LUMINAL LOCALIZATION OF A CFTR-GFP FUSION CONSTRUCT IN INTACT KILLIFISH (*FUNDULUS HETEROCLITUS*) RENAL PROXIMAL TUBULES

David S. Miller[1], Timothy J. Jensen[2] and Jack R. Riordan[2]
[1]Laboratory of Pharmacol & Chemistry, NIH/NIEHS, Research Triangle Pk, NC 27709
[2]Mayo Graduate School of Medicine, Mayo Clinic, Scottsdale, AZ 85259

Cystic fibrosis is an autosomal recessive human genetic disorder that is caused by altered or lost function of a cyclic AMP-regulated chloride channel, the cystic fibrosis transmembrane conductance regulator (CFTR). One aspect of the disease appears to involve impaired trafficking of CFTR and failure to deliver the protein to its proper cellular location, the plasma membrane. Indeed, a single mutant allele (ΔF508), found in ~ 90% of patients causes mislocalization of CFTR and is associated with a severe form of the disease. Several approaches have been used to study CFTR trafficking, including, immunocytochemistry, subcellular fractionation and epitope tagging.

Here we describe a new experimental approach to the study of CFTR trafficking. It is based on green fluorescent protein (GFP) technology and a unique assay system using intact killifish (Fundulus heteroclitus) renal proximal tubules (Sweet et al, Am. J. Physiol. 276:F864-F873, 1999). Each component of the system offers distinct advantages. GFP is ideal for use in a wide variety of organisms, since fluorescence is stable and species independent. Moreover, GFP expression and localization can be monitored in living cells and tissues. The teleost kidney provides a good source of intact renal tubules (mostly proximal segment) that retain polarity and transport function for long periods of time (Miller, Environ. Health Perspect. 71:59-68, 1987).

GFP was ligated to the C-terminus of CFTR and cloned into the pCDNA3 expression plasmid. Freshly dissected tubules were transfected using Effectine reagent. After 1 hour, excess cell culture medium was added, the preparation was incubated overnight and protein expression in living tubules was examined using confocal microscopy.

Figure 1 shows representative confocal micrographs of transfected tubules. Those transfected with GFP alone show a diffuse fluorescence, consistent with cytoplasmic localization (Fig. 1A). Tubules transfected with the CFTR-GFP construct show a distinct, but somewhat diffuse, band of fluorescence at the luminal membrane of the tubular epithelial cells (Fig. 1B). In contrast, tubules transfected with the ΔF508-CFTR-GFP showed no such luminal localization (not shown). These results show insertion of GFP-labelled CFTR in the luminal membrane of the cells in an intact, native renal epithelium, indicating that signals directing the localization of human CFTR are recognized in teleost cells. Furthermore, the endoplasmic reticulum quality

control apparatus which can distinguish wild-type human CFTR from ΔF508 is also conserved in the cells of teleost kidney. Hence this preparation may be of value in further studies of CFTR trafficking.

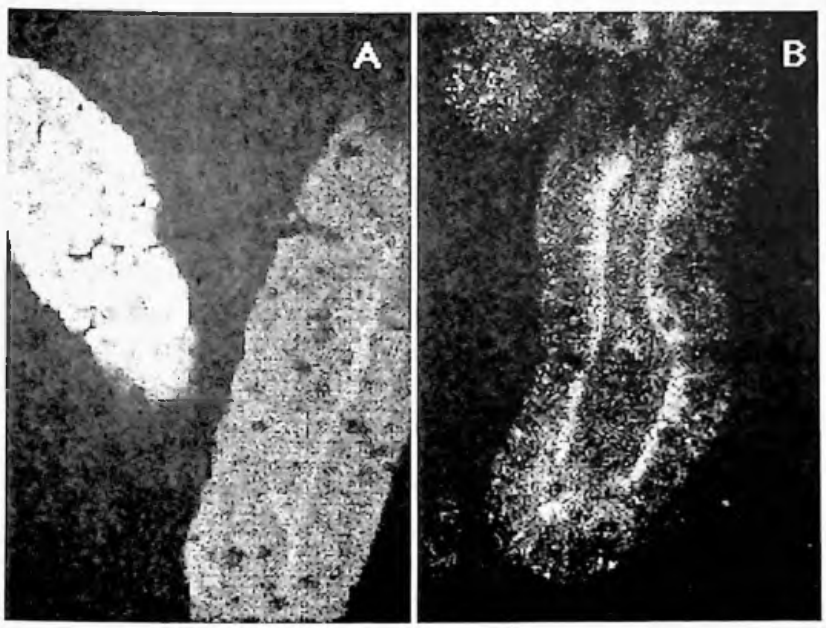

Figure 1. Confocal micrographs of living killifish renal tubules transfected with wild-type GFP (A) and CFTR-GFP (B).