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Review

The role of aquaporin 3 in teleost fish ☆

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Received 26 April 2006; received in revised form 1 September 2006; accepted 4 September 2006
Available online 4 October 2006

Abstract

The aquaporin isoform, AQP3 has now been identified in a number of different teleost fish species, with additional DNA sequence information on AQP3 genes in further fish species available in genome databases. In zebrafish (*Danio rerio*), the AQP3 gene is present as two duplicate isoforms resulting from a teleostean fish genome-wide duplication. A further splicoform/isoform has also been identified in rainbow trout (*Oncorhynchus mykiss*). The identification of these AQP3 isoforms in other fish species is consequently explored. The role of AQP3 in physiological/osmoregulatory processes, in various teleost organs is then described. In teleost gill, AQP3 is expressed in 'chloride' cells, and in some species, in other epithelial cell types, where it may have a number of different functions including the prevention of dehydration. In eel esophagus, immunohistochemistry shows that AQP3 is expressed in surface epithelial cells in the anterior esophagus, but in mucus cells within the epithelium of the posterior esophagus. In eel intestine, AQP3 is found in macrophage-like cells and probably plays no part in osmoregulatory processes. In the rectum, as in the posterior esophagus AQP3 is expressed in mucus cells. In eel kidney, AQP3 is expressed in a subset of renal tubules, and localizes to the apical pole of tubule cells. There is no apparent change in the location or protein abundance of renal AQP3 following the acclimation of eels from freshwater to seawater.
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Keywords: Aquaporins; Water transport; Osmoregulation; Teleost fish; Gill; Esophagus; Intestine; Kidney

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1. AQP3 isoforms

Aquaporin 3 (AQP3) is a member of an extended family of water and small solute channels known as aquaporins or major intrinsic proteins. In mammals, where aquaporins have been studied most extensively, there are currently 13 aquaporin homologues with 3 main sub-groups, the aquaporins (AQP0, 1, 2, 4, 5, 6 and 8), the superaquaporins (AQP11 and 12) and the

Abbreviations: AQP, aquaporin; NKCC, sodium potassium chloride cotransporter; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; NCBI, National Center for Biotechnology Information; FW, freshwater; SW, seawater; RT-PCR, reverse transcriptase-polymerase chain reaction; kDa, kilo daltons; cAMP, cyclic adenosine mono-phosphate.

☆ This paper was presented in the session "Water transport" at the Society of Experimental Biology's Annual Meeting at the University of Kent, Canterbury, UK April 2nd–7th 2006.

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1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.09.022

