

A TALE OF TWO STRESSORS: CROSS PROTECTION IN FISH

Anne E. Todgham
Faculty of Agricultural Sciences
University of British Columbia
Vancouver, BC V6T 1Z4, Canada
phone: (604) 822-4910/ fax: (604) 822-9274/
email: todgham@interchange.ubc.ca

George K. Iwama
Faculty of Agricultural Sciences, University of British Columbia
and Institute for Marine Biosciences, National Research Council
Halifax, NS B3H 3Z1, Canada
email: george.iwama@nrc.ca

EXTENDED ABSTRACT ONLY – DO NOT CITE

The focus of stress research in fish has recently grown to encompass the cellular stress response in addition to that of the whole animal, broadening our understanding of the mechanisms allowing an animal to tolerate stress. Much of this expanded interest has focused on heat shock proteins and their role as molecular chaperones in the cellular response to stress (Iwama et al, 1998; Feder and Hofmann, 1999). Although HSPs have a relatively short half-life, their levels remain elevated in the whole organisms long after the stressor is removed, indicating a role in long term adaptation and increased stress tolerance (Parsell and Lindquist 1993; Morimoto and Santoro 1998).

By artificially enhancing a fish's stress tolerance both *in vivo* and *in vitro*, through exposure to a mild heat shock, we have been able to explore some of the pathways involved in the protection against stress in fish hepatocytes, and to relate these to the physiological changes that occur in the whole animal. This phenomenon of cross protection, or the ability of a mild stressor to transiently increase the tolerance of an animal to a subsequent heterologous stressor, has not been well documented in fish. We have begun to define and describe cross protection in fish *in vivo* and more recently have used cross protection as a model to identify some of the critical pathways regulating the cellular stress response in fish *in vitro*.

Cross Protection *in vivo*

This study was designed to investigate the capacity of a mild heat shock to increase the tolerance of tidepool sculpins (*Oligocottus maculosus*) to a subsequent heterologous stressor, focusing on the functional role of heat shock proteins (Hsps). Survival of tidepool sculpins exposed to severe osmotic and hypoxic stressors increased from 68% to 96%, and from 47% to 76% respectively when 10°C acclimated fish were exposed to a 22°C heat shock. The magnitude of this heat shock was critical for protection (Fig.1). A 20°C heat shock was insufficient to confer cross protection while a 25°C heat shock impaired the ability of the sculpin to tolerate a second stressor. Further experiments demonstrated that cross protection was present in a defined temporal window between 12 and 48h following the 22°C heat shock. Western blot analysis revealed that hepatic Hsp70 levels were significantly elevated 12h following exposure to the mild heat shock, corresponding to the increase in stress tolerance.

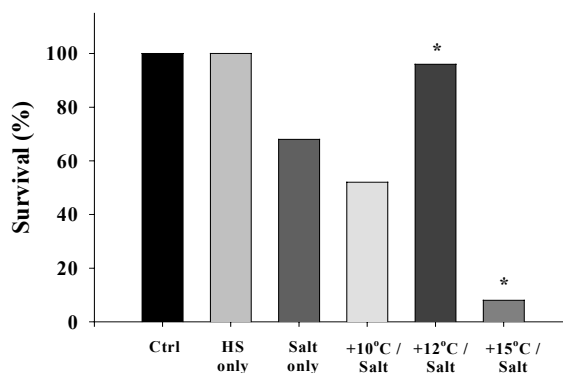


Figure 1. Survival of tidepool sculpins exposed to a heat and/or osmotic shock. * signifies significant difference from Salt only ($p < 0.01$).

In nature, a fish's thermal history is important in structuring its cellular response to stress (Feder and Hofmann, 1999). In their natural environment there is approximately 12 hours of high tide between low tide cycles. The time frame of this cross protection may provide evidence of the tidepool sculpin's ability to invoke a protective mechanism from one low tide period for the unpredictable nature of the next.

Cross Protection *in vitro*

Recently we have begun to investigate some of the underlying mechanisms that are responsible for the protection conferred by a mild heat shock in primary cultures of Rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Hepatocytes were

exposed to a 28°C heat shock (HS) for 2h, then allowed to recover for 14h at 13°C, and then exposed to a 24h oxidative shock (OxS, 2.0mM H₂O₂). Pretreatment with a 28°C heat shock significantly increased viability of cells

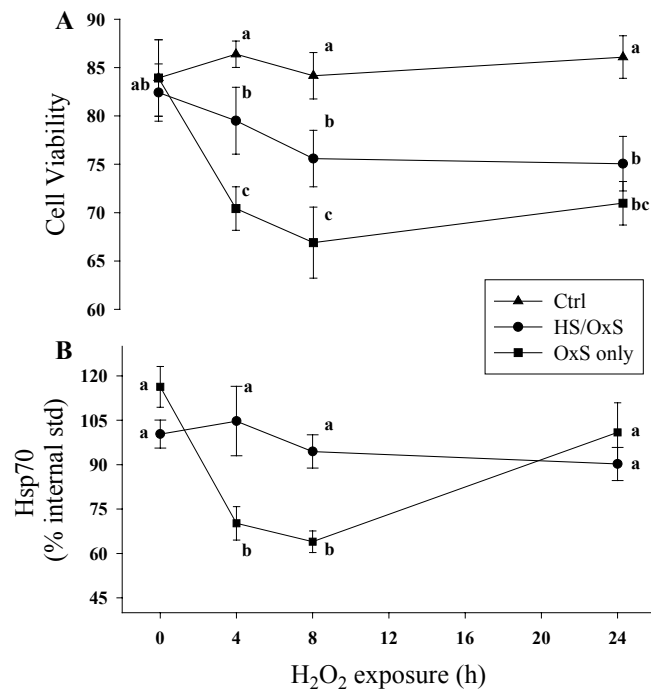


Figure 2. Viability (A) and Hsp70 (B) levels of hepatocytes exposed to a heat and/or oxidative shock. Different letters signifies significant difference ($p < 0.05$). Cell viability of HS only group did not differ significantly from controls.

exposed to an oxidative shock at 4h and 8h of exposure (Fig. 2A). However heat shock was unable to restore cell viability to that of controls and was unable to maintain protection against oxidative shock at 24h of exposure. Exposure to the oxidative shock alone significantly decreased Hsp70 levels at 4h and 8h of exposure. Pretreatment with heat shock eliminated this oxidative stress induced decrease in Hsp70 levels (Fig. 2B). Following 24h exposure to H₂O₂, heat shocked cells (HS only and HS/OxS groups) had significantly lower levels of

glucose in their media compared to the other groups, with HS/OxS cells having the lowest levels. This depletion of glucose suggests that cross protection may represent an energetic cost to the fish.

In summary, exposure to a mild heat shock increased the tolerance of hepatocytes exposed to a subsequent more severe stressor, and this tolerance persisted for a defined time period. Hsp70 levels correlated with the protection conferred by the heat shock; however, their exact involvement requires further investigation.

Conclusions

These results suggest that patterns of cross protection are similar whether studied *in vivo* or *in vitro*. *In vivo* experiments provide an appropriate model to investigate the relationship between the cellular and physiological stress responses, while *in vitro* studies will be invaluable in probing the mechanisms underlying the cellular stress response.

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