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Heat shock proteins, Importance and expression in fishes

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Abstract
A unique class of proteins (heat shock proteins) have evolved to resist with the potential for proteins to become unstable or denatured when stressed and thus act as a defense against protein damage. Heat shock proteins are collectively the only molecular mechanisms that animals utilize to tolerate stress. These proteins have pleiotropic effects interacting with multiple systems in diverse ways regulated by the endocrine system. HSPs have critical roles in helping fish cope with environmental change. The crucial role of HSPs is in thermo - tolerance. HSP70 is mostly expressed protein in response to thermal stress. HSPs enable fish to adapt to environmental stressors including temperature and osmotic stress and exposure to a variety of xenobiotic compounds. HSPs trigger immune response through activities that occur both inside the cell (intracellular) and outside the cell (extracellular).

Keywords: Pleiotropic; HSP70; Thermal stress; xenobiotic.

Introduction

Heat shock proteins (HSP) are a class of functionally related proteins involved in the folding and unfolding of other proteins. Heat shock proteins are a family of highly conserved cellular proteins present in all organisms including fish. Their expression is increased when cells are exposed to elevated temperatures or other stress (Maio, 1999) [10]. This increase in expression is transcriptionally regulated. The dramatic upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (HSF) (Wu, 1995) [58]. HSPs are found in virtually all living organisms, from bacteria to humans. Heat shock proteins (Hsps) play a pivotal role in protein homeostasis and cellular stress response within the cell (Feder and Hofmann, 1999; Iwama *et al*., 2004; Mao *et al*., 2005; Multhoff, 2007; Keller *et al*., 2008) [13, 14, 23, 35, 42, 27]. Disruption of normal cellular processes may cause rapid increase in the synthesis of a group of proteins which belong to the Hsp families. These proteins have been classified into several families based on their molecular weight such as Hsp90 (85-90 kDa), Hsp70 (68-73 kDa), Hsp60, Hsp47, and small Hsps (12-43 kDa) (Park *et al*., 2007; Hallare *et al*., 2004) [46, 18]. The heat shock response is an evolutionarily conserved mechanism for maintaining cellular homeostasis following sublethal noxius stimuli (Lindquist, 1986; Lindquist and Craig, 1988) [34]. Several heat shock proteins act as molecular chaperones which mediate the correct assembly and localization of intracellular and secreted polypeptides and oligomeric protein structures. The importance of Hsps in the protein folding pathway is reflected in the fact that a number of heat shock genes are expressed at high levels during normal cell growth. Oxygen radicals, toxicants, and inflammatory stress enhance the synthesis of Hsps and often give rise to an accumulation of denatured and aberrantly folded proteins within the cell. Thus the interaction of Hsps with abnormal proteins during stress is thought to be an extension of their role under normal, non-stress conditions (Hightower *et al*., 1994; Morimoto and Santoro *et al*., 1998) [21, 50, 39]. Fish are an excellent vertebrate model to investigate the physiology, function and regulation of Hsps, because they are exposed to thermal and other stressors in their natural environment. The functions of Hsps affect various aspects of fish physiology, including development and aging, stress physiology and endocrinology, immunology, environmental physiology, stress tolerance and acclimation (Basu *et al*., 2003) [5]. In the unstressed cell, heat shock proteins have constitutive functions that are essential in protein metabolism (Morimoto *et al*., 1994; Hightower *et al*., 1999) [40, 22]. Hsps have been proposed as biomolecular biomarkers for toxicity associated with physical and chemical stressors (Sanders, 1993; Ryan and Hightower 1994; Ovelgonne *et al*., 1995) [51, 21, 50, 45] since the expression of their genes may be activated be heat shock heavy metals (Airaksinen *et al*., 2003) [3]. There have been several efforts to validate the use of the Hsp response as an indicator of stressed states in fish. It has been shown that several forms of environmental stressors may induce the Hsp response in fish. For example, increased levels of various Hsps have been measured in tissues of fish exposed to industrial effluents, polycyclic aromatic hydrocarbons (Vijayan *et al*., 1998) [24, 56], several metals such as copper, zinc and mercury (Sanders, 1993; Williams *et al*., 1996) [51, 57], pesticides (Hassanein *et al*., 1999) and arsenite (Grosvik and Goksoy, 1996). These studies and others revealed the use of Hsp as an indicator of stressed states in fish is a complex issue. The Hsp response can vary according to tissue (Smith *et al*., 1999; Rabergh *et al*., 2000) [53, 48], distinct Hsp families (Smith *et al*., 1999) [53] and stressors (Airaksinen *et al*., 2003; Iwama *et al*., 1998) [3, 24, 56] and the sensitivity of Hsp expression may also vary with the species (Basu *et al*., 2002; Nakano and Iwama, 2002) [43] developmental stage (Lele *et al*., 1997; Santacruz *et al*., 1997; Martin *et al*., 2001) [31, 52, 36], and season (Fader *et al*., 1999) [12]. The crystallin small heat shock protein (sHsp) family plays a major role in cell homeostasis, injury responses, and disease. The functions of sHsps presumably have their

