The evolution of cyclooxygenase in ancestral chordates

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In the mammalian kidney, prostaglandins control vascular tone, osmotic homeostasis, hormonal action, and osmotic stress responses at the cellular level[6]. Cyclooxygenase (COX, prostaglandin synthase; G_2/H_2) is the enzyme responsible for the initial rate-limiting conversion of arachidonic acid to prostaglandin G_2 and then to prostaglandin H_2. In fishes, the gills are the primary site for osmoregulation[4], and prostaglandins are produced by gill tissue[1]. In addition, prostaglandin E_2 has been shown to inhibit salt extrusion by the killifish opercular epithelium, which is a model for the teleost branchial epithelium[5]. Mammals have a COX1 isoform that is constitutively expressed in many tissues as well as a COX2 isoform that is constitutively expressed in the kidneys. In the tunicate (*Ciona intestinalis*), there are two ancestral COX genes (COXA and COXB) that predate the divergence of the vertebrate COXs. We have previously determined that a COX2 orthologue is present in the gill of the euryhaline killifish (*Fundulus heteroclitus*) where it may be involved in ion transport[2]. In this study, we obtained COX cDNA sequences from the lancelet (*Branchiostoma lanceolatum*), hagfish (*Myxine glutinosa*) and lamprey (*Petromyzon marinus*) as well as the COX1 sequence from the killifish in order to assess the evolution of the vertebrate COX genes.

Killifish, hagfish and lampreys were acquired at the Mount Desert Island Biological Laboratory (MDIBL), and lancelets were purchased from Gulf Marine Specimens (Panacea, FL). Gills from killifish, hagfish and lampreys and whole lancelets were frozen in liquid nitrogen. Total RNA was then isolated with TRI-reagent (Sigma, St. Louis, MO), and first-strand cDNA was synthesized from 2 μg of total RNA with a Superscript II reverse transcriptase kit (Invitrogen, Carlsbad, CA) using oligo-dT as a primer. Degenerate primers were designed that could promote amplification of all known chordate COX transcripts. To ensure that the COX1 isoform was isolated in killifish, additional degenerate primers were designed to amplify areas of COX1 that are not conserved with COX2. PCR reactions were performed with Hot Start Ex-Taq (Takara Bio Inc., Japan) in a PCR Express thermocycler (ThermoHybaid, Franklin, MA) with standard cycling parameters. PCR products were visualized by ethidium bromide staining in 1.0 to 1.5% agarose gels, ligated into pCR4- TOPO vectors, and transformed into TOP10 chemically competent cells using a TOPO TA 9 Cloning Kit for sequencing (Invitrogen). Plasmid DNA was then sequenced in both directions at the Marine DNA Sequencing Facility at MDIBL. Initial sequences were extended with the procedures above using specific primers and then aligned using GeneTools software (BioTools Inc., Edmonton, Alberta) and searched for open reading frames. PepTools software (BioTools Inc.) was used to align predicted amino acid sequences with full-length COX sequences from other chordates. MEGA software was used with a minimum evolution and bootstrap (500 replicates) analysis to generate phylogenetic trees for each novel COX sequence with other known chordate COX sequences[7].

COX orthologues were cloned from all chordates studied as was the COX1 ortholgue from killifish. Degenerate primers supported the amplification of a 1335 bp product from killifish gill cDNA that was found to be 74.4% identical to the zebrafish (*Danio rerio*) COX1 and 65.4% identical to killifish COX2. Similarly, a 1454 bp product from the hagfish was found to be 46.9% identical to COXA and 46.5% identical to COXB from the tunicate. Two 532 bp products from the lancelet were

shown to be 50.9% and 58.8% identical to COXA and COXB from the tunicate, respectively. Finally, a 466 bp product from the lamprey was found to be 68.4% identical to zebrafish COX1. Phylogenetic trees for each novel sequence were combined to generate a likely evolutionary scenario for COX (Figure 1). This scenario places the origin of COX1 and COX2 between the evolution of the hagfish and lamprey. This period corresponds to a gene duplication event that occurred around the evolution of vertebrates (2R hypothesis)[3].

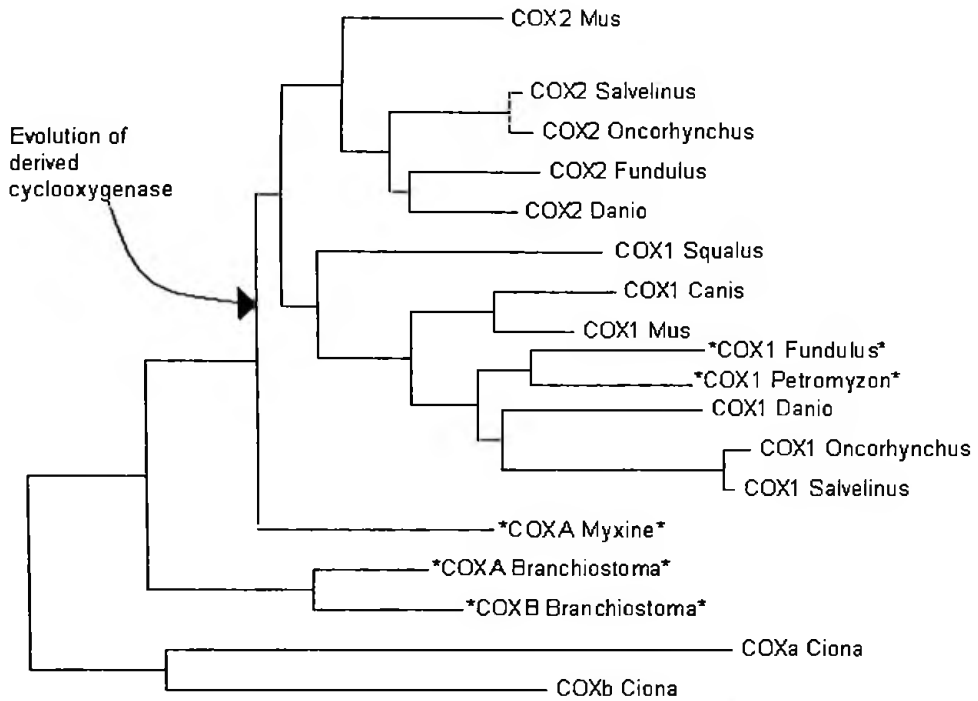

Figure 1. A phylogenetic analysis of cyclooxygenase sequences with our killifish, lamprey, hagfish, and lancelet sequences added (*denotes novel sequences). The amino acid sequences were aligned with Peptool (Biotools 2.0) and MEGA was used in a minimum evolution analysis with a bootstrap of 500 replicates to generate phylogenetic trees for each sequence that were then combined to make this consensus tree.

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