CONSTRUCTION OF A *Squalus acanthias* CFTR EXPRESSION VECTOR TO STUDY EVOLUTIONARILY CONSERVED CFTR FUNCTIONS

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Cystic fibrosis (CF), a lethal genetic disorder, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). The protein has been described as a chloride channel as well as a regulator of the outwardly rectified chloride channel (ORCC) and the epithelial sodium channel (ENaC). CFTR is a member of the ATP-binding cassette (ABC) family of transporters, and is composed of two repeated motifs, each with a transmembrane domain and nucleotide binding fold, separated by a regulatory region unique to CFTR. The last twenty to thirty amino acids of the carboxy terminus are also CFTR-specific and highly conserved among species. The dogfish transmembrane conductance regulator (dCFTR) from *Squalus acanthias* exhibits CFTR-like chloride conductance in primary cultures of the dogfish rectal gland, but its ability to regulate other channels is unclear.

An expression vector containing the full-length dCFTR cDNA is being constructed to determine if dogfish CFTR can regulate human ORCCs. A 4.5 Kb cDNA reverse transcribed from dogfish rectal gland derived mRNA (pCRII-dCFTR) was obtained from Dr. John Forrest. Restriction enzyme analysis of pCRII-dCFTR revealed the loss of a *Bam* HI restriction site at nucleotide 3942 in the cDNA compared to the consensus sequence. An additional 1.5 Kb of the plasmid cDNA was sequenced and revealed ten missense mutations, four which would alter the predicted amino acid (T350A, I498T, C525R and L595P). The number of differences from wild-type sequence indicated the need for a different strategy utilizing partial dCFTR cDNA fragments from a dogfish rectal gland library (gifts of Dr. John Marshall). The dCFTR partial cDNAs are currently being sequenced to identify restriction sites suitable for ligation with the 5' cDNA region in pCRII-DFTR to create an accurate full-length dCFTR cDNA.

We are also investigating the functional role of the evolutionarily conserved C-terminus of CFTR. A chimera of green fluorescent protein (GFP) and residues 1370-1480 from human CFTR was created by a fusion PCR technique and cloned into a pCR 2.1 TA cloning vector. Sequencing confirmed the absence of amino acid changes. The construct was then subcloned into a RSV-driven expression vector, pAVS6, using the *Cla* I and *Xba* I restriction sites common to both plasmids. DH5α competent cells were transformed with pAVS6-GFP/CFTR and plated on LB/amp to isolate colonies. Subsequently, pAVS6-GFP/CFTR plasmid DNA was purified, and the DNA was quantitated by gel electrophoresis. Human IB3-1 airway epithelial cells were transfected with 3μg of DNA using lipofectin. Cells transfected with pAVS6-GFP/CFTR displayed punctate fluorescence compared to the cytoplasmic fluorescence characteristic of IB3-1 cells transfected with pAVS6-GFP. The cellular location of the carboxy-terminus chimera is currently being investigated by confocal microscopy.

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