Cloning of the 5' end of Na, K-ATPase cDNA in the rectal gland of the *Squalus acanthias*

Ana Blakaj[1], Rachel B. Plattus[1], Nancy Berliner[1], and Edward J. Benz, Jr[2]

[1]Department of Medicine, Yale University School of Medicine, New Haven, CT 06510
[2]Dana-Farber Cancer Institute, Boston, MA 02147

Na, K-ATPase is an integral transmembrane protein found in almost all eukaryotic cells. It plays a critical role in the regulation of cell volume, the maintenance of the high intracellular potassium concentration, the generation of sodium gradients which drive the transport of amino acids, glucose and calcium ions, and the regulation of the electrochemical gradients in many tissues of the body. It is a heterodimeric protein, consisting of an alpha subunit, of which there are three isoforms (A1, A2, A3), and a beta subunit. The alpha subunits are each expressed in different cells and tissues[1].

The 3' end of the cDNA encoding for the Na, K-ATPase gene in the shark (*Squalus acanthias*) rectal gland was cloned in 1997[2]. At that time, a previously isolated cDNA fragment of the gene was used to screen a rectal gland cDNA library cloned into the lambda-ZAP vector. These clones were isolated by phagemid rescue, excised, and subcloned, and sequenced. The 1.4kb EcoRI fragment was confirmed to have high sequence homology to previously isolated Na, K-ATPase cDNAs from human and *Drosophila*. A close homology was found to the A3 and A2 isoforms of the alpha subunit[2].

The goal for this project was to isolate the remainder of the 5' end of the full-length cDNA encoding for the *Squalus acanthias* rectal gland Na, K-ATPase cDNA. Shark rectal gland was homogenized and total RNA was isolated using TRIzol reagent (Life Technologies, USA). The 5' end was then isolated using the SMART RACE method (Clontech, Palo Alto, CA). This technique automatically incorporates a synthetic adaptor sequence on both the 5' and 3' ends of a partially cloned sequence of cDNA. A specialized oligo (dt) primer selectively primes mRNA in the total RNA sample. Using reverse transcriptase and a specialized SMART oligo, RACE-ready cDNA is obtained in which the ends of the cDNA are ligated to a synthetic adaptor sequence. Two antisense primers about 100bp apart were made from known cDNA sequences of Na, K-ATPase, both with melting points of over 80°C to allow for touchdown PCR. A PCR reaction was carried out using the 3'-most antisense primer and a RACE universal primer. A second reaction was then performed using the nested antisense primer encoding sequences 100bp upstream. The nested PCR yielded a band at 1.7-2 kb by gel electrophoresis. This PCR product was cloned into a TOPO vector (Invitrogen, Carlsbad, CA), confirmed by enzyme digestion with Eco RI, and sequenced. Sequence analysis confirmed the identity of the insert as the Na, K-ATPase cDNA, with sequence homology to the A3 isoform. In summary, we have obtained sequence for a full-length *Squalus acanthias* rectal gland cDNA encoding Na, K-ATPase. Further studies will be directed at determining whether there are multiple isoforms of the gene as found in higher animals, and to characterize tissue-specific ATPase expression. Data analysis of multiple cDNAs have revealed no evidence for alpha subunit heterogeneity. This may indicate that the predominant isoform in shark rectal gland epithelium is most like the A3 form, which is not abundant in epithelia of mammals.

1. Devarajan P. Differential Translation of the Na, K-ATPase Subunit mRNAs. J Biol Chem 1992; 257:22435-22439.
2. Yagoda N, Berliner N, Benz EJ, Jr.. Isolation of the cDNA Encoding Na, K-ATPase from Squalus acanthias. MDIBL Bulletin. 36 1997; 36:21.