GENETIC POLYMORPHISMS AND POPULATION DIVERSITY IN THE Na,K-ATPase GENE OF THE SPINY DOGFISH, SQUALUS ACANTHIAS

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The goal of our work this past summer was to develop the methodology to survey genetic diversity and population structure in the spiny dogfish, Squalus acanthias. As a specific target vehicle for this analysis, we chose the Na,K-ATPase gene, portions of which had been cloned previously at MDIBL by Benz et al. (Bull. MDIBL 31:103-104, 1992). The significance of this gene is that it serves as a prototypic ion transporter whose genetic polymorphisms may affect individual sharks' viability and thus selection in response to heavy metal and other environmental toxicants. Widely distributed Squalus acanthias might ultimately serve as a sentinel species with which to ascertain genetic toxicant contamination and ecological/population changes in the three-quarters of the world covered by ocean.

The previously cloned and sequenced portion of shark Na,K-ATPase cDNA included parts of exons 9 and 12 as well as complete exons 10 and 11. Using these precisely known shark sequences, plus information from the literature on highly phylogenetically conserved regions from other species, Polymerase Chain Reaction (PCR) primers were designed and synthesized for exons 4, 9-12 and 16. Exons 4, 9, 12 and 16 contain structural motifs thought to be essential for function of the Na,K-ATPase gene product, and thus might reveal changes with biological significance for the survival of individual animals. Reaction conditions were optimized such that all primer pairs gave servicable PCR amplification products from initial target shark genomic DNA.

After designing and testing the PCR reagents, genomic DNA was prepared from more than 50 individual animals that were sacrificed for physiological experiments by other MDIBL investigators. Work to be continued during the winter and next summer at MDIBL will examine the genetic heterogeneity of the Na,K-ATPase gene in these 50 randomly collected animals. Polymorphisms have already been detected in terms of the success or failure of amplification of specific exons from individual animals, which presumably reflects heterogeneity in the binding sites for specific PCR primers. Sequence variations within the amplified regions will be sought via the Single-Stranded Conformational Polymorphism (SSCP) and Heteroduplex (HD) techniques.

If these studies demonstrate genetic polymorphisms in the Na,K-ATPase gene of Squalus acanthias, further research directions will be (1) sequencing the variant alleles to see if structural (i.e. functional) polypeptide changes would result, (2) correlating these genetic polymorphisms with physiologic data from other MDIBL investigators working with the same animals, and (3) investigating shark population structures in the ocean environment in response to suspected toxicant insults. (Supported by an NSF EPSCoR grant and a Grass Foundation Fellowship to J. Rock.)