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EXPRESSION ANALYSIS OF HEAT SHOCK GENES IN THE SKIN, SPLEEN AND BLOOD OF COMMON CARP *(CYPRINUS CARPIO)* AFTER CADMIUM EXPOSURE AND HYPOTHERMIA

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Heat shock proteins are chaperones that play a pivotal role in controling multiple regulatory pathways such as stress defense, hormone signaling, cell cycle control, cell proliferation and differentiation, and apoptosis. In this study, the expression patterns of four well-known heat shock genes *(hsp70, hsc70-1, hsc70-2* and *hsp90α)* were characterized in the skin, spleen and blood cells of the common carp, under unstressed conditions and after Cd^{2+} treatment or hypothermia. The examined genes were expressed in a tissue-specific manner: *hsc70-2* was expressed constitutively, and was at best only slightly inducible; *hsp90α* exhibited a high basic expression in all three tissues, whereas *hsc70-1* did so only in the blood cells, the expression of *hsp70* proved to be below the level of detection in unstressed fish. Cold shock induced the expression of *hsp* genes in the spleen *(hsp90α)* and blood cells *(hsp70, hsc70-1* and *hsp90α),* while Cd^{2+} treatment has no effect on the expression pattern. The highest inducibilities were detected in the skin: for *hsp70* an induction of at least 20-fold after cadmium exposure, for *hsc70-1* of at least 30-fold and for *hsp90α* of 3-fold after hypothermia.

Keywords: Cadmium treatment – carp – heat shock genes – RT-PCR

INTRODUCTION

The heat shock proteins (Hsps) are a family of highly conserved cellular proteins present in all organisms examined to date [10, 28], including fish [19]. Under both normal and stress conditions, the Hsps display constitutive functions that are essential from various aspects of the protein metabolism, e.g. *de novo* protein folding, membrane translocation, and the formation or degradation of misfolded proteins [11, 17, 19, 39]. Some family members (Hsp) are at best weakly expressed under normal conditions and are inducible by heat and other forms of stress, allowing cells to cope with acute stressor insults. Others (Hscs) are expressed constitutively, and at best are only slightly inducible; they play essential roles in the protein metabolism under normal conditions [37, 43]. Most of the cited studies have demonstrated a correlation between increased levels of Hsps and exposure to stressors within an ecologically

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relevant range, including temperature changes, hormones, chemicals and heavy metals [3, 31, 35].

Many heat shock genes have been identified and characterized from different fish species. The Hsp90 proteins play essential housekeeping functions, such as controling the activity, turnover and trafficking of various proteins, promoting cell survival through maintenance of the structural and functional integrity of client proteins which control cell survival, proliferation and apoptosis, and playing an important role in the progression of malignant disease [5, 14, 33, 42]. Genomes of higher animals encode two closely-related *hsp90* genes (*α* and *β*). Both have also been sequenced in zebrafish, and both have been shown to be differentially regulated in developing embryos [22].

The Hsp70s interact with a number of other proteins to assist the folding of nascent polypeptide chains, act as molecular chaperones, and mediate the repair and degradation of altered or denatured proteins. *Hsp70* expression is regulated by environmental and physiological stress and non-stressful conditions such as growth and development [21, 26, 32, 41].

The response of Hsps, highly conserved throughout evolution, is observed universally, from bacteria through lower eukaryotes to human. Elevated levels of various Hsps have been measured in tissues of fish exposed to environmental contaminants such as heavy metals [3, 7, 40], industrial effluents [29, 38], pesticides [16, 23, 36], and polycyclic aromatic hydrocarbons [25, 38]. Pathogen exposure and chronic diseases also influence the Hsps.

