

Membrane Transport in Cystic Fibrosis

a report by

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A brief analysis of transport via cystic fibrosis transmembrane conductance regulators (CFTRs) in various organ systems can highlight the importance of basic membrane transport processes across epithelial cells in the pathophysiology of cystic fibrosis (CF).

CF—The Disease

According to data from the Cystic Fibrosis Foundation (CFF), more than 10 million white Americans are unknowing, asymptomatic carriers of a defective gene for CF, and one out of every 3,200 live Caucasian births in the US has CF.

One of the main problems for many individuals with CF is blockage of the outflow of digestive enzymes from the exocrine pancreas into the small intestine, and the resultant pancreatitis that can lead to the cystic changes in the pancreas previously noted by Dorothy Andersen in 1938. A second major difficulty in CF (and probably the most devastating for the long-term health of the individual) is the accumulation of heavy dehydrated mucus in the airways and the resultant changes in the capability of the lungs to fight infections. A third major change—which leads to the ability to use the sweat chloride test as the standard diagnostic test for the disease—is the abnormally salty sweat of CF patients. A fourth clinical manifestation is sterility in both males and females; this is particularly prevalent in males and may be irreversible. A fifth manifestation, which may be recognized less frequently than some of the others, is abnormal secretion in the small intestine.

Mutations Causing CF

How do the various CF mutations affect the function of the CFTR proteins? The various mutations that have been shown to cause CF have been categorized into four classes:

- Class I mutations cause defective protein production, with a total loss of functional CFTRs. One defect produces a truncated CFTR due to premature stop mutants. This defect may be corrected by certain antibiotics that bypass the shortened protein to make

full-length functional CFTR. These antibiotics have restored 25–35% CFTR protein function when only 10% correction appears to be enough for noticeable improvement in patients.

- Class II mutations cause defective protein processing leading to CFTR that is not in its correct location in the cell or that is different from CFTRs in normal individuals (fewer glycoproteins and gangliosides on the cell surface in CF cells). The most common mutation found in 70% of CF patients (the delta F508 deletion) is one of these class II mutations.
- Class III mutations cause defective regulation of channel opening of CFTR by changes in the nucleotide binding fold or R domain of CFTR.
- Class IV mutations cause defective ion conduction through CFTRs. These mutations are in the membrane-spanning domains of the protein and thus affect the pore that normally allows ion fluxes.

Class I and II mutations generally lead to the more serious phenotype of the disease and have accompanying pancreatic insufficiency. Class III and IV mutations generally lead to a less serious phenotype with normal pancreatic function.

Regulation of CFTR

How are CFTRs in normal, healthy people regulated? Various studies of CFTR protein function have shown that, in the absence of phosphorylation of the regulatory (R) domain, the channel is closed and chloride ion transport ceases. Cyclic adenosine monophosphate (cAMP) stimulates protein kinase A to phosphorylate one to four serine residues on the R domain and, depending upon how many sites are phosphorylated, the CFTR may be activated to varying degrees. Sequential phosphorylation of at least three sites increases the likelihood of high-affinity binding of adenosine triphosphate (ATP) to the nucleotide binding fold and enhances the open probability of the channel. When the nucleotide binding folds (NBFs) on CFTR bind ATP, a conformational change occurs. ATP hydrolysis has been

shown to be necessary for the conformational change in the pore and may be located in or near the membrane-spanning portion of the pore. These changes may lead to as many as three different gating states of the pore, including closed, open only briefly, and open for long periods of time. In addition, the open probability and voltage-dependent fast gate of CFTR may be dependent upon tyrosine phosphorylation.

CFTRs are cAMP and protein kinase A-regulated chloride channels that also regulate numerous other ion channels. Recent studies have shown that at least 13 different transporters interact with CFTR by being inhibited or activated, and that a common motif for protein interaction called the PDZ-binding domain is likely involved.⁵ Evidence indicates that CFTRs play important roles in the transcellular secretion of bicarbonate by serving as the conductive pathway for hydrocarbonate (HCO_3^-) exit across the apical membranes in HCO_3^- -secreting cells.

Transport Problems

At the cellular level, how do mutations leading to CF cause the transport problems in the various organs/systems that are recognized symptoms of CF? Briefly, in the lung upper airways, there is decreased Cl^- secretion into the lumen, increased serum sodium (Na^+) absorption through epithelial Na^+ channels (ENaC) out of the lumen, possibly due to loss of an inhibitory influence of functional CFTRs and/or ENaCs stimulating the activity of CFTR up to six-fold, and normal but not CF CFTR may activate water permeability through aquaporins there.

In sweat glands, sweat is normally produced at the base of the glands and then passes through a narrow duct where reabsorption of salt occurs (in CF abnormal Cl^- absorption out of the duct via defective CFTRs leads to excessive Na^+ and Cl^- (three to five times the normal concentration) in sweat. In the liver of CF patients, plugging of small bile ducts based on lack of Cl^- secretion impedes digestion and disrupts liver function; however, liver involvement may be asymptomatic and may be slowly or not at all progressive. In the pancreas, occlusion of ducts prevents pancreatic enzymes from reaching the lumen of the intestine and may lead to premature activation of digestive enzymes inside the pancreas, causing pancreatitis that may also cause diabetes mellitus.

The lack of digestive enzymes in the intestinal lumen can cause symptoms including frequent, loose, oily, and malodorous stools caused by steatorrhea (fat in stool due to lack of active pancreatic lipases). Eighty to 85% of CF patients have pancreatic insufficiency, which leads to decreased availability of both digestive enzymes and

bicarbonate in the intestinal lumen and clinical maldigestion and malabsorption based on the lack of Cl^- secretion. Fortunately, oral pancreatic enzyme supplementation after meals appears to partially override the digestive problems. In the intestine, obstruction of the gut by a “thick, dehydrated, rubbery, tarry, tenacious, mucoid plug”⁹ leads to meconium ileus, which necessitates surgery in 10% of newborns with CF. In addition, knockout mice with no CFTRs (model developed in 1992) will die from intestinal obstruction by five weeks of age and their intestines exhibit an inflammatory state leading to an innate immune response that may cause additional tissue destruction and pathogenesis. Constipation and distal intestinal obstruction syndrome also occur in older CF patients (CF patients have been shown to have loss of CFTR function in their colons) with rectal prolapse being common (usually prior to pancreatic enzyme therapy).

All of these situations are based on lack of Cl^- secretion. In the reproductive tract, absence of fine ducts (i.e. vas deferens) developmentally renders 95% of CF males infertile. The speculation is that the faulty development is due to blockage of the ductules *in utero*, so that most adult CF males are aspermic or hypospermic. CF women show a significantly lower fertility rate due to physical mucus plugs in the fallopian tubes, which block sperm from fertilizing ova. These transport problems are based on the lack of Cl^- secretion. Thus, in multiple organs and systems, ion and water imbalance contributes to the pathophysiology by failing to dilute the native mucus sufficiently to maintain its normal fluidity. This dehydrated mucus becomes very viscous and relatively immovable.

It is now known that CFTR is the transporter required for chloride (and accompanying water) secretion both in the intestines and in the upper airways of the lungs. Mutations in CFTR lead to various expressions of CF, which is still a lethal genetic disease. CFTR inhibitors might be useful to establish the CF phenotype in cells and animal models in order to investigate more specifically the mechanisms of recurrent lung infections and lung tissue destruction eventually leading to death in patients with cystic fibrosis. A more detailed version of this analysis including the physiological relevance of treating CF symptoms is found elsewhere. ■

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A version of this article containing references can be found in the Reference Section on the website supporting this briefing (www.touchrespiratorydisease.com).