsp synthesis at the level of translation. The transcription initiation site was identified as a result of cloning and sequencing of gene encoding hsp 70 of the mulberry silkworm (Titareka et al., 1990). Eventually, Kobayashi and Sudawan (1993) opined that, rearing of silkworms on farms at sustained high temperature might be useful in preventing infectious viral diseases.

Lohmann and Riddiford (1992b) reported several heat shock proteins, one at approximately 84 kDa, 2 proteins near 70 kDa, 3 proteins of 31, 30 and 29 kDa and 3 proteins near 26 kDa which, belongs to 3 hsp families from epidermis of fifth instar larvae of B. mori and 42°C as the maximum tolerable temperature. Wu and Hou, (1993) observed that the relationship between thermotolerance and heat stable esterase in silkworm, B. mori varies with strains and it is positively related to the activity of the heat stable esterase (Hs EST).

Hsieh et al. (1995) observed 70 kDa protein from fat body cells and haemocytes of fifth instar larvae of Chinese and Japanese races heat shocked at 40°C for 1 hour.
The heat shock response and thermal sensitivity of C. Nichi, Pure Mysore and NB₄D₂ has been reported. The 43°C proved to be lethal for all the silkworm strains. Expression of 93 kDa protein was observed in all the stages. 93, 89 and 70 kDa proteins in fat body, 70 kDa protein in haemolymph 93, 46 and 28 kDa in the cuticle was discernible (Joy and Gopinathan, 1995).

2.4 Biological and Commercial traits of silkworm

Lohmann and Riddiford (1992b) observed 10 percent mortality at 42°C, 66.6 percent at 44°C and 100 percent mortality in fifth instar B. mori larvae at 46°C heat shocked for 1 hour.

Chinese and Japanese silkworm races subjected to heat shock at 44, 45, 45.5 and 46°C for 1 hour showed 0, 2, 87 and 100 percent mortality respectively (Hsieh et al., 1995). The Indian multivoltine races (C. Nichi and Pure Mysore) exhibited better survival rate than the bivoltine (NB₄D₂) strain when exposed to 41°C and above for 1 hour. V instar larvae and pupae exhibited maximum tolerance compared to early larval instars, adults or the eggs. Treatment of larvae at 41°C for 1 hour resulted in a variety of physiological alterations including increased heartbeats, differential haemocyte count, enlargement of granulocytes and the presence of additional proteins in tissues and haemolymphs. Whereas, heat shock at 43°C and above proved to be lethal for all the stages and strains. Further, Joy and Gopinathan (1995) opined that there is no impact of heat shock on rearing parameters.

Hsieh et al. (1995) reported that among different bivoltine strains investigated the Chinese race Feng was the most tolerant followed by Japanese
races Kuo and J-09. Whereas the cocoon shell weight percentage was not
significant in polyvoltine strains but varies in bivoltine strains. However,
reduction in silk yields was observed in response to high and fluctuated
temperatures.