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Proteome changes in rainbow trout (*Oncorhynchus mykiss*) fertilized eggs as an effect of triploidization heat-shock treatment

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ABSTRACT

The aim of the present study was to explore proteome changes in rainbow trout (*Oncorhynchus mykiss*) fertilized eggs as an effect of triploidization heat-shock treatment. Eggs and milt were taken from eight females and six males. The gametes were pooled to minimize the individual differences. After insemination, the eggs were incubated at 10 °C for 10 min. Half of the fertilized eggs were then subjected to heat shock for 10 min submerged in a 28 °C water bath to induce triploidy. The remainder were incubated normally and used as diploid controls. Three batches of eggs were randomly selected from each group and were incubated at 10–11 °C under the same environmental conditions in hatchery troughs until the fry stage. Triplicate samples of 30 eggs (10 eggs per trough) from each group were randomly selected 1.5 h post-fertilization for proteome extraction. Egg proteins were analyzed using two-dimensional electrophoresis (2-DE) and MALDI-TOF/TOF mass spectrometry. Based on the results from the statistical analyses, 15 protein spots were found to decrease significantly in abundance in heat-shock treated group and were selected for identification. Out of 15 protein spots showing altered abundance, 14 spots were successfully identified. All of the egg proteins identified in our study were related to vitellogenin (vtg). Decreased abundance of vitellogenin in heat-shock treated eggs in our study may either be explained by (i) higher utilization of vtg as an effect of increased cell size in triploids or (ii) changed metabolism in response to heat-shock stress and (iii) diffusion of vtg through chorion due to incidence of egg shell damage. Decreased abundance of vitellogenin in heat-shock treated eggs was associated with reduced early survival rates and lowered growth performance of triploid fish.

1. Introduction

Triploid fish with three sets of homologous chromosomes are produced with the objective of producing sterile populations for aquaculture or fisheries management (Benfey, 1999). Because fish eggs are released at

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the metaphase stage of meiosis II, induction of triploidy can be achieved by blocking the second meiotic division and preventing extrusion of the second polar body (Ihssen et al., 1990). Retention of the second polar body is occurred through thermal or hydrostatic pressure treatment of eggs shortly after fertilization (Pandian and Koteeswaran, 1998).

Thermal shocks including both heat and cold shocks have been successfully applied for induction of triploidy in fish species (reviewed in Piferrer et al., 2009). As sterility makes triploids of interest for aquaculture purpose, much of the research on triploid fish has been focused on economically important culture traits (reviewed in Tiwary et al., 2004). While stress associated with the induction of triploidy may also affect performance of triploids in early life stages, there has been no investigation on effects of thermal shocks on fertilized eggs.

Proteomics has emerged as a powerful tool toward a deep understanding of aquatic organisms' biology and provides data at a mechanistic level. Compared to genomics, it can also reveal changes in protein activity identified as post-translational modifications (PTMs) (Rodrigues et al., 2012). Recent proteomics analyses were successful in determining the protein map of fish oocytes (Keyvanshokooh and Vaziri, 2008; Ziv et al., 2008). However, as indicated above, effect of temperature shocks on proteome of fish fertilized eggs is not yet studied. Therefore, the aim of the present study was to explore proteome changes in rainbow trout (*Oncorhynchus mykiss*) fertilized eggs as an effect of heat-shock applied for triploidy induction. Proteome analysis of fertilized eggs in response to heat-shock will offer a basic background in order to trace the molecular alterations and may help to improve the thermal treatments for obtaining better quality in triploidization. Therefore, triploidy was inducted through the application of a heat shock treatment and proteome of heat-shock treated and untreated fertilized eggs were compared.