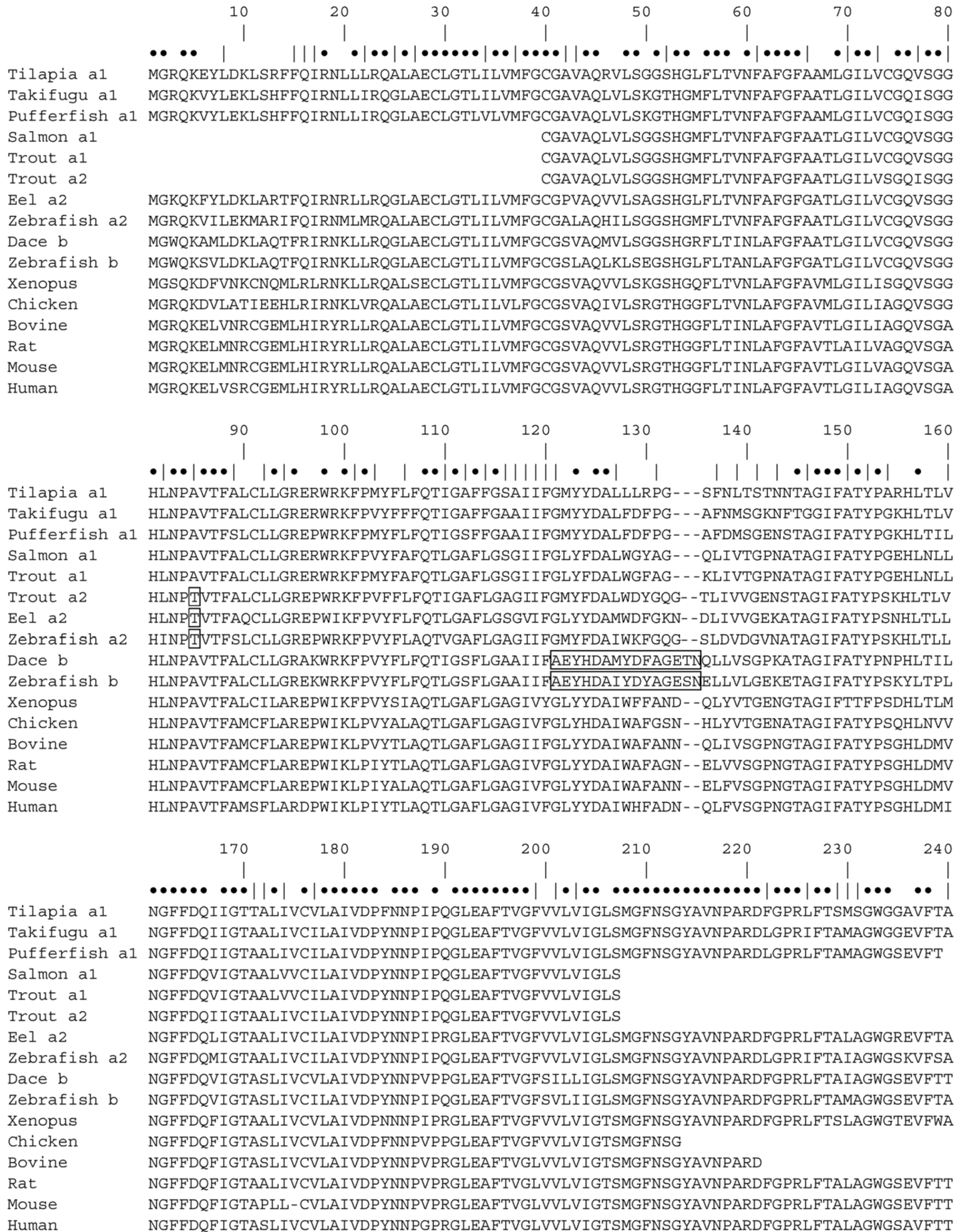

Fig. 1. Alignment of AQP3 derived amino acid sequences from teleost fish and other vertebrates. – have been inserted to align the sequences as optimally as possible. • symbol represents positions in the sequence alignment that are completely conserved in every case where the sequence is known. Numbers represent the position within the alignment of each amino acid. Boxed motifs highlight some of the regions of sequence that are the most significantly different between the different forms of teleost AQP3 (here indicated as a1, a2 or b respectively). All sequences were obtained from the NCBI gene bank (http://www.ncbi.nlm.nih.gov/) or using the Ensembl genome browser (http://www.ensembl.org/index.html), except previously unpublished data from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*).

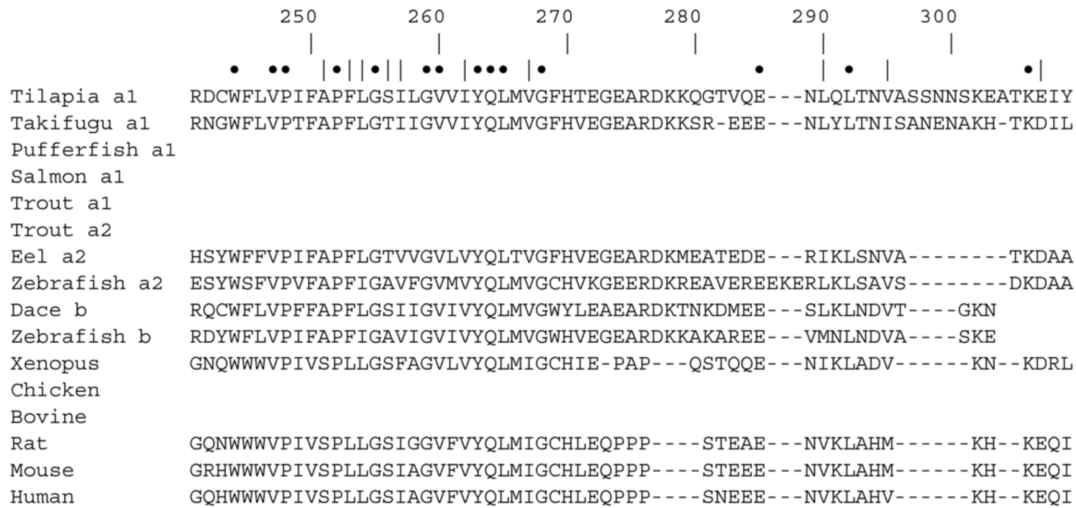

Fig. 1 (*continued*).

aquaglyceroporins (AQP3, 7, 9 and 10). Members of this third sub-group, which includes AQP3, are usually permeable to small solutes such as glycerol and urea as well as water. The role of aquaporins in mammals has been extensively reviewed many times (Recently: Nejsum, 2005; Verkman, 2005; Agre, 2006). Mammalian AQP3 is expressed in several locations including renal collecting duct, epidermis, lung airways, conjunctiva and urinary bladder (Verkman, 2005). AQP3 expression is known to be regulated by vasopressin, cAMP, and hypertonicity (Nejsum, 2005; Wang et al., 2006) and may be regulated by corticosteroids (King et al., 1997; Tanaka et al., 1997; Liu et al., 2003). AQP3 transport can be blocked by nickel, copper and acidification (Zeuthen and Klaerke, 1999; Zelenina et al., 2003, 2004).

In teleostean fish, AQP3 was initially identified in the European eel (*Anguilla anguilla*: Cutler and Cramb, 2000, 2002a) but has subsequently also been characterized in Osorezan dace (*Tribolodon hakonensis*: Hirata et al., 2003) and Mozambique tilapia (*Oreochromis mossambicus*: Watanabe et al., 2005). Additionally, genome sequencing efforts have led to the availability of sequence data from three species: fugu (*Takifugu rubripes*), spotted green pufferfish (*Tetraodon nigroviridis*) and zebrafish (*Danio rerio*). Further previously unpublished data has also been included from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Alignment of these sequences (Fig. 1) sheds some light on one possible factor that may have an impact on the role of AQP3 in teleosts, namely the effect of genome duplication. The presence of an additional genome duplication event (compared to terrestrial vertebrate and other fish species) in teleost evolutionary history, was originally suggested by Ohno (1999; see also Jaillon et al., 2004). The point at which this duplication event occurred during evolution has still not been fully determined (Christoffels et al., 2004; Hurley et al., 2005) although some recent evidence suggests that the duplication event occurred after the appearance of basal teleosts such as the *Semionotiformes* (gars) but before the development of more derived teleosts such as the *Osteoglossiformes*, (bony tongues etc), the *Elopiformes* (eels etc), or the *Euteleostei* (Hoegg et al., 2004). The apparent result of this genome duplication is that some genes in the more-derived teleost fish are present as two copies with different functions or patterns of expression or regulation (Cutler and Cramb, 2001). Duplication events may lead to the subdivision of the functions of the original gene copies or to the development of novel functionality of either duplicate (Hoegg et al., 2004).

