FUNDULUS HETEROCLITUS 14-3-3.a: FULL-LENGTH cDNA CLONING, PHYLOGENETIC ANALYSIS, AND OSMOTIC REGULATION

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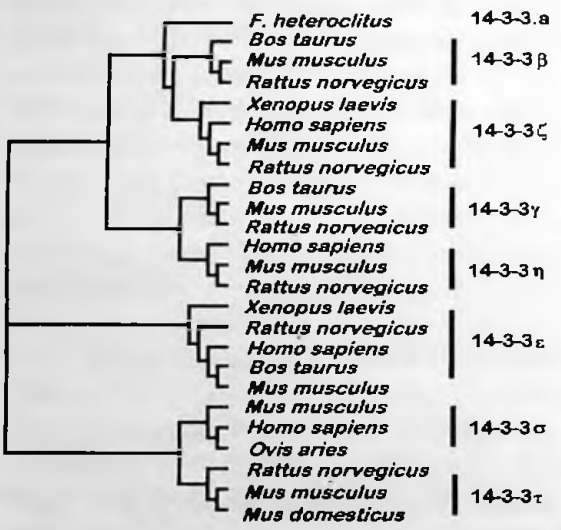
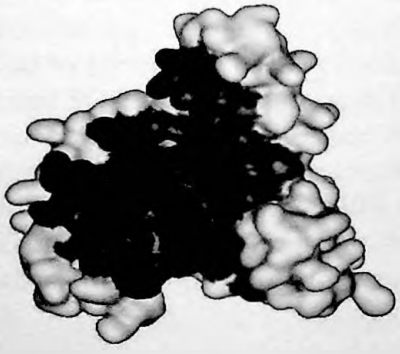
The direction and molecular machinery of ion transport across gill epithelium of euryhaline fish differs markedly in fish exposed to seawater versus fresh water. Such differences are the result of very dramatic changes in differentiation patterns of gill epithelial cells. For instance, the size, morphology, and epithelial location of mitochondria-rich cells differ in FW- versus SW-acclimated fish. 14-3-3 proteins control such adaptive cell differentiation in response to environmental change in many tissues. Therefore, we hypothesize that 14-3-3 proteins also control adaptive remodeling of fish gill epithelium during salinity change. We cloned the full-length cDNA including the complete 3' UTR of a novel 14-3-3 gene from the euryhaline fish *Fundulus heteroclitus* and named it 14-3-3.a (1659 bp, GB AF302039). Phylogenetic analysis of the deduced amino acid sequence revealed that the 14-3-3.a gene product is most similar to vertebrate 14-3-3ζ. However, fish 14-3-3.a is also closely related to 14-3-3β of higher vertebrates. A phylogenetic tree depicting this relationship is shown in Figure 1. Fish 14-3-3.a mRNA expression is highest in gill epithelium, even higher than in brain, where vertebrate 14-3-3 proteins are normally most highly expressed. Moreover, 14-3-3.a mRNA levels increase fourfold within 24h of salinity transfer of fish from SW to FW. The N- and C-termini of fish 14-3-3.a are most unique, whereas the amino acids forming the amphipathic ligand-binding groove are highly conserved, indicating that fish 14-3-3.a binds phosphorylated proteins. The surface structure model of fish 14-3-3.a in Figure 2 (right) clearly shows that the most highly conserved regions (black, frontal side) form the inner substrate binding groove, while the outer face of 14-3-3.a is made up of less conserved amino acids (light gray, back side). Because 14-3-3 proteins are important modulators of many phospho-proteins and signaling complexes the high abundance and osmotic regulation of 14-3-3.a in gills of euryhaline fish suggests that it plays an important role in the remodeling of gill epithelium during salinity adaptation of euryhaline fish. The cloning and the phylogenetic and structural analysis of fish 14-3-3.a provide the basis for the identification of phospho-proteins and signal transduction pathways that contribute to the adaptive remodeling of gill epithelium during salinity change.





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