

**AN ANALYSIS OF AQUAPORIN CHANNELS AND THEIR  
POTENTIAL ROLES IN OSMOREGULATION IN  
EELS AND OTHER TELEOST FISH.**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

**Introduction**

To date, thirteen aquaporin water and small solute channel isoforms have been identified in mammals (Ishibashi *et al.*, 2000; Hatakeyama *et al.*, 2001). However, in vertebrates, one of a few notable absences from the list of species known to possess these small membrane proteins, was until recently, the teleost fish. The presence of aquaporin gene homologues in teleost fish was first described in Cutler *et al.*, (2000), but recently further information has become available on the role of aquaporins in this phylogenetic group (Cutler *et al.*, 2001; Virkki *et al.*, 2001). However with the recent release of the draft sequence of the pufferfish (*Fugu*) genome (October 2001) it has become possible to identify further aquaporin homologues in the teleost genome, and to compare and review this additional information with that already available.

**Aquaporins in the Pufferfish Genome.**

The current pufferfish draft genome contains 13 genes sharing homology with aquaporins (Table 1.). Amongst these, pufferfish appear to possess orthologous copies of AQP0, 1, 3, 4, 7 and 8, as well as apparent duplicate copies of AQP0 and 1 (which may have arisen from an ancient genome duplication event; Cutler *et al.*, 2001). In addition, there are also genes which share sequence homology to aquaporins but which are not sufficiently similar to individual isoforms to be described as direct orthologues. One of these is most similar to the sub-group of

aquaporins which are generally recognised to be solely water permeable (aquaporins 0, 1, 2, 4, 5, 6), the other four, belong to the glycerol-aquaporin sub-group (which are additionally known to be able to transport urea and glycerol).

Type	Mammalian Nomenclature	<i>Fugu</i> Orthologues	Eel (and Other Teleosts) Orthologues
<b>Aquaporins</b>	AQP1	AQP1 AQP1 Duplicate	AQP1 AQP1 Duplicate
	AQP2	-	-
	AQP0	AQP0 AQP0 Duplicate	AQP0 ( <i>Fundulus</i> ) -
	AQP5	-	-
	AQP4	AQP4	-
	AQP6	-	-
		AQP?	-
<b>Glycero-Aquaporins</b>	AQP7	AQP7	-
	AQP3	AQP3	AQP3 (and <i>Tribolodon</i> )
	AQP10	-	-
	AQP9	-	-
		AQP? AQP?	AQP? -
		AQP? AQP?	- -
<b>Divergent Aquaporins</b>	AQP8	AQP8	AQP8
	AQPX1	-	AQPX1?
	AQX2	-	-

Table 1. Nomenclature of aquaporin channels, in mammals, as well as in pufferfish (*Fugu*), eel (*Anguilla*) and other teleost species.

### **Water Transport in Osmoregulation.**

In teleost fish, there are several potential roles for water transporters in osmoregulatory processes. These include the absorption of water across the intestine following drinking in marine teleosts, absorption/loss of water across the gills, and the concomitant re-absorption of water together with ions in the kidney of freshwater fish.

### **Aquaporin3 in Eel Gill.**

The object of this study was to identify aquaporins within osmoregulatory tissues of the eel, and characterise any changes in their gene expression associated with salinity acclimation. Initial experiments led to the isolation of an AQP3 orthologue; its mRNA is expressed at high levels in the gill with lower levels in the eye, oesophagus and intestine. Expression experiments using yellow or silver eels showed that branchial AQP3 mRNA abundance decreased to 3% (of freshwater values) in seawater acclimated fish. Using an eel AQP3 antibody, the level of branchial AQP3 protein abundance was also shown to be 3-fold lower in seawater acclimated eels, and localisation studies identified chloride cells as a major site of gill AQP3 expression. The data suggests that AQP3 may play a role in similar changes in gill osmotic water permeability previously demonstrated.

### **Aquaporin1 in Eel Intestine.**

Subsequent experiments identified an orthologue of AQP1 expressed in the intestine, but some mRNA expression was also found in oesophagus, gill and kidney and other tissues. Initial quantitative studies revealed that the level of intestinal AQP1 mRNA expression increased 10-25x following seawater transfer, in contrast mRNA abundance decreased by 72% in yellow eel kidney. The high level of AQP1 mRNA expression in the intestine of marine eels suggests AQP1 may play a role in water absorption in this tissue.

### **Aquaporins in Eel Kidney.**

Further experiments with eel kidney led to the discovery of AQP1 dup, which is a duplicate copy of AQP1. AQP1 dup mRNA expression was only identified in the intestine, oesophagus and kidney. The level of AQP1 dup kidney mRNA expression decreased following seawater transfer of eels. The presence of higher

levels of expression of both the AQP1 and AQP1 dup isoforms in freshwater eels suggests that they may play a role in the re-absorption of water associated with salt retention in this environment, although other roles in other cells types can by no means be excluded.

#### **Aquaporins in Eel Oesophagus.**

The presence of expression of AQP3, AQP1 and AQP1 dup in the oesophagus was on the face of it somewhat puzzling as this tissue is particularly impermeable in marine fish, with only a small net osmotic water movement across this epithelium. However, as uni-direction fluxes have not been measured, one possibility is that there is a balance in the oesophagus between the absorption of water (and ions), with an opposing and nearly equivalent loss of water by osmosis to the imbibed seawater in the oesophageal lumen. The aquaporin channels present in this tissue may play a role in either of these opposing water transport phenomena.

#### **Other Aquaporins.**

Data on the characteristics of the other three eel aquaporin homologues also isolated (see Table 1.) will also be presented if at all possible.

#### **References**

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