It has been speculated that there may be more than one AQP3 isoform in teleosts, such as the European eel (Cutler and Cramb, 2002a), as the level of expression of this aquaporin in eel kidney was exceedingly low and this is a major site of AQP3 protein expression in mammals (Nejsum, 2005). Analysis of AQP3 gene sequences (Figs. 1 and 2) shows that there appears to be two AQP3 homologues in the zebrafish genome (arbitrarily here called a2 and b) that are present on different chromosomes (5 and 21; note:—there additionally appears to be a second, near-identical tandem repeat of the AQP3 isoform located on chromosome 21). The derived amino acid sequences of these zebrafish genes are around 74% homologous, which is similar to, but somewhat higher than, that shared for example, by AQP1 duplicate copies but lower than that shared by NKCC1 duplicates in the European eel (AQP1 69%, Martinez et al., 2005c; NKCC1 80%, Cutler and Cramb, 2002b). The duplication of both eel AQP1 and NKCC1 is likely to have happened during the teleost-wide genome duplication event (Cutler and Cramb, 2001). The slightly higher level of homology between zebrafish AQP3 compared to between eel AQP1 isoforms, may simply reflect the higher level of conservation in teleost AQP3 sequences (in comparison to those of mammals; 67–69%; Cutler and Cramb, 2002a) than in teleost AQP1 sequences (56–58%; Martinez et al., 2005c). Further analysis of the amino acid sequences shows that one of the main substantive differences between the two zebrafish AQP3 isoforms lies at amino acids 121–135 of the alignment, and this region includes a deletion of 2 amino acids in AQP3a(2) compared to AQP3b. Another possible difference between the two isoforms is the truncation of the C-terminal tail of AQP3b, the amino acid sequence of which is at least 6 amino acids shorter than AQP3a′s (as they are aligned).

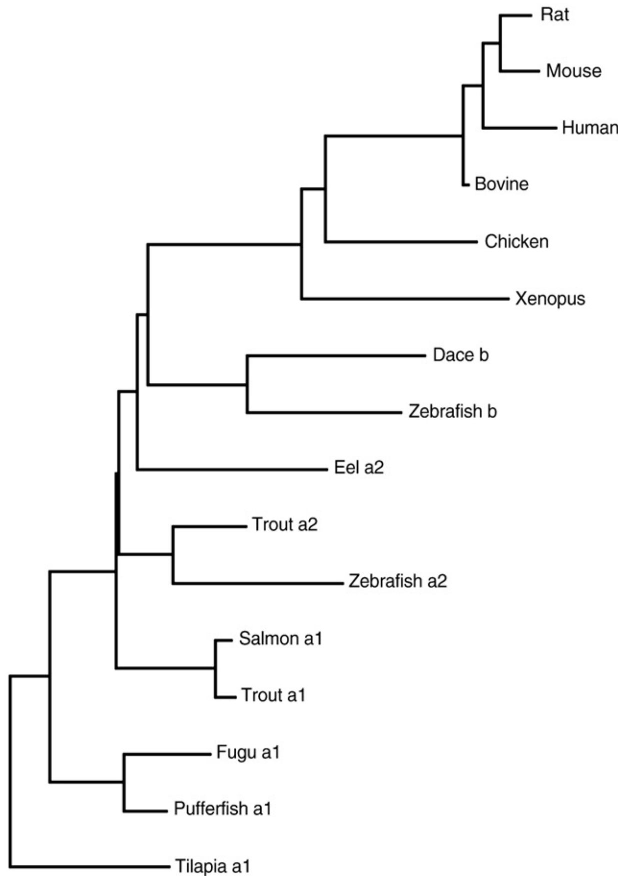

Fig. 2. A gene tree showing the relationship between AQP3 sequences produced using PAUP 4.0 software employing a Neighbour Joining method, which analysed the total distance between each sequence. Alignment data used as the basis for the tree was exactly as shown in Fig. 1. Gaps in the sequence alignment and missing data were both incorporated into the analysis.

Another question is, what type of isoforms do the AQP3 sequences from other teleost species represent? As can easily be seen in the alignment and the gene tree, AQP3 from Osorezan dace is of the AQP3b type, whereas the other AQP3 sequences from fugu, European eel, spotted green pufferfish, tilapia, Atlantic salmon and rainbow trout, are more similar to zebrafish AQP3a(2).

One aspect of the teleost genome duplication is that many of the duplicate genes that were present immediately after the event have subsequently been lost, such that today, only around 20% of genes in zebrafish and 10% of genes in fugu have additional paralogues (or isoforms without comparable orthologues in higher vertebrates; Fishman, 2001). Consequently, as different teleost species may have lost a different assortment of the original complement of duplicate copies of every gene, it is impossible to know whether a particular species will have retained a duplicate copy of a particular gene, such as AQP3, or not. One species where this is relatively clear is in *T. rubripes* where only AQP3a(1) seems to be present in the genome. Another explanation for the presence of the duplicate AQP3b gene in zebrafish and Osorezan dace is that this gene is derived from an individual duplication event rather than from genome-wide duplication. In favor of this, is the fact that zebrafish and Osorezan dace are closely related species, as they are members of the *Cyprinidae* taxonomic family. The other species with known AQP3 sequences are members of different taxonomic orders, the *Anguilliformes* (eel), *Perciformes* (tilapia), and *Tetraodontiformes* (pufferfishes) rather than the *Cypriniformes* (zebrafish and dace). Evidence against the individual duplication hypothesis is firstly that the two isoforms are on entirely different chromosomes and secondly that the level of homology between them is relatively low, suggesting that the duplication event occurred quite some time ago and this is consistent with having occurred at the same time as the duplication of other genes in teleosts i.e. during the genome-wide duplication event. Only additional data, such as the discovery of AQP3b-like isoforms in species in other teleost taxonomic orders, will resolve this issue.

