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Heat Shock Protein Genes in Fish

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Abstract

Heat shock proteins are a family of highly conserved cellular proteins present in all organisms including fish. Fish represent an ideal model organism to understand the regulation and functional significance of heat shock proteins (Hsps). The mechanism regulating the expression of Hsp genes in fish have not been studied in detail. In this review, the function, genomic structure and environmental adaptation of the major fish Hsps were discussed. Future research evaluating the functional genomics of Hsps in fish will provide substantial insight into the physiological and ecological roles of these highly conserved proteins.

Keywords: Heat shock protein, fish, environment, genomic structure, function.

Balıklarda Isı Şoku Proteinleri

Özet

Isı şoku proteinleri balıklarda dahil olmak üzere tüm organizmalarda bulunan yüksek oranda korunmuş hücresel bir protein ailesidir. Balıklar, ısı şoku proteinlerinin düzenlenmesi ve fonksiyonel önemlerinin anlaşılmasında ideal model organizmalardır. Balıklarda ısı şoku protein gen anlatımlarını düzenleyen mekanizmalar ayrıntılı şekilde çalışılmamıştır. Bu derlemede, temel balık ısı şoku proteinlerinin fonksiyon, genomik yapı ve çevresel adaptasyonları tartışılmıştır. Balıklarda gelecekte yapılması planlanan fonksiyonel genomik araştırmalar, yüksek oranda korunmuş ısı şoku proteinlerininin fizyolojik ve ekolojik rollerinin anlaşılmasını sağlayacaktır.

Anahtar Kelimeler: Isı şoku proteini, balık, çevre, genomik yapı, fonksiyon.

Introduction

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). 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). The Hsp genes are highly conserved and have been characterized in a wide range of organisms. The heat shock response is an evolutionarily conserved mechanism for maintaining cellular homeostasis following sublethal noxius stimuli (Lindquist, 1986; Lindquist and Craig, 1988).

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).

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 relationship between Hsp synthesis and the development of thermotolerance has been studied by some investigators (Mosser *et al*., 1987; Chen *et al*., 1988). The effects of daily and seasonal temperature fluctuations as well as acclimation temperature have also been examined, especially in fish species (Koban *et al*., 1987; White *et al*., 1994).

Function

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). In the unstressed cell, heat shock proteins have constitutive functions that are essential in protein metabolism (Morimoto *et al*., 1994; Hightower *et al*., 1999). 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) since the expression of their genes may be activated be heat shock heavy metals (Airaksinen *et al*., 2003).

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), several metals such as copper, zinc and mercury (Sanders, 1993; Williams *et al*., 1996), 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), distinct Hsp families (Smith *et al*., 1999) and stressors (Airaksinen *et al*., 2003; Iwama *et al*., 1998) and the sensitivity of Hsp expression may also vary with the species (Basu *et al*., 2002; Nakano and Iwama, 2002) developmental stage (Lele *et al*., 1997; Santacruz *et al*., 1997; Martin *et al*., 2001), and season (Fader *et al*., 1999).

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). Thus Elicker and Hutson (2007) 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).

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 (Baek *et al*., 2000; Escobedo *et al*., 2004), metal toxicity (Somji *et al*., 1999; Leal *et al*., 2002), and anoxia/ischemia (Shelden *et al*., 2002; Hollander *et al*., 2004), cancer (Ciocca *et al*., 1993; Ciocca and Vargas-Roig, 2002), cardiac hypertrophy (Knowlton *et al*., 1998; Scheler *et al*., 1999), and muscle myopathies (Benndorf and Welsh, 2004) 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) 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).

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). 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).

Genomic Structure

Little is known about the sequence, genomic structure, or organization of the genes encoding heat shock proteins in fish because studies have been performed exclusively at the protein level. Heat shock protein genes have only been cloned from a restricted number of different fish species. At present, limited knowledge is present about the genomic organization of the genes encoding Hsps in fish.

