# ISOFORMS OF THE $Na^+/H^+$ ANTIPORTER (NHE) IN GILL mRNA OF THE MARINE LONG-HORNED SCULPIN (*MYOXOCEPHALUS OCTODECIMSPINOSUS*)

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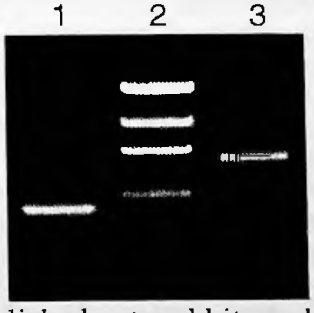
Recent experiments have shown an amiloride sensitive $Na^+/H^+$ exchanger, thought to be primarily involved with acid-base regulation, in the gills of the long-horned sculpin, *Myoxocephalus octodecimspinosus* (Claiborne, Campbell, and Long, Bull. MDIBL 35:48-49, 1996). Using Northern blot analysis, mRNA transcripts that are homologous to a cloned human NHE-1 isoform of this $Na^+/H^+$ antiporter were shown to be present in the gills of this species (Harris, Claiborne, Pouyssegur, and Dawson, Bull. MDIBL 32:128-130, 1993). There are five known NHE isoforms that have been cloned and sequenced in vertebrates: NHE-1, ßNHE, NHE-2, NHE-3, NHE-4 (Bianchini and Pouyssegur, J. exp. Biol. 196:337-345, 1994). The isoform of the $Na^+/H^+$ exchanger, NHE-3, found in the proximal convoluted tubule of mammals, is thought to aid in systemic pH regulation by secreting $H^+$ in exchange for uptake of luminal $Na^+$ (Gluck and Nelson, J. exp. Biol. 172:205-218, 1992). We speculate that in marine fish an antiporter homologous to this isoform is located on the apical side of the branchial epithelium.

Isolation of total RNA from gill homogenate of the sculpin was performed using an acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, Anal. Biochem. 162:156-159, 1987). Reverse transcriptase was used to isolate mRNA and transcribe cDNA (Perkin Elmer GeneAmp RNA PCR Kit). Oligo $d(T)^{16}$ primer was used as a template to specifically select for mRNA. Polymerase chain reaction (PCR) was then performed to amplify the cDNA. Degenerate primers from conserved NHE regions, found in other vertebrate isoforms of NHE (generously provided by Dr. D. Towle), were used to locate the cDNA specific for the $Na^+/H^+$ antiporter. The length of the strand expected with these primers is approximately 700 base pairs long. Figure 1 shows the band in lane 3 of an ethidium bromide stained, 1% agarose gel. The sample was then run on a low melting point agarose gel, the band was cut out, digested with Agarase (Sigma), and then sequenced at the University of Maine DNA Sequencing Core Facility. The resulting sequence showed a significant (~63%) nucleotide homology to the ßNHE sequence found in trout cephalic kidney (Borgese et al., Proc. Natl. Acad. Sci. USA. 89:6768-6769, 1992).

Figure 1.




Ethidium bromide stained 1% agarose gel of RT-PCR product. Lane 1 shows a positive control band of 308 bp; lane 2 is a DNA Mass ladder showing sizes, from the top, of 2000, 1200, 800, 400, and 200 bp; lane 3 shows the band of ~700 bp that was sequenced; a negative control on the same gel showed no band.

Using degenerate primers from conserved regions of published rat, rabbit, and human NHE-3 mRNA, an attempt to find sequences specific for this NHE-3 isoform in the sculpin gill resulted in a PCR product of the correct size (~405bp). Likewise, these same primers have identified similar sized mRNA in the killifish (*Fundulus heteroclitus*) and dogfish (*Squalus acanthias*). Sequencing of these cDNAs is currently underway. This research was supported by NSF RUI-94-19849 to J.B.C.