In addition to the two isoforms from zebrafish, the data from rainbow trout are interesting, in that there are two sequences expressed in this species, the first one of which resembles the AQP3a isoforms from tilapia, pufferfish and salmon and these have been designated AQP3a1, whereas the second form is similar to the AQP3a isoforms from European eel and zebrafish and these have been designated AQP3a2. The two rainbow trout sequences only represent a part of the AQP3 coding region and so it is difficult to tell if these represent two fully independent isoforms or exon splice variants (splicoforms) of a single isoform. The nucleotide sequences of the two rainbow trout AQP3 forms suggests that the latter is the case, as the last 101 nucleotides of the two cDNAs were identical (data not shown). However, it is not inconceivable that trout AQP3a1 and a2 could be completely separate isoforms. The gene tree (Fig. 2) supports this notion, as the trout a2 sequence is more similar to zebrafish a2 (88.0%) than to trout a1 (84.9%) and the sequences consequently line up that way in the tree. The higher level of homology shared by the trout forms (84.9%) than the zebrafish isoforms (76.8% in the comparable region) suggests that the trout duplication event occurred after the zebrafish event and, one duplication event is likely to be the result of the teleost genome-wide duplication (probably the one in zebrafish) the other due to a tandem duplication of an individual gene or chromosome segment (or exon if the two trout forms turn out to be splice variants). Another possible explanation for the existence of the trout sequences is that the salmonids as a group, are known to have undergone an additional, more recent genome duplication event (McKay et al., 2004). However, the duplicate genes generated from this more recent event tend to have relatively high levels of homology to each other, as for example evidenced by the CFTR gene duplicates from salmon, where the two copies are 95% similar at the amino acid level (Chen et al., 2001). Additionally, the sequences of duplicate genes stemming from the salmonid-specific duplication event would be expected to diverge independently of sequences in other groups of teleost species. So it is highly unlikely that one salmonid duplicate copy would be, significantly more homologous to a sequence from another non-salmonid teleost species, than the other duplicate copy (as is the case with the two trout AQP3 forms).

One of the key differences between the two AQP3a forms appears to be a change to the (normally) highly conserved 1st

NPA site (amino acids 83-5) such that the AQP3a2 forms have a NPT rather than a NPA amino acid motif. Additionally, one of the main regions of difference between AQP3a and b isoforms (amino acids 133-5) is also different between AQP3a1 and a2 forms. Here, AQP3a1 forms have three amino acids less than AQP3b isoforms whereas the AQP3a2 forms have two amino acids less. Although it is not clear that the two rainbow trout forms represent fully independent isoforms, the full length copies of the isoforms classified as AQP3a1 (tilapia and *T. rubripes*) and AQP3a2 (European eel and zebrafish) do appear to have different length C-terminal ends (see around amino acid 300 of the alignment), adding further weight to the idea that AQP3a1 and a2 are completely different AQP3 isoforms.

2. Teleost osmoregulation

Teleosts in freshwater (FW) have body fluids that are hyper-osmotic to the external environment, and continuously gain water by endosmosis and lose ions by diffusion across their permeable body surfaces, principally the gills. As with terrestrial vertebrates, water homeostasis is achieved by excreting excess water through the production of relatively large quantities of dilute urine in the kidney, and additionally ions are absorbed, by specialized ion transporting chloride cells in the gill, or from the diet. Teleosts in the marine environment face the opposite osmoregulatory problem to their FW counterparts, in that they are constantly losing water to the external hyper-osmotic seawater (SW) across permeable body surfaces such as the gills. To counteract this dehydration, marine fish employ a regulated drinking response. Imbibed SW is first partially desalinated in the esophagus and stomach before entering the intestine. The absorption of water across the intestinal epithelium takes place with the concomitant uptake of salts. Renal urine production is severely reduced in comparison to FW teleosts and is mainly concerned with the excretion of divalent cations (for a more extensive review of fish osmoregulation see Marshall and Grosell, 2006).

3. AQP3 expression in teleosts

European eel AQP3 is expressed in a number of tissues such as gill, intestine, oesophagus, and eye. Although AQP3 expression in kidney was not seen on Northern blots, it was reported to be present sporadically, at a low level, in this tissue (Cutler and Cramb, 2002a) and can be easily detected using RT-PCR (data not shown). AQP3 mRNA expression was not found in eel brain. In tilapia, AQP3 is detectable (by RT-PCR) in a wider range of tissues in addition to those found in the European eel. These additional tissues include brain, pituitary, spleen and skin (Watanabe et al., 2005). In dace, AQP3 was only detected in Northern blots in gill, but not in brain, kidney or intestine (Hirata et al., 2003). Differences in the tissue distribution of AQP3 between these three fish species may have as much to do with differences in the sensitivities of the techniques employed rather than any real differences in expression. The possible exception to this is that AQP3 expression in tilapia brain appears to be very strong, as strong as in other tissues such as the gill, whereas in the European eel and in dace there is no sign of any expression in brain, despite clearly identifiable levels in the gill.

