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The effect of heat and cold exposure on *HSP70* expression and development of deformities during embryogenesis of Atlantic salmon (*Salmo salar*)

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Abstract

Temperature stress is recognized as a teratogenic factor that induces deformities during the embryonic development of teleosts. In order to further elaborate the mechanisms involved, Atlantic salmon (*Salmo salar*) embryos were heat (16 °C) and cold (1 °C) shocked at eight and four different embryonic stages, respectively, during the period from gastrulation until the completion of somitogenesis. Additionally, embryos were subjected to a long-term heat exposure at 12 °C from the ~1st until the ~20th somite stage. Real-time RT-PCR results showed that the *HSP70* mRNA expression was dependent on the stage of development. Whereas temperature shock was found to induce *HSP70* mRNA up-regulation at the gastrula stage, the ~9th, the ~15th, the ~20th and the ~45th somite stage, the additional three investigated stages showed no up-regulation. The highest *HSP70* expression levels were induced at the ~45th somite stage as shown by a 12- and 4-fold increase after heat and cold shock, respectively. Embryos subjected to the prolonged heat exposure showed a stronger *HSP70* expression than embryos that were given a 1-h heat shock. Intriguingly, a high incidence (17%) of *situs inversus* of abdominal organs was found in fry subjected to the long-term exposure, supporting that early somitogenesis is an important period of left–right determination in teleosts. In general, the 1-h temperature shock was not sufficient to induce high frequencies of deformities. Though, a 14% incidence of vertebral deformities was observed both at the ~45th somite stage and at the completion of somitogenesis after cold shock. The results provide new insight regarding the tolerance of high and low temperature stress in Atlantic salmon embryos.

Keywords: Cold shock; Deformities; Embryos; Expression; *HSP70*; Heat shock; *Situs inversus*

1. Introduction

Deformities are recognized as a recurring problem in fish aquaculture and represent both ethical and economical challenges for the industry. Deformities

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of skeletal structures as well as soft tissues are observed, including aplasia of *septum transversum* and anomalies of the heart and the swimbladder (Kaada and Hopp, 1995; Poppe et al., 1997; Vågsholm and Djupvik, 1998; Kvellestad et al., 2000). Factors that induce deformities in fish include temperature (Baeverfjord, 1998), nutrition (Sandnes et al., 1992; Cahu et al., 2003), infections (Madsen et al., 2001), antibiotics (Toften and Jobling, 1996), genetics (Sadler et al., 2001) and intensive aquaculture conditions (Koumoundouros et al., 2001). Fish embryos are particularly vulnerable to development of temperature-induced deformities (Koo and Johnston, 1978; Sato et al., 1983; Wiegand et al., 1989; Wang and Tsai, 2000; Hansen and Falk-Pedersen, 2001; Ornsrud et al., 2004a,b), and the teratogenic effect is dependent on the developmental stage of the exposed embryos (Baeverfjord, 1998; Roy et al., 1999; Werner et al., 2003). In salmon aquaculture, the teratogenic effects of temperature are of particular interest, as temperature manipulations are a powerful and commonly used tool to increase developmental rate in this cold-water species. A range of malformations was induced in Atlantic salmon exposed to high temperatures (>8 °C) during egg incubation, and a variation in temperature sensitivity within the embryonic period was demonstrated (Baeverfjord, 1998). Current recommendations for incubation of Atlantic salmon eggs identify 8 °C as an upper limit for normal embryonic development for this species, while the lethal temperature is approximately 12 °C (Gunnes, 1979).

The amount of heat shock proteins (HSP) has been shown to play an important role in protecting embryos against temperature-induced damage (Edwards, 1998; Mirkes et al., 1999). HSPs are a highly conserved group of genes that are divided in three major families: HSP90 (85–90 kDa), HSP70 (68–73 kDa) and low molecular weight proteins (16–47 kDa). HSP70 is known to assist the folding of nascent polypeptide chains, act as a molecular chaperone, and mediate the repair and degradation of altered or denatured proteins (Kiang and Tsokos, 1998; Basu et al., 2002; Hartl and Hayer-Hartl, 2002). A number of heat shock genes, including *HSP70*, exhibit complex patterns of constitutive expression during early embryonic development (Lele et al., 1997; Krone et al., 1997; Santacruz et al., 1997; Krone et al., 2003). Furthermore, *HSP70* has been shown to be heat inducible at different embryonic stages in several species, including zebrafish (*Danio rerio*) (Lele et al., 1997), medaka (*Oryzias latipes*) (Werner et al., 2003), sea urchin (Sconzo et al., 1995), *Xenopus* (Heikkila et al., 1997) and rat (Mirkes and Doggett, 1992).

The aim of this study was to identify temperature sensitive developmental stages in Atlantic salmon embryos. This was conducted by quantification of the *HSP70* mRNA expression in embryos subjected to high or low temperature stress and by relating the *HSP70* transcription level to the subsequent incidents of deformities in juvenile salmon.

2. Materials and methods

2.1. Egg material, egg incubation and fish rearing

Eggs and milt were collected from mature Atlantic salmon from a commercial egg producer (Aakvik settefisk, Halsa, Norway) in December 2001 and transported separately to the AKVAFORSK research station (Sunndalsøra, Norway). The fish were of AquaGen breed, a farmed stock subjected to selective breeding for 6 generations prior to the experiment (Gjedrem et al., 1991). Transport time was 2 h and the temperature in the egg buckets was 4.5 °C at arrival in the hatchery. At arrival, the pooled egg batch from five females was fertilized with the pooled milt from three males and rinsed in saline water (0.9% NaCl).

The eggs were incubated in a hatchery designed for incubation of small egg volumes. The incubation set-up consisted of longitudinally cut styrofoam insulated plastic tubes, which were subdivided into separate compartments with separate water inlets and outlets. The eggs were placed in wire baskets covered with styrofoam lids, which were painted black to reduce exposure to light. Water supply was continuous from a temperature controlled tank stabilized at 8 °C monitored twice daily. Approximately 0.2 l of non-water-hardened eggs (about 1500–2000) were incubated in each unit.

One-hour temperature shock was performed by turning off the 8 °C water supply and gradually replacing the water volume of the compartment with either warmer or colder water. Care was taken to avoid any mechanical stress to the eggs. A stable exposure