Nucleotide repeats in gill NHE3 of longhorn sculpin (*Myoxocephalus octodecimspinosus*)

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Gill Na^+/H^+ exchangers (NHE) are thought to be responsible for acid excretion across the gills in marine fish[1]. We previously reported the preliminary PCR detection and partial 1.0 kb cDNA sequence of longhorn sculpin (*Myoxocephalus octodecimspinosus*) gill Na^+/H^+ exchanger isoform 3 (NHE3)[3] and Northern blot analysis[4]. We have now used 5' and 3' RACE (Generacer Invitrogen) to obtain the full length 3.1 kb cDNA sequence of this NHE3. Bioinformatic analysis of sculpin NHE3 led to the discovery of two sets of nucleotide repeats. A 27 bp repeat (5'-GACACGGGACACGAGACGGGCAC TGCA-3'; typically thought of as a minisatellite) was found in the 5' region (starting at bp 105) and a single trinucleotide repeat (5'-GCC-3') was found in the 3' sequence (bp 2543) of the coding region of NHE3 (when numbered without repeats). To our knowledge, this is the first report of a large repeat area within the coding region of a fish protein. The effect of these repeats on protein function is unknown. To determine if these repeat areas varied between individual animals, gill RNA was collected from 8 sculpin and the NHE3 CDS was amplified, sequenced and analyzed.

TRI Reagent (Molecular Research Center, Inc.) was used to isolate gill RNA from seawater adapted sculpin. Total RNA was reverse transcribed at 55°C using Thermoscript (Invitrogen) to generate cDNA. RT-PCR amplification (FastStart Taq; Roche) with primers designed around the repeat areas was used to generate products that were then analyzed by agarose gel electrophoresis. ExoSAP-IT (USB Corp.) was applied to the PCR products to prepare for direct sequencing at the MDIBL Marine DNA sequencing center. Bioinformatic analysis was used to align and count the number of repeats.

Both the trinucleotide and minisatellite repeats in sculpin NHE3 were found to be polymorphic. The trinucleotide repeats varied in lengths from 15 bp (1 of 5 fish), 27 bp (n=1) and 30 bp (n=3). The minisatellite repeats were found in lengths of 108 bp (3 of animals), 135 bp (n=2) and 189 bp (n=1). For example, a minisatellite of 108 bp is composed of 4 sets of the 27 bp repeat. Expansions of trinucleotide repeats are correlated with 14 diseases in humans, including Huntington's disease[2]. Minisatellites have been found to be associated with the regulation of transcription, interference with slicing, control of imprinting and chromosome fragile sites[5]. We speculate that the observed variations may alter the function and/or regulation of the NHE3 in the sculpin gill. To understand the effect of repeat variations we hope to functionally express NHE3 CDS which includes selected repeat combinations. Funding was provided by Hancock County Scholars Program to G.L., REU Fellowship to M.F. at MDIBL (NSF DBI-0453391) and NSF IBN-0111073 to J.B.C.

1. **Claiborne J.B., Edwards S., Morrison-Shetlar A.** Acid-base regulation in fishes: cellular and molecular mechanisms. *J. Exp. Zool.* 233: 302-31, 2002.
2. **Cummings C. J. and H. Y. Zoghbi.** Trinucleotide repeats: mechanisms and pathophysiology. *Annu Rev Genomics Hum Genet.* 1: 281-328, 2000.
3. **Lanier, C. E. and J.B. Claiborne.** Evidence of gill Na^+/H^+ exchanger (NHE3) in long-horned sculpin (*Myoxocephalus octodecimspinosus*). *Bull. Mt. Desert Isl. Biol. Lab.* 42: 80, 2003.
4. **Lanier, C. E. and J.B. Claiborne.** Analysis of branchial longhorn sculpin (Myoxocephalus octodecimspinosus) Na^+/H^+ exchanger isoform 3 using Northern hybridization). *Bull. Mt. Desert Isl. Biol. Lab. 43:* 107, 2004.
5. **Vergnaud G. and F. Denoeud.** Minisatellites: mutability and genome architecture. *Genome Res.* 10(7): 899-907, 2000.