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HEAT SHOCK PROTEINS: WHAT ARE THEY, AND DO THEY HAVE A ROLE IN ASSESSING THERMAL TOLERANCE?

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Abstract

Heat shock proteins (HSPs) are a suite of evolutionary conservative proteins of varying molecular weight produced across phylogenies. HSPs exist at latent "baseline" levels but induction accelerates under stress. Stressors that can induce the production of HSPs are several, including altered thermal regimes, hypoxia, exposure to toxic pollutants, and exposure to pathogens. Regarding exposure to thermal stress, the principal functions of induced HSPs are: (1) restore the original folding of polypeptides (proteins) that are altered, thus restoring function; (2) suppress the aggregation (agglutination) of proteins; and (3) delay or accelerate protein catabolism. The response of HSP induction to thermal stress in aquatic organisms has been investigated in the laboratory and, to a lesser extent, in the field. In recent years, the relationship between HSP induction and phenotypic expression (e.g., thermal tolerance) has been documented for some fish species. The finding that HSP expression is often linked to physiological, biochemical, and/or behavioral responses suggests the potential utility of HSPs as a biological indicator. One advantage of monitoring HSP expression in field settings is nonlethal handling techniques. Further research is needed to expand the faunal representation of HSP responses, and evaluate the sensitivity of induction as compared to other responses to thermal exposure at higher levels of biological organization.

Basic Biochemistry

HSPs, often called stress proteins and extrinsic chaperones, are a class of polypeptide molecules that are ubiquitous and cross across phylogenies (bacteria, plants, animals). HSPs are categorized by their molecular weight (in the range 16 – 100 kDa), with their nomenclature dictated by the molecular weight of the protein (e.g., the HSP90 family contains heat shock proteins having molecular weights ranging between 82 – 96 kDa). A key feature of HSPs is their evolutionary conservatism; even among diverse organisms, the amino acid sequence of HSPs is surprisingly similar. For example, the amino acid sequence similarity in the heat shock protein HSP70 among all eukaryotic organisms ranges between 60-80% [1]. HSPs were first discovered in fruit flies (*Drosophila* sp.) when chromosome "puffs" were observed after the flies were exposed to high temperatures [2].

There is a diverse variety of stress proteins, including some unrelated to HSPs. These molecules serve some kind of biochemical response (function) to stress, and the specificity of the response

is variable. Some stress proteins are induced due to exposure to contaminants. Examples of these include metallothioneins and cytochrome P450 enzymes. These two particular stress proteins have been studied in a wide variety of exposure conditions, often in field settings where aquatic life is chronically exposed to pollutants [3, 4].

Mechanistic Function

For many HSPs, the precise function (biochemical pathways, induction stimuli) has yet to be delineated. What seems to be clear is that HSPs have functional roles for normal cellular function, and during periods of stress. For the well-studied HSP70 class, the development of thermal tolerance in animals has been documented by numerous researchers. In general, the induction of HSP70 molecules is correlated with increased thermal tolerance (depending, of course, on the regime of a sublethal exposure), and experimental manipulations that either block HSP70 accumulation or deliberately "over-express" their induction are directly related to thermal tolerance [1].

The principal function of HSPs is to modify a cellular response to heat stress. Since protein function is directly related to protein configuration, any disruption of the protein molecule (e.g., altered amino acid sequence, structural derangement) will cause aberrant function. Extreme heat stress in animals may lead to irreversible protein function loss resulting in the loss of homeostatic equilibrium (which may lead to mortality). HSPs – that are induced themselves by the heat stress – affect protein assembly and translocation, repair protein folding, prevent proteins from agglutinating, and delay or accelerate protein catabolism [5]. While the biochemical pathways of how HSPs affect cellular processes after heat stress are complicated (and insights to these processes have only recently been elucidated), the basic process of HSP function is summarized below (summarized from Figure 2 of Iwama et al. [5]):