evolutionary roots in chaperoning proteins, many have additional functions. For example, Hspb1 (Hsp27) regulates actin filament dynamics, its exact role depends on phosphorylation state (Liang and MacRae, 1997; Mounier and Arrigo, 2002). Zebrafish Hsp27 (zfHsp27) contains three conserved phosphorylaable serines and a cysteine important for regulation of apoptosis, but lacks much of a C-terminal tail domain and shows low homology in two putative actin interacting domains that are features of mammalian Hsp27. zfHsp27 mRNA is most abundant in adult skeletal muscle and heart and is upregulated during early embryogenesis. ZfHsp27 expressed in mammalian fibroblasts was reported to be phosphorylated in response to heat stress and anisomycin, and this phosphorylation was prevented by treatment with SB202190, an inhibitor of p38 MAPK. Expression of zfHsp27 and human Hsp27 in mammalian fibroblasts promotes a similar degree of tolerance to heat stress. ZfHsp27 fusion proteins enter the nucleus and associate with the cytoskeleton of heat stressed cells in vitro and in zebrafish embryos (Mao *et al*., 2005) [35]. Thus Elicker and Hutson (2007) [11] revealed conservation in regulation and function of mammalian and teleost Hsp27 proteins and defined zebrafish as a new model for the study of Hsp27 function (Elicker and Hutson, 2007) [11]. Altered expression and phosphorylation of Hsp27, the most widely distributed and well-studied sHsp, is observed in cells and tissues responding to numerous sublethal injuries including those associated with hyperthermia and oxidative damage, metal toxicity, and anoxia/ischemia, cancer, cardiac hypertrophy, and muscle myopathies have also been associated with changes in Hsp27 regulation or expression. Scientific data suggest that Hsp27 and other small heat shock proteins play role in development and aging. Mao *et al.* (2005) [35] published the sequence of a zebrafish mRNA coding for a heat shock protein homologous to human Hsp27/HSPB1 and characterized the phosphorylation, thermoprotective activities, and intracellular distribution of the derived protein in zebrafish and cultured mammalian cells under control conditions and after application of heat stress (Mao *et al*., 2005) [35]. Hsp70 is known to assist the folding of nascent polypeptide chains, acts as a molecular chaperone, and mediates the repair and degradation of altered or denatured proteins (Kiang and Tsokos, 1998) [28]. Hsp90 is activated when supporting various components of the cytoskeleton and steroid hormone receptors (Csermely *et al*., 1998; Pearl and Prodromou, 2000; Young *et al*., 2001) [8, 47, 59].