To date, we have identified and characterized five members of the *hsp* family *(hsp70, hsc70-1, hsc70-2, hsp90α* and *hsp90β)* from *Cyprinus carpio.* The *hsp* genes in the carp exhibit specific patterns of expression. Carp *hsp70* mRNA has not been detected in the brain or muscle, and it is around the limit of detection in the kidney and liver of unstressed animals [2]. The proteins Hsc70 in the carp are the first examples in lower vertebrates of Hsc70 isoforms with substantially lower identities than their counterparts in zebrafish or mammals. In the unstressed carp liver, *hsc70-2* is highly expressed, whereas the expression of *hsc70-1* is virtually undetectable in this tissue [1]. The *hsp90β* is constitutively expressed at a fairly high level in the carp brain, liver and kidney, and is slightly inducible by elevated temperature. *Hsp90α* mRNA is present in the brain, but is hardly detectable in the kidney and liver of unstressed animals. *Hsp90α,* but not *hps90β,* responds to an elevated level of Cd^{2+} in a dose-, time- and tissue-dependent manner in the liver and kidney [18].

Studies of the expression of *hsps* genes of fish have focused on the liver and kidney, organs most involved in the processing and excretion of toxic agents. However, little is known of the manner of expressions in the skin, spleen or blood cells; in the elements of the defense system. The skin is the largest organ in the body, forming the interface between the animals and their environment. The spleen is well developed in fish, with important roles as regards the red blood cells and via the melanomacrophages, the immune system. In the spleen, the new blood cells enter the blood stream, and the degenerating blood cells (especially the erythrocytes) are sorted out [9]. Blood performs many important functions within the body, including the supply of

nutrients and oxygen to tissues; the removal of waste materials such as carbon dioxide, urea and lactic acid; immunological functions; the transport of hormones; and the signaling of tissue damage [8]. In contrast with most mammals, the erythrocytes of lower vertebrates are nucleated and able to synthesize proteins [12].

In this study, our aims were the characterization of expressions of *hsp70, hsc70-1, hsc70-2* and *hsp90α* genes in organs with no immediate role in detoxification (skin, blood cells and spleen) of common carp. The common carp, an omnivorous fish commonly used in commercial aquaculture, has been proposed as a test organism in toxicologic assay due to its economic importance and wide geographic distribution [30]. In addition, we studied the gene- and tissue-specific effects of a high-dose in Cd^{2+} loading and the hypothermia. Our experiments may allow the better detection of the transcriptional characteristics of fish *hsp* genes.

MATERIALS AND METHODS

Animals and treatments

Carp weighing 800–1000 g, obtained from the Tisza Fish Farm, Szeged, were acclimatized under fasting conditions in well-aerated 400-L water tanks over a 3-week period at 12 °C. During the acclimatization, the water was changed twice a week. In cold shock treatments, the fish were transferred from 12 °C to 5 °C for 1 to 5 h. Samples were taken from the tissues either immediately after the cold treatment or after a 1-h recovery period at the acclimatization temperature. For metal treatment, the carp were transferred into 100-L water tanks (2 fish/tank) containing 10 mg/L Cd^{2+} ($Cd(CH_3COO)_2 \times 2H_2O$, Fluka) under static conditions. Cd^{2+} at this concentration is not lethal to common carp for 21 days. In all experiments, 4 animals were sacrificed at each time point for organ harvesting, frozen immediately in liquid nitrogen and stored at –80 °C.

RNA extraction, reverse transcription and PCR amplification

Frozen, intact hearts were homogenized in RNAzol B reagent (Tel-Test, Inc., Friendswood, Texas, USA) and the total RNA was prepared according to the procedure suggested by the manufacturer. To detect carp *hsp*-specific mRNAs, an RT-PCR-based strategy was employed. First-strand cDNA synthesis, PCR amplification and primers, specific to *hsp70, hsc70-1, hsc70-2, hsp90α* and *β*-actin genes of carp were used as described earlier [1, 2, 16]. Amplification was performed in a PTC 200 Peltier Thermal Cycler (MJ Research). The number of amplification cycles during which PCR product formation was limited by the template concentration was determined in pilot experiments: for *hsp*s 30 cycles were used. The amplified products were electrophoresed on 2% agarose (Sigma) gel.