- Before induction of a stress, heat shock factor molecules (HSF) are present in latent monomeric (unchained, not polymerized) form in the cell cytoplasm or nucleus.
- Following organism exposure to stress (e.g., elevated temperature), HSF molecules enter the nucleus and undergo trimerization (polymerization of three HSF molecules); the polymerized HSFs bind to amino acids on a HSP70 gene promoter site.
- Transcription of the bound amino acid sequences begins, resulting in the expression (translation) of HSP70 polypeptides, which are released to the cytoplasm.
- Cytosolic HSP70 proteins increase, which can be used to repair misfolded proteins or suppress agglutination of proteins (cellular damage). Once protein repair has taken place, the HSP70 proteins are released and these can either repair other deranged proteins or return to the nucleus.
- Once in the nucleus, HSP70 proteins bind to polymerized HSF molecules, causing the disassociation of the HSF molecules and release of monomeric HSF proteins into the nucleus or back into the cytosol.

The cellular function of HSPs (protein repair during heat stress) can be inactivated by any process that disrupts the gene transcription/translation cycle. The timing of HSP induction is variable (is highly dependent on the magnitude and duration of the heat stress), and can last between hours or days following the incipient thermal stress. Over-expression of HSPs has been

documented in many cases. This is probably an adaptive evolutionary tactic, since ectothermic organisms – unable to escape the heat stressor through behavioral or physiological means – must use all cellular mechanisms to survive the present exposure conditions.

Heat Shock Protein Studies in Fish

Fish are ideal ectothermic organisms to study the function of heat shock proteins due to their relatively large size, well-known life history attributes, and (in some cases) the documentation of genome sequences. A review of HSP studies in aquatic organisms other than fish (freshwater and marine invertebrates) was summarized by Sanders [6]. Studies using fish involve three levels of organization: (1) fish cell lines; (2) primary cell cultures; and (3) whole fish, in laboratory or field settings [5]. In this section, a review of representative studies involving whole fish – in both laboratory and field settings – is provided.

Laboratory studies documenting the induction and expression of HSPs in fish exposed to manipulated thermal regimes involve several species, with some of these being mummichog [7], two gobiid species [8], four marine species [9], cutthroat trout [10], fathead minnow [11], and desert topminnows [12]. Most of these studies evaluated HSP induction using a rapid (acute) heating regime, which may not be representative of many *in situ* conditions where a point source of heat discharges to a water body. In contrast, Kikuchi et al. [13] exposed groups of goldfish to a constant temperature of both 10°C and 30°C, providing a five-week acclimation period. A novel 65kDa protein was isolated only in fish exposed to the 30°C water. The protein was chemically distinct from heat shock proteins in the HSP70 class.

In the vast majority of laboratory studies, the induction of specific HSPs cannot be definitely linked to some kind of phenotypic expression, such as increased thermal resistance or morphological changes. In a study using larval green sturgeon exposed to three different thermal regimes, the expression of specific HSPs was evaluated and compared to survival and development [14]. Newly hatched larvae were exposed to one of three temperature conditions: (1) constant control temperature of 17°C; (2) a short-term (3-day) exposure to an elevated temperature (26°C), followed by a return to the control temperature; and (3) constant exposure to 26°C up through yolk-sac absorption. Specific HSP70 proteins were assayed in both control and exposed fish. One-third of the fish exposed to the short-term elevated temperature developed deformed notochords. When these fish were returned to the lower control temperature, only 16.5% of the original 33% showed deformed notochords, suggesting a morphological recovery from the stress. In the fish that were returned to cooler water, the induction of HSPs continued for at least nine days. The percentage of deformed larvae, and the expression of two HSP70 proteins (HSP72 and HSP78), were highest in fish exposed to the most stressful thermal regime (continuous exposure to 26° C). Fish with irreversibly deformed notochords had significantly higher expression levels of HSP72 and HSP78, and lower HSP60 levels compared to normal larvae. Thus, the variation in phenotypic expression (normal or deformed notochord) was clearly linked to the over-expression, or under-expression, of certain HSP70 proteins.