Discovery

The discovery of HSPs is often attributed to the reporting of chromosomal puffs in the salivary gland cells of the fruit fly, Drosophila busckii, soon after a heat shock (Ritossa, 1962) [49]. As Mitchell and Peterson (1982) [37]. State in their review, stress effects on specific gene expression leading to certain phenotypes have been reported since the mid-1930s. The special nature of the HSPs came to light in the decade after Ritossa's observations, as various investigators described special characteristics of this group of proteins. Investigators such as Berendes *et al*. (1965) [6], Ashburner (1970) [4], and Mitchell *et al*. (1974) [38, 54] showed that the heat-induced puffs in the Drosophila salivary gland chromosomes occurred within minutes of the heat shock, and that the novel group of proteins were associated with those puffs. Mitchell *et al*. (1974) [38, 54] also reported that a number of constitutive proteins disappeared as the novel HSPs were being produced. Tissieres *et al*. (1974) [38, 54] showed that these proteins did not occur exclusively in the salivary glands. They reported HSP expression also in brain, Malphigian tubules, and wing marginal discs of the fruit fly. Definitive evidence that specific HSPs are expressed from genes in the chromosome puffs came in the late 1970s when it was shown that purified mRNA which hybridized to the heat shock puffs were translated to specific HSPs when added to in vivo.

Classification

The naming of HSPs are generally based on their molecular mass (kilodaltons, kDa) as determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS± PAGE). Heat shock proteins are also grouped according to function (e.g. chaperonin), DNA sequence, and antibody cross-reactivity. The commonly used categories are: 100 kDa; 90 kDa; 70 kDa; 60 kDa; and the 16±30 kDa group, and are usually referred to as HSP100, HSP90, HSP70, HSP60, and the low molecular weight (LMW) class of proteins, respectively (Morimoto *et al*., 1994) [40].

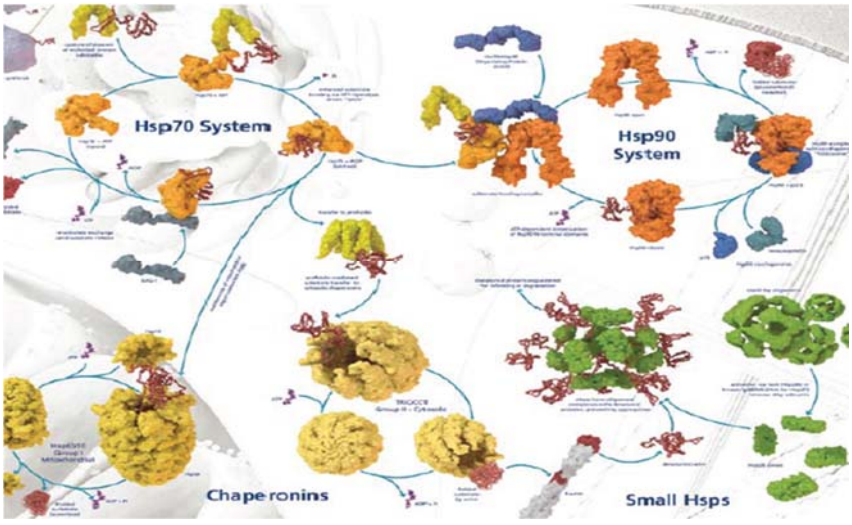


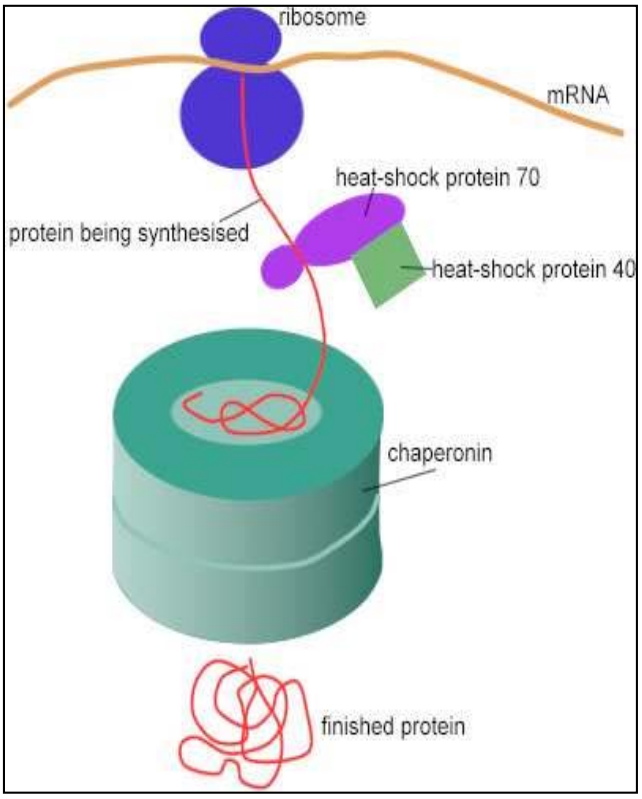
Fig 1: Hsp chaperone complexes. Hsps and their associated cofactors often function together in complexes, acting in concert as molecular chaperones to facilitate the proper folding and activation of many cellular proteins.

