Partial functional characterization of an aquaporin 3 ortholog from the European eel, *Anguilla anguilla*.

Bryce MacIver[1], Zachary S. Karim[2], Mark L. Zeidel[3], Christopher P. Cutler[4] and Warren G. Hill[1]

[1]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA15261
[2]New Mexico Highlands University
[3]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School Boston, MA 02215
[4]Department of Biology, Georgia Southern University, Georgia GA 30460

Maintenance of body fluid composition and osmoregulation are essential for metazoan animals to survive. Aquatic animals face the particular problem of directly interacting with an aqueous environment that differs markedly in osmolality from their internal physiology. Freshwater (FW) fish experience driving forces for osmotic water uptake across the exposed epithelial surfaces of the gill and the gut. They compensate by drinking little and producing large volumes of dilute urine. Conversely, marine fish experience osmotic water loss to the sea and compensate by drinking seawater (SW) and excreting the excess salts ingested via the gills and the kidney. A number of species are euryhaline, exhibiting remarkable physiological adaptation as they move at some stage of their life cycle between FW and SW environments. One such example is the European eel *Anguilla anguilla,* a euryhaline teleost. In order for species such as the eel to acclimate to environments of different salinity, water and ion transport pathways in epithelia which interface with the environment must be under tight regulation.

Aquaporins are a family of water channel proteins now known to be important for volume homeostasis in humans. The recent discovery of aquaporin water channels in the gill, intestine and esophagus of the eel[1,3] led us to speculate on the role they play in osmoregulation in this highly adaptable species. Of particular interest is the observation that RNA transcripts of an aquaporin 3 (AQP3) ortholog present in fresh water adapted gills, is drastically downregulated in SW adapted animals (76 - 97%). This was supported by immunolocalization studies with specific antisera to the protein[3]. Since it is present in much higher concentrations in the FW gill than in any other tissue, it is likely to be important to gill function and overall osmoregulation in this environment. Human AQP3 belongs to the family of aquaglyceroporins which transport glycerol preferentially to water and are universally found basolaterally in mammalian epithelia. Thus it is tempting to speculate that the function of this protein in the FW eel may be to transport solutes other than water. It is possible that eel AQP3 (eAQP3) may also transport urea across the gills. This correlates with observations showing that urea and water excretion appear to occur in parallel[4,5]. It is also possible that NH_3, an abundant nitrogenous waste product could be a transported substrate[2].

To begin to examine the substrate specificity and conductance properties we expressed the eAQP3 ortholog in *Xenopus* oocytes. Optimal expression in oocytes was achieved by subcloning cDNA into a T7 RNA polymerase based vector that incorporates 5' and 3' untranslated sequences from the *X. laevis* globin gene. We have used the pXT7 vector although other variants exist. An in vitro translation kit (mMessage mMachine, Ambion) was used to produce cRNA from the T7 promoter. Microinjection of 10ng cRNA per oocyte produced optimal expression 3 days post-injection. Oocytes injected with water, human AQP1 and eAQP3 were placed in Modified Barth's Saline (MBS) diluted to 75% with water, time lapse imaged on an Olympus XZS7 trinocular microscope equipped with digital camera and image capture software. Oocyte images were thresholded to black and white and the cross-sectional pixel area quantitated using ImageJ NIH software.

Figure 1 shows that oocytes injected with water swelled slowly in response to the osmotic gradient as a result of passive water permeation through the plasma membrane. Oocytes expressing AQP1 and eAQP3 swelled much more rapidly indicating the presence of functional water conducting channels present in the membrane. eAQP3 appeared to have lower water conductance than AQP1, however in the absence of data about relative expression levels of the two transporters this conclusion cannot be confirmed at present. The observation that eAQP3 swelled at a lower rate than hAQP1 is however consistent with findings showing other aquaglyceroprins exhibit lower fluxes than aquaporins[6]. When oocytes expressing eAQP3 were incubated with 100 μM $HgCl_2$ for 30 minutes at room temperature, water transport was inhibited, consistent with the inhibitory activity of Hg^{2+} shown for many other AQPs.

