TRAFFICKING OF ABC TRANSPORTERS

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OBJECTIVES: ABC (ATP Binding Cassette) transporters, including CFTR and P-glycoprotein (Pgp), have received considerable attention because of their role in human disease including cystic fibrosis (CF) and multidrug resistance of cancer cells. CFTR is a cAMP-activated Cl channel. Pgp transport xenobiotics, including chemotherapeutic drugs, toxins, and steroids out of cells. Pgp is expressed in the brush border membrane of kidney proximal tubules where it mediates the transport of organic cations and xenobiotics into tubule fluid. Recent studies demonstrate that the intracellular trafficking of CFTR and Pgp is regulated. However, little is known about the amino acid motifs and accessory proteins involved in directing the regulated and polarized expression of CFTR and Pgp in the apical plasma membrane of epithelial cells. Thus, our goal is to identify accessory proteins involved in regulating CFTR and Pgp trafficking in epithelial cells.

PDZ proteins are accessory proteins that organize signaling complexes in well-defined regions of the cell, retain proteins in specific domains via interaction with the actin-based cytoskeleton, and regulate protein trafficking. We have identified two PDZ proteins that bind to CFTR via a PDZ interacting domain in the C-terminus of CFTR: NHERF (i.e., EBP50) and CAL (CFTR Associated Ligand) (Moyer, B.D., et al., J. Clin. Invest. 104:1353-1361, 1999, Moyer, B.D., et al., J. Am.Sco. Neph., 11:53A, 2000). However, little is known about the subcellular location and role of these PDZ proteins in regulating ABC transporter trafficking and function. NHERF is expressed in the brush border membrane of the proximal tubule, a nephron segment that also express Pgp and CFTR. NHERF binds cytoskeletal proteins and may retain CFTR in the apical plasma membrane (Moyer, B.D., et al., J. Clin. Invest. 104:1353-1361, 1999).

APPROACH: Western blot analysis was performed on shark rectal gland and Killifish operculum obtained from the Animal Core courtesy of our collaborator Dr. Forrest. COS and MDCK cells were maintained in the Instrumentation Core by standard techniques (Moyer, B.D., et al., J. Clin. Invest. 104:1353-1361, 1999). Co-immunoprecipitation and immunofluorescent microscopy were conducted in the Imaging Core using the Olympus Fluoview laser scanning confocal microscope (Moyer, B.D., et al., J. Clin. Invest. 104:1353-1361, 1999).

ACCOMPLISHMENTS AND RELEVANCE TO HUMAN HEALTH: We report here that CAL, a PDZ protein recently cloned, is expressed in kidney proximal and distal tubules, in the trans-Golgi network (Figure 1) and regulates export of CFTR from the trans-Golgi network (Moyer, B.D., et al., J. Am.Sco. Neph., 11:53A, 2000). We also conducted studies to determine if CAL and NHERF are expressed in Killifish operculum and shark rectal gland, organs that express high levels of CFTR. CAL, but not NHERF, was detected in Killifish operculum by Western blot analysis. By contrast, neither CAL nor NHERF could be detected in shark rectal gland. These studies suggest that CAL may regulate CFTR trafficking in Killifish operculum.

Pgp also has a potential PDZ interacting domain in the C-terminus. However, it is not known if Pgp interacts with PDZ proteins or if PDZ proteins regulate Pgp trafficking. Thus, we conducted studies to determine if PDZ proteins interact with and regulate Pgp trafficking. CAL and Pgp were co-immunoprecipitated in MDCK and COS cells. However, overexpression of CAL had no effect on the amount of Pgp expressed in the plasma membrane in COS cells. By contrast, although NHERF could not be co-immunoprecipitated with Pgp, NHERF reduced the amount of

Pgp in the plasma membrane of COS cells. These preliminary studies suggest that NHERF may regulate Pgp trafficking to the plasma membrane by an indirect mechanism and thus regulate the renal excretion of xenobiotics.

These studies suggest that Killifish operculum may be a useful model for studying the regulation of CFTR trafficking by PDZ and other accessory proteins. Moreover, these studies show for the first time that Pgp trafficking is regulated by PDZ proteins. Because CFTR and Pgp play an important role in human disease including CF and multidrug resistance of cancer cells these studies should increase our understanding of these human diseases. (Supported by a Pilot Project from the Center for Membrane Toxicity Studies, a Roy P. Forster Fellowship to BAS and Hancock Young Scholars Awards to ME and LE).

