Poster #16

Assessment of Molecular Interaction between Low Oxygen and Estrogen in Fish Cell Culture

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Exposure of fish to low oxygen levels (hypoxia) is associated with retarded gonadal development, reduced spawning success, fertilization success, hatching rate, and larval survival. Here, we test for specific interaction between hypoxia and estrogen in mediating gene expression in cultured fish cells. The cellular responses to hypoxia and estrogen are mediated, in part, by the hypoxia-inducible factor (HIF) and the estrogen receptor (ER), respectively.

HIF is a heterodimeric transcription factor that plays a central role in oxygen-regulated gene expression in mammals. During hypoxia, the HIF-α subunit accumulates, dimerizes with ARNT (aryl hydrocarbon receptor nuclear translocator), and together with accessory proteins forms a transcriptional complex that regulates the expression of a number of genes associated with anaerobic metabolism, erythropoeisis, and angiogenesis. Upon estradiol stimulation, the estrogen receptor is activated expressing specific genes such as vitellogenin in the liver of the fish.

Homologs of HIF subunits and estrogen receptors are present in fish. In previous work, we have characterized oxygen-dependent reporter gene expression in two cell lines from rainbow trout, RTG-2 (gonad) and RTH-149 (hepatic). When transiently transfected with a plasmid containing a putative hypoxia response element upstream of the luciferase gene, reporter gene expression was maximal at the lowest oxygen level tested (0.5%) at 48 h. In current experiments, both cell lines are exposed to hypoxia in the presence of an agonist (17- β estradiol) or an antagonist (ICI 182.780) of the estrogen receptor. We will measure levels of mRNA and protein of HIF-α, ARNT, estrogen receptors, and selected down-stream genes (e.g., vitellogenin).

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