There are many anecdotal observations made by biologists, in field studies where fish populations are exposed to limiting thermal regimes, suggesting that younger individuals of a species (juveniles) are more thermally tolerant than adults. Thermal tolerance studies with the fruit fly, *Drosophila*, demonstrated that heat-shock resistance decreases with age in these insects, which was associated with decreased expression of HSP70 proteins [15]. A biochemical

mechanism of these observations in fish was lacking, until Fowler et al. [16] showed that the induction of HSP70 proteins in heart tissues of rainbow trout were significantly higher in fingerlings compared to adults when fish were exposed to a rapid heat stress (1 hr at 25°C). Juvenile fish also had a greater induction of constitutive (heat shock factor) proteins.

In a novel study, two subspecies of the common killifish (*Fundulus heteroclitus*) were collected from streams differing in latitude, and tested for thermal resistance [17]. Adult individuals of the northern subspecies (*F. heteroclitus macrolepidotus*) and the southern subspecies (*F. heteroclitus heteroclitus*) were collected from three stream sites each in lower and higher latitude regions. The critical thermal maxima (CTmax) and minima (CTmin);temperature at which 50% of test fish died following a slow heating or temperature lowering regime was determined, and tissue samples for analyzed for HSP70 profiles. Killifish collected from southern latitudes had significantly higher CTmax values compared to fish from northern latitudes; a temperature differential of about 1.5°C occurred within a wide range of acclimation temperatures. Both northern and southern fish showed significantly greater HSP70-2 levels compared to controls at a heat shock temperature of 33°C, however the magnitude of expression was higher in northern fish. Levels of HSP70-1 proteins during thermal trials, in contrast, did not differ between the two groups. Lastly, levels of the constitutive HSP70 protein were significantly elevated by heat shock in southern fish, but not in the northern fish. The variation in specific HSP expression between the southern and northern fish was closely linked to whole organism phenotypic expression (thermal tolerance).

Collectively, studies conducted to date have shown that, at minimum, the variability in thermal resistance in fresh and marine fish (especially between disjunct populations) is associated with the duration and magnitude of HSP expression. Advances in molecular assays and techniques will likely provide more insights into the role of HSPs in thermal acclimation and tolerance. It has been argued that the regulation and expression levels of HSPs are of major evolutionary and ecological importance, and that the expression of HSPs represent a balance of benefits (short-term resistance) and costs (cellular constituents taken away from growth and development) [18]. Clearly, HSPs have played an important role in the selection of taxa that can adapt and survive during conditions of climate change, which may be highly episodic but severe in terms of magnitude.

Heat Shock Factors as Biological Indicators (Biomarkers)

The use of HSPs as non-destructive biomarkers of thermal exposure and/or effect is appealing, however there are many factors – unrelated to temperature – that affect HSP expression [19]. The influence of confounding factors can be problematic when assessing HSP induction in field-collected aquatic life. As with most other biomarkers used for the assessment of stressor exposure, temporal and spatial variability of the assay endpoint (in both exposed and reference organisms) needs to be carefully evaluated before a conclusion can be made that a specific stressor caused a specific biological response.

References

1. Kregel, K.C. “Heat Shock Proteins: Modifying Factors in Physiological Stress Responses and Acquired Thermotolerance.” Journal of Applied Physiology. Vol. 92, p. 2177 (2002).

2. Ritossa, F. "A New Puffing Pattern Induced by Temperature Shock and DNP in *Drosophila*". Experientia. Vol. 18, p. 571 (1962).
3. Hamilton, S.J., and P.M. Mehrle. "Metallothionein in Fish: Review of its Importance in Assessing Stress from Metal Contaminants." Transactions of the American Fisheries Society. Vol. 115, p. 596 (1986).
4. Roberts, A.P., J.T. Oris, G.A. Burton, Jr., and W.H. Clements. "Gene Expression in Caged Fish as a First-Tier Indicator of Contaminant Exposure in Streams." Environmental Toxicology and Chemistry. Vol. 24, p. 3092 (2005).
5. Iwama, G.K., P.T. Thomas, R.B. Forsyth, and M.M. Vijayan. "Heat Shock Protein Expression in Fish". Reviews in Fish Biology and Fisheries. Vol. 8, p. 35 (1998).
6. Sanders, B.M. "Stress Proteins in Aquatic Organisms: an Environmental Perspective". Critical Reviews in Toxicology. Vol. 23, p. 49 (1993).
7. Koban, M., A.A. Yup, L.B. Agellon, and D.A. Powers. "Molecular Adaptation to Environmental: Heat-Shock Response of the Eurythermal Teleost *Fundulus heteroclitus*". Molecular Marine Biology and Biotechnology. Vol.1 , p. 1 (1991).
8. Dietz, T.J., and G.N. Somero. "The Threshold Induction Temperature of the 90 kDa Heat Shock Protein is Subject to Acclimatization in Eurythermal Goby Fishes (Genus *Gillichthys*)". Proceedings of the National Academy of Science. Vol. 89, p. 3389 (1992).
9. Dietz, T.J., and G.N. Somero. "Species- and Tissue-Specific Synthesis Patterns for Heat-Shock Proteins HSP 70 and HSP 90 in Several Marine Teleost Fishes". Physiological Zoology. Vol. 66, p. 863 (1993).
10. Mazur, C.F. "The Heat Shock Protein Response and Physiological Stress in Aquatic Organisms". Doctoral Thesis, University of British Columbia. 175 pp. (1996).
11. Dyer, S.D., G.L. Brooks, B.M. Sanders, and E.G. Zimmerman. "Tissue Specific Patterns of Heat Shock Protein Synthesis and Thermal Tolerance of the Fathead Minnow (*Pimephales promelas*)". Canadian Journal of Zoology. Vol. 69, p. 2021 (1991).
12. White, C.N., L.E. Hightower, and R.J. Schultz. "Variation in Heat-Shock Proteins among Species of Desert Fishes (Poeciliidae, *Poeciliopsis*)". Molecular Biology and Evolution. Vol. 11, p. 106 (1994).
13. Kikuchi, K., S. Watabe, Y. Suzuki, K. Aida, and H. Nakajima. "The 5-kDa Cytosolic Protein Associated with Warm Temperature Acclimation in Goldfish, *Carassius auratus*". Journal of Comparative Physiology. Vol. 163B, p. 349 (1993).
14. Werner, I., J. Linares-Casenave, J.P. Van Eenennaam, and S.I. Doroshov. "The Effect of Temperature Stress on Development and Heat-Shock Protein Expression in Larval Green Sturgeon (*Acipenser medirostris*)". Environmental Biology of Fishes. Vol. 79, p. 191 (2007).
15. Sorensen, J., and V. Loeschcke. "Decreased Heat-Shock Resistance and Down-Regulation of Hsp70 Expression with Increasing Age in Adult *Drosophila melanogaster*". Functional Ecology. Vol. 16, p. 379 (2002).

16. Fowler, S.L., D. Hamilton, and S. Currie. “A Comparison of the Heat Shock Response in Juvenile and Adult Rainbow Trout (*Oncorhynchus mykiss*) – Implications for Increased Thermal Sensitivity with Age”. Canadian Journal of Fisheries and Aquatic Sciences. Vol. 66, p. 91 (2009).
17. Fangue, N.A., M. Hofmeister, and P.M. Schule. “Intraspecific Variation in Thermal Tolerance and Heat Shock Protein Gene Expression in Common Killifish, *Fundulus heteroclitus*”. Journal of Experimental Biology. Vol. 209, p. 2859 (2006).
18. Sorensen, J.G., T.N. Kristensen, and V. Loeschcke. “The Evolutionary and Ecological Role of Heat Shock Proteins”. Ecology Letters. Vol. 6, p. 1025 (2003).
19. Roberts, R.J., C. Agius, C. Saliba, P. Bossier, and Y.Y. Sung. “Heat Shock Proteins (Chaperones) in Fish and Shellfish and their Potential Role in Relation to Fish Health: A Review”. Journal of Fish Diseases. Vol. 33, p. 789 (2010).