Measurements and statistical analysis

At each experimental time point, 3–4 fish were used to prepare RNA. RT-PCR reactions for each animal were performed in triplicate in order to increase the reliability of the measurements. For normalization of the amount of *hsp* mRNAs, the carp *β*-actin mRNA level was used as internal standard. Images of ethidium bromide-stained agarose gels were digitized with a GDS 7500 Gel Documentation System and analyzed with GelBase/GelBlot™ Pro Gel Analysis Software (UVP). The relative levels of *hsp* mRNAs are expressed as ratios [10 × *hsp/β*-actin]. Statistical differences were calculated with one-way analysis of variance (ANOVA) (MedCalc Statistical Software version 9.4.2.0, Broekstraat, Belgium) with a Student-Newman-Keuls follow-up test. Significant difference was accepted at $P < 0.05$.

RESULTS

Basal expressions of heat shock genes

In the skin, the level of *hsc70-2* mRNA was found to be highly expressed, but that of *hsp90α* was low and those of *hsp70* and *hsc70-1* were below the limit of detection.

In the spleen, the *hsc70-2* mRNA level was again expressed highly, that of *hsp90α* was relatively high, and those of *hsp70* and *hsc70-1* were below the limit of detection.

In the blood, the *hsc70-2* mRNA level was lower than in the other examined tissues, while *hsp70* mRNA was virtually undetected. As concerns the three examined tissues, the *hsc70-1* and *hsp90α* mRNA levels were highest in the blood (Fig. 1).

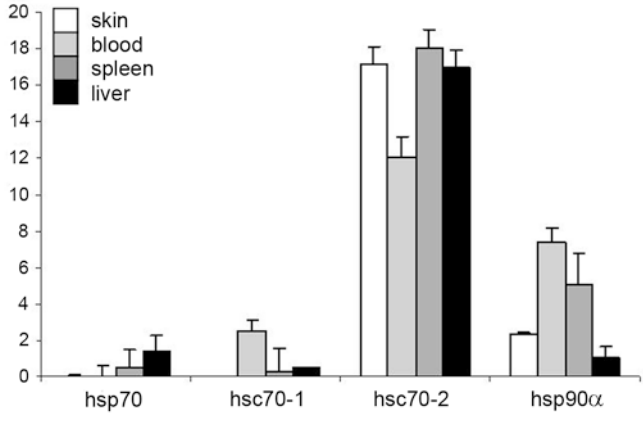

Fig. 1. The relative levels of *hsp* transcripts in untreated carp tissues. All data are means ± S.D. of the results of measurements on 3–5 fish at each time point

Inducibility of heat shock genes

After Cd^{2+} treatment, the expressions of *hsc70-1* and *hsc70-2* in the skin were only insignificantly changed. The *hsp90α* and *hsp70* mRNA levels were induced transiently. After the 24-h treatment, the induction of *hsp90α* was 2.5-fold and *hsp70* was induced at least 20-fold. After hypothermia, the *hsp70* and *hsc70-2* mRNA levels were unchanged. The expressions of *hsc70-1* and *hsp90α* were increased significantly, with the highest levels (3-fold of *hsp90α* and at least 30-fold of *hsc70-1*) after the 5-h cold treatment (Fig. 2).

In the spleen, the mRNA levels of the examined four heat shock genes were changed only insignificantly after the 24-h Cd^{2+} exposure. After the cold shock, the expression of *hsc70-2* proved to be significantly decreased (~65% of the control level), while the level of *hsp90α* mRNA was increased 2-fold after the 5-h treatment (Fig. 3).