4. AQP3 in teleost gill

One of the major differences between AQP3 mRNA expression levels in European eel and tilapia, is the very large decrease in expression (up to 97%) that occurs when European eels are transferred from FW to SW (Cutler and Cramb, 2002a). A recent study on Japanese eels has shown a similar, although smaller (around 33%), decrease in branchial AQP3 mRNA levels, following FW to SW transfer, (Tse et al., 2006). This difference in AQP3 mRNA expression levels was not detected in tilapia gill when comparing FW- to SW-adapted fish (Watanabe et al., 2005). There did appear to be a slightly lower level of mRNA expression in the SW-adapted tilapia, however this was not quantified presumably because results obtained from the use of single samples are not reliable. The decrease in eel AQP3 mRNA expression correlates with a decrease in osmotic water permeability in the gill following transfer to SW (Isaia, 1984). However other teleost fish species do not always show such changes in osmotic water permeability (rainbow trout; Isaia, 1984). It may be that if there is a real connection between gill AQP3 expression levels and branchial osmotic water permeability, then the level of osmotic water permeability in FW-adapted tilapia may not be much different to SW-adapted fish and this is reflected in the levels of gill AQP3 mRNA. This is of course speculative and in any case the measurement of osmotic water permeability is also potentially unreliable (Rankin and Bolis, 1984).

Dace AQP3 is expressed in the gill where mRNA levels were seen to increase following exposure of FW fish to acidic environmental conditions (pH 3.5; Hirata et al., 2003). This increase in expression may be a response to concomitant changes in the requirement for ion, water and/or osmolyte (such as glycerol or urea) transport under these conditions. However, mammalian AQP3 is gated by protons, such that AQP3's glycerol and water channel permeability′s, are reduced in acid conditions (Zeuthen and Klaerke, 1999; Zelenina et al., 2003). If dace AQP3 behaved similarly, then the reduced AQP3 permeability that would occur when plasma pH decreased (as has been demonstrated in dace following pH 3.5 transfer) may stimulate the production of additional AQP3 units to recover the original overall level of transport capacity. Hirata et al. (2003) speculate that AQP3 may be required to provide the enzyme carbonic anhydrase with water as a substrate, where water and carbon dioxide are combined and form bicarbonate and protons, and the protons are subsequently excreted across the surface of the gills. This is a possible reason to upregulate AQP3 mRNA levels in the extremely acidic environments such as those employed in the dace study where higher than normal quantities of water might be utilized as a substrate for carbonic anhydrase. However, even under these circumstances, from a purely enzymatic point of view this would still seem unlikely, as the effective approximate concentration of water in aqueous solutions is around 55 M and therefore if ever there was an

enzyme whose substrate would never be rate limiting, carbonic anhydrase should be it. Furthermore, if water was being supplied by AQP3 for use by carbonic anhydrase for the purpose of proton excretion across the gills, it would be expected that the level of AQP3 should correlate with that of branchial proton excretion. Evidence suggests that branchial proton excretion 1) declines in marine fish when the environmental salinity is decreased, 2) is lower in FW compared to SW teleost fish species, and 3) reduces in euryhaline teleost fish acclimated from SW to FW (Claiborne, 1997). Far from this correlating with a decrease in AQP3 in FW, in the European eel, AQP3 shows around a 4–30 fold higher level of mRNA and a three-fold higher level of protein in fish acclimated to FW when compared to SW fish (see elsewhere in this article; Cutler and Cramb, 2002a; Lignot et al., 2002a). This suggests that while AQP3 might provide water, some of which may ultimately be utilized by carbonic anhydrase, this appears to be insignificant for AQP3 expression except possibly in extremely acidic environments.

In addition to the decrease in European eel branchial AQP3 mRNA abundance following SW-acclimation (as mentioned above), it has been reported that there was a 3-fold decrease in the level of gill AQP3 protein with SW-acclimation, as assessed using Western blotting (Lignot et al., 2002a). Furthermore, the level of Japanese eel branchial AQP3 protein was also reported to decrease following SW acclimation, although the extent of the decrease was not quantified (Tse et al., 2006). This correlates well with the changes in mRNA expression and changes in gill osmotic water permeability following the SW-acclimation of eels. Although a Western blot of AQP3 protein was also performed on tilapia gill samples, no comparison between FW and SW fish was made (Watanabe et al., 2005). Interestingly the reported molecular weights of the European eel AQP3 (24 kDa; also Japanese eel; 23 kDa) and tilapia AQP3 (26 or 28 kDa) proteins are significantly lower than those that can be calculated from the derived amino acid sequences of their genes (European eel 32 kDa; tilapia 33 kDa). This is likely to be due to incomplete denaturation of AQP3 proteins during electrophoresis, however it is also possible that AQP3 may additionally be post-translationally processed, presumably at the N-terminal end (as the European eel and tilapia antibodies were both raised against and detect the C-terminal ends of these proteins).

Antibodies raised against European eel, tilapia and dace AQP3 have also been used for immunohistochemistry and electron microscopy (European eel and tilapia antibodies only) to localize the expression of AQP3 proteins. Additionally, a heterologous mammalian AQP3 antibody has been used to localize the protein in the Japanese eel gill.

In the European eel, immunohistochemistry revealed that similar levels of intense AQP3 staining were present within branchial chloride cells of both FW- and SW fish (Lignot et al., 2002a,b). Some expression was found in the basal regions within these cells, and this co-localized with an antibody raised against Na,K-ATPase, suggesting that the AQP3 was located in the basolateral tubular network (which is an extensive invagination of the basal membrane). Further intense AQP3 staining was also seen towards the apical pole of chloride cells which did not co-localize with Na,K-ATPase. As the apical AQP3 staining was shown by immuno-gold electron microscopy not to be present in apical membranes of chloride cells, it was suggested the most likely location of expression was in the tubulo-vesicular system also present in the apical region of these cells. This localization was not possible to confirm due to the poor morphology present in electron micrographs. The level of AQP3 protein expression (relative to Na,K-ATPase) in chloride cells was quite variable both within and between chloride cells. Most cells expressed both AQP3 and Na,K-ATPase but the occasional cell exhibited virtually no Na,K-ATPase but had high levels of AQP3 and conversely, some cells exhibiting high levels of Na,K-ATPase had no discernable AQP3. The reason for this variability is unclear. In addition to the AQP3 staining in branchial chloride cells, more basal epithelial cells within the primary lamellae also showed AQP3 staining on their plasma membranes. Cells within the epithelium covering the branchial arch also showed significant levels of staining. The staining in both of these non-chloride cell locations was significantly reduced in SW-acclimated European eels suggesting that this reduction, rather than any changes in AQP3 protein levels in chloride cells, might be responsible for the down regulation of AQP3 mRNA and protein in SW.