FUTURE DIRECTIONS: We plan on examining the role of CAL in the trafficking of CFTR in Killifish operculum during adaptation from fresh to seawater. Moreover, we plan on examining the expression and possible role of NHERF in regulating Pgp trafficking in killifish proximal tubule.

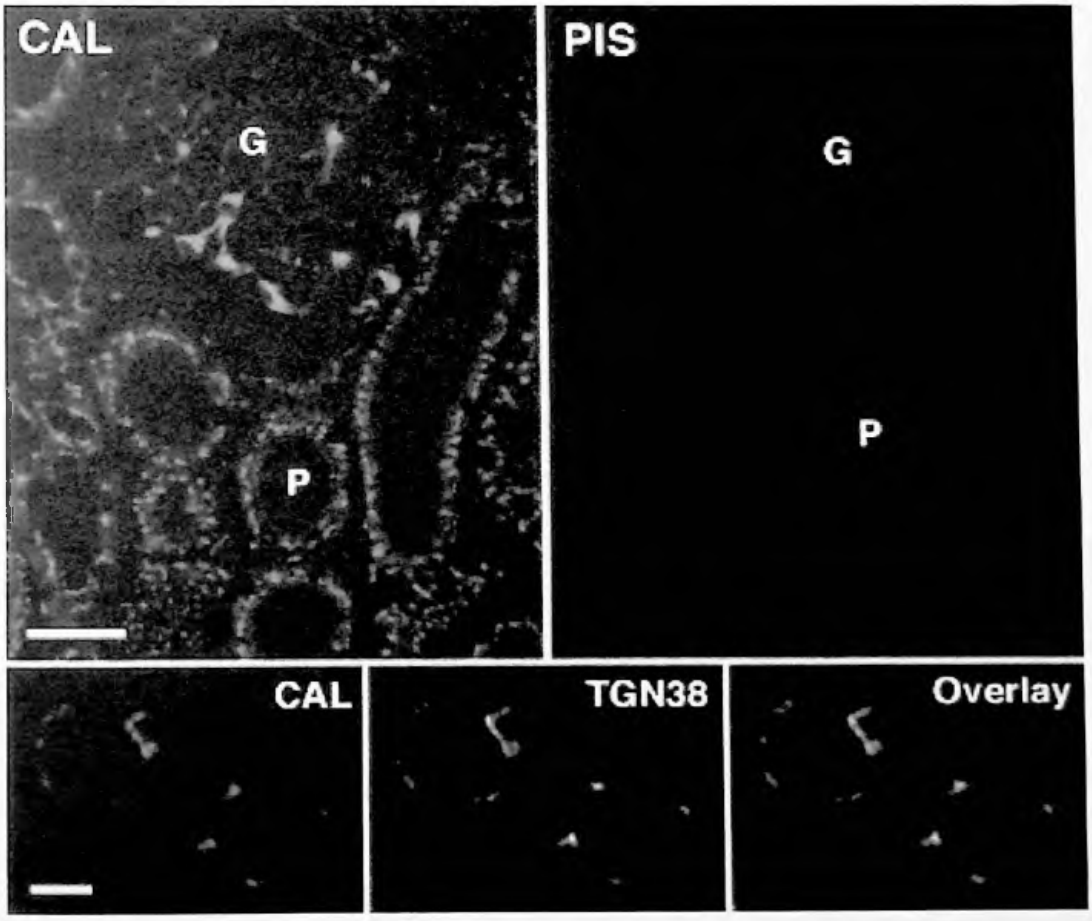

FIGURE 1. CAL was detected in rat kidney cortex using an anti-CAL polyclonal antibody and an anti-rabbit Texas red conjugated secondary antibody. G is glomerulus, P is proximal tubule. PIS is preimmune serum, TGN indicates the trans-Golgi network detected using an anti-TGN38 monoclonal antibody and an anti-mouse Alexa 488 conjugated secondary antibody. Upper two images: cortex. Scale bar = 50 microns. Lower three images: same section depicting the localization of CAL (left), TGN38 (center) and co-localization of CAL and TGN38 (right). Scale bar = 5 microns.