Function of Hsps

The HSPs are known to play vital cellular roles including protein assembly, correct folding, and translocation, as well as regulating interactions between hormones and their receptors (Welch, 1993). Research in this area continues to grow at an ever-increasing rate, and applications to problems and opportunities in human health and environmental monitoring are developing rapidly. Heat shock protein studies in fish are still in the early stages compared with those in bacteria, yeast and mammals. Studies in fish are still in the descriptive stages of documenting novel proteins that are produced in various tissues in response to a variety of biological and abiotic stressors. The primary objective of this review is to summarize what is currently known about HSP expression in fish cell lines, in fish cells in primary culture, and in whole animals. The current models of the molecular processes that are involved in HSP expression as well as the molecular characteristics of fish HSPs are described. There is a discussion of what is known about the relationship between the generalized stress response and HSP expression in fish and other animals. A section on the commonly used methodology in HSP research is included to benefit those new to this field. The review concludes with speculations as to the feasibility of using the expression of HSPs in biomonitoring of the aquatic environment, through the detection of stressed states in fish.

HSPs in protein folding

The diagram shows the role of heat-shock proteins and a chaperonin in protein folding. As the ribosome moves along the molecule of messenger RNA, a chain of amino acids is built up to form a new protein molecule. The chain is protected against unwanted interactions with other cytoplasmic molecules by heat-shock proteins and a chaperonin molecule until it has successfully completed its folding.



HSP70 and other HSPs in rainbow trout

Stress responses have been well characterized in rainbow trout and its cultured cells. Currie and Tufts (1997) observed that erythrocytes synthesized HSP70 both constitutively and in response to an increase in temperature. Airaksinen *et al.* (1998) [2]. examined the effects of heat stress (from 18 to 26°C) and low oxygen tension (1% O2 = 1 kPa) on protein synthesis in primary cultures of hepatocytes, gill epithelial cells, and RTG- 2 cells of rainbow trout. All of these cells displayed elevated levels of 67-, 69-, and 92-kDa proteins, whereas a 104-kDa protein was induced only in RTG- 2 cells. Hypoxia induced a cell-type-specific response, increasing the synthesis of 36-, 39-, and 51-kDa proteins in the gill epithelial cells.

Rainbow trout HSF1

Two distinct cDNA clones encoding HSFs have been isolated from RTG-2 cells of rainbow trout and subsequently denoted HSF1a and HSF1b (Ojima and Yamashita 2004). The predicted amino acid sequence of HSF1a shows 86.4% identity to that of HSF1b. The two proteins contained the general structural motifs of HSF1, i.e., a DNA-binding domain, hydrophobic heptad repeats, and nuclear localization signals. Southern blot analysis showed that each HSF1 is encoded by a distinct gene. The two HSF1 mRNAs were coexpressed in unstressed rainbow trout RTG-2 cells as well as in a variety of tissues. An electrophoretic mobility shift assay revealed that each *in vitro* translated HSF1 binds to the HSE, and a chemical cross-linking and immunoprecipitation analysis showed that HSF1a and HSF1b form heterotrimers as well as homotrimers. Based on these results, two distinct HSF1 isoforms that can form heterotrimers are present in rainbow trout cells, suggesting that a unique molecular mechanism regulated by a combination of distinct HSF1 isoforms underlies the stress response in rainbow trout. These HSF1 isoforms may have diverged during the evolution of tetraploid fish.

