PRELIMINARY IDENTIFICATION OF Na^+/H^+ EXCHANGER mRNA IN GILL AND COXAL GLAND OF THE HORSESHOE CRAB *LIMULUS POLYPHEMUS*

David W. Towle[1,2] and Jennifer Litteral[2]
[1]Lake Forest College, Lake Forest, IL 60045
[2]Mount Desert Island Laboratory, Salsbury Cove, ME 04672

The horseshoe crab *Limulus polyphemus* encounters dilute salinities during its spawning migration, displaying a modest osmoregulatory capacity that may be mediated by epithelial ion transporters in the gill or coxal gland. Unlike the true crabs, the coxal gland of *Limulus* is able to produce anisosmotic urine (Towle, D.W., et al., *Physiology and Biology of Horseshoe Crabs*, J. Bonaventura et al., eds., A.R. Liss Inc., New York, pp. 147-172, 1982). In the gill, Na^++K^+-ATPase activity is most concentrated in the central region of each leaflet, coinciding with a highly developed epithelial cell layer containing extensive basolateral membrane interdigitations (Henry, R.P. et al., *Biol. Bull.* 191:241-250, 1996). Specific Na^++K^+-ATPase activities in homogenates of coxal gland increase as environmental salinity declines, suggesting that active Na^+ transport is augmented in conditions of salinity stress (Towle et al., 1982). The ultrastructure of the coxal gland indeed supports its role in ultrafiltration and osmoregulation (Briggs, R.T., and Moss, B.L., *J. Morphol.* 234:233-252, 1997). No information exists, however, concerning candidate apical transporters that may be involved in NaCl regulation in *Limulus*.

We collected horseshoe crabs from the tidal Bagaduce River on the western side of the Blue Hill (Maine) peninsula at low tide during the June 1998 full moon. The salinity of the adjacent waters was 30 ppt. A male crab was chilled on ice and bled. The brick-red nephridial lobes of the coxal gland as well as central and peripheral regions of gill leaflets were dissected and immediately homogenized under RNAse-free conditions. Total RNA extracts were prepared using the Qiagen RNeasy mini kit. Poly-A^+ mRNA was reverse transcribed with the Gibco/BRL Superscript Preamplification system, using oligo-dT as primer. Putative Na^+/H^+ exchanger (NHE) cDNA was amplified by PCR using degenerate primers designed to detect NHE in decapod crustaceans (Towle, D.W. et al., *J. Exp. Biol.* 200: 1003-1014, 1997). On the basis of previous results with crustaceans and teleosts, an 800-bp product is expected with the primers employed. Our preliminary data suggest that the nephridial lobes of the coxal gland and the central region of the gill leaflets of *Limulus* express an NHE-like mRNA (Fig. 1). Interestingly, the peripheral region of gill leaflets appears to be devoid of NHE-like RNA, indicating a specificity of gene expression that is correlated with Na^++K^+-ATPase distribution and gill ultrastructure. Supported by the National Science Foundation (IBN-9807539).

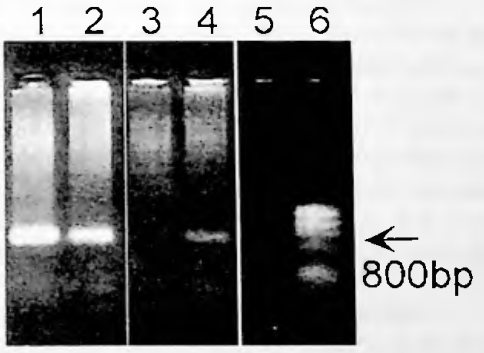

Fig. 1. Amplification of Na^+/H^+ exchanger cDNA using degenerate PCR primers. Templates: Lane 1, *Callinectes sapidus* gill (positive control); Lane 2, *Limulus* gill central region; Lane 3, gill peripheral region; Lane 4, nephridial lobes of coxal gland; Lane 5, negative control; Lane 6, DNA markers.