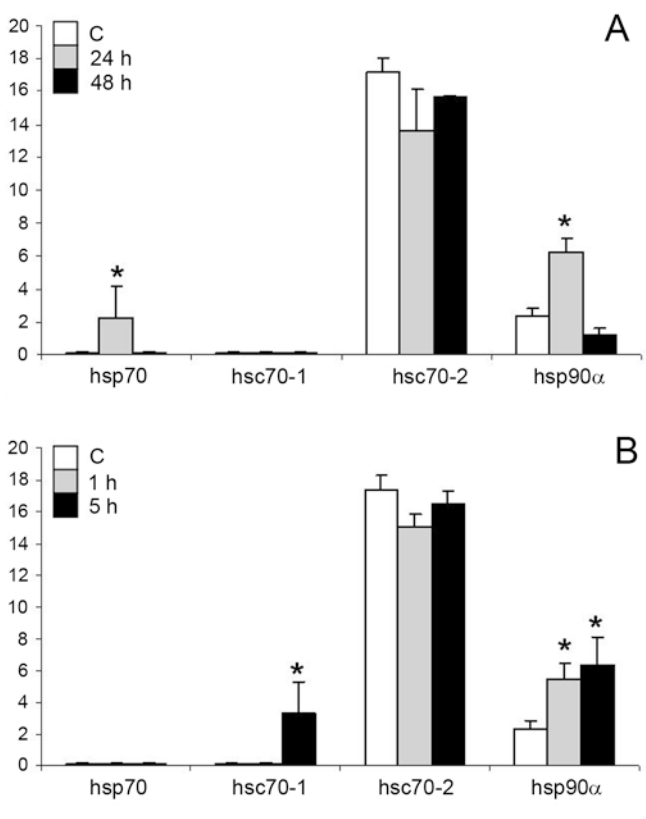

Fig. 2. Hsp expression levels in the skin following Cd^{2+} treatment (A) and hypothermia (B). The colors of columns indicate the time points of treatments. Values are means ± S.D. of the results of measurements on 3–4 animals at each time point. Data labeled by an asterisk differ significantly from the control value at the $P < 0.05$ level

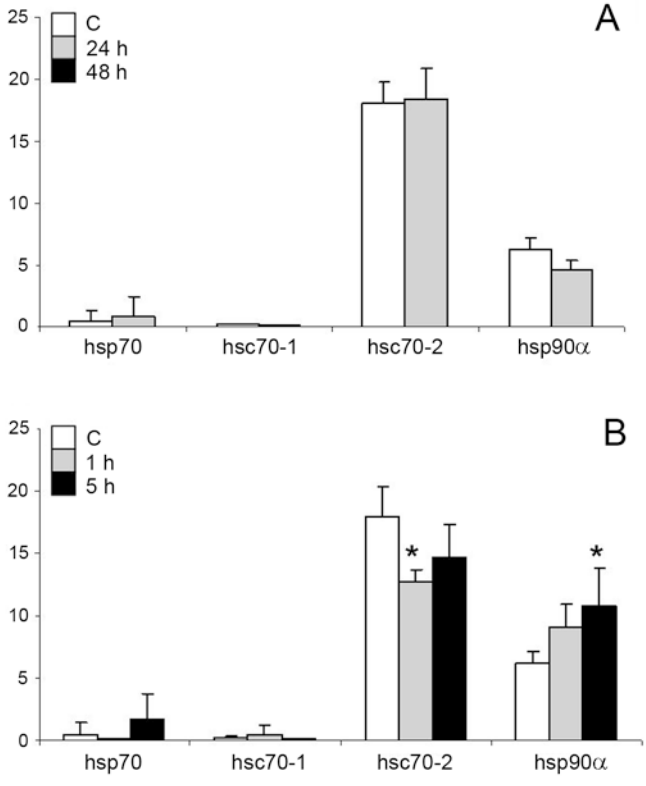

Fig. 3. Hsp expression levels in the spleen following Cd^{2+} treatment (A) and hypothermia (B). The colors of columns indicate the time points of treatments. Values are means ± S.D. of the results of measurements on 3–4 animals at each time point. Data labeled by an asterisk differ significantly from the control value at the $P < 0.05$ level

In the blood cells, the expression of *hsp90α* after Cd^{2+} treatment significantly decreased, to ~55% of the control level. The *hsp70* and *hsc70-1* mRNA levels were increased. A fast response occurred in the case of *hsp70;* after the 24-h Cd^{2+} exposure, the mRNA levels were similar to that of *hsp70* in the spleen. The *hsc70-1* mRNA level reached the maximum (2.5-fold) after the 48-h exposure. Following the 1-h cold shock exposure, the *hsp70* mRNA level was transiently increased and the expression of *hsc70-1* demonstrated a 2-fold induction. The expression of *hsp90α* was induced 1.5-fold after the 5-h cold shock, whereas the *hsc70-2* mRNA level was decreased to ~75% of the control level (Fig. 4).

