CFTR ACCESSORY PROTEIN IDENTIFICATION VIA COMPARATIVE PROTEOMICS

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Cystic fibrosis (CF) is an inherited life-limiting lung disease caused by mutations in the CF transmembrane conductance regulator (CFTR). However, the nature of a mutation in the CFTR gene does not fully account for the phenotypic heterogeneity; instead, other genetic factors contribute to the etiology (Mickle, J.E. & G.R. Cutting, Clinics in Chest Medicine 19:443-458, 1998). Identification of these factors from airway epithelia is difficult because native tissue is relatively inaccessible and proteins such as CFTR are not abundantly expressed. Thus, to discover genetic modifiers that affect CF phenotype a comparative proteomic approach was employed.

CFTR has been identified in numerous species; one of the most divergent forms is from killifish, *Fundulus heteroclitus* (Singer, T. *et al.*, Am. J. Physiol. 43:C715-C723, 1998). Like human (h)CFTR, killifish (kf)CFTR is an apical membrane-associated ion channel in native epithelia (Lankowski, A. *et al.*, MDIBL Bulletin 41:7-78, 2002). In addition, kfCFTR is abundantly expressed and easily accessible in native gill and opercular epithelia for molecular and functional studies (Mickle, J.E. *et al.*, 39:75-77, 2000). Moreover, kfCFTR functions similar to hCFTR upon heterologous expression in mammalian epithelial cells (Mickle, J.E. *et al.*, Am J Hum Genet 65:2126, 1999a), which demonstrates that protein interactions of functional significance are conserved (Mickle, J.E. *et al.*, MDIBL Bulletin 41:29-30, 2002). To identify these proteins that interact with CFTR, Green Fluorescent Protein (GFP) and Glutathione-S-Transferase (GST) fusion constructs were utilized.

To function properly, CFTR first must be directed to the plasma membrane. The carboxyl terminus (C-ter) of hCFTR harbors sequence motifs that are important for localization to the apical membrane (Milewski, M.I. *et al.*, J Cell Sci 114:719-726, 2001). KfCFTR has similar motifs (Mickle, J.E. *et al.*, MDIBL Bulletin 38:66-67, 1999b). To determine if the corresponding motifs in kfCFTR affect apical trafficking two GFP-kfCFTR C-ter plasmid constructs were synthesized, namely GFP-kfCFTR C-ter delta 1 and GFP-kfCFTR C-ter delta 1/ TRL→AAA. Each construct was transiently and separately expressed in polarized mammalian epithelial cells (MDCK II) cultured at 37^0C and 5% CO_2 for 48 to 96 hours prior to immunolocalization and confocal microscopy. The C-ter motifs of kfCFTR (Mickle, J.E. *et al.*, 1999b *ibid*) affect apical localization (Figure 1) like those of hCFTR (Milewski, M.I. *et al.*, *ibid*). Consequently, functional conservation of the C-ter provides a basis to identify proteins involved in apical trafficking.

Accordingly, GST-CFTR C-ter fusion constructs were generated to isolate such proteins. In particular, GST-hCFTR C-ter fusions (courtesy of J. LaRusch and G. Cutting) were used to isolate accessory proteins from killifish. Firstly, killifish were collected from Northeast Creek (Bar Harbor, ME), and adapted to running seawater at MDIBL for two weeks before tissue isolation. Next, opercular epithelia were isolated, homogenized and the lysate passed through a

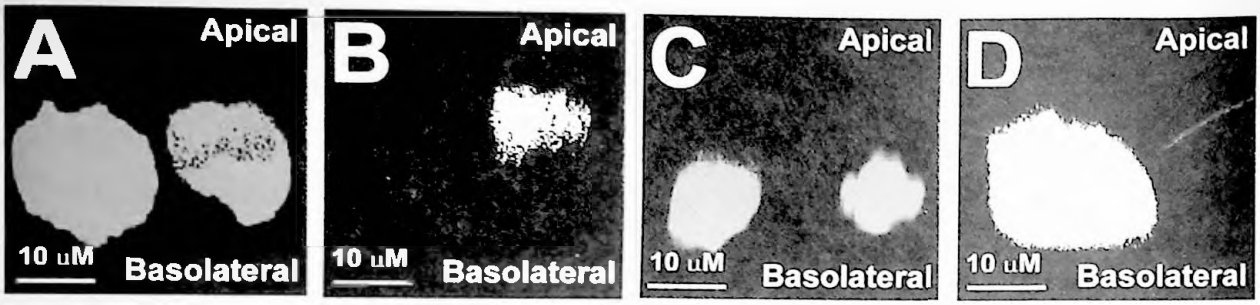

Figure 1. *Heterologous expression of kfCFTR C-ter in mammalian epithelial cells.* Confocal imaging reveals GFP-kfCFTR C-ter delta 1 is localized to the apical surface of polarized MDCK II cells (panel B). Alteration of the last three amino acids disrupts apical trafficking such that the GFP-kfCFTR C-ter delta 1/TRL→AAA peptide is expressed throughout the cytoplasm (panel D). Nuclei are stained with DAPI (Panels A and C, which correspond to the same cells in B and D, respectively).

column containing GST conjugated hCFTR C-ter delta 1 to capture accessory proteins that interact with the C-ter. Bound proteins were subsequently eluted and size separated by polyacrylamide gel electrophoresis. After staining the gel, select bands were excised then analyzed by mass spectrometry (MALDI-TOF). The resulting sequence signatures of killifish proteins that bound the hCFTR C-ter were queried in various databases to determine protein identity. One of the accessory proteins found by this approach shares homology to an actin-interacting protein, which complements earlier observations that CFTR activation is functionally linked to actin organization (Cantiello, H.F., Pflugers Arch 443:S75-S80, 2001). Overall, this comparative approach provides a means to identify CFTR accessory proteins, which are candidates for modifying phenotype and potential targets for therapeutic intervention. This research was supported by Cystic Fibrosis Foundation Student Traineeship Awards (JTB and CRS), a Salisbury Cove Research Fund Award (JEM) and a NIH grant DK027363 (JEM).