

Cloning of an aquaporin 9 gene orthologue from the hagfish (*Myxine glutinosa*)

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Information concerning the first elasmobranch aquaporin water channel was recently published¹. Additionally, expressed sequence tag (EST) sequencing efforts have led to the presence of several EST entries in the gene bank. However, despite these sequence data and other reports on aquaporin water channels in teleost fish², the presence (and role) of aquaporin water channel genes in more evolutionarily ancient fish lineages such as agnathans, remains unexplored. This study utilized the new sequence data that had then recently become available, to identify an orthologue of mammalian aquaporin 9 (AQP9) in the hagfish (*Myxine glutinosa*).

The nucleotide and derived amino acid sequence of an EST in the genebank (Accession No., CV720323) encoding an AQP9 orthologue from the dogfish shark (*Squalus acanthias*) was utilized to redesign oligonucleotide primers that were targeted at amplifying AQP9 homologues in the hagfish. This primer pair was either used alone or in combination with other pre-existing primers designed for amplifying AQP9 homologues in teleost fish. All these primers used the nucleotide analogue, inosine, at positions of uncertainty in the sequence and were made using regions of the amino acid sequence that are the most conserved in AQP9 orthologues or paralogues from a variety of species. Initial PCR amplifications resulted in products of approximately the correct size, as estimated in agarose gel electrophoresis. Successful amplification was achieved in cDNA samples made from total RNA extracted from hagfish esophagus. Complementary DNA (cDNA) was made from RNA samples (for PCR), using Invitrogen's Superscript III reverse transcriptase. Putative positive DNA bands from agarose gels were purified using a GeneClean DNA purification kit (Bio 101, Irvine, CA) and cloned into a TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). The initial product sequenced for hagfish AQP9 was located in the center of the coding region of the gene. Amplification of the 5' and 3' ends of both genes was attempted using cDNA made for that purpose, with a Marathon cDNA Cloning kit (Clontech, Mountain View, CA). The 5' and 3' ends of hagfish AQP9 were successfully amplified (using PCR), cloned and sequenced. Overlapping sequences were pieced together using GeneJockey DNA analysis software (Biosoft, Cambridge, UK).

The cDNA obtained for hagfish AQP9 was 1196 nucleotides in length, encoding an open reading frame of 271 amino acids. The cDNA had a 5' untranslated region of 346 nucleotides and a 3' untranslated region of 34 nucleotides. This is a somewhat smaller amino acid sequence than that of human AQP9 (295 Amino Acids). The shorter length of the hagfish AQP9 amino acid sequence was due to the presence of two short deletions (relative to all other vertebrate AQP9 sequences) in the central portion of the cDNA. The carboxyl terminal of the amino acid sequence was also truncated in comparison to other AQP9 sequences from other species.

As might be expected from one of the most evolutionarily ancient extant fish species, hagfish AQP9 had the lowest level of amino acid homology of any AQP9 sequence known, sharing only 42-43% homology with mammalian sequences (Table 1). The homology of the hagfish AQP9 amino acid sequence is also around 10% more divergent compared to AQP9's from other species than is the dogfish AQP9 sequence. Exactly what difference the sequence divergence and deletions in hagfish AQP9 make to its functional characteristics, will await functional expression of the cDNA.

Table 1. A comparison of AQP9 orthologues and paralogues from various vertebrate species. The sequences compared are derived partial amino acid sequences, where the only regions utilized were those with corresponding segments in all the sequences employed. This resulted in the use of 176 amino acids of sequence from the center of each gene's coding region. Fugu a and Fugu b are arbitrary names used to indicate the two AQP9 paralogues present in the Fugu (*Fugu rubripes*) genome. Figures in the body of the table represent the percentage of identical amino acids in the alignment between any two sequences.

	Hagfish	Dogfish	Fugu a	Fugu b	Chicken	Mouse	Human
Hagfish	-	50.0	50.0	52.3	47.2	42.6	43.2
Dogfish	50.0	-	59.1	58.0	56.8	55.7	54.5
Fugu a	50.0	59.1	-	76.1	55.7	54.0	58.0
Fugu b	52.3	58.0	76.1	-	57.4	51.7	56.3
Chicken	47.2	56.8	55.7	57.4	-	62.5	69.3
Mouse	42.6	55.7	54.0	51.7	62.5	-	77.3
Human	43.2	54.5	58.0	56.3	69.3	77.3	-

The characteristics of aquaporin genes such as hagfish AQP9, tell us nothing about its physiological role. Experiments to assess the physiological role of this gene, have been initiated, and these seek to influence the extracellular ionic environment of these fish by manipulation of the external environmental salinity. Despite the fact that hagfish are osmo-conformers, changes in interstitial fluid concentrations may affect intracellular ion concentrations inducing potential concomitant changes in ion and water transporter gene expression. Tissue samples obtained from hagfish held in different environmental salinities will be used for total RNA extraction to assess any changes in the AQP9 gene expression.

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