The Impacts of Osmotic Stress During the Critical Window of Development on Protein and Histone PTM Alterations in California grunion

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Abstract

The California Grunion, Leuresthes tenuis, can experience a broad range of salinities during their early life stages, with their nursery grounds measuring salinities of 21 to 42ppt under normal conditions. Due to the unique subterrestrial incubation of their eggs within the sand of California beaches, it is not unlikely for this marine fish to hatch in non-seawater conditions, whereafter the larvae may transition to relatively diluted estuaries or concentrated harbors to develop as juveniles for several months. Consequently, they are an ideal system to study the impacts of ecologically relevant salinity stress on the alteration of proteins and histone post-translational modifications (PTMs) during early development. The study of the relationship between the critical window of development (CWD), in which sexual bipotential is lost, and the longevity of protein landscape and histone PTM changes has not been widely explored. The CWD is of interest as this is when the gametes of this species become set. Therefore, we hypothesize that histone PTM changes that occur during this time may thus be a form of heritable epigenetics. Thus, hypo-and-hyperosmotic exposures of L. tenuis hatchlings during this window, and past it, attempt to identify if the CWD is relevant to the presence and persistence of protein and histone PTM changes under salinity stress. We hypothesize that exposure to osmotic stress during the CWD will result in induced compensatory mechanisms in the protein landscape of the California grunion larvae, as well as persistent alterations in the histone PTMs in the larvae. To elucidate the relationship between these factors, California grunion larvae were exposed in replicate to one of three chronic salinity treatments (16ppt, 32ppt, or 40ppt) for the duration of their CWD (68dph), with 16ppt and 40ppt inducing hypoosmotic and hyperosmotic stress respectively. Chronic post-CWD exposures and recovery times until 98dph were used to determine whether the CWD or prolonged salinity exposures were key to alterations in the histone PTM and protein landscape of the larvae. After each timepoint, L. tenuis were culled and processed for histone PTM enrichment and proteomic analysis. At the time of culling, there were no significant differences in survivorship between replicates and experimental groups, with statistically significant different deaths between groups occurring only shortly after hatching. Skyline and MSstats were used in the analysis of the statistical significance of differential regulation of proteins and histone PTMs under stress conditions versus the controls. Of special interest is the osmoregulatory mechanisms involved in the myo-inositol biosynthesis pathway, with analysis focusing on the abundance of histone acetylation, amidation, ubiquitylation, and 4-hydroxynonelation

in histone PTMs, as the accumulations of these have been found to be correlated with osmotic stress in some fish tissues.

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