2. Materials and methods

2.1. Fish, eggs and experimental conditions

The mature rainbow trout from a commercial farm (Aligoudarz, Lorestan province, Iran) were used as broodstock for this study. The broodstock were maintained under natural photoperiod. Eggs and milt were taken from eight females (average weight 1600 ± 246 g) and six males (average weight 1393 ± 186 g) selected after visual and microscopic inspection for quality of eggs and semen as described previously (Aegerter and Jalabert, 2004; Labbe and Maisse, 2001). The gametes were pooled to minimize the individual differences that may affect triploid yields. After insemination, the eggs were rinsed and incubated at 10 °C for 10 min. Half of the fertilized eggs were then (10 min post-fertilization) subjected to heat shock for 10 min submerged in a 28 °C evenly heated, aerated water bath (Pandian and Koteeswaran, 1998) in order to induce triploidy. The remainder were incubated normally and used as diploid controls. Three batches of eggs were randomly selected from each group (heat-shocked and control) and were incubated at 10–11 °C under the same environmental conditions in hatchery troughs (about 1650 eggs per trough) until the fry stage (76 days post-fertilization, dpf). The first-feeding offspring (38 dpf) were also reared under the same environmental conditions (mean water temperature of 14 °C, pH of 7.5 and dissolved oxygen of 8.1 mg/l) and were fed on a rainbow trout commercial diet (BioMar, France) 12 times a day at the rate of 7% of their body weight. Dead eggs and alevins were counted and removed at the eyed egg stage and afterward. Survival rates in early life phases including eyed egg stage (18 dpf), hatching stage (25 dpf) and swim-up stage (38 dpf) were obtained. The fish in the different experimental groups were weighed at the onset of first feeding and at the end of rearing period for estimation of growth.

Fish ploidy level was determined by erythrocyte size measurement (Benfey et al., 1984). Briefly at the end of rearing period (76 dpf), when the size of fishes was reached about 2 g, blood samples were collected by cutting the caudal fin of thirty fish from each experimental group. Blood smears fixed in 95% methanol for 3 min, left to air-dry and stained with 10% Giemsa solution for 15 min. Twenty erythrocytes per slide were studied at 400× magnification to measure the size of erythrocytes using following formula. The letter "*S*" was used to designate the nuclear or cell area, "*V*" the nuclear or cell volume, "a" and "b" the small and large axis of the nucleus or cell. The formulae used for calculations, therefore, were: $S = a \times b \times \pi/4$; and $V = [a/2] \times [b/2]^2 \times \pi \times 4/3$.

2.2. Sampling and protein extraction

Triplicate samples of 30 fertilized eggs (10 eggs per trough) from each experimental group were randomly selected 1.5 h post-fertilization, snap frozen in liquid nitrogen and stored at −80 °C until further analysis. The egg samples were washed in 40 mM Tris–HCl buffer (pH = 7) and finely ground in the presence of lysis buffer. The lysis solution consisted of 7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 50 mM Tris, 0.2% carrier ampholyte, 1 mM PMSF, 0.25% RNase, and 1% DNase. The homogenates were maintained for 1 h at room temperature for protein release before centrifugation at 12,000 × *g* for 10 min at 20 °C. Protein concentration of the extract was determined using the Bradford method with BSA as the standard.

2.3. Two dimensional gel electrophoresis (2-DE)

Isoelectric focusing was performed using the IEF Cell (BioRad, USA). 17 cm IPG (pH 3–10 L) strips were rehydrated in a buffer containing 8 M urea, 4% CHAPS, 50 mM DTT, 0.2% ampholytes pH 3–10, and 2 mg of the protein extracts. Strips were focused at 20 °C with the following program: 20 min with a linear ramp (0–250 V), 5 h with a linear ramp (250–10000 V) and 50000 Vh with a rapid ramp. Once the IEF was completed, the IPG strips were equilibrated for 15 min in equilibration solution (50 mM Tris–HCl pH 8.8, 6 M urea, 20% glycerol, 2% SDS, 0.01% bromophenol blue) containing 2% DTT and then alkylated for a further 15 min in equilibration solution without DTT and supplemented with 2.5% iodoacetamide.