The immunohistochemical localization of AQP3 protein in Japanese eel gill revealed staining in chloride cells in a similar fashion to the European eel, although by contrast, staining in FW Japanese eels showed the apparent presence of AQP3 in all of the cells of the primary and secondary lamellae epithelia, including pavement cells (Tse et al., 2006). The reason for this marked difference between two closely related eel species is unclear, although the Japanese eel study used a heterologous mammalian AQP3 antibody. If two AQP3 isoforms were expressed in eels then a heterologous antibody might detect both of them, as cross-reactions with different isoforms is more likely to occur when using a heterologous rather than a homologous antibody.

In tilapia gills, stained with a homologous AQP3 antibody, AQP3 staining was reported only in chloride cells in either FW- or SW-acclimated fish, with no specific immunoreaction in other parts of the gill. It was also reported that the immunoreaction for AQP3 and Na,K-ATPase completely coincided with each other, and localization of AQP3 to the basolateral tubular network was confirmed by electron microscopy (Watanabe et al., 2005). Contrary to these assertions, the staining in the basal part of tilapia chloride cells appeared to exhibit similar variability to European eel chloride cells, with additionally the occasional cell staining for AQP3 but with no apparent Na,K-ATPase staining and with some parts of cells staining with the Na,K-ATPase antibody without any coincidental AQP3 staining. Furthermore there also appeared to be a small amount of AQP3 staining on more basal cells within the primary lamellae that did not coincide with Na,K-ATPase staining and was absent in control sections. The level of this AQP3 staining within tilapia basal epithelial cells did appear to be considerably less than that shown for the European eel, but similar to the European eel, the extent of AQP3 staining was also somewhat reduced in SW-acclimated tilapia gill (in the

single section shown). Despite the AQP3 expression in basal cells, it is clear that the vast majority of AQP3 expression in tilapia gill was found in the chloride cells, where its apparent abundance did not change between FW- and SW-acclimated fish. This result agrees with that suggested by RT-PCR experiments where there appeared to be only a very slightly lower level of AQP3 mRNA expression in SW-acclimated tilapia. If the presence of AQP3 really is (at least partly) responsible for the level of osmotic water permeability in the gill (a possibility suggested for European eel; see above) then this would again suggest that there would likely be little difference between the osmotic water permeability of the gills of FW- and SW-tilapia.

AQP3 protein expression was also localized to cells within the gill of dace acclimated to an acidic environment (Hirata et al., 2003). In dace, as in European eel and tilapia, the most intense AQP3 staining was found in chloride cells, although significant staining also appeared to be present in surrounding epithelial cells. The specificity of this AQP3 staining is rather difficult to judge, as control sections were not included.

Concerning the function of AQP3 in the gill and elsewhere, there has been much speculation that it may be functionally involved in water flows into or out of the basolateral tubular network of chloride cells in order to prevent swelling or dehydration, and that it may be involved in urea and/or ammonia excretion across the gills (Cutler and Cramb, 2002a; Lignot et al., 2002a,b). A role for AQP3 in preventing dehydration in surface epithelial cells has been shown previously in mammals (Matsuzaki et al., 1999, 2000). In dace, it has also been suggested that AQP3 may be used to provide water as a substrate for the enzyme carbonic anhydrase (see above; Hirata et al., 2003). In tilapia, it was suggested that AQP3 may play a role in osmo-sensing both in the chloride cells of the gill and in the pituitary (Watanabe et al., 2005). All these functional roles are possible but there is currently little except circumstantial evidence to support them.

The transport functions of teleost AQP3 proteins have recently begun to be studied. Expression of tilapia AQP3 mRNA in *Xenopus* oocytes was recently shown to enhance the oocyte′s water permeability (Watanabe et al., 2005). The enhanced water permeability was inhibitable by the reversible non-specific aquaporin inhibitor, mercury (Hg^{2+}). Subsequently, preliminary studies expressing European eel AQP3 mRNA in *Xenopus* oocytes, have also shown that eel AQP3 expression similarly enhances water permeability in a mercury-inhibitable fashion (MacIver et al., 2006). This data also suggests that eel AQP3′s functional characterisitics may include additional glycerol and urea permeabilities in a similar fashion to mammalian AQP3 (Nejsum, 2005).

5. AQP3 in teleost esophagus

The esophagus is a particularly important osmoregulatory organ in marine teleost fish, as they have a regulated drinking response that counteracts dehydration. This results in full strength SW entering the esophagus, where it is thought to be desalinated down to a concentration equivalent to roughly 50% SW (Hirano and Mayer-Gostan, 1976; Parmelee and Renfro, 1983). As the net flows of water across the esophageal epithelial were negligible, it has long been suggested that the esophagus has a low permeability epithelium (Hirano and Mayer-Gostan, 1976). However, unidirectional flows of water across the esophagus may be much greater than the net flows suggest and there is some evidence that this may be the case in flounder (Parmelee and Renfro, 1983). The possibility of larger unidirectional flows of water across the esophagus leaves open a role for aquaporins in water transport in this organ. It is possible to speculate that as SW enters the esophageal lumen, some water may be lost by osmosis from the surrounding tissue and this water may then be recovered together with salts as the desalination process progresses. For some time now it has been known that at least 3 aquaporin isoforms are expressed in the European eel esophagus, namely AQP1, AQP1dup (an AQP1 paralogue) and AQP3 (Cutler and Cramb, 2000, 2001, 2002a; Lignot et al., 2002b; Martinez et al., 2005a). Little is known about the role of AQP1 and AQP1 dup in the esophagus other than that their expression is up regulated in FW-acclimated silver eels following perfusion with the steroid hormone, cortisol

