

## Evolutionary and comparative analysis of aquaporin water channel genes in fish

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The role of aquaporin water channels in some teleost fish has been described for some (orthologues of mammalian) aquaporins, especially aquaporins<sup>1</sup> (AQP) 0, 1 and 3, however to date the role of aquaporins in other more ancient fish lineages such as the Elasmobranches and Agnathans remains almost completely uninvestigated. The goal of this project is to identify homologous aquaporin genes from Elasmobranches (dogfish shark; *Squalus acanthias* and bullshark; *Carcharhinus leucas*) and Agnathan (hagfish; *Myxine glutinosa*) species, and from ancient teleost fish species (eels; *Anguilla anguilla* and *Anguilla rostrata*) in order to answer a number of questions regarding the kind of aquaporin homologues that are present in these species and their relationship to aquaporins found in higher vertebrates. A longer term objective is begin to discern the physiological role, that any aquaporins found in Agnatha, Elasmobranches or Teleosts play.

The initial approach to identifying aquaporin genes in the dogfish shark and in hagfish, using degenerate PCR cloning was unsuccessful. However, a partial cDNA fragment of an aquaporin gene had previously been isolated using this approach in the bullshark. Cloning and sequencing of the remainder of this cDNA was completed using 5' and 3' RACE PCR and bullshark kidney total RNA. The bullshark aquaporin (AQP1e) cDNA sequence isolated was ~1.1kb in size and contained an open reading frame of 256 amino acids. Comparison of the central conserved region of the bullshark amino acid sequence with the sequences of the various aquaporins found in mammals (humans), revealed that bullshark AQP1e had equally similar levels of homology to human AQP1 (47.9%), AQP2 (47.8%) and AQP5 (48.9%) with slightly lower levels of homology to other water-selective aquaporins, AQP0, 4 and 6 (39-43%). Still lower levels of homology were seen between bullshark AQP1e and human AQP8 (27.9%), aquaglyceroporins (AQP3, 7, 9 and 10; 20-26%) and more distantly related aquaporin homologues (AQP11 and 12; 14-23%). The similar level of homology between bullshark AQP1e and human AQP's 1, 2 and 5, suggests that bullshark AQP1e may represent an Elasmobranch descendent of a common ancestral gene, which was subsequently duplicated to become AQP1, 2 and 5 during the further evolution that led to higher vertebrates. Further information that lends some weight to this idea comes from the analysis of teleost sequence data. Analysis of the current fugu (*Fugu rubripes*) and zebrafish (*Danio rerio*) databases as well as cDNA sequence data for eel (*Anguilla anguilla*) aquaporins (unpublished), reveals that while there are homologues of AQP1 genes present in these fish, no orthologues of either AQP2 or AQP5 appear to exist. This further suggests that the ancestral gene may have been an orthologue of AQP1 from which the AQP2 and AQP5 genes developed presumably by gene duplications and accumulation of subsequent mutations. Clearly more information from other Elasmobranches, Agnathans or Teleosts (or even from Actinistia or Dipnoi species) is required to confirm these hypotheses. With the increase in sequence data from bullshark AQP1e, as well as in other gene databases, a further attempt has been made to amplify aquaporin fragments from the dogfish shark and hagfish cDNA using redesigned degenerate PCR primers, this has resulted in the production of three potential aquaporin cDNA fragments. Subsequent work will focus on cloning and sequencing these fragments to determine if they encode aquaporin homologues.

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1. Cutler C. P. and G. Cramb. Molecular Physiology of Osmoregulation in Eels and Other Teleosts: The Role of Transporter Isoforms and Gene Duplication. *Comp. Physiol. Biochem.* 130: 551-564, 2001.