Sequencing of a putative COX-2 from the killifish (*Fundulus heteroclitus*) gill

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We have shown previously that the prostanoid PGE_2 is a potent dilator of the aortic vascular smooth muscle in a variety of fish species[1] and also can inhibit the transport of NaCl across the opercular epithelium of the killifish as part of an endothelin-generated cascade[2]. To more carefully delineate the role(s) of this prostanoid in gill physiology, we decided to clone the relevant synthetic enzyme, cyclooxygenase (COX).

Gills were isolated from *Fundulus heteroclitus* and homogenized in Tri-Reagent (Sigma, St. Louis, MO) on ice. RNA was isolated using BCP followed by a series of alcohol washes to precipitate and wash the RNA. Total RNA was reverse transcribed using Invitrogen's Super-Script First Strand Kit (Carlsbad, CA) for RT PCR. Gill cDNA was then used in RT-PCR with primers derived from known shark cyclooxygenase-2 sequence[3]. The product from this initial RT-PCR was cloned into *Escherichia coli* cells using a TOPO-TA kit (Invitrogen). This initial 600 bp sequence was then used to design killifish specific primers that were used with a GeneRacer™ kit (Invitrogen) to amplify the 3' and 5' ends of the transcript. All PCR products were cloned into *E. coli* cells and sequenced at the MDIBL Marine DNA sequencing center. The entire COX gene was sequenced using this method, with only 10 bp that remain to be sequenced.

The final sequence includes 2701 bp that codes for a protein that is most homologous to COX-2 sequences of other vertebrates (Fig. 1) (GenBank accession #AY532639), which supports our physiological evidence that COX-2 is the enzyme involved in prostanoid production in the killifish operculum, and by analogy the gill epithelium[2]. We can now use this gene sequence to generate primers for qPCR and the deduced peptide sequence to generate antibodies, both important in our further exploration of the role of COX in the physiology of the fish gill. (Supported by NSF IBN-0089943 and an REU Supplement to DHE)

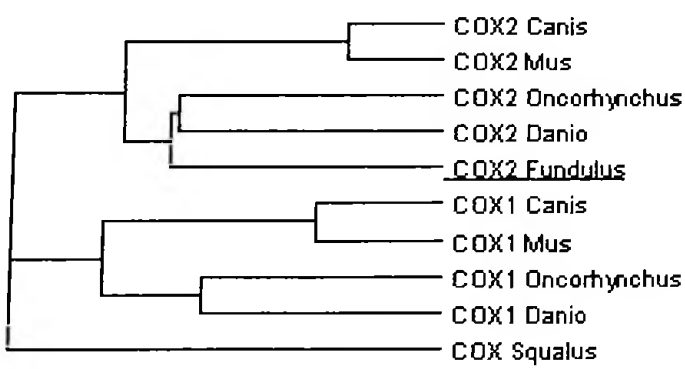

Figure 1. Phylogenetic tree of representative vertebrate COX sequences using the neighbor-joining method (MEGA version 2.1; Tempe, Arizona). The GeneBank accession numbers are, from top to bottom: AAK97783, Q05769, AY028585, AJ238307, AY532639,AAN33049,MP032995 , AY028584,AJ299018,and AF420317.

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3. Yang T, Forrest SJ, Stine N, Endo Y, Pasumarthy A, Castrop H, Aller S, Forrest JN, Jr., Schnermann J, and Briggs J. Cyclooxygenase cloning in dogfish shark, Squalus acanthias, and its role in rectal gland Cl secretion. *Am J Physiol Regul Integr Comp Physiol* 283: R631-637, 2002.