Factors regulating heat shock proteins in fish

1. Abiotic factors and their effects on heat shock proteins

The majority of studies on heat shock proteins in fish have been limited to in vitro examinations conducted in laboratory environments. Furthermore, most of these studies reported the induction of heat shock protein families following exposure to stress, without elucidating the functional significance underlying their observations (Iwama *et al*., 1998) [24, 56]. Studies in fish have demonstrated that heat stress can induce various heat shock proteins in cell lines (Kothary *et al*., 1984), primary cell culture (Koban *et al*., 1987), and in tissues from whole animals (Koban *et al*., 1991). Osmotic stress has recently been demonstrated to induce hsp90 mRNA in Chinook salmon (Oncorhynchus tshawytscha), and Hsp54 and Hsp70 in Atlantic salmon (Smith *et al*., 1999) [53]. Elevated levels of various heat shock proteins have been measured in tissues of fish exposed to environmental contaminants, such as heavy metals (Heikkila *et al*., 1982), industrial effluents (Janz *et al*., 1997), pesticides (Sanders, 1993) [51], and polycyclic aromatic hydrocarbons (Vijayan *et al*., 1997) [55]. It is noteworthy that while many indicators of fish stress (e.g. plasma cortisol concentrations) are altered by handling and sampling procedures, Vijayan *et al*. (1997) [55] demonstrated that handling stress does not alter levels of hepatic hsp70 in

rainbow trout (Oncorhynchus mykiss). The effects of abiotic factors on heat shock protein expression in fish have been extensively reviewed (Sanders, 1993) [51].

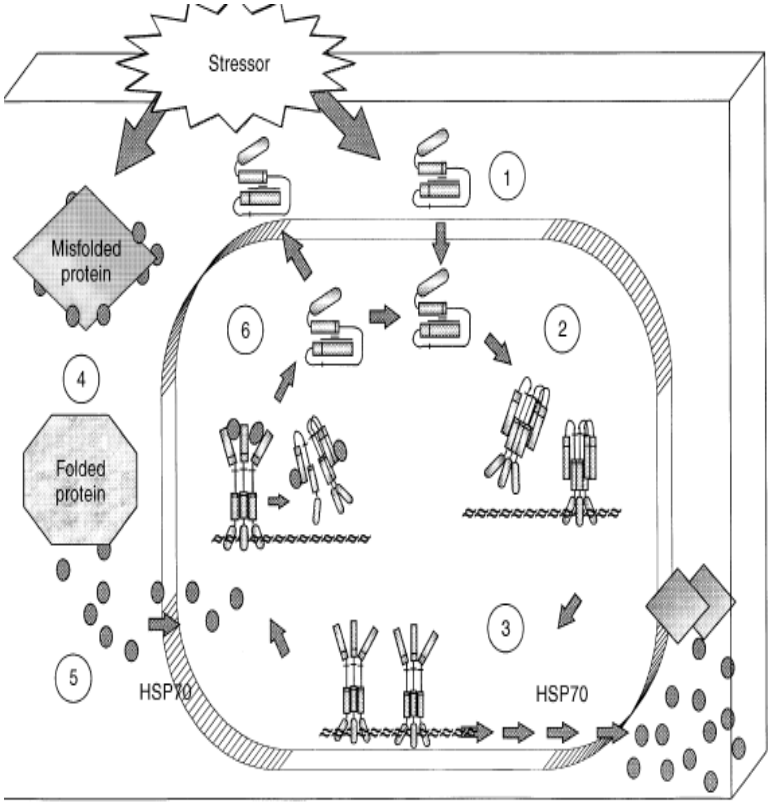
2. Biotic factors and their effects on heat shock proteins:

