# TRAFFICKING OF DOGFISH, KILLIFISH AND HUMAN CFTR

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated Cl channel. Nearly seventy percent of individuals with cystic fibrosis (CF) are homozygous for the CFTR-ΔF508 mutation. CFTR-ΔF508 is trapped in the endoplasmic reticulum and does not reach the apical plasma membrane in airway epithelial cells. However, CFTR-ΔF508 retains function as a Cl channel. Therefore, identification of strategies for increasing CFTR-ΔF508 in the plasma membrane would have important therapeutic implications for the treatment of CF. To devise strategies for increasing CFTR-ΔF508 in the apical plasma membrane it is first necessary to identify the sorting determinants that direct CFTR to the apical membrane in polarized epithelial cells. However, little is known about the motifs involved in directing the polarized expression of CFTR in the apical plasma membrane.

PDZ domains, which are named for three proteins in which this domain was first described (PSD-95, Dlg and ZO-1), play an essential role in determining the polarized expression of proteins in neurons. A role for PDZ domains in the localization of proteins to the apical membrane of epithelial cells is also emerging. The C-terminal amino acids of CFTR (T-R-L) are highly conserved across species, including dogfish, killifish and human, and comprise a PDZ interacting domain which binds to PDZ domains in ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50), a protein which is polarized to the apical membrane in MDCK and other epithelial cells. We recently reported that the PDZ interacting domain of CFTR is required for the polarization of CFTR to the apical plasma membrane in MDCK and human airway epithelial cells (1). Our data also suggest that apical polarization of CFTR requires interaction with EBP50. Deletion of the C-inal amino acids of CFTR (<50 amino acids) does not affect the Cl channel function of CFTR, thus, C-terminal deletions of CFTR may result in defective vectorial chloride transport and cause CF in part by altering the polarized distribution of CFTR.

In addition to the PDZ interacting domain, there are several other potential sorting and trafficking motifs in CFTR. For example, a dileucine motif (LL: 1431-1432 in human CFTR) and a tyrosine based motif (YDSI: 1424-1427 in human CFTR) are potential sorting signals and may be involved in CFTR endocytosis. However, because these motifs are not conserved across species, their role in CFTR trafficking and the polarized expression in the epithelial cells are not clear. In dogfish shark CFTR (sCFTR) the tyrosine based motif is not conserved (i.e., the sequence is FDAL), yet the dileucine based motif is conserved. In killifish, the tyrosine based motif is conserved whereas the dileucine based motif is not conserved (i.e., the sequence is LM).

Our goal was to elucidate the role of the dileucine and tyrosine based motifs in the polarized expression of human, dogfish and killifish CFTR. To these ends we made chimeric constructs in which the green fluorescent protein (GFP) was linked to either wild-type (wt) CFTR or CFTR with mutations in the dileucine or tyrosine based motifs and expressed these proteins in polarized kidney epithelial cells (MDCK). Scanning confocal fluorescent microscopy was used to assess the cellular distribution of GFP-CFTR. Mutation of the dileucine motif to AA had no effect on the polarized expression of CFTR. Moreover, mutation of the tyrosine-based motif from YDSI to ADSI also had no effect on the polarized expression of CFTR. Thus, the dileucine and tyrosine motifs play no role in the polarization of CFTR to the apical plasma membrane in epithelial cells.

## REFERENCE

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