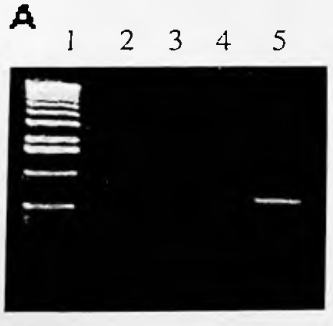
CLONING OF A 14-3-3 GENE FRAGMENT FROM GILL EPITHELIUM OF FUNDULUS HETEROCLITUS

Dietmar Kültz
The Whitney Laboratory
University of Florida, St. Augustine, FL 32086-8623

Here, we report the cloning of a 14-3-3 gene fragment from gill epithelial cells of the euryhaline teleost *Fundulus heteroclitus*, which provides the basis for further investigation of 14-3-3 function during environmental stress in a euryhaline teleost fish. 14-3-3 genes encode phylogenetically highly conserved signaling proteins in all eukaryotes. They were discovered in mammalian brain extracts based on their strong induction in response to environmental stress (Aitken, A., Trends Biochem. Sci. 20:95-97, 1995). Several 14-3-3 isoforms are known to regulate the activity of osmosensory kinases and phosphatases, including MAP kinases (Xing et al., EMBO J. 19:349-358, 2000) and cdc25 (Peng et al., Cell Growth Diff. 9, 197-208, 1998; Kumagai et al., Mol. Biol. Cell 9:345-354, 1998). The major (central) portion of a *F. heteroclitus* 14-3-3 gene was cloned using a homology PCR strategy and degenerate primers. RNA from gill epithelium of eight fish was isolated, reverse transcribed, and the resulting cDNA used for construction of a cDNA library and as a template for PCR. A single PCR band was obtained with no detectable background. Figure 1A depicts the results of the PCR reaction (Lane 1 = 1kb ladder, Gibco BRL, Lane 2 = 25 cycles, Lane 3 = 30 cycles, Lane 4 = 35 cycles, Lane 5 = 40 cycles). The size of the PCR product matched the expected size of ca. 500 bp, which is the length of a conserved region of 14-3-3 flanked by sites that were the basis for construction of our degenerate primers. This region represents the central and major portion of 14-3-3 genes, whose open reading frames are ca. 750 bp long. The PCR product was cloned and sequenced. Phylogenetic sequence analysis of the 529 bp fragment revealed that the deduced amino acid sequence of this partial 14-3-3 coding sequence is most similar to 14-3-3 ζ. An alignment of the most highly conserved portion of the deduced amino acid sequence with mouse and frog 14-3-3 ζ is depicted in Fig. 1B.



B

Fundulus heteroclitus:	Ile Ser Lys **Ala** **Asp** Met **His** Pro Thr His Pro Ile Arg Leu
Xenopus laevis:	Ile Ser Lys Ser Glu Met Gln Pro Thr His Pro Ile Arg Leu
Mus musculus:	Ile Ser Lys Lys Glu Met Gln Pro Thr His Pro Ile Arg Leu

530 bp

Cloning of the full-length coding sequence of this 14-3-3 gene provides the necessary basis for thoroughly probing the function of 14-3-3 during osmotic and other environmental stresses in a euryhaline teleost fish. In the future, we plan to test if 14-3-3 is required for the activation of osmosensory MAP kinase pathways and for cell cycle regulation in response to osmotic stress to further characterize the molecular basis for osmosensory signal transduction in epithelial cells of a euryhaline animal. This work was supported by the Salisbury Cove Research Fund.