PARTIAL CDNA SEQUENCE OF PERIPHERAL AQUAPORIN EXPRESSED IN THE FOOT OF *LIMAX MAXIMUS*.

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The terrestrial gastropod, *Limax maximus*, has served as a model system to understand the role that the CNS plays in regulating osmoregulatory responses in central and peripheral targets (e.g. Welsford et al., J. Exp. Zool., 253, 229-234, 1990; Matanock and Welsford, Can Jl. Zool. 73, 607-609, 1995). We are currently investigating the role aquaporins might play in both peripheral water uptake mechanisms as well as central osmosensation in *Limax*. As a prelude to investigating the transcriptional regulation of water channels and the stress response gene families, which may regulate them, we report the partial cloning of the cDNA putatively encoding foot AQPs in *Limax*.

RNA was isolated from the foot epithelium of cold-anesthetized slugs using Trizol reagent (GibCo BRL) and mRNA was isolated from this pool using a Message Maker kit from GibCo. RT-PCR was performed on this mRNA using degenerate primers corresponding to highly conserved regions of known aquaporins. Based on nucleotide preferences of *Limax* determined from previous sequencing runs on *Limax*, the sequence of the upstream primer used was 5'GGNGSNCACNTNCAAYCCNGC 3' and the downstream primer was 5' GGNCGNKCBWSNRAVCSDGG 3'. Using a Pfu polymerase kit (Access RT-PCR kit from Promega) a high yield product was obtained of approximately 390 base pairs (based on regression analyses of agarose gels), close to the predicted size (based on published sequences of AQPs) of 400 bp. The band was extracted from the gel and placed into PCR 2.1 vector (InVitrogen Inc.) after first treating reaction product with Taq polymerase to add 3' overhang regions. Ligation products were quantified by OD_{260} and used to transform competent INVαF cells (InVitrogen Inc.). Vector was isolated from 10 positive colonies using either a Qiagen Miniprep kit or standard alkaline lysis technique. Vector was analyzed for presence of insert by digestion with ECOR1 and vectors positive for insert were sent to the Marine DNA Sequencing Facility at the MDIBL. Sequences were analyzed for similarity to known AQPs using BLAST and ClustalW.

The cDNA sequence was found to be most similar the AQP superfamily of genes and shared highest homology with yeast AQY1 (71.43%) and human AQP1 (72.35%). Experimentation is underway to clone the cDNA encoding putative CNS AQPs in *Limax*. Preliminary RT-PCR analyses suggest that this gene is structurally distinct from foot AQP since experiments using the same primers as noted above generated a band of approximately 436 bp, significantly different from foot ($p < 0.05$).

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