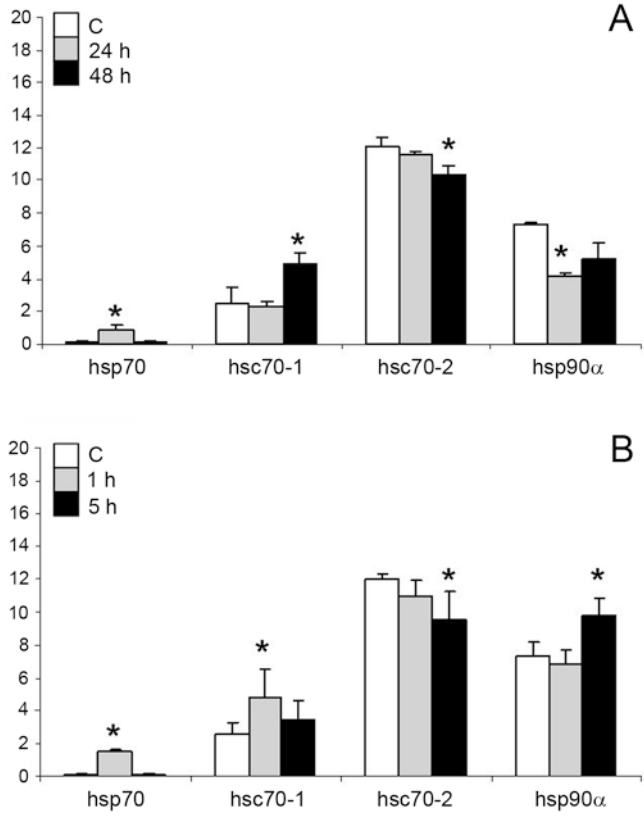

Fig. 4. Hsp expression levels in the blood following Cd^{2+} treatment (A) and hypothermia (B). The colors of columns indicate the time points of treatments. Values are means ± S.D. of the results of measurements on 3–4 animals at each time point. Data labeled by an asterisk differ significantly from the control value at the $P < 0.05$ level

DISCUSSION

The fish represent an ideal organism to resolve the regulation and functional significance of heat shock proteins, as they are naturally exposed to thermal and other complex stressors in their natural environment. Generally, Hsp proteins are thought to provide the cell with protection by preventing aggregation or improper folding of proteins [15]. In addition, Hsps play crucial roles in the long-term adaptation of animals to their environment [27]. Numerous biotic and abiotic factors regulate the expression of the heat shock proteins in fish (thermal stress, osmotic stress, or environmental contamination) [1–3, 18, 28]. In this study hypothermia and heavy metal exposure were selected to follow the transcriptional regulation of hsp genes in three organs; blood, skin, spleen.

The skin is the largest organ in the body. It acts as a barrier, provides physical protection for the body, is the site of coloration, contains sensory receptors, and in some fish, functions in respiration [20, 34]. The importance of skin in the antioxidant defense system is indicated by the high levels of the cytokine genes INF-γ and IL-1β, glucose transport genes and catalase in the skin of the Atlantic cod indicate [4].

In the skin of the unstressed common carp, *hsc70-1* and *hsp70* mRNA were not detected, but the control *hsp90α* mRNA level was higher than in the previous examined carp tissues [8]. A moderate *hsp70* mRNA level was measured in the skin of the Atlantic cod [4], and in human keratinocytes [20]. In addition, one of the highest *hsp90α* mRNA level was measured in the skin of the flatfish [25]. After Cd^{2+} treatment and cold shock, changes in gene-specific expression were detected in the skin. The *hsp70* mRNA levels were transiently induced after Cd^{2+} exposure, but remained lower than those of the other *hsp* genes. The *hsp90α* mRNA levels were also increased in the skin after these experiments. In previous studies, the *hsp90α* expression was influenced only in the kidney of common carp by Cd^{2+} exposure [18].

Blood performs many important functions within the body, including the supply of nutrients and oxygen to tissues; the removal of waste materials such as carbon dioxide, urea and lactic acid; immunological functions; the transport of hormones; and the signaling of tissue damage [8]. In contrast with most mammals, the erythrocytes of lower vertebrates are nucleated and able to synthesize proteins, including the *hsp* proteins [6, 12].