Less is known regarding the effects of biotic factors on heat shock proteins in fish. Kagawa *et al*. (1999) [26] reported that levels of Hsp70 were significantly raised in the brains of goldfish (Carassius auratus) that were reared in the presence of a predator, the bluegill sunfish (Lepomis macrochirus). More is known about the effects of pathogenic exposure on heat shock proteins. Pathogens are common in natural environments and can have detrimental impacts on the health of fish populations. Heat shock proteins are known to be involved in the immune response following pathogenic exposures in mammals (Young, 1990) [60]. Cho *et al*. (1997) [7] were the first to observe a cellular stress response (Hsp90) in fish cells, following exposure of cells to infectious haematopoietic necrosis virus (IHNV). Forsyth *et al*. (1997) [15, 55] observed increased Hsp70 in hepatic and head kidney tissues of coho salmon (Oncorhynchus kisutch) infected with Renibacterium salmoninarum, the causative agent of a slowly developing, chronic disease (bacterial kidney disease) of salmonids. Subsequent experiments demonstrated that juvenile rainbow trout (Oncorhynchus mykiss) infected with Vibrio anguillarum, the causative agent of the acute disease vibriosis, had elevated levels of Hsp70 in hepatic and head kidney tissues prior to clinical signs of disease (Ackerman and Iwama, 2001) [1]. Collectively, these data provide early evidence that a relationship exists between heat shock proteins and disease in fish.

Heat Shock Protein Gene in Fishes

Model of the role of heat shock factor (HSF1) in the regulation of heat shock protein expression. Drawing was made after diagrams in Morimoto *et al*. (1996).

1) Latent monomeric form of HSF1 in cytoplasm or nucleus.
2) Activation by stressor leads to trimerization of HSF1, which facilitates binding to heat shock element of the HSP70 gene promoter.
3) Phosphorylation and activation of HSP70 transcription results in HSP70 expression.
4) Increase in cytosolic HSP70 repairs misfolded proteins, along with other HSPs.
5) Subsequent repair of damaged proteins results in higher levels of free HSP70 in cytosol.
6) Higher concentration of HSP70 results in binding to HSF1, facilitating release from DNA, and dissociation of HSF1 back to the monomeric form.



Conclusion

Heat shock proteins are known to play a pivotal role in protein homeostasis and the cellular stress response within the cell (Feder and Hofmann, 1999) [13, 14]. However, despite decades of extensive investigations a number of outstanding questions remain. Feder and Hofmann (1999) [13, 14] suggested that future experiments are required to: (a) resolve how heat shock protein genes, their regulation, and function have co-evolved in response to environmental change, and (b) how the action of heat shock proteins at the molecular level leads to whole-organismal stress tolerance. We propose that one of the fundamental questions about the role of heat shock proteins is the functional relationship between the cellular stress response, the organismal stress response, and physiological processes at higher levels of biological organization. This linkage between genomics and physiology has seldom been

addressed, but will be critical for understanding the responses of organisms to their environment. Fish are ideal models for addressing this question as they are naturally exposed to Thermal and other complex stressors in their natural environment, and offer an excellent vertebrate model to investigate the physiology, function, and regulation of heat shock proteins. DNA sequences are now becoming available for heat shock proteins in fish, providing the tools necessary to begin functional genomic research in fish. Even in a context where complete sequence information is not known, it is possible to carry out preliminary functional genomic experiments. Gracey *et al*. (2000) [16] used DNA microarrays to study gene expression in Gillichthys mirabilis, even though little prior sequence information was available for this species. This indicates that functional genomics studies can be carried out in any species. With the development of new tools, we will be able to revisit old questions regarding the roles and regulation of heat shock proteins and gain new insights into their functional importance in fish. Heat shock proteins are collectively only one of the molecular mechanisms that animals utilize to tolerate stress, and these proteins can have pleiotropic effects, interacting with multiple systems in diverse ways. Thus, the cellular stress response has impacts on, and is influenced by, processes at all levels of biological organization.

Functional and evolutionary genomics approaches will be critical for understanding the complex and integrative regulatory mechanisms that animals invoke in order to cope with changes in their natural environment.

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