IDENTIFICATION OF THE SNARE PROTEIN SYNTAXIN IN THE SHARK (*SQUALUS ACANTHIAS*) BRAIN

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The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is an important chloride channel in humans and model organisms, including the dogfish shark. We have previously shown that CFTR is expressed in several shark tissues, including rectal gland, brain, intestine, testis and spleen (Hemminger et al., Bull. MDIBL 36:33-35, 1997). The function of this Cl^- channel has been well characterized in the rectal gland, but is less clear in other tissues, including brain. Recently, it was shown that CFTR can be inhibited by the SNARE protein syntaxin 1A by direct protein-protein and domain specific interactions in human T84 cells (Naren et al., PNAS 95:10972-7, 1998). Using degenerate RT-PCR, we tested the hypothesis that syntaxin-like proteins are present in CFTR-expressing tissues in the shark. We report here the partial cloning of a syntaxin-like protein from dogfish brain.

Total RNA was isolated from fresh dogfish shark brain and rectal gland using TRIZOL® Reagent (GIBCO). RNA was subjected to a DNAse digest and reverse transcription was carried out with oligo(dT) primers (Clontech Advantage™). Degenerate primers were designed from two highly conserved regions found in several mammalian syntaxin proteins. The sense primer 5'-ATGGANGARTTYTTYGARCARGT-3' and antisense primer: 5'-TCNCCYTGNSWY TCNACNARCAT-3' were used to amplify a fragment of shark syntaxin. PCR was performed for 35 cycles (95 °C for 30 sec; 51 °C for 30 sec; 68 °C for 60 sec) using Expand™ Long DNA polymerase (Boehringer Mannheim). PCR products from brain of the expected size (~ 600 bp) were cloned (TA-cloning, Invitrogen), recombinants were screened utilizing blue-white selection, and clones were sequenced at the MDIBL Marine DNA Sequencing Facility.

Figure 1 shows the PCR results from shark brain and rectal gland using degenerate primers designed from mammalian syntaxin sequence. A weak but distinct product was amplified from shark brain cDNA, but not from rectal gland. This band was cloned, sequenced and determined to have highest identity (80%) to sheep syntaxin 1B using BLASTN. The putative protein sequence of the shark fragment had greatest similarity to rat syntaxin 1B with 94% identity.

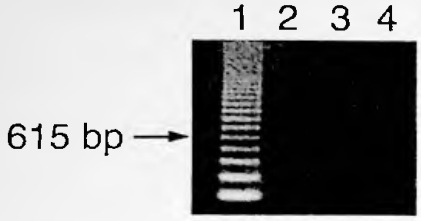
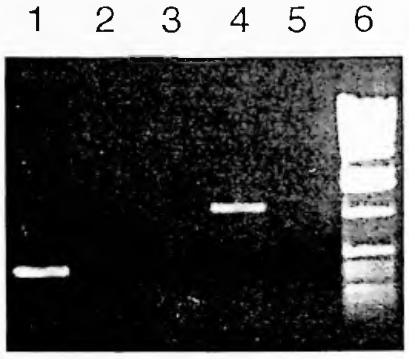


Figure 1. Degenerate PCR using primers designed from mammalian syntaxins. Lane 1: 123 bp DNA ladder, Lane 2: shark rectal gland cDNA template, Lane 3: shark brain cDNA template, Lane 4: negative control of primers (no cDNA present in the reaction).

To determine whether the shark syntaxin 1B is expressed in rectal gland, we designed specific oligonucleotide primers from the brain sequence for further PCR. Sense (5'-CTCGCCGCACCTAACCCTGATGA-3') and antisense primers (5'-GGGCCTGTTTTGTGA TCTTGGAGT-3') were designed from the shark sequence and used in a PCR reaction with rectal gland and brain cDNA as template. The cycles used were: 35x (95 °C for 30 sec; 61 °C for 30 sec; 68 °C for 45 sec). Figure 2 shows the results of the PCR.

Figure 2. Amplification products of shark syntaxin 1B using specific PCR primers designed from brain sequence. All syntaxin 1B PCRs were performed with shark specific primers. Lane 1: syntaxin primers and shark brain cDNA; Lane 2: syntaxin primers and no cDNA (negative control); Lane 3: syntaxin primers and shark rectal gland cDNA; Lane 4: shark rectal gland cDNA template and A_0 adenosine receptor specific primers (positive control). Lane 5: no cDNA present and A_0 adenosine receptor specific primers. Lane 6: 1 kb DNA ladder.



In summary, we have cloned and sequenced a fragment of syntaxin from shark brain having highest homology to syntaxin 1B. By RT-PCR, this form is not expressed in shark rectal gland. We are determining the full-length sequence of syntaxin 1B from brain and are searching for other isoforms in the shark rectal gland to characterize the regulatory role of syntaxin in the function of CFTR.

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