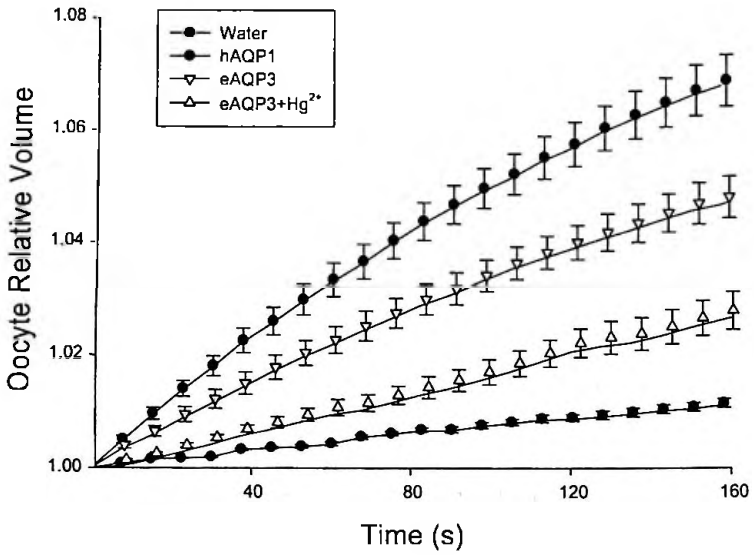

Fig. 1. Oocytes injected with the indicated RNA constructs or water were exposed to a hypotonic solution, imaged over time and cross sectional area quantitated and normalized to starting volume. For each experiment n=4-6 oocytes and data are shown as mean±SEM.

To determine if eAQP3 might conduct glycerol and/or urea, a flux assay was employed in which the oocyte was placed into a solute solution containing 180 mM urea or glycerol and 10 mM Hepes, pH7.4. The osmolality of these solutions was matched to that of MBS, so oocytes experienced a chemical gradient for the solute but not an osmotic gradient. Influx of solute then induces a solvent drag resulting in water uptake and swelling. Fig. 2 shows representative results from one experiment. After 500 s there was little evidence for significant urea uptake by the eAQP3 expressing oocytes (left panel), however there was a suggestion that glycerol may permeate through this transporter as evidenced by the swelling kinetics shown in Fig. 2 right panel. Swelling induced by solvent drag is probably not the optimal system for examining these permeation kinetics and other approaches including isotope uptake and plasma membrane vesicle preparations in combination with stopped-flow fluorometry will be employed for future experimentation.

These studies demonstrate that the AQP3 ortholog from the European eel is a mercury-inhibitable channel capable of transporting water and possibly glycerol. It remains a possibility that other solutes such as urea, NH_3 and CO_2 are also substrates for this transporter. Further definition of this channels transport kinetics and specificities will enable a clearer understanding of its physiological role in the gill where it is dramatically downregulated upon salt water acclimation.

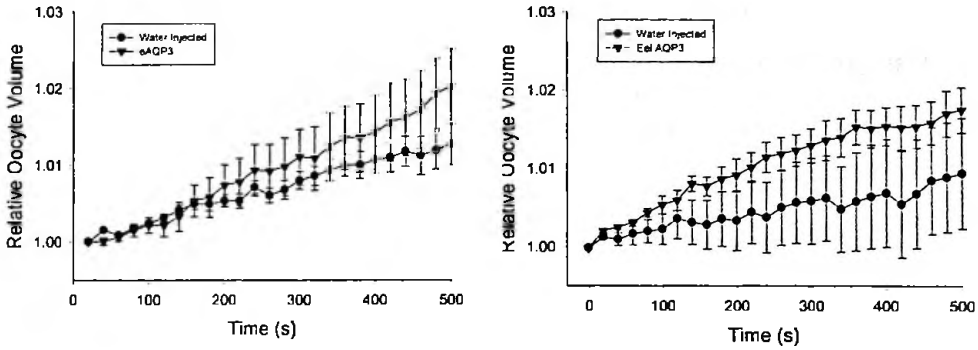

Fig. 2. Oocytes exposed to a 180 mM gradient for urea (left) and glycerol (right) compared to water injected controls (n=4-6 oocytes/condition and data are mean±SEM).

This work supported by an REU Fellowship to Zachary Karim, NIDDK Grant DK-43955 to Mark Zeidel and by an Epstein Fellowship and MDIBL New Investigator Award to Warren Hill.

1. **Cutler CP and Cramb G.** Branchial expression of an aquaporin 3 (AQP-3) homologue is downregulated in the European eel Anguilla anguilla following seawater acclimation. *J Exp Biol* 205: 2643-2651, 2002.
2. **Jahn TP, Moller AL, Zeuthen T, Holm LM, Klaerke DA, Mohsin B, Kuhlbrandt W, and Schjoerring JK.** Aquaporin homologues in plants and mammals transport ammonia. *FEBS Lett* 574: 31-36, 2004.
3. **Lignot JH, Cutler CP, Hazon N, and Cramb G.** Water transport and aquaporins in the European eel (Anguilla anguilla). *Symp Soc Exp Biol*: 49-59, 2002.
4. **Masoni A and Payan P.** Urea, inulin and para-amino-hippuric acid (PAH) excretion by the gills of the eel, Anguilla anguilla L. *Comp Biochem Physiol A* 47: 1241-1244, 1974.
5. **Motais R and Isaia J.** Temperature-dependence of permeability to water and to sodium of the gill epithelium of the eel Anguilla anguilla. *J Exp Biol* 56: 587-600, 1972.
6. **Yang B and Verkman AS.** Water and glycerol permeabilities of aquaporins 1-5 and MIP determined quantitatively by expression of epitope-tagged constructs in Xenopus oocytes. *J Biol Chem* 272: 16140-16146, 1997.