Identification of aquaporin mRNA in the rectal gland of *Squalus acanthias*

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The membranes that envelop the cell are made out of a double layer of lipids that does not permit water to go in or out of the cell. There are a family of molecules called aquaporins that allow water to flow in and out of the cell. This report identifies for the first time that the rectal gland of the spiny dogfish has the capacity to make these molecules.

The secretion of the rectal gland of the shark is isoosmotic with the shark blood or the solutions used to perfuse it *in vitro*. The secretion of chloride varies as much as forty fold when stimulated with peptide agonists or cyclic AMP. This secretion of chloride requires the rapid and simultaneous movement of water across the rectal gland. To date there is no information on the type of aquaporin molecules that must be present in the cell membranes of the rectal gland. There are expressed sequence tag (EST) reports for aquaporin 1, 4, and 9, in normalized cDNA clones of various tissues of *S. acanthias*[1-4] and aquaporin 3 and 9 in normalized cDNA clones of various tissues of skate. We used the reported EST to determine the type of aquaporins present in the rectal gland of *S. acanthias*.[1-4]

A portion of a rectal gland, kidney, and spiral valve from a single dogfish were homogenized in lysis buffer from Qiagen using a Tekmar tissue homogenizer. The homogenate was passed through a Qiagen shredder column, and messenger RNA was prepared using Qiagen RNAeasy minikit and treated with DNase. Single strand cDNA was then prepared using an Invitrogen First-Strand synthesis kit. PCR amplification was done using RedTaq ready mix from Sigma and the degenerate primers shown in Table I. The amplified products were separated using 2% agarose gel in TAE. The products were eluted from the gel using MinElute Gel extraction kit from Qiagen, purified and sequenced at the MDIBL DNA Sequencing Core.

The primers for aquaporins were designed using the reported sequences for the normalized cloned cDNA for *S. acanthias*. The primer sequences used are reported in Table I. Figure 1 shows an agarose gel with the amplification results for aquaporin 1, 4, and 9 in kidney, rectal gland and spiral valve. The two set of primers for aquaporin 1 yielded products of the expected number of bases, 401 and 679 respectively, and were present in all tissues. Aquaporin 4 was present in kidney and rectal gland, but not in the spiral valve. Aquaporin 9 was present only in kidney.

Table I			
		primer sequence	Predicted # bases
Aquaporin 1	Left	5'-gggtgaccatcttcgtcttt -3'	401
	Right	5'-agagaacgagctggaaggtg -3'	
	Left	5'-cagagaagtccagcgcaag -3'	679
	Right	5'-cgtggtgaacacaaagtcgt -3'	
Aquaporin 4	Left	5'-gagttacaatgcaccgctca -3'	462
	Right	5'-ttaacgtgacccccactgat -3'	
Aquaporin 9	Left	5'-atcctgcgatttccctttct -3'	401
	Right	5'-ctgggttatgggacaaccac -3'	
Na-K-ATPase	Left	5'-gacagctctttggtggcttc -3'	657
	Right	5'-gcttcaagccagctgtatcc -3'	

The amplified product yielded the sequences for aquaporin 1 and 4 shown in Figures 2 and 3. The sequences are 67% to 80% similar to that of aquaporin 1 and 4 in a variety of tissues in many species ranging from invertebrates to vertebrates. The product for aquaporin 9 was not sequenced.

The results reported here show that aquaporin 1 was expressed in all three tissues examined, kidney, rectal gland and spiral valve. Aquaporin 9 was expressed only in the kidney. The surprising finding was that aquaporin 4 was expressed in kidney and rectal gland but not in the spiral valve. Since the rectal gland is an appendix of the intestine, it was expected that aquaporins expressed in the rectal gland would also be found in the intestine.

