Water absorption in the spiral intestine of *Leucoraja erinacea*

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The emergence of aquatic animals onto land 370 million years ago presented immense challenges for adapting to terrestrial life. Life on land required novel mechanisms for the absorption and retention of water. Terrestrial vertebrates retain body water through the kidneys and absorb water through the colon. In contrast, Chondrichthyes do not absorb water from ingested food as they are nearly iso-osmotic with their ocean environment [3]. Thus, the development of a water-absorbing colon in the digestive tract was essential for the adaptation of animals to a terrestrial niche. The general aim of my work is to understand the origin of the terrestrial vertebrate colon taking both morphological and genetic approaches.

Previously we characterized the water-absorption potential of the little skate, *Leucoraja erinacea*, digestive tracts by analyzing the distribution of acid mucins contained in discreet regions along the gut tube [7]. The presence of acid mucins in the large intestine of mammals has been linked to the water absorptive property of this organ [4,5,6]. We found the concentration of acid mucins in the distal-most region of the little skate spiral intestine at levels comparable to those found in the terrestrial vertebrate colon [7]. The presence of acid mucins in the spiral intestine presents the possibility that the posterior region of the spiral intestine may itself be an organ that reabsorbs water that expanded over time to become the terrestrial colon. We have made initial efforts to verify that the distal spiral intestine of *L. erinacea* has the ability to absorb water.

To determine whether the distal spiral intestine is a region of water absorption, we are taking two approaches. First, molecular tools are used to characterize aquaporin gene and protein expression. This will confirm our previous results that the distal spiral intestine expresses water channel proteins necessary to absorb water. Second, we are developing physiological methods to measure water uptake in the little skate digestive tract. Briefly, the little skate digestive tract is filled with Elasmobranch Ringer's solution (in mM, 270 NaCl, 4 KCl, 1 KH_2PO_4, 8 $NaHCO_3$, 350 Urea, 0.5 Na_2SO_4, 3 $MgCl_2 \cdot 6H_2O$, 2.5 $CaCl_2 \cdot 2H_2O$, 5 Glucose, 5 HEPES/Tris pH 7.5) to a hydrostatic pressure of 1.0 kPa. The intestinal sac is tied at both ends and incubated for 3 hours in Ringer's and weighed every 30 minutes. The amount of water absorbed by the intestine is calculated as a decrease in mass per cm of intestine. Similar methods have been used to measure water absorption in the digestive tract of the Japanese eel, *Anguilla japonica,* and European eel, *A. anguilla* [1].

For molecular studies, fresh digestive tract tissue was harvested from *L. erinacea* adult animals. Regions were removed from stomach, proximal and distal spiral intestine, rectal gland and sphincter, and flash frozen for protein or RNA extraction. Total RNA was isolated from each isolated region and single-stranded cDNA was synthesized with qScript cDNA Supermix (Quanta Biosciences) for 5 minutes at 22°C, 30 minutes at 42°C and 5 minutes at 85°C, for later use in amplifying aquaporin family members using degenerative primers. Protein was extracted from the tissues by homogenizing in TG lysis buffer (20 mM HEPES pH 7.2, 1% Triton-X, 10% glycerol, 1 μg/ml aprotinin, 100 μg/ml PMSF, 1 μg/ml pepstatin and 1:100 dilution of phosphatase inhibitor cocktail II) for immunoblot analysis. In addition, tissues from *L. erinacea* were fixed in 4% paraformaldehyde, dehydrated in an ethanol series and prepared for paraffin embedding. Paraffin was removed from 6 μm sections by washing in xylene and hydrated in an ethanol series to PBS. Sections were blocked in 10% goat serum

and stained with a polyclonal antibody against rat AQP4 (Millipore AB2218). AQP4 was detected in the basolateral membrane of the distal spiral intestine epithelium in *L. erinacea* (Fig. 1). AQP4 has been shown to regulate water permeability of the proximal colon in mice [2]. Thus, the expression of AQP4 in the distal spiral intestine is the first indication that this organ has the capacity to absorb water. Further investigation of the initial experiments outlined here will help elucidate the origin of a water uptake organ (colon) in the vertebrate lineage.

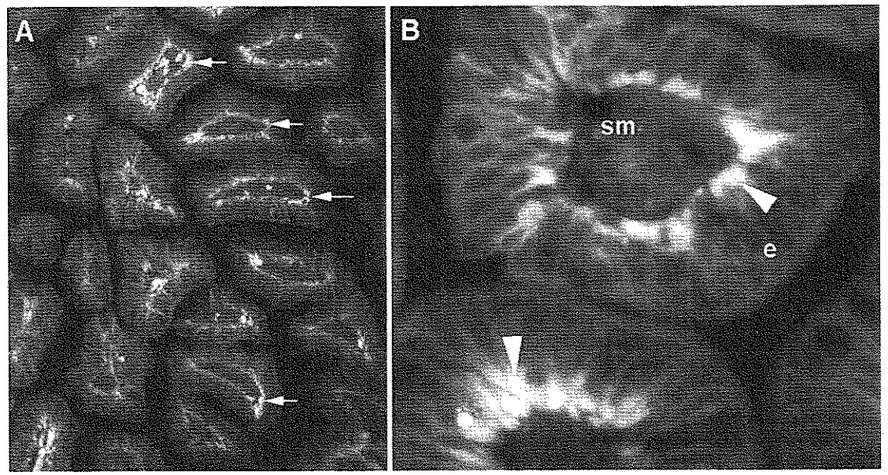

Fig. 1. AQP4 is expressed in the distal spiral intestine. (A) AQP4 is localized to the epithelium (arrows) in the distal spiral intestine. (B) Expression of AQP4 is confined to the basolateral epithelium (arrowheads), and is absent from the submucosa. Sm, submucosa, e, epithelium.

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