In the blood cells of common carp, the *hsc70-1* and *hsp90α* mRNA levels were higher, while that of *hsc70-2* was lower than in the liver [1, 2, 18]. The ratio *hsp90α* : *hsc70-2* was ~1 : 6 in the skin, and ~2 : 3 in the blood. After Cd^{2+} treatments the *hsp90α* mRNA level was decreased in the blood cells. *Hsc70-1* may be of great importance for the blood cells, as the mRNA level of *hsc70-1* was highest in the blood, and inductions of this gene were detected after both metal exposure and hypothermia. The *hsp70* mRNA levels were transiently induced after treatments, the increased of levels were at least 15-fold. *Hsp70* mRNA has been shown to be actively produced in the red blood cells of the brook trout subjected to heat shock [24], and has been induced in red blood cells of rainbow trout after 25 °C heat exposure [6].

The spleen is well developed in fish, with important roles as regards the red blood cells and via the melanomacrophages, the immune system. In the spleen, the new blood cells enter the blood stream, and the degenerating blood cells (especially the erythrocytes) are sorted out [9]. In the spleen of the striped bass, the transcriptions of metallothionein and TGF-β, but not the expressions of *hsp70* and *hsp90,* are strongly influenced by Cu^{2+} treatment [13].

Overall, the expression patterns of the four examined *hsp* genes in the spleen of unstressed carp proved similar to those in the skin or liver [1, 2, 18]. The spleen was the only tissue in which significant changes in expression of the examined heat shock genes were not found after 10 mg/l Cd^{2+} treatment. Likewise, the *hsp70* expression was unchanged after heavy metal treatments in the spleen of the striped bass [13]. After cold shock, diminished *hsc70-2* mRNA levels were measured in the spleen, while the *hsp90α* mRNA levels were increased with 25%. Our results indicate that the

hsp90α gene may play a significant role in the examined tissues: in all cases, high control levels (2–8-fold of the levels in the liver) and also 2–3-fold inductions were detected after hypothermia.

Heat shock proteins will be critical for understanding the responses of organisms to their environment [3]. A fundamental question about the role of Hsp proteins may be the functional relationship between the cellular stress response, the organismal stress response and physiological processes of different organisms.

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REFERENCES

1. Ali, K. S., Dorgai, L., Ábrahám, M., Hermesz, E. (2003) Tissue- and stressor-specific differential expression of two *hsc70* genes in carp. *Biochem. Biophys. Res. Com. 307,* 503–509.
2. Ali, K. S., Dorgai, L., Gazdag, A., Ábrahám, M., Hermesz, E. (2003) Identification and induction of hsp70 gene by heat shock and cadmium exposure in carp. *Acta Biol. Hung. 54,* 323–334.
3. Basu, N., Todgham, A. E., Ackerman, P. A., Bibeau, M. R., Nakano, K., Schulte, P. M., Iwama, G. K. (2002) Heat shock protein genes and their functional significance in fish. *Gene 295,* 173–183.
4. Caipang, C. M., Lazado, C. C., Brinchmann, M. F., Rombout, J. H., Kiron, V. (2011) Differential expression of immune and stress genes in the skin of Atlantic cod *(Gadus morhua). Comp. Biochem. Physiol. Part D Genomics. Proteomics.* [Epub ahead of print]
5. Csermely, P., Schnaider, T., Soti, C., Prohaszka Z., Nardai, G. (1998) The 90-kDa molecular chaperone family: Structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther. 79,* 129–168.
6. Currie, S., Tufts, B. L. (1996) Synthesis of stress protein 70 (Hsp70) in rainbow trout *(Oncorhynchus mykiss)* red blood cells. *J. Exp. Biol. 200,* 607–614.
7. Duffy, L. K., Scofield, E., Rodgers, T., Patton M., Bowyer, R. T. (1999) Comparative baseline levels of mercury, hsp70 and hsp60 in subsistence fish from the Yukon-Kuskokwim delta region of Alaska. *Comp. Biochem. Physiol. Toxicol. Pharmacol. 124,* 181–186.
8. Ellett, F., Lieschke, G. J. (2010) Zebrafish as a model for vertebrate hematopoiesis. *Curr. Opin. Pharmacol. 10,* 563–570.
9. Fänge, R., Nilsson, S. (1985) The fish spleen: structure and function. *Experientia 41,* 152–158.
10. Feder, M. E., Hofmann, G. E. (1999) Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annu. Rev. Physiol. 61,* 243–282.
11. Fink, A. L., Goto, Y. (1998) *Molecular Chaperones in the Life Cycle of Proteins: Structure, Function, and Mode of Action.* Marcel Dekker, New York.
12. Fulladosa, E., Deane, E., Ng, A. H., Woo, N. Y., Murat, J. C., Villaescusa, I. (2006) Stress proteins induced by exposure to sublethal levels of heavy metals in sea bream *(Sparus sarba)* blood cells. *Toxicol. In Vitro 20,* 96–100.
13. Geist, J., Werner, I., Eder, K. J., Leutenegger, C. M. (2007) Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass *(Morone saxatilis)* following treatment with copper and esfenvalerate. *Aquat. Toxicol. 85,* 28–39.
14. Hao, H., Naomoto, Y., Bao, X., Watanabe, N., Sakurama, K., Noma, K., Motoki, T., Tomono, Y., Fukazawa, T., Shirakawa, Y., Yamatsuji, T., Matsuoka, J., Takaoka, M. (2010) HSP90 and its inhibitors. *Oncol. Rep. 23,* 1483–1492.

