PROCESSING AND LOCALIZATION OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR IN GILL AND OPERCULUM FROM *FUNDULUS HETEROCLITUS*

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Cystic fibrosis (CF) is a disorder of epithelial cell ion transport caused by mutations in the CF transmembrane conductance regulator (CFTR). In human airway epithelial cells CFTR functions as a chloride channel and a regulator of several different ion channels such as outwardly rectifying chloride channels (ORCCs). Both functions implicate CFTR as a key component in the coordination of ion movement across apical membranes of airway cells. Absence or dysfunction of CFTR alters the salinity of airway surface fluid which initiates a cascade of events that culminate in pulmonary disease. CFTR is highly conserved among species, and CFTR from *Fundulus heteroclitus* (killifish) is the most divergent form cloned to date (Singer T. D. *et al.*, Am. J. Physiol. 43:C715-C723, 1998). Killifish are euryhaline estuarine teleost capable of rapid acclimation from fresh water to salt water. The adaptation is mediated in a biphasic manner through specialized cells (chloride cells) in gill and opercular epithelium of killifish (Zadunaisky, J.A. Kidney Intl. 49:1563-1567, 1996). Functional studies reveal that a CFTR-like anion conductance is present at the apical membrane of isolated chloride cells, but an associated outward rectifying anion conductance is not appreciable (Marshall, W.S. *et al.*, Am. J. Physiol. 268: R963-R969, 1995). To determine whether the functional profile from chloride cells is consistent with CFTR expression, we investigated the synthesis and localization of CFTR in killifish gill and opercular epithelia.

Killifish CFTR (kfCFTR; 1503 a.a.) shares the highest degree of amino acid identity, 60%, with the shark homologue (sCFTR; 1492 a.a.). Among mammalian homologues, kCFTR is most like human (hCFTR; 1480 a.a.), 59% identical. Since kfCFTR-specific antibodies have not been developed, we tested whether antibodies developed for sCFTR (60.1.2 and 76.1.2) and hCFTR (24.1, M3A7, 155 and L12B4) would also detect kfCFTR. Gill and operculum were dissected from seawater adapted killifish for Western analysis. The samples were homogenized under denaturing conditions and protein was quantitated by Lowry assay prior to separation by 6% PAGE, transfer to PDVF membrane (Amersham, Piscataway NJ) and incubation with mouse monoclonal anti-CFTR 1° antibodies (1:2000 dilution). Subsequent incubation with horseradish peroxidase (HRP) conjugated anti-mouse 2° antibody (1:5000 dilution, Amersham) provided a means for chemiluminescent detection (Pierce, Rockford IL) of immobilized kfCFTR. The membranes were exposed to imaging film and signal was sized against molecular markers

(RPN800, Amersham) run in parallel. Specificity of 1° antibodies was confirmed using hCFTR and sCFTR controls. kfCFTR was detected with 24.1 and, to a lesser extent, 60.1.2 and 76.1.2. The protein from gill migrated as two forms approximately 160 kDa and 210 kDa in size. CFTR from the operculum was detected as a single band at 160 kDa.

To identify the location of kfCFTR in gill and operculum, tissue was collected from seawater adapted fish for immunocytochemistry. Specimens were fixed, permeabilized, and incubated with mouse monoclonal anti-CFTR 1° antibodies (24.1, 60.1.2 and 76.1.2; 1:100 dilution) as whole tissues. Anti-mouse 2° antibodies (1:100 dilution) conjugated to a fluorophore (FITC or Cy3) were used to detect kfCFTR by confocal microscopy. kfCFTR was detected along the perimeter of gill filaments, particularly at the base of respiratory lamellae. Mitochondria-rich chloride cells are known to occur in the secretory epithelium between respiratory lamellae (Karnaky, K.J. Jr. and W.B. Kinter, J. Exp. Zool. 199:355-364, 1977). To determine whether chloride cells are the sites of kCFTR expression, tissue samples were frozen in embedding medium, sectioned and incubated simultaneously with 1° antibodies for CFTR (60.1.2) and Hsp60, a mitochondrion-specific marker (rabbit polyclonal at 1:100 dilution). Different fluorophores were conjugated to the 2° antibodies (anti-mouse/FITC [1:100] and anti-rabbit/Cy3 [1:300]) to enable detection of each antigen upon epifluorescent microscopy. Images were captured with a cooled charged coupling device (CCD) camera and overlaid. CFTR and Hsp60 co-localize at the base of respiratory lamellae which suggests that chloride cells are the site of kfCFTR expression (Figure 1).

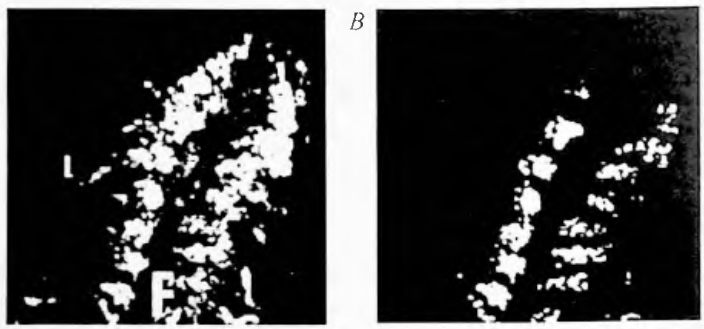

Figure 1. Immunolocalization of CFTR in killifish gill. A. Longitudinal section of gill filament stained for Hsp60. The area in white, which is conspicuous at the base of respiratory lamella where chloride cells prevail, depicts the presence of the mitochondrial marker Hsp60. The fluorescent image was taken at 100x magnification with a cooled CCD camera and shown in grayscale contrast. The section is 10μM thick. *B.* Same section counter-stained for CFTR. The sites of CFTR expression overlap the area where mitochondria-rich chloride cells are abundant. F: filament, L: respiratory lamella.

In killifish gill, CFTR appears to be expressed, predominantly in chloride cells, as two forms (160kDa and 210 kDA). In operculum only the 160kDa form was detected; CFTR localization in this tissue awaits further refinement. The different forms may be a consequence of alternative RNA splicing or partial glycosylation; both possibilities are being investigated. Perhaps these forms exhibit different functional properties that account for the absence of an associated outward rectifying anion conductance in chloride cells. Identification of CFTR alterations that impact function may elucidate integrative mechanisms of ion regulation relevant to CF pathophysiology.

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