ISOLATION OF THE cDNA ENCODING NA+K+-ATPase FROM *SQUALUS ACANTHIUS*

Nancy Berliner[1], Edward J. Benz, Jr.[2], and Nicholas Yagoda[3]
[1]Yale University School of Medicine, New Haven, CT
[2]Johns Hopkins University School of Medicine, Baltimore, MD
[3]Brown University, Providence, RI

The Na+K+-ATPase is an essential transmembrane protein required to maintain sodium and potassium gradients within cells by the efflux of sodium and the uptake of potassium at the expense of ATP breakdown. There are two subunits of this protein, α and β. There are three distinct α subunit isoforms in vertebrate species (A1, A2, and A3), each of which is expressed in specific cells and tissues (Mercer, Schneider and Benz, *Prog Clin Biol Res* 268B: 119-126, 1988).

During the past two summers, we have attempted to isolate the full-length cDNA encoding the Na+K+-ATPase of the dogfish shark (*Squalus acanthius*). There is evidence that the dogfish shark has only one isoform of the Na+K+-ATPase, and that this isoform most closely resembles the A3 isoform of humans (Benz et al, *Bull. MDIBL* 32: 101-103, 1992.

We screened a dogfish shark rectal gland cDNA library with a previously isolated 400bp PCR fragment of the dogfish shark Na+K+-ATPase gene as probe. Seven cDNA clones were isolated. Using PCR with primers directed at flanking vector sequences, we amplified the inserts of these clones, which resulted in fragments ranging from 500 bp to 4kb in length. The human Na+K+-ATPase is approximately 4kb in length; this past summer we characterized the longest of the clones, which was approximately 3kb.

The cDNA clone was excised from the ZAP phage vector through an excision and phagemid rescue procedure which sequesters the clone in the plasmid pBK-CMV. The isolated clone was analyzed by restriction enzyme digestion, and Eco RI and HindIII fragments were subcloned back into the pBK-CMV vector and sequenced along with the 3kb full length clone in order to obtain as much sequence as possible. The results revealed that only a single 1.4kb EcoRI fragment contained sequences from the dogfish shark Na+K+-ATPase; the remaining portions of the insert are an EcoRI cDNA fragment which was inserted with the ATPase fragment during construction of the library.

Sequence analysis of the cDNA revealed striking homologies to the A3 (78%) and A2 (77%) a subunits of vertebrate species, but considerably less homology to the A1 subunit. These results are striking because the dogfish shark rectal gland is an epithelial transport tissue that would be expected, by analogy to mammalian, amphibian, and avian transport epithelia, to express only the A1 isoform. Our finding confirms and extends our previous results and strengthens our suggestion that the Na+K+-ATPase isoform present in dogfish shark rectal gland most strongly resembles the A3 and A2 forms. Since the single isoform present in insects also resembles the A3 form (Varadi, Gilmore-Hebert and Benz EJ, *FEBS Letter* 258(2): 203-207, 1989), we suggest that the primordial isoform was an A3 or mosaic of an A2/A3 isoform, and that the A1 isoform emerged at a later stage of vertebrate evolution.

Funding Source: This project was funded by a New Investigator grant from the Center for Membrane Toxicology Studies to NB. NY was funded by a Maine American Heart Association Summer Student Research Fellowship.