15. Hartl, F. U. (1996) Molecular chaperones in cellular protein folding. *Nature 381,* 571–580.
16. Hassanein, H. M. A., Banhawy, M. A., Soliman, F. M., Abdel-Rehim, S. A., Muller, W. E. G., Schroder, H. C. (1999) Induction of Hsp70 by the herbicide oxyfluoren (goal) in the Egyptian Nile fish, *Oreochromis niloticus. Arch. Environ. Contam. Toxicol. 37,* 78–84.
17. Hendrick, J. P., Hartl, F. U. (1993) Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem. 62,* 349–384.
18. Hermesz, E., Ábrahám, M., Nemcsók, J. (2001) Identification of two hsp90 genes in carp. *Comp. Biochem. Physiol. Part C. 129,* 397–407.
19. Iwama, G. K., Thomas, P. T., Forsyth, R. B., Vijayan, M. M. (1998) Heat shock protein expression in fish. *Review. in Fish Biol. Fish 8,* 35–56.
20. Jonak, C., Klosner, G., Trauringer, F. (2006) Heat shock proteins in the skin. *Int. J. Cosm. Sci. 28,* 233–241.
21. Kiang, J. G., Tsokos, G. C. (1998) Heat shock proteins 70 kDa: Molecular biology, biochemistry, and physiology. *Pharmacol. Ther. 80,* 183–201.
22. Krone, P. H., Sass, J. B. (1994) Hsp90α and hsp90β genes are present in the zebrafish and are differentially regulated in developing embryos. *Biochem. Biophys. Res. Commun. 204,* 746–752.
23. Lertkiatmongkol, P., Pethuan, S., Jirakanjanakit, N., Rongnoparut, P. (2010) Transcription analysis of differentially expressed genes in insecticide-resistant *Aedes aegypti* mosquitoes after deltamethrin exposure. *J. Vector. Ecol. 35,* 197–203.
24. Lund, S. G., Phillips, M. C., Moyes, C. D., Tufts, B. L. (2000) The effects of cell ageing on protein synthesis in rainbow trout (*Oncorhynchus mykiss*) red blood cells. *J. Exp. Biol. 203,* 2219–2228.
25. Manchado, M., Salas-Leiton, E., Infante, C., Ponce, M., Asensio, E., Crespo, A., Zuasti, E., Cañavate, J. P. (2008) Molecular characterization, gene expression and transcriptional regulation of cytosolic HSP90 genes in the flatfish Senegalese sole *(Solea senegalensis Kaup). Gene 416,* 77–84.
26. Mladineo, I., Block, B. A. (2010) Expression of cytokines IL-1beta and TNF-alpha in tissues and cysts surrounding *Didymocystis wedli* (Digenea, Didymozoidae) in the Pacific bluefin tuna *(Thunnus orientalis). Fish Shellfish Immunol. 29,* 487–493.
27. Morimoto, R. I., Santoro, M. G. (1998) Stress-inducible responses and heat shock proteins: New pharmacological targets for cytoprotection. *Nat. Biotechnol. 16,* 833–838.
28. Morimoto, R. I., Tissieres, A., Georgopoulos, C. (1990) *Stress Proteins in Biology and Medicine.* Cold Spring Harbor Press, New York.
29. Mukhopadhyay, I., Nazir, A., Saxena, D. K., Chowdhuri, D. K. (2002) Toxicity of cypermethrin: hsp70 as a biomarker of response in transgenic Drosophila. *Biomarkers 7,* 501–510.
30. Oruc, E. U., Usta, D. (2007) Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ. Toxicol. Pharmacol. 23,* 48–55.
31. Padmini, E. (2010) Physiological adaptations of stressed fish to polluted environments: role of heat shock proteins. *Rev. Environ. Contam. Toxicol. 206,* 1–27.
32. Park, J. H., Lee, J. J., Yoon, S., Lee, J. S., Choe, S. Y., Choe, J., Park, E. H., Kim, C. G. (2001) Genomic cloning of the Hsc71 gene in the hermaphroditic teleost *Rivulus marmoratus* and analysis of its expression in skeletal muscle: Identification of a novel muscle-preferred regulatory element. *Nucleic Acids Res. 29,* 3041–3050.
33. Pearl, L. H., Prodromou, C. (2000) Structure and in vivo function of hsp90. *Curr. Opin. Struct. Biol. 10,* 46–51.
34. Rakers, S., Gebert, M., Uppalapati, S., Meyer, W., Maderson, P., Sell, A. F., Kruse, C., Paus, R. (2010) 'Fish matters': the relevance of fish skin biology to investigative dermatology. *Exp. Dermatol. 19,* 313–324.
35. Roberts, R. J., Agius, C., Saliba, C., Bossier, P., Sung, Y. Y. (2010) Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. *J. Fish. Dis. 33,* 789–801.
36. Sanders, B. M., Nguyen, J., Martin, L. S., Howe, S. R., Coventry, S. (1995) Induction and subcellular localization of two major stress proteins in response to copper in the fathead minnow *Pimephales promelas. Comp. Biochem. Physiol. C Pharmacol. Toxicol. 112,* 335–343.