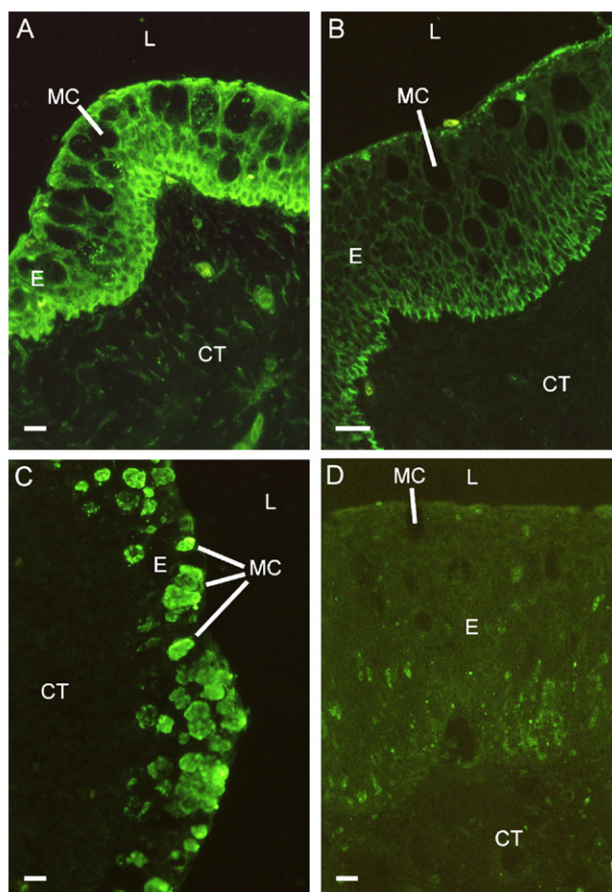

Fig. 3. Immunolocalization of AQP3 in the esophagus of silver European eels. Typical AQP3 immunofluoresence images of the anterior esophagus (A and B) were taken from SW-acclimated eels, and of the posterior esophagus (C) were taken from FW-acclimated eels. Esophageal section D was instead incubated with AQP3 antibody pre-immune serum. Bars in the bottom left corner of each image represent 10 μm. The esophageal lumen (L), epithelium (E), mucus cells (MC) and connective tissue (CT) are indicated. All sections were processed as indicated in Martinez et al. (2005b,c).

(Martinez et al., 2005a). AQP3 mRNA is also expressed in SW-acclimated eel esophagus (Cutler and Cramb, 2002a).

Initial studies, investigating the localization of AQP3 expression in the European eel esophagus suggested that there may be a change in the site of AQP3 expression from FW-acclimated fish (where AQP3 was found in mucus or goblet cells) and SW-acclimated fish (where AQP3 was found in plasma membranes of non-mucus epithelial cells; Lignot et al., 2002b). More detailed immunohistochemical analysis using the European eel AQP3 antibody (Fig. 3) indicates that the difference in AQP3 localization is heavily dependent on the location in the esophagus used for experiments. AQP3 was indeed expressed in the plasma membranes of non-mucus epithelial cells throughout this tissue layer, but only in anterior regions of the oesophagus. The level of AQP3 expression in the anterior region was quite variable, being greater in some fish (Fig. 3A) than others (Fig. 3B). In the posterior region of the esophagus, AQP3 was expressed in mucus cells (Fig. 3C). These localizations were similar between FW- and SW-acclimated eels (data not shown). It is not yet clear what roles aquaporins play in the teleost esophagus, but it is clear that AQP3 is expressed in epithelial cells in anterior esophagus and may play a role in replacing any water lost from these cells to the hyperosmotic fluid in the lumen. In the posterior esophagus it is likely that AQP3 is playing a role in mucus fluid secretion.

6. AQP3 in teleost intestine and rectum

AQP3 mRNA expression occurs in the intestine/rectum of both European eels and tilapia (Cutler and Cramb, 2002a; Watanabe et al., 2005). However, there was no sign of any intestinal expression of AQP3 in dace (Hirata et al., 2003). In eels, AQP3 mRNA was usually detectable by Northern blotting and showed that there was no difference in mRNA levels between FW- and SW-acclimated fish. AQP3 protein expression on Western blots could not be detected in FW-acclimated fish and this situation was unchanged following seawater-acclimation. AQP3 protein could be detected using immunohistochemistry where it was apparent that in the intestine, AQP3 was expressed in large macrophage-like cells present within the epithelial cell layer. However in the rectum, AQP3 was expressed in mucus/goblet cells. In FW-acclimated fish there were small numbers of long thin goblet cells expressing AQP3, whereas following SW acclimation the abundance of AQP3 expressing goblet cells increased and there was additionally a morphological change as these cells were wider or rounder in shape. AQP3 was postulated to be involved in mucus fluid secretion from these cells (Lignot et al., 2002a). The absence of a role for AQP3 in water uptake across the surface of the intestine may be explained by the presence of other aquaporins in this tissue (Martinez et al., 2005b).