Small heat shock proteins are present in nearly

every species and have low-molecular weight Hsps. (Narberhaus, 2002). Several members of the sHsps family have been cloned in fish. sHsps range in size from 12–43 kD and are characterized by a single conserved domain of approximately 80 residues known as the α-crystallin domain. While humans have ten sHsps (Fontaine *et al*., 2003; Kappe *et al*., 2003), it has recently been suggested that the common ancestor to teleosts had as many as thirteen. sHsps have been identified in the zebrafish, ten of which are likely orthologs of human sHsps, and each of which corresponds to one of the thirteen teleost sHSPs (Franck *et al*., 2004). Through searching all available expressed gene and genomic sequence databases, seven additional zebrafish sHsps exist (Hspb1, Hspb2, Hspb3, Hspb4, Hspb5a, Hspb5b, and Hspb12) (Posner *et al*., 1999; Franck *et al*., 2004; Mao *et al*., 2005; Smith *et al*., 2006). The zebrafish protein is 57% similar to human Hsp27, 56% similar to mouse Hsp25 and 64% similar to an Hsp27 protein cloned from the desert topminnow, *Poeciliopsis lucida* (Norris *et al*., 1997). The nearest human homologs to fish specific genes; Hspb13, Hspb14 and Hspb15 are HSPB6, HSPB9, and HSPB1, respectively. Assuming that fish do not have HSPB6 or HSPB9, which suggests that the common ancestor to teleosts had all four genes (HSPB6, HSPB13, HSPB9, and HSPB14), with HSPB6 and HSPB9 having been lost during the evolution of the teleost. zfHsp27 is 22% similar to Hsp30 identified in *Poeciliopsis lucida* and 16% similar to an Hsp30 sequence from rainbow trout.

Hsp30 proteins in zebrafish, zfHsp27 appear to be a member of the Hsp27 family of proteins. Hsp30 has been cloned from the chinook salmon (Kondo *et al*., 2004). Pearson *et al*. (1996) cloned and characterized an hsp47 in zebrafish. Norris *et al.* (1997) cloned two small heat shock proteins, hsp27 and hsp30, in the desert pupfish, *Poeciliopsis lucida*. Phosphorylated serines present in human Hsp27 at positions 15, 78 and 82 are conserved in zfHsp27 at positions 15, 85 and 89. This cysteine is also predicted at position 144 in zfHsp27. Interestingly, a second cysteine, not found in the human or other mammalian sequences, is predicted at position 163 of zfHsp27. Like Hsp27 from *Poeciliopsis lucida*, zfHsp27 appears to lack much of C-terminal tail domain of about 18 amino acids characterizing mammalian Hsp27 proteins. Similarity between zfHsp27 and mammalian proteins within the carboxyl domain is similar to that of the total protein (53% similarity with human) (Norris *et al*., 1997).

The synteny is strongly conserved between four zebrafish and human sHsp genes (two or more immediate gene neighbors in common), hspb1, hspb2, hspb5b, and hspb7. Conservation of synteny is less strong for hspb3, hspb4, hspb5a, and hspb8 (Sun *et al*., 2004). While zebrafish hspb5b and human HSPB5 share the same two immediate neighbors, zebrafish hspb5a and human HSPB5 share only one of two nearby neighbors. Of the six genes that map to within 100 kbp of zebrafish hspb5a, however, five are within 6.7 Mbp of HSPB5, supporting the argument for their orthology.

Hsp70 has been cloned from rainbow trout (*Oncorhynchus mykiss*) (Kothary *et al*., 1984; Airaksinen *et al*., 1998), medaka (*Oryzias latipes*) (Arai *et al*., 1995), zebrafish (Lele *et al*., 1997; Santacruz *et al*., 1997), tilapia (*Oreochromis mossambicus*) (Molina *et al*., 2000), carp (*Cyprinus carpio*) (Yin *et al*., 1999) and pufferfish (*Fugu rubripes*) (Lim and Brenner, 1999) and heat stress-related increases in mRNA levels have been investigated. The fish hsp70 genes are highly conserved at the amino acid level (Molina *et al*., 2000; Deane and Woo, 2006). Keller *et al.* (2008) explored that heat stress-induced Hsp70 expression was altered by activation of ERK (Extracellular signal regulated kinase) in the zebrafish Pac2 fibroblast cell line as occurs in mammalian cells. Heat stress induced both Hsp70 mRNA expression and phosphorylation of both ERK1 and ERK2 (ERK1/2) in Pac2 cells. ERK inhibitors, PD98059 and U0126 we reported to block both heat stress-induced and platelet-derived growth factor (PDGF)-induced ERK1/2 phosphorylation, and also diminished heat-induced Hsp70 expression. Pac2 cell viability was not affected by either the ERK inhibitors or heat stress. This knowledge demonstrates that induction of Hsp70 as a response to heat stress is dependent on ERK activation in Pac2 cells. The available knowledge suggests that the heat shock response in zebrafish utilizes a similar signaling pathway to that of mammals (Elicker and Hutson, 2007; Keller *et al*., 2008).

