MOLECULAR MECHANISMS FOR CONTENDING WITH OSMOTIC STRESS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Atlantic salmon (*Salmo salar*) must contend with hyperosmotic stress as juveniles on a seaward migration from their natal freshwater stream. This osmotic stress is more acute in routine aquaculture practice, in which juveniles are directly transferred from freshwater hatcheries to netpens in full salinity seawater (≅ 1100 mOsm). Plasma chloride can rise from 110 to $\geq$ 200 mMol/L in 12 hours (Handeland et al., 1996). Individuals incapable of regaining osmotic/ionic homeostasis die, or grow at a reduced rate (Björnsson et al., 1988; Duston, 1994; Koch and Evans, 1959). In this report, we present investigations of the molecular mechanisms by which salmon adapt to hyperosmotic stress. At least 6 genes have been found to be upregulated in tissues of salmon exposed to osmotic stress *in vitro* or *in vivo*. Accumulation of mRNA for heat shock protein (hsp) 70, the major stress protein of fish, increased as much as 500 % in branchial lamellae, hepatic tissues and erythrocytes. Incubation of branchial lamellae in medium containing the membrane permeable solute, glycerol, also caused a prominent increase in the concentration of hsp70. A 54 kDa protein, Osp54, also was found to be induced in branchial lamellae and erythrocytes by osmotic stress caused by NaCl or by the membrane impermeable solute, mannitol. Similar to hsp70, mRNA coding for Hsp90 accumulated in the branchial lamellae in response to

osmotic stress *in vitro* and *in vivo.* Although, hsp90 was upregulated in both branchial lamellae and kidney by thermal stress, osmotic stress did not stimulate expression in the kidney. Two novel genes, expressed in branchial lamellae and kidney in response to osmotic stress, were isolated using differential expression analysis and cloned. Nucleotide sequence analysis indicates that one of the cDNAs codes for a protein 160 amino acids in length and rich in glycine. Hydropathy analysis revealed two domains with the second (amino acids 83 to 160) containing G_2-Y-G_2 repeats. Of significant relevance to osmotic stress, the protein exhibited a high degree of similarity (58% at the deduced amino acid level) to a glycine-rich RNA binding protein upregulated by drought stress in plants. Partial sequence analysis revealed that the other cDNA contains a RING box protein motif.

The results of these investigations suggest that a complex array of mechanisms, similar to those observed in the renal tubules of terrestrial mammals, are involved in adaptation of salmon to seawater. Research is underway in our laboratory to identify additional genes and the mechanisms involved in adaptation to osmotic stress.

References

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