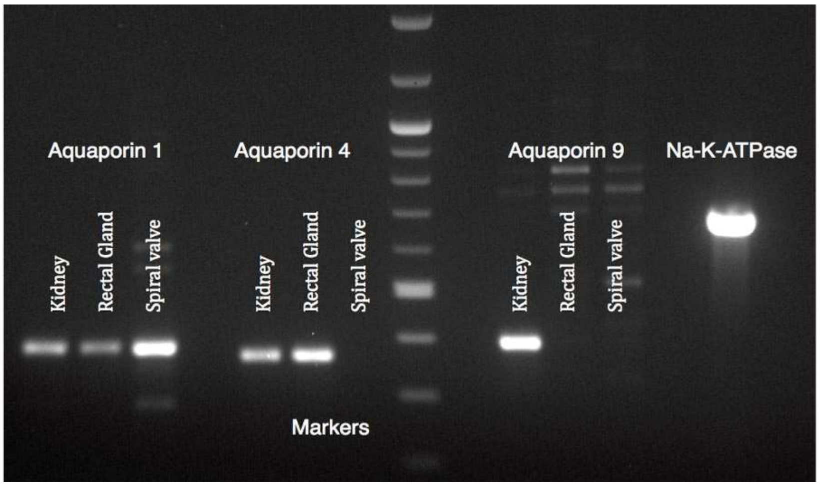

Figure 1. RT-PCR amplification of aquaporin 1,4, and 9 from *S. acanthias* kidney, rectal gland, and spiral valve. All products are of the expected size. Aquaporin 1 was present in all tissues, aquaporin 4 in kidney and rectal gland. Aquaporin 9 was only present in kidney. The control Na-K-ATPase resulted in a product of the expected size

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ATGGCGGGCTGTGCTCGCCGAGTTCTTGGGGGTGACCATCTTCGTCTTTCTCAG
CATCGGGTCGGCCACCAAGTGGACGCCCAGCGGCTTCCCCGCCGACGTGGTGCA
GATCGCCCTGACCTTCGGGCTGTCCATCGCCACCCTGGCCCAGAGCATCGGCCA
CATCAGCGGGGCGCACCTCAACCCGGCCGTCACCCTGGGGCTGCTGGTGGGCTG
CCAGATCAGCGTGCTGCGGGCGGTCATGTACATGGTCTCCCAGCTGCTGGGCGC
CGTGGCAGCCAGCGCCATCCTCTTCGGTGTCACCCCCAACTCCAGGAACGGGAC
CCTCGGAGTCAACGCGCTGGGAGAAGGGGTTACCCCGGGGCAGGGTCTGGGCGT
TGAAATTATCATCACCTTCCAGCTCGTTTTCTGCGTCTTTGCAACCACAGATAA
ACGAAGGACGGATCTCTCCGGCTCCGGCCCTCTAGCCATTGGACTTTCGGTTGC
TATTGGCCACTTAATGGCGATTGGCTTCACCGGATGTGGGATGAACCCTGCCCG
TTCCTTCGGGCCAGCTGTCATTACCGGCAACTTCAAGGATCACTGGCTGTACTG
GGTGGGCCCCATGATCGGAGGATCCGTCGCCGCCCTCCTCTACGACTTTGTGTT
CACCACAATCCAACAA
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Figure 2. Partial nucleotide sequence of aquaporin 1 from the rectal gland of *S. acanthias*. The sequence contains 664 bases.

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CGCCAGACTCTTGAGTTACAATGCACCGCTCACNCCAGGGTATCGAGCCGGAGT
CTCAGTCCCAGCCCCTGTTCCACTCTCTGTCTACCGGCAGTTCGATCACACCCT
CGCAGCCACCGGCAGCCAGTAAGCGCTCGCCATTGTGCAGCTTTGGGGCAGCCA
TGACGGCATTCAAAGGGATCTGGACGCAGCAGTTCTGGAGAGCAGTTTCTGGGG
AGTTCCTGGCGACGCTCATCTTTGTCCTTCTCAGCCTAGGTTCCACCATTGGCT
GGAGCGGTGAAGGTGGACCTTTGGACATTGTCCTCATCTCTCTCTCCTTCGGAC
TCAGCATCGCGACCATGGTCCAGTGCTTTGGTCACATCAGTGGGGGTCACGTTA
ACCCTGTGGTCCCCGCTGCCTGCGTCTGTACCAGGAAGCTCAGCCTAACTTATA
AT
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Figure 3. Partial nucleotide sequence of aquaporin 4 from the rectal gland of *S. acanthias*. The sequence contains 434 bases.

These results are the first demonstration of aquaporin messenger RNA in the rectal gland. Prior functional analysis based on permeability measurements of isolated membranes failed to provide evidence for an involvement of water channels in fluid secretion. Thus, the mechanisms of coupling between salt and water movement through and by the cells of the rectal gland have to be reconsidered.[5]

1. GenBank: ES605906 EST 30-JUN-2009

2. GenBank: ES324936.1 EST 30-JUN-2009
3. GenBank: ES324160 EST 30-JUN-2009
4. GenBank: CV720323 EST 30-JUN-2009
5. **Zeidel, JD, Mathai, JC, Campbell, JD, Ruiz, WG, Apodaca, GL, Riordan, J and Zeidel, ML.** Selective permeability barrier to urea in shark rectal gland. *Am. J. Physiol. Renal Physiol.* 289:F83-F89, 2005.