Hsp70 response in rainbow trout red blood cells (Currie *et al*., 1999) corresponds to Hsp90. Mammalian genomes encode two closely related Hsp90 genes, namely as alpha and beta. Both have been sequenced in zebrafish, both have been shown to be differentially regulated in developing embryos (Krone and Sass, 1994). A complete sequence of an hsp90a has also been obtained from the chinook salmon (*Oncorhynchus tshawytscha*) (Palmisano *et al*., 2000; Eder *et al*., 2009). The expression of Hsp90a gene was studied in a chinook salmon embryonic cell line and it was shown to be heat inducible (Palmisano *et al*., 2000). A fragment of Hsp90a has been cloned from the Japanese flounder (*Paralichtys olivaceus*) (Nam *et al*., 2003). An hsp90 sequence from Atlantic salmon (*Salmo salar*) was characterized by Pan *et al.* (2000) which corresponded to the Hsp90b of zebrafish with a 92% amino acid identity. Atlantic salmon hsp90b expression, in vitro and in vivo, was shown to be upregulated in gill and kidney tissues, but the magnitude of induction was not as great as for the inducible Hsp70 gene (Basu *et al*., 2002; Pan *et al*., 2003).

Partial cDNA sequences encoding Hsp30, Hsp70, Hsp90 beta and heat shock cognate70

(HSC70), and full-length cDNA sequences encoding Hsp27, Hsp47 and Hsp60 were also cloned from goldfish (*Carassius auratus*). A significant up-regulation in Hsp30 and Hsp70 transcripts was exhibited in goldfish collected in winter in Gaobeidian Lake. Hsp27, Hsp30 and Hsp90 beta transcripts were upregulated on the day of collection in summer. The increase in expression of Hsp30 was found to be more prominent among the fishes in Gaobeidian Lake than at the cleaner reference site (Huairou Reservoir). In the latter case, the Hsp30 expression was almost non-detectable, suggesting the possibility of using it as a biomarker for complex environmental pollution (Wang *et al*., 2007).

Environmental Adaptation

The regulation of Hsps in fish has both a genetic and environmental component. Strong evidence suggests that Hsps have critical roles in helping fish cope with environmental change. Their involvement in inducible stress tolerance raises some fundamental questions regarding the regulation of this protection and whether fish in nature can be conditioned by one stressor to better tolerate a subsequent insult. Organisms respond to environmental stress by synthesizing a small number of highly conserved Hsps. The role of Hsps in thermotolerance appears to be crucial, since the inhibition of Hsp synthesis prevents the development of thermotolerance in rainbow trout (*Oncorhynchus mykiss*) fibroblasts (Mosser *et al*., 1987). During or following perturbation of the intracellular environment (e.g., thermal shock, heavy metal exposure), Hsps restore structure and function to denatured proteins, where such denaturation is reversible, or target proteins for removal from the cell, where denaturation is irreversible.

Most studies on Hsps in an environmental context have focused on the effects of heat stress; however, natural environments are highly complex and fish are often exposed to multiple stressors. Hsps enable fish to adapt to environmental stressors including temperature and osmotic stress and exposure to a variety of xenobiotic compounds. Exposure of salmon to a mild thermal shock capable of inducing Hsp70 significantly enhances survival of fish subjected to osmotic stress (Dubeau *et al*., 1998). Cross-protection, also known as cross-tolerance is the ability of one stressor to transiently increase the resistance of an organism to a subsequent heterologous stressor. This cross-protection may be a critical feature of cellular stress response in an environmental context. Studying fish in natural environments may tease out the complex and highly integrated genetic and environmental relationship, and give information on the relative significance of recent or long-term environmental history in regulating the cellular stress response. Hsp70 is the most commonly expressed protein in response to thermal stress. The extent of its expression is associated with differences in environmental temperatures.

Conclusions and Future Perspectives

In conclusion, heat shock proteins are collectively the only one of the molecular mechanisms that animals utilize to tolerate stress, and these proteins have pleiotropic effects, interacting with multiple systems in diverse ways regulated by the endocrine system. The utility of fish as a model system to address the unknown questions regarding the functional, ecological, and evolutionary roles of heat shock proteins, and the relevant studies of heat shock protein genes and the regulation of their expression in fish is discussed. Future experiments are needed to resolve heat shock protein genes regulation, function, response to environmental change, and their action at the molecular level leading to aquatic organismal stress tolerance. Evolving functional genomics approaches will provide the tools to gain a comprehensive understanding of the significance of heat shock proteins in the cellular stress response, in the physiological processes at higher levels of organization, and in the whole animal in its natural environment.

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