TRAFFICKING OF CFTR IN KILLIFISH (*FUNDULUS HETEROCLITUS*) OPERCULAR MEMBRANE: RESPONSE TO ELEVATIONS IN cAMP AND ADAPTATION TO SEAWATER

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CFTR is a cAMP-activated Cl channel that mediates transepithelial Cl transport in a variety of mammalian epithelia including airway, intestine, pancreas, sweat duct and the opercular membrane of killifish. It is well accepted that stimulation of CFTR-mediated Cl secretion by cAMP involves protein kinase A-mediated phosphorylation of CFTR. In addition, in some but not in all epithelial cells, cAMP also stimulates CFTR-mediated Cl secretion by stimulating the insertion of CFTR into the plasma membrane from a cytoplasmic vesicular pool (Jilling, et al., *Int Rev Cytol.* 172:193-241, 1997). However, with the exception of studies in shark rectal gland (Lerich et al., *J. Clin. Invest.* 101:737-745, 1998), studies on CFTR trafficking have been conducted in immortalized cells in culture and/or heterologous cell lines over-expressing CFTR. The biological significance of CFTR trafficking in these models is unknown. Accordingly, the goals of this project were to: (1) Develop an *in vivo* model to study CFTR trafficking and (2) Test the hypothesis that physiological stimuli enhance CFTR mediated Cl secretion by inducing the insertion of CFTR into the plasma membrane from a cytoplasmic vesicular pool. To this end, studies were conducted on killifish opercular membrane, an epithelium that expresses high levels of endogenous CFTR (Marshall and Bryson. *Comp. Biochem. Physiol.* 119A(No1):97-106, 1998, Singer et al., *Am J Physiol.* 274:C715-23, 1998). Elevations in cAMP and an increase in the salinity of the swim water stimulate CFTR mediated Cl secretion by the operculum by an undefined mechanism.

Killifish were collected from Northeast Creek (Bar Harbor, ME) and held in aquaria containing running seawater at the MDIBL (August, 2001) or artificial seawater (Instant Ocean) at the Dartmouth Medical School (September, 2001 to January, 2002). Fish were kept in seawater for at least two weeks to acclimate. Adaptation to fresh water was achieved by gradually (~1 hr) reducing the aquaria salinity to 10% and maintaining the fish in 10% seawater for two weeks. Subsequently, the water was changed gradually (~1 hr) to dechlorinated tap water. Fish were maintained in fresh water for at least two weeks before the fish were returned to 100% seawater. The amount of CFTR in the plasma membrane of the opercular membrane was determined by cell surface biotinylation and SDS-PAGE (Loffing et al. *Am. J. Physiol.* 275: C913-920, 1998) using a monoclonal antibody that recognizes shark and killifish CFTR (60.1.2: J. Riordan, unpublished). Opercular membranes were isolated and mounted in Ussing chambers and the CFTR Cl current was measured as described (Ernst, et al., *J. Cell Biol.* 87:488-497, 1980). Similar results were obtained at the MDIBL and Dartmouth.

In fish adapted to 100% seawater, isoproterenol (10 μM) increased CFTR mediated Cl secretion across opercular membranes from 153 to 237 μA/cm^2 (15 minutes) and dramatically increased the amount of CFTR in the plasma membrane by 4.1-fold as determined by western blot analysis and densitometry (Figure 1). A cocktail of compounds that increase cAMP levels (db-cAMP, 500 μM; IBMX, 0.1mM and Forskolin, 1 mM) elicited a similar result. A chronic change in salinity from 100% seawater to freshwater reduced CFTR protein expression in cell lysates and the plasma membrane and reduced CFTR Cl secretion to ~0 μA/cm^2. 24 hours after the aquaria water was changed from fresh to 100% seawater CFTR Cl currents increased from ~0 to 93 μA/cm^2 and the amount of CFTR in cell lysates and the plasma membrane increased dramatically. Taken together, these data demonstrate that the opercular membrane of killifish is an excellent *in vivo* model to study CFTR trafficking and that physiological stimuli enhance CFTR mediated Cl secretion, in part, by inducing the insertion of CFTR into the plasma membrane from a cytoplasmic vesicular pool. (Supported by a MDIBL New Investigator Award to B.A.S., a NSF REU program DBI 9820400 award to M.E., a MDIBL New Investigator Award to J. E. M, and a Cystic Fibrosis Foundation award to A. L.).

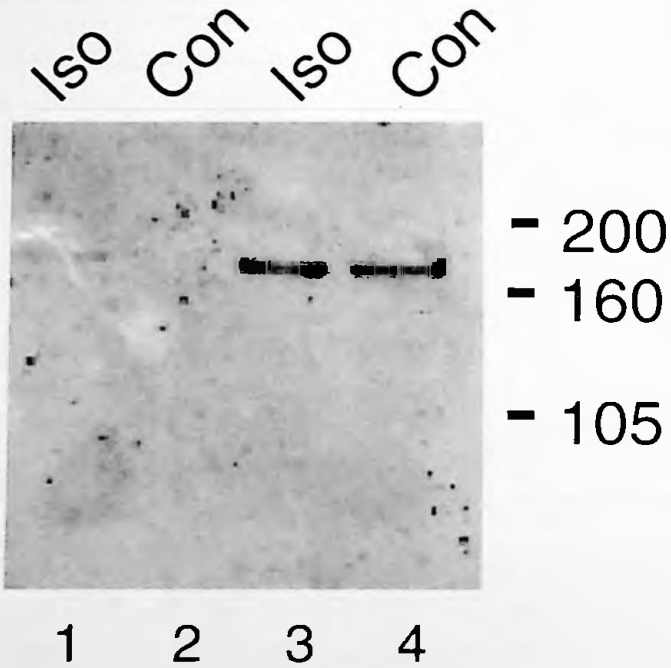

Figure 1. Isoproterenol stimulates CFTR-mediated Cl secretion in killifish opercular membranes, in part, by increasing the amount of CFTR in the plasma membrane. Representative Western blot of CFTR in the plasma membrane (lanes 1 and 2) and whole cell lysates (lanes 3 and 4) in killifish opercular membrane treated with isoproterenol (Iso: 10^{-5} M) for 15 minutes or vehicle (Con). CFTR was detected with monoclonal antibody 60.1.2 (1:1,000) and an anti-mouse HRP secondary antibody (1:5,000) by enhanced chemiluminescence (ECL: Pierce). Similar results were obtained in a total of three experiments. In each experiment the left opercular membrane was treated with isoproterenol and the right opercular membrane of each fish was treated with vehicle (four fish/experiment). 50 μg of protein was loaded in each lane. Overexposure of the blot reveals CFTR in the plasma membrane of control membranes (not shown).