7. AQP3 in teleost kidney

AQP1, AQP1 dup and a further aquaglyceroporin AQPe (AQPe is a novel teleost AQP although it is orthologous to other teleost AQP genes sometimes considered to be orthologues of mammalian AQP10 in genome databases; see Martinez et al., 2005b,c), are all expressed, at least at the mRNA level, in the kidney. Messenger RNA abundances of these aquaporins were generally decreased following acclimation of FW European eels to SW (Martinez et al., 2005c). As the more distal parts of renal tubule are thought to have low permeability in FW fish but higher permeabilities in SW fish where water needs to be conserved (Marshall and Grosell, 2006), the changes in AQP mRNA abundances might seem the opposite of what would be expected. The most likely answer is that these aquaporins (or at least AQP1 and AQP1 dup) are involved in fluid secretion occurring in the early proximal tubule segment (Beyenbach, 2004). In agreement with this, it has been suggested that fluid secretion would be most important when there is a need to excrete excess water, i.e. when fish are in FW (Braun and Danzler, 1997; Danzler, 2003). Although, in the case of fluid secretion, the few data that are available do not suggest that fluid secretion occurs at a higher rate in FW than SW fish, despite the higher urine flow rates for fish in the FW environment (Beyenbach, 2004). Initial Northern blots for

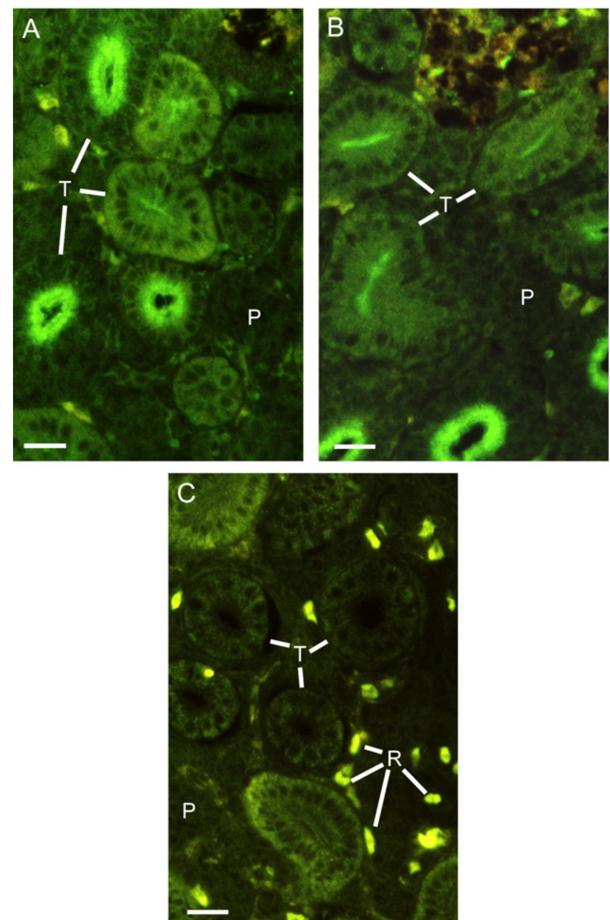

Fig. 4. Immunolocalization of AQP3 in the kidney of silver European eels. Typical AQP3 immunofluoresence images of the kidney were taken from a FW-acclimated eel (A), and a SW-acclimated eel (B). Sections were also incubated with AQP3 antibody pre-immune serum (C). Bars in the bottom left corner of each image represent 10 μm. The renal tubules (T), Parenchyma (P), and Red Blood Cells (R) are indicated. All sections were processed as indicated in Martinez et al. (2005c).

European eel AQP3, did not indicate that this gene is expressed in renal tissues (Cutler and Cramb, 2002a,b). Low levels of AQP3 expression was seen in some experiments and this was subsequently corroborated by RT-PCR (data not shown). Similarly tilapia AQP3 mRNA was detected in the kidney using RT-PCR and there seemed to be a higher level of expression in SW-acclimated fish than in FW fish (at least in one sample). This is the opposite of what was found for eel AQP1, AQP1dup (and possibly AQPe) and may suggest a role for AQP3 in water/fluid re-absorption in the distal nephron, particularly in marine teleosts. A distal localization for teleost renal AQP3 correlates with the location of AQP3 expression in mammals where it is found in the distal segments of the nephron (Nejsum, 2005). Staining of eel kidney sections with the AQP3 antibody (Fig. 4) showed that like AQP1, AQP3 is expressed only in a subset of renal tubules (Martinez et al., 2005c). However there appeared little difference between AQP3 staining seen in FW-acclimated fish compared to SW-acclimated fish. AQP3 expressed in tubules with open lumens was located in the apical pole of tubule cells, however whether any was localized to the apical brush border membrane of those cells was difficult to ascertain. AQP3 expression in renal tubules with closed lumens showed expression in the apical brush border. There may be somewhat less staining in the closed-lumen apical brush border membranes of FW-acclimated fish than SW-acclimated fish. Some tubules were unstained. The significance of these renal localizations is difficult to determine without markers for each renal tubule segment. However, in the light of these results one highly speculative possibility presents itself. It maybe that AQP3 is involved in the process of opening and closing the lumens of renal tubules (that are presumably no longer filtering). The role of AQP3 might be to aid withdrawal or replacement of fluid from or into the lumen during this process and it would therefore have to be inserted into the apical membrane for this to happen.

Although the amount of information concerning the role of aquaporins, and AQP3 in particular, in fish has increased considerably in recent years, there is still a long way to go before the various roles that AQP3 plays are fully understood. Despite this, it is already clear that AQP3 plays a complex role in cells within the major osmoregulatory organs of teleost fish.

Acknowledgements

The authors would like to acknowledge the contributions of Dr Quentin Fang (Georgia Southern University, USA) who helped in the production of the gene tree and Dr Jean Lignot (Université de Strasbourg, France) who performed preliminary experiments in the oesophagus with the eel AQP3 antibody, information on which was reported previously (Lignot et al., 2002b).

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