37. Santacruz, H., Vriz, S., Angelier, N. (1997) Molecular characterization of a heat shock cognate cDNA of zebrafish, hsc70, and developmental expression of the corresponding transcripts. *Dev. Genet. 21,* 223–233.
38. Vijayan, M. M., Pereira, C., Kruzynski, G., Iwama, G. K. (1998) Sublethal concentrations of contaminant induce the expression of hepatic heat-shock protein 70 in 2 salmonids. *Aquat. Toxicol. 40,* 101–108.
39. Welch, W. J. (1993) How cells respond to stress. *Sci. Am. 269,* 56–64.
40. Williams, J. H., Farag, A. M., Stansbury, M. A., Young, P. A., Bergman, H. L., Petersen, N. S. (1996) Accumulation of hsp70 in juvenile and adult rainbow trout gill exposed to metal-contaminated water and/or diet. *Environ. Toxicol. Chem. 15,* 1324–1328.
41. Young, J. C. (2010) Mechanisms of the Hsp70 chaperone system. *Biochem. Cell. Biol. 88,* 291–300.
42. Young, J. C., Moarefi, I., Hartl, F. U. (2001) Hsp90: A specialized but essential protein-folding tool. *J. Cell Biol. 154,* 267–273.
43. Zafarullah, M., Wisniewski, J., Shworak, N. W., Schieman, S., Misra, S., Gedamu, L. (1992) Molecular cloning and characterization of a constitutively expressed heat-shock-cognate hsc71 gene from rainbow trout. *Eur